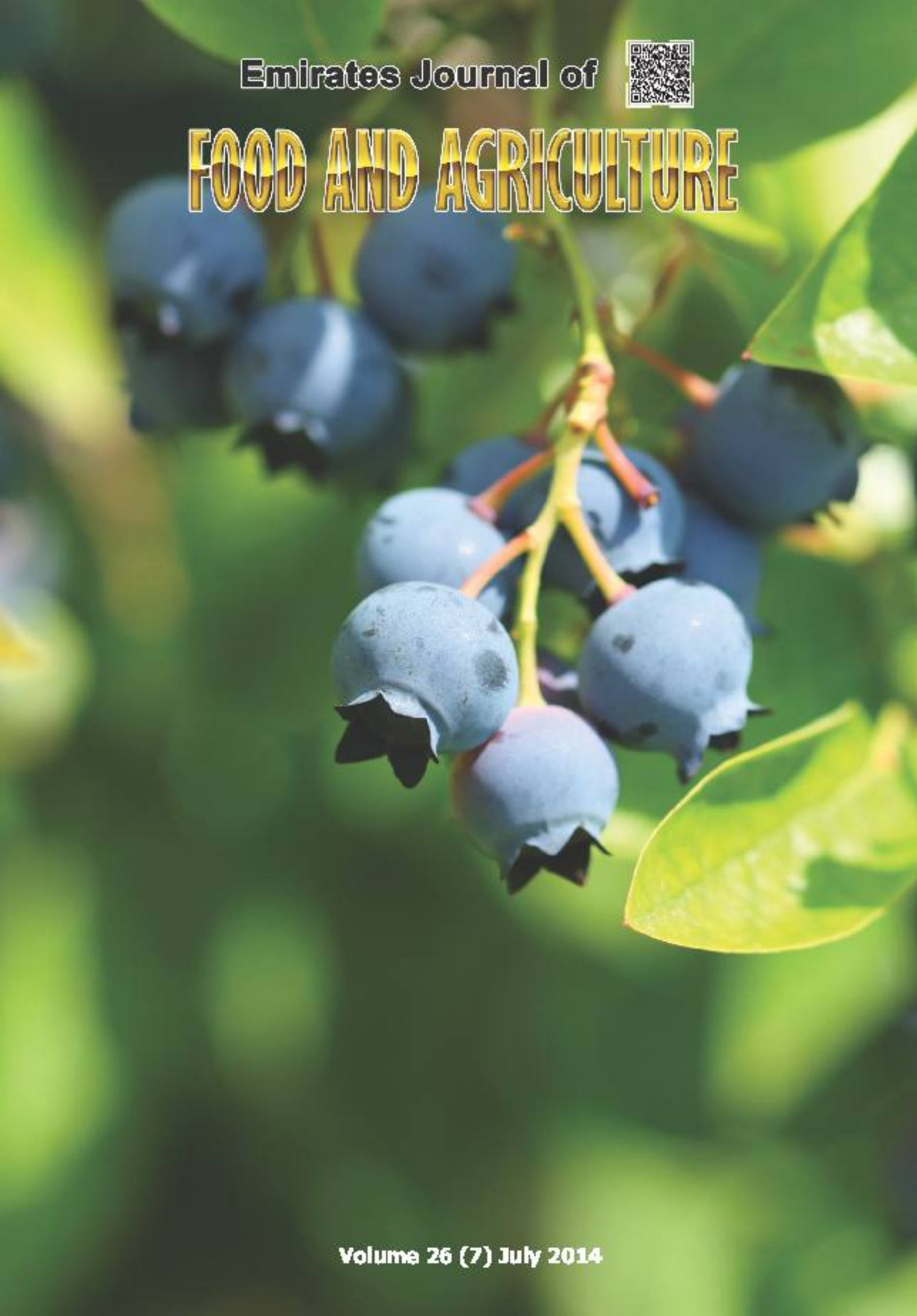

Volume 26 (7) July 2014

College of Food and Agriculture
جامعة الإمارات العربية المتحدة
United Arab Emirates University

EMIRATES JOURNAL OF FOOD AND AGRICULTURE

[Formerly known as EMIRATES JOURNAL OF AGRICULTURAL SCIENCES]

ISSN 2079-052X (Print)
ISSN 2079-0538 (Online)

(Monthly)

Volume 26 No. 7
July 2014

Special Issue on "Medicinal and Edible Plants and Their Application"

An International Refereed Monthly Research Journal Published By:

College of Food and Agriculture
United Arab Emirates University
P.O. Box 15551, Al Ain
United Arab Emirates
Phone: +971-3-7134576
Email: ejfa@uaeu.ac.ae
Website: http://www.ejfa.info/

Subscription Information: Emirates Journal of Food and Agriculture is published in both online and print form. Full text is available as open access through online in the website http://www.ejfa.info/. The printed copies can be obtained free of charge, by requesting through email ejfa@uaeu.ac.ae.

JOURNAL RANKING

Ranked in SCIMago	**:**	**H Index: 3**
SJR (SCImago Journal Rankings)	**:**	**0.153 [2012]**
SNIP (Source Normalized Impact per Paper)	**:**	**0.220 [2012]**
Index Copernicus / IC Value	**:**	**7.61 [2012]**
National Academy of Sciences / NAAS Rating	**:**	**5.34 out of 6 [2013]**
Google Scholar Metrics	**:**	**h5-index: 7, h5-median: 10**

ABSTRACTED / INDEXED / LISTED IN

1. ABI/INFORM Complete
2. ABI/INFORM Trade & Industry
3. Academic Index
4. Academic Keys
5. Academic Search Complete
6. Academic Research Library
7. Academic Resources
8. AFFRIT-ALIS
9. AgBiotech News and Information
10. AgBiotechNet
11. AGORA
12. AGRICOLA
13. Agricultural Econ. Database
14. Agriquest
15. AGRIS/ CARIS (FAO)
16. Agroforestry Abstracts
17. Animal Breeding Abstracts
18. Animal Science Database
19. Animal Production Database
20. ASCI Database
21. Arab Repository
22. BASE
23. Biblioteca
24. Biocontrol News & Information
25. Biofuels Abstracts
26. Biological Sciences Database
27. Birmingham Public library
28. Botanical Pesticides
29. CAB Abstracts
30. CAB Full Text Repository
31. CAB Direct
32. CardCat
33. Career and Technical Education
34. Catalyst
35. Crop Physiology Abstracts
36. Crop Science Database
37. CrossRef
38. CrossCheck
39. Dairy Science Abstracts
40. DOAJ
41. EBSCO Publishing
42. EBSCO*host*
43. E-Journal Consortium
44. Environmental Impact
45. Environmental Science Database
46. Env. Sci. Poll. Manage Database
47. EVISA
48. Field Crop Abstracts
49. Forest Products Abstracts
50. Forest Science Database
51. Forestry Abstracts
52. FSTA (IFIS Publishing)
53. Gale | Cengage Learning
54. Genamics Journal Seek
55. GeonD
56. GFMER
57. Global Health
58. Global Issues
59. GOAP-UNESCO
60. Google Scholar
61. Grasslands and Forage Abstracts
62. Hinari (WHO)
63. HKULib
64. Horticultural Science Abstracts
65. Horticultural Science Database
66. Index Copernicus
67. Index Veterinarius
68. infoSAT
69. InfoTrac-Academic OneFile
70. InfoTrac - Agriculture Collection
71. InfoTrac Custom
72. Invasive Species Compendium
73. Irrigation and Drainage Abst.
74. ISC Master Journals List
75. iSEEK
76. I-Share
77. IUCat
78. J-Gate
79. Journal Finder
80. Journals for Free
81. JournalTOCs
82. Koha Journal Finder
83. LISGateway
84. LivRe!
85. Lupton Library
86. Maize Abstracts
87. Mathewson-IGT K Cent.
88. MeLCat
89. Melvyl
90. MU Libraries
91. NAAS
92. NAL
93. NewJour, USA
94. Nutr. Abst. A: Human & Exp.
95. Nutr. Abst. B: Livestock Feeding
96. Nutr. Food Sci. Database
97. OAJSE
98. OhioLINK
99. OJS Database
100. Open J-Gate
101. OpenPub
102. ORBi Database
103. Ornamental Horticulture
104. PASTIC
105. Peter Scott's Library
106. Pig News and Information
107. PLEIADI
108. Plant Breeding Abstracts
109. Plant Genetic Resources Abst.
110. Plant Gen. Breeding Database
111. Plant Growth Regu. Abst.
112. Plant Protection Database
113. Postharvest News & Information
114. Potato Abstracts
115. Poultry Abstracts
116. Prof. ABI/INFORM Compl.
117. Professional ProQuest Central
118. ProQuest Agriculture Journals
119. ProQuest Central
120. ProQuest Engineering Journals
121. ProQuest Nat. Science Journals
122. ProQuest SciTech Journals
123. ProQuest Technology Journals
124. PRORCH
125. RefSeek
126. ResearchBib
127. Research Library
128. Review of Agric. Entomology
129. Review of Arom. Med. Plants
130. Review of Med. & Vet.
131. Entomology
132. Review of Plant Pathology
133. RICeST
134. Rice Abstracts
135. RoMEO
136. Rural Development Abstracts
137. Scirus
138. SCIMago
139. SCOPUS
140. Seed Abstracts
141. SJSU
142. SOCOLAR, China
143. Soils and Fertilizers Abstracts
144. Soybean Abstracts
145. SU-Landesbibliothek Saarb.
146. Summon
147. TIBORDER
148. The British Library
149. The Open Access Digital Lib.
150. Soil Science Database
151. TROVE
152. trueserials.com
153. UlrichsWeb
154. Veterinary Bulletin
155. VetMed Resource
156. Wageningen URLC
157. Weed Abstracts
158. Wheat Barley Triticale Abst.
159. World Ag. Econ. R. Sc. Abst.
160. WorldCat
161. Zeitschriftendatenbank
162. ZDB
163. Zurich Open Repository
164. OneSearch
165. GSSD
166. ROAD

EDITORIAL BOARD

CONTENTS

Emir. J. Food Agric. 2014. 26 (7)
http://www.ejfa.info/

EDITORIAL

SILAE_EJFA Special Issue: Medicinal and Edible Plants and Their Application

Luca Rastrelli

Dipartimento di Farmacia, University of Salerno, Italy
Email: rastrelli@unisa.it

SILAE is a Free Scientific Web Community at www.silae.it dedicated to advancing science around the world by serving as an educator, leader, spokesperson and professional association.

In this new SILAE_Special Issue of the Emirates Journal of Food and Agriculture it is my pleasure to highlight a review, an ethnobotanical paper; six analytical studies, a pharmacological paper and an agronomic paper.

Vaccinium (*Ericaceae*) is a cosmopolitan well known genus that reaches 450 species. In different places its berries are consume as food and, currently there are in the market *Vaccinium* spp. based herbal medicine and functional. In their review, Abreu et al. (2014) focused on its therapeutical potential. They found reports of medicinal use of 36 *Vaccinium* species, mainly from North America, Asia and Europe countries. More than 70 diverse uses were compiled, mainly from the systems: digestive, genitourinary and endocrine/metabolic.

Etnobotanical data are very important base for further pharmacological tests. Kozuharova et al. (2014) have investigated the not yet documented therapeutic effect of some popular Bulgarian medicinal plants.

The year 2013 has been declared "The International Year of the Quinoa" (IYQ). *C. quinoa* and *C. pallidicaule* (quinoa and cañihua) have been cultivated as a food crop for centuries in Latin America. Currently, descendants of the Inca Empire still use its seeds as an important component in their diet, and by-products are partially used in animal nutrition. Gallego et al. (2014) reported the chemical composition of cañihua and quinoa (*Chenopodium quinoa*) in relationship with wheat, corn, rice, rye and evaluated the nutritional aspects in human and animal.

Three papers present the compositions of different compounds using analytical techniques such as GC, GC/MS, Arazo et al. (2014) identified 61 constituents from the hexane extract of *Garcinia tinctoria* fruits, used in Cuba for culinary purposes and as folk medicine to treat skin infections, wounds, and diarrhea. Rios et al. (2014) reported the essential oil composition of a propolis sample collected in Falcon state of Venezuela. Twenty-three compounds were identified (93.6%), of which the three major compounds were germacrene D (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%). Elaloui et al. (2014) identified thirteen fatty acids from the pulps of four tunisian *Ziziphus jujuba* ecotypes, deciduous shrub which belongs to Rhamnaceae family. This plant is used in in Algerian and Tunisian traditional medicine for its anti-diabetic, sedative, analgesic, anti-inflammatory and hypoglycaemic activities.

An aqueous decoction of mango (*Mangifera indica* L.) stem bark has been developed in Cuba on an industrial scale to be used as a nutritional supplement, cosmetic, and phytomedicine and mangiferin was found to be the predominant component. Salomon et al. (2014) reported the microwave-assisted extraction of mangiferin and the improved extraction efficiencies whereas Castellanos et al. (2014) reported a new LC method for the analysis of mangiferin in the *Mangifera indica* L. stem bark extract and pharmaceuticals. Method showed good selectivity, repeatability and linearity. Validation of the method has shown its usefulness in the quantitative routine analysis of mangiferin in extracts and pharmaceutical form of this Mango extract.

Martinez et al. (2014) validated the hypoglycemic effect of *Azadirachta indica* (neem) in human blood cells in a normoglycemic medium.

Barley production in Brazil has been used by beer makers for malting and food. During the barley development several fungal diseases have been detected. Spot blotch is caused by *Bipolaris sorokiniana* (asexual fungi), and is the most serious deleterious diseases for the producers that can be affect the ears, darkening the grains and impairing the quality of malt and beer. Bach et al. (2014) reported the activity of *Bauhinia variegata ecxtract* as elicitor of local and systemic resistance in barley plants against *Bipolaris sorokiniana* upon 90% of protection.

The editor would like to thank the contributors who gave so generously their time and experience and who made this publication a valuable tool for scientists in the field of edible and medicinal plants chemistry, analysis and biology.

Thanks are also due to the referees for their valuable comments and for the very detailed and accurate review of manuscripts; their comments certainly helped to improve the papers.

The editor is also very grateful to the Editorial Board of EJFA for embracing this project with interest and enthusiasm, and for the opportunity to publish this new SILAE Special Issue in this attractive and interesting journal.

References

Abreu, O. A., G. Barreto and S. Prieto. 2014. *Vaccinium* (Ericaceae): Ethnobotany and pharmacological potentials. Emir. J. Food Agric. 26(7):577-591.

Arazo, M., M. Hernández, A. Bello, D. Marrero and A. Al-Shagdari. 2014. Gas Chromatography-Mass Spectrometry study of the pulp of *Garcinia tinctoria* fruit. Emir. J. Food Agric. 26(7):643-646.

Bach, E. E., S. G. Costa, H. A. Oliveira, J. C. Lima, M. B. F. Oliveira, V. O. Cardoso and N. S. Y. Wadt. 2014. *Bauhinia variegata* Linn. leaf extract controls spot blotch in barley plants. Emir. J. Food Agric. 26(7):630-638.

Castellanos, E. G., R. G. Hernández, L. Nuevas Paz and J. Hoogmartens. 2014. Determination of mangiferin in *Mangifera indica* L. stem bark extract (Vimang®) and pharmaceuticals by liquid chromatography. Emir. J. Food Agric. 26(7):592-601.

Elaloui M., A. Laamouri, A. Albouchi, M. Cerny, C. Mathieu, G. Vilarem and B. Hasnaoui. 2014. Chemical compositions of the Tunisian *Ziziphus jujuba* oil. Emir. J. Food Agric. 26(7):602-608.

Gallego, D. Y. V., L. Russo, K. Kerbab, M. Landi and L. Rastrelli. 2014. Chemical and nutritional characterization of *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds. Emir. J. Food Agric. 26(7):609-615.

Kozuharova, E., N. Benbassat and I. Getov. 2014. Ethnobotanical records of not yet documented therapeutic effects of some popular Bulgarian medicinal plants. Emir. J. Food Agric. 26(7):647-651.

Martínez, N., Y. Rodríguez, O. Salguero, D. Requena, L. Triana and L. Pérez-Ybarra. 2014. A study of hypoglycemic effects of *Azadirachta indica* (Neem) in human blood cells. Emir. J. Food Agric. 26(7):623-629.

Rios, N., C. Yánez, L. Rojas, F. Mora, A. Usubillaga and P. Vit 2014. Chemical composition of essential oil of *Apis mellifera* propolis from Falcón State, Venezuela. Emir. J. Food Agric. 26(7):639-642.

Salomon, S., I. Sevilla, R. Betancourt, A. Romero, L. Nuevas-Paz and J. A. Esquijarosa. 2014. Extraction of mangiferin from *Mangifera indica* L. leaves using microwave-assisted technique. Emir. J. Food Agric.. 26 (7): 616-622

Emir. J. Food Agric. 2014. 26 (7): 577-591
doi: 10.9755/ejfa.v26i7.16404
http://www.ejfa.info/

REVIEW ARTICLE

Vaccinium (Ericaceae): Ethnobotany and pharmacological potentials

Orlando A. Abreu[1*], Guillermo Barreto[1] and Sylvia Prieto[2]

[1]*Department of Foods Science and Technology, Faculty of Chemistry, Universidad de Camagüey, Cuba*
[2]*Centro de Química Farmacéutica, La Habana, Cuba*

Abstract

Vaccinium (Ericaceae) is a cosmopolitan genus that reaches 450 species. In different places its berries are being consumed as food and, currently *Vaccinium* spp. based herbal medicines and in the market. Mainly functional foods are from *V. mirtillus* and *V. macrocarpon* from Europe and North America respectively. Hence, for reviewing the ethnobotanical interests of this genus, available literature and specialized databases were accessed. There are reports of medicinal uses or food uses of 36 *Vaccinium* species, mainly from North America, Asia and Europe. More than 70 diverse uses were compiled, mainly from the systems: digestive, genitourinary and endocrine/metabolic. Most commonly reported uses were: antidiabetic, diuretic, urinary antiseptic, antidiarrheal, antipyretic and astringent. In 22 species, berries are used as food. Fruits and leaves were the most cited useful parts of the plant. Geographic distribution of published researches pointed out that, except *V. floribundum* and *V. meridionale*, most of autochthonous Latin-American species has not been studied yet as part of a research work, therefore ethnobotanical, phytochemical and biological activity are needed to be studied in this region. According to pharmacological investigation, that in many cases corroborated traditional uses: presence of biologically active compounds in this genus (mainly polyphenolics) could mean a potential field for development of herbal medicines or nutritional supplements from this plant.

Key words: *Vaccinium*, Ethnobotany, Medicinal plants, Cranberry, Blueberry, Mortiño, Agraz

Introduction

Genus *Vaccinium* (Ericaceae), subfamily *Vaccinioideae*, tribe *Vaccinieae* comprises 36 sections and, reach more than 450 species. It is cosmopolitan, primarily of the Northern Hemisphere, but especially in Southeast Asia and Malaysia and mostly montane in the tropics, represented with more than 40 species in the Neotropics, ranging from Mexico to northern Argentina, and eastwards to Guyana and the Caribbean (Berazaín, 1992; Lutein, 2007).

Most known species are, in Europa: *V. mirtillus*, named "Heildelbeere", "Blaubeere" (Ger.), "Blaebar" (Dan.), "Airelle", "Myrtille", "Raisin des bois", "Brembelle ou brimbelle" (Fr.), "Arándano", "Arándano Negro", "Mirtilo" (Sp.), "Blaauwe bessen" (Dutch), "Common blueberry" (En.), "Mirtillo" (It.), "Borowkie zarna" (Pol.) and "Blabær" (Sw.), which is commonly used as medicinal plant and in potted fruit (Dorvault, 1875) and, *V. macrocarpon*, (cranberrry) from North America also employed traditionally as food and medicinal, mainly to treat and to prevent Urinary Tract Infections (UTI) (Yarnell and Abascal, 2008).

Others species of interest are: *V. oxycoccus* "Cranberry" (En.), "arándano trepador europeo" (Sp.); *V. vitis-idaea*, "Cowberry", "Lingen", Lingonberry" (En.); *V. corymbosum*, "highbush", "Blueberry" (En.), "arándano americano", "arándano alto" (Sp.); *V. ashei* "rabbiteye" (En.), "arándano ojo de conejo" (Sp.) and *V. angustifolium* "lowbush" (En.), these later three species also are mainly agronomically developed (Finn, 1999). Most important species growing in the Neotropics are *V. floribundum* and *V. meridionale* consumed as food and marketed from Ecuadorian paramus and Colombia as "Andean Blueberry" and "Agraz" respectively. *V. meridionale* also grow in Blue Mountains of Jamaica, where is used as food (Blue and John Crow Mountains, 2004).

Farming is mainly distributed in Europe (Alps, Apennines Central, and Pyrenees), Asia, Central America, U.S. and Canada. U.S. is the greatest producer, consumer, exporter and importer of these

Received 03 July 2013; Revised 05 October 2013; Accepted 10 February 2014; Published Online 18 May 2014

*Corresponding Author

Orlando A. Abreu
Department of Foods Science and Technology, Faculty of Chemistry, Universidad de Camagüey, Cuba

Email: orlando.abreu@reduc.edu.cu

berries in the world and with Canada comprise 90% of total productive area, followed by Chile (pioneer in this farming at the south hemisphere), Argentina, New Zeeland and South Africa. Main European producers are: France, Holland, Poland and Spain. Countries demanding this kind of fruits are: Japan, Italy, UK, Belgium and Holland (Arándano, 2004).

Cranberry fruit, beside nutritional values, is claimed for UTI prevention and it is well ranked in the herbal products market in U.S. (Siciliano, 1998). This market success, partly is due to scientific results accumulated all over past century related mechanism by which cranberry have effect on UTI. Interference on bacterial fimbrial adhesion is mechanism of action that better demonstrated cranberry activity against *Escherichia coli*, the most frequent bacteria causing UTI (Schmidt and Sobota, 1989; Zafriri et al., 1989; Avorn et al., 1994; Ahuja, et al., 1998; Foo et al., 2000a,b; Stothers, 2002). UTI conventional therapy is based on chemotherapy, but bacterial resistance frequently cause clinical fail, mainly for ß_lactamics, trimethoprim-sulfamethoxazole, and more recent drugs like fluoroquinolones (von Baum and Reinhard-Marre, 2005; Mediavilla et al., 2005; Marcusson et al., 2009); that is why some alternatives to bactericide or bacteriostatic activities, among of which are the use of plant extract, has been under investigation (Barreto et al., 2001; Abreu and Barreto, 2012).

In Cuba there are described six endemic *Vaccinium* species, characterized as shrub or little trees, with coriaceous leaves, urseolate inflorescens and black berry fruits or brown when ripe, growing on high lands (100-2000 m) under pine trees (Berazaín, 1992). According literature and ethnobotanical inquires (Roig, 1975; Seoane, 1984; Fuentes and Expósito, 1995), plants of this genus seem to be unknown by population in Cuba, since there is not reported any vernacular name in the localities where it is grown (Roig and Acuña, 1957; Berazaín, 1992).

Because of the increase of research and commercial production of *V. macrocarpon* as food and dietetic supplement claimed to avoid UTI, it was established *Vaccinium* spp. as objective for its ethnobotanical review as food and medicine and its pharmacological potential.

Review related ethnobotany of *Vaccinium* spp. and biological activity was made on available literature and specialized databases: NAPRALERT (Farsnworth, 2003), Native American Ethnobotany Database (Moerman, 2012), Phytochemical and Ethnobotanical Database (Duke, 2008) and recent articles indexed on Pubmed, Hinari and Scielo. Ethnomedical information was tabulated and analyze.

There are more than 70 registered ethnomedical and food uses in 36 *Vaccinium* species, mainly from the digestive, genitourinary and endocrine/metabolic systems. Besides food use, most reported uses were: antidiabetic, diuretic, urinary antiseptic, antidiarrheal, antipyretic and astringent. Species with more number of uses were *V. vitis_idaea*, *V. mirtillus*, *V. macrocarpon* and *V. uliginosum,* in the properly order. Fruit and leave were the most cited part of the plant, in almost all species fruit was reported as food, while leave or aerial part are most mentioned as medicinal (Table 1).

Table 1. Traditional use of *Vaccinium* spp.

No.	Specie	Part	Use	Country
1.	*V. alpinum*	fruit	food	U.S.
2.	*V. angustifolium*	fruit	ceremonial medicine, food	Canada
		fruit	food	U.S.
		leave	flavouring, blood purifier	U.S.
		root	colic in infants, after a miscarriage, to induce labor	U.S.
		flower	food, for "craziness"	Canada
3.	*V. arboreum*		diarrhea, dysentery, poultice, sore (throat), swelling, tonic	U.S.
4.	*V. caespitosum*	fruit	food	Canada
		fruit	food, source of vitamin C	U.S.
		root	used as a tobacco	U.S.
5.	*V. bancanum*	root	post-parturition aid	Malaysia
6.	*V. berberidifolium*		tea	
7.	*V. bracteatum*	entire	cancer	China
8.	*V. calycinum*		tea	

Table 1. Contd…

No.	Specie	Part	Use	Country
9.	*V. caespitosum*	fruit	food	U.S.
10.	*V. corymbosum*	fruit	food	Canada
		fruit	food, flavouring	U.S.
11.	*V. deliciosum*	fruit	food	U.S.
		fruit	food	Canada
12.	*V. dentatum*		tea	
13.	*V. floribundum*	fruit	food, astringent, diuretic, stomach	Ecuador
14.	*V. globulare*		arthritis, heart, kidney, rheumatism	U.S.
15.	*V. keysseri*	leave	internal sores	Papua-N Guinea
16.	*V. macrocarpon*	fruit	food, cancer, scurvy, to allay fervour, minor throat irritations,blood purifier, fiber, liver, asthma, gall bladder, mild stimulant, cystitis, prevent urinary infection	U.S.
		branches	pleurisy	U.S.
		fruit	food	Canada
17.	*V. maingayi*		lactogogue	
18.	*V. membranaceum*	fruit	food	Canada
		fruit	food, source of vitamin C	U.S.
		fruit	food	U.S.
		root	rheumatism, arthritis, heart	U.S.
		branches	rheumatism, arthritis, heart	U.S.
		fruit	antiseptic, astringent, carminative, diabetes	
19.	*V. meridionale*	fruit	food	Jamaica Colombia
20.	*V. myrsinites*	leave	food, ceremonial medicine, colds, fever, emetic, sun sickness: eye disease, headache, high fever and diarrhea	U.S.
		root	bath for hog sickness: unconsciousness	
21.	*V. myrtilloides*	fruit	food	Canada
		fruit	food	U.S.
		flower	food, preserves	Canada
		aerial part	food, to bring menstruation, contraceptive, diaphoretic, gynecological aid, menstrual bleeding, prevent miscarriage	Canada
22.	*V. myrtillus*		antidote, antiseptic, astringent, catarrh, diabetes, diuretic, dysentery, hemostat, tonic, refrigerant, inflammation, intestine, scurvy, typhoid	
		aerial part	intestinal diseases	Turquía
		fruit	stomaquic	Francia
		fruit	food, cutaneous maladies, collutory,	Italia
		fruit	food, diarrhea, diuretic	U.S.
		fruit	to stay women's courses	England
		leave	diarrhea, diabetes	U.S.
		leave	diabetes, mild worm remedy	England
		leave	diabetes, anthelmintic moderate	Canada
		leave	antispasmodic, inflammation, diarrhea, diabetes	France
		leave	diuretic, urinary disturbances, diabetes	Italia
		leave	diabetes mellitus	URSS
		leave	diabetes	Puerto Rico
		leave	stomach ache, tonic cardiac	Tunisia
		leave	diabetes	Yugoslavia
		root	diabetes	Yugoslavia
			diabetes	Portugal
23.	*V. oxycoccos*	aereal part	cancer, refrigerant, tea	U.S.
		fruit		
		fruit	antiemetic, tea, food	U.S.
			food	Canada

Table 1. Contd…

No.	Specie	Part	Use	Country
24.	*V. oldhamii*	fruit	food	Korea
25.	*V. ovalifolium*	fruit	food, source of vitamin C	U.S.
		fruit	food	Canada
		leave	after childbirth	U.S.
		leave	mixed with other plant leaves and smoked.	Canada
26.	*V. ovatum*	fruit	food	U.S.
		fruit	food	Canada
		leave	to mothers after childbirth to gain their strength, diabetes	U.S.
	V. pahalae		tea	
27.	*V. padifolium*	fruit	bronchitis, cold, cough	Madeira
28.	*V. parvifolium*	bark	colds	U.S.
		fruit	food	U.S.
		fruit	food, wine	Canada
		branches	used as a broom	U.S.
		leaves	smoke	Canada
29.	*V. peleanum*		tea	
30.	*V. pennsylvanicum*	leave	diabetes	U.S.
31.	*V. reticulatum*		tea	
32.	*V. scoparium*	leave	antiemetic, to increase appetite	U.S.
		fruit	food	U.S.
		fruit	food	Canada
		braches	used as a broom	U.S.
33.	*V. uliginosum*		catarrh, cystitis, enteritis, gastritis, intestine, liqueur, narcotic, stomachic	
		leave	to mothers a few days after childbirth to gain their strength food, source of vitamin C	U.S.
		fruit	food	U.S.
		fruit		Canada
34.	*V. vacciniaceum*	fruit	food	India
35.	*V. vitis-idaea*	fruit	antiseptic, antiseptic (urethra), diuretic, appetitive, astringent, cancer (breast), debility, depurative, diabetes, disinfectant, nervine, rheumatism, styptic, urethra, urogenital	
		fruit	colds, coughs, sore throat, urogenital	Canada
		fruit	food, colds, coughs, sore throat	U.S.
		fruit	diuretic, astringent, diarrhea, dyspepsia, hemoptysis, menses, digestant, tonic, appetitive, antipyretic, to breasts in treat engorgement	Iran
		fruit	food, astringent, dyspepsia	Italia
		leave	beverage, diuretic, coughs, gout, blood rectifier, catharsis, rheumatoid arthritis, pain due to kidney stones, antipyretic, disinfectant, toxic (high doses)	Iran
			diabetes	England
36.	*V. whitfordii*		tongue	

Geographical distribution of *Vaccinium* spp. published studies indexed in accessed databases include: Europe (France, Italy, UK, Turkey, URSS, Yugoslavia), Asia (China, Korea, India, Iran, Malaysia, Papua-Nueva Guinea) and America (Canada, U.S., Puerto Rico). While ethnobotanical information of Neotropical species *V. floribundum* ("Mortiño") and, *V. meridionale* ("Agraz") from the Andean Zone, were founded in other sources (Hinari, Pubmed, Scielo).

Some of the medicinal traditional use of *Vaccinium* spp. have been pharmacologically demonstrated *in vitro* or *in vivo*, for instance: antidiabetic (Allen, 1927a,b; Kit et al., 1972; Zozulya, Museva and Kuzmina, 1975; Dhawan, 1977; Gato and Calleja, 1982; Neef, Declercq and Laekeman, 1995; Wilson, 2010), antimicrobial

(Brantner and Grein, 1994; Hamilton-Miller, 1994; Ofek, Goldhar and Sharon, 1996; Lee, 2000; Puupponen-Pimiä et al., 2001), interfering bacterial virulence factors like: quorum sensing (Vattem et al., 2007), fimbrial adhesion in *Helicobacter pylori* (Burger et al., 2000), *Porphyromonas gingivalis* (Bonifait and Grenier, 2010), *E. coli* (see epigraph below about *V. macrocarpon*); diuretic (Dhawan, 1977) and against cancer and cardiovascular diseases (Neto, 2007; McKay and Blumberg. 2007).

Bioactivity demonstrated on *Vaccinium* spp. give credit to ethnobotanical criteria as a valid source for investigation on new herbal medicine, drug or dietetic supplement (Abreu and Cuéllar, 2008). In the case of antioxidant effect that has been founded in some species (Costantino et al., 1994; Yelioglu et al., 1996; Pietta et al., 1998; Prior et al., 1998; Wilson, Porcari and Harbin, 1998; Martin-Aragon et al., 1999; Yan et al., 2002; Su and Silva, 2006; Wang and Ballington, 2007; Zafra-Stone et al., 2007; Rojano et al., 2009; Gaviria et al., 2009; 2012; Schreckinger et al., 2010; Garzón et al., 2010; Wang et al., 2011), this activity could be related with traditional claims as hepatic depurative and blood purifier.

Some use like astringent, anti-infectious, diuretic, diabetes, colds, coughs or tonic were founded in more than one specie, even from different latitudes (Table 1), this coincidence it is not casual, beside cultural appropriation among peoples of different places, it could be due to chemotaxonomic factors, since nature of metabolites present in *Vaccinium* spp. are similar (Abreu et al., 2008).

V. mirtillus fruit are use commonly on Europe as food and, it is valued by Nordics people for improving nocturnal vision, particularly by drivers, since anthocyanosides increased rhodopsin regeneration rate in the retin, while anthocyanosides have protective effect on gastric mucus in experimental ulcerogenesis and, because of vasoprotective, coronary vasodilatation and anti-free radicals activity, it is indicated by phytotherapists to treat diabetic microangiopathy, prevent coronary disease, phlebitis, thrombophlebitis and circulatory problems related vision illnesses (Peris et al., 1995). Matsunaga et al. (2011) determined that *V. mirtillus* extracts inhibit angiogenesis *in vitro* and *in vivo*, suggesting this activity may be effective against retinal diseases involving angiogenesis.

Ahmet et al. (2009) evaluated a blueberry-enriched diet as a myocardium protective for induced ischemic damage. Lipid-lowering activity of blueberry leaves has been demonstrated in rats (Cignarella et al., 1996).

Use of leaves of *V. mirtillus* as antidiabetic has been related to presence of chromium salts (9 ppm) (Peris et al., 1995) but, in a recent review Helmstädter and Schuster (2010), expose that investigations on blueberry leaves against diabetes mellitus not support traditional use since evidence it is not conclusive.

In 22 *Vaccinium* species it is reported the use of its berries as a source of food. From data of Native American Ethnobotany Database it is noticeable the quantity and diversity of forms of preparation of dishes with these berries by North Americans different native people: it can be eaten fresh or dried into cakes and stored, canned and used as a winter food, stewed and made into a sauce, and used for dumplings, pies, puddings and toppings in pancakes and muffins.

Considering C vitamin present among nutrient reported in some fruit species like: *V. caespitosum, V. corymbosum, V. macrocarpon*, *V. membranaceum*, *V. mirtillus*, *V. ovalifolium*, *V. oxyccocos, V. uliginosum* and *V. vitis_idaea* (Farsnworth, 2003; Native American Ethnobotany Database, 2012), there is no doubt about its antiscorbutic potential.

In a previous review on phytochemistry of *Vaccinium* spp. (Abreu and Cuéllar, 2008) benzenoids, flavonoids (cyanidines, hyperoside, epi-cathequine and proanthocyanidines) and phenylpropanoids were the predominant metabolites, the majority of which were reported in fruits and leave. Polyphenolic compounds have been the most studied in *Vaccinium* sp., flavonoids are predominant compounds isolated in fruit. Those phytochemical researches are usually related to studies of antioxidant and antimicrobial activity on UTI, mainly on cranberry and blueberry.

Some *Vaccinium* spp. of economic interest are under farm condition, but still some other species are wild harvested. In order to develop better cultivars, hybridization research and biotechnological procedures has been undertaken (Paal, 1993; Debnath and McRae, 2001; Meiners et al., 2007; Morozova, 2007; Debnath, 2007; Rache and Pacheco 2010; Magnitskiy et al., 2011; Castro et al., 2012; Song and Hancock, 2012).

In many other related subjects have been carried out research on *Vaccinium* spp., ie: cultivars of *V. ashei* and antioxidant capacities (Wang et al., 2011); genotype and growing season of *V. mirtillus* (Wang et al., 2012a); ecological influences on phenolic composition and antioxidant capacity,

sampling *V. mirtillus* leaves along a geographical gradient in the boreal zone that indicated that leaves from higher latitudes and higher altitudes had greater soluble phenolic and flavonol levels, higher antioxidant capacity, and lower contents of chlorogenic acid derivatives (Martz et al., 2010); phenotypic plasticity of *V. meridionale* in wild populations (Ligarreto et al., 2011); research on bioavailability of anthocyanin (Talavéra et al., 2006) or technological procedures for production of herbal medicine, food or functional food products (Su and Silva, 2006; Ávila et al., 2007; Bononi and Tateo, 2007).

V. macrocarpon

Description

V. macrocarpon is an evergreen trailing shrub, with rhizomatous habits when young; with pink, simple and axillar flowers, ovaries have four locules. Wind or insect are needed for polinization. Fruit is a shining red epiginous berry, ripening is 60-120 days after fertilization (Mark's Fruit Crops. 2002).

Distribution

East bogs of North America, from Newfoundland to Manitoba, South of Virginia, Ohio and North of Illinois (Mark's Fruit Crops. 2002).

Ethnobotany

Cranberry fruit is reported as food by native people of North America and prepared in different ways: raw or cooked, fruit sun or fire dried and stored for future for example. dried fruit cakes soaked in warm water and cooked as a sauce or mixed with corn bread and, as medicinal for erysipelas, tonsillitis, scarlatina sore throat, ulcers, pleurisy (leaves) (Moerman (2012); cancer and scurvy (Duke, 2008) and to treat cystitis and prevent UTI (Farnsworth, 2003). It is cultivated in U.S. since the early nineteenth century.

Antimicrobial activity

Most biological effects evaluated on cranberry fruit are antimicrobial activity: antiviral (Konowalchuk and Speirs, 1978; Weiss et al., 2005), antifungal (Marwan and Nagel, 1986; Cipollini and Stiles, 1992; Patel et al., 2011), antibacterial: *E. coli, Pseudomonas aeruginosa, P. fluorescens, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecalis, Salmonella enteritidis, Micrococcus luteus, Listeria monocytogenes*, *Streptococcus mutan*, *Bacillus cereus* and *Micrococcus luteus* (Marwan and Nagel, 1986; Lee et al., 2000; Leitão et al., 2005; Magariños et al., 2008; Viskelis et al., 2009).

Cranberry can exert effect on Cell–Cell Signaling System of *Vibrio harveyi* (Feldman et al, 2009) and as quorum sensing disrupting in *Chromobacterium violaceum* (Vattem et al., 2007). Enache and Chen (2007) determined effect of cranberry juice concentrates at different ^{o}Brix levels on *E. coli* (O157:H7), *Salmonella*, and *L. monocytogenes*.

Particular interest has been focussed on cranberry effect on oral microflora related dental and periodontal diseases by inhibiting: biofilms formation in *Streptococcus mutans* on saliva-coated apatitic surface and on dental caries development *in vivo* (Koo et al., 2010); biofilm and adhesion of *Porphyromonas gingivalis*, the main etiologic agent in chronic periodontitis (Labrecque et al., 2006; Bonifait and Grenier, 2010); the coaggregation capabilities of oral bacteria: *Streptococcus mutans* with *Fusobacterium nucleatum* or *Actinomyces naeslundii* (Riihinen et al., 2010) and matrix metalloproteinases produced by resident and inflammatory cells in response to periodontopathogens that play a major role in periodontal tissue destruction (La et al., 2009). Another possible cranberry use is against formation of contact lens biofilm by *Staphylococcus epidermidis* (Leshem et al., 2011).

No activity of cranberry juice (pH- 3,5 and 6,9) and cranberry proanthocyanidin-rich fractions against *E. coli* and other bacteria were found by Leitão et al. (2005), it is well established that cranberry use in UTI prevention or UTI treatment it is not mainly due to bacteriostatic/bactericide activity instead; there are many *in vitro* and *in vivo* research related antiadhesive effect of cranberry on uropathogenic *Escherichia coli* with P fimbriae (UPEC P^{+}) (Farsworth et al., 2003), P fimbriated strain are the most common bacteria isolated in UTI. This activity of cranberry fruit is sustained too by report of cranberry as fimbrial antiadhesive in other bacteria (Burger et al., 2000; Toivanen et al., 2010).

First studies to support antimicrobial effect cranberry at the beginning of XX century were focused on the possibility of acidification of urine or hypuric acid excretion, a potent bacteriostatic associated to the fruit ingestion (Blatherwick, 1923; Moen, 1962); but other results questioned this mechanism (Fellers, 1933; Nickey, 1975). It is in the 80' that research on bacterial adhesion began to be consider as a mechanism of action of cranberry in UTI (Sobota, 1984; Schmidt and Sobota, 1989; Zafriri et al., 1989), since then, dozens of articles

has been reporting *in vitro*, *ex vivo* and *in vivo* experiences of antiadhesion activity in guinea pig erythrocytes, human erythrocytes, uroepithelial cells, bladder cultured cell lines and laboratory animal models (Nowack and Schmitt, 2008).

Pili or fimbriae are hair-like polymeric proteinaceous appendages expressed on the outer surfaces of bacteria that enable pathogens to recognize host receptors, anchor and begin infection (Johnson, 2003; Niemann et al., 2004). Fimbriae is determinant in early steps of *E. coli* colonization of most of *E. coli* pathovars, in UPEC they has the significance of avoid urine mechanical flow, attach to urinary tract mucosa and triggering signals to start the disease. This virulence factor is associated to invasion, biofilm formation, cell motility and transport of proteins and DNA across membranes (Johnson, 2003; Kaper et al., 2004; Gal- Mor and Finlay, 2006; Wiles et al., 2008). Adhesin at the fimbriae tip are considered as the most important determinant of pathogenicity in *E. coli*., thus fimbrial adhesion is a critical event, if fimbria-receptor interaction is not well established UTI symptoms never occurs (Niemann et al., 2004; Le Bouguénec, 2005; Wiles et al., 2008; Croxen and Finlay, 2010).

Cranberry antifimbrial effect was corroborated by Ahuja et al. (1998) by means of electronic microscopy, no fimbrial expression or loose of them, and change in *E. coli* morphology were clear. More recently, isolated cranberry proanthocyanidins (PACs) at 60 µm/ml were tested on UPEC P^+ resulting in a potent antiadhesive activity (Foo et al., 2000a, 2000b, Howel et al., 2005).

Although, in clinical practice even several researches in human has been done to validated cranberry for UTI prevention, results still are controversial, since metaanalyses of human clinical trials of cranberry for UTI prevention are not conclusive. Jepson & Craig (2008) state: "There is some evidence that cranberry juice may decrease the number of symptomatic UTIs over a 12 month period, particularly for women with recurrent UTIs. It's effectiveness for other group is less certain"... "The evidence is inconclusive as to whether it is effective in older people (both men and women), and current evidence suggest that it is not effective in people with a neuropathic bladder."... "Further properly designed studies with relevant outcomes are needed".

Similarly, Wang et al. (2012-b) in other metaanalysis showed that: "cranberry-containing products were effective in preventing urinary tract infections", "Subgroup analyses showed that cranberry-containing products were most effective in women with recurrent urinary tract infection (two trials); females (four trials); children (mean age under 18 years; two trials) those using cranberry juice (five trials); and when products were used more than twice a day (four trials).", concluding that: cranberry-containing products were associated with protective effect against urinary tract infection, though "advised cautious interpretation of this result given substantial variation across the included trials".

But, further clinical trials allowed Jepson, Williams and Craig (2012) to state: "that cranberry juice is less effective than previously indicated. Although some of small studies demonstrated a small benefit for women with recurrent UTIs, there were no statistically significant differences when the results of a much larger study were included". Thus, research in clinical trial continues.

Phytochemistry

V. macrocarpon is phytochemically among most investigated *Vaccinium* spp. (Abreu et al., 2008). Cranberry fruit contain predominantly organic acids: citric, chlorogenic, malic, quinic and shiquimic (Duke, 2008; Jensen, 2002), and polyphenols like: flavonoids and anthocyanic pigment glycosides of cyanidin and paeonidin (Duke, 2008; Yan, 2002; Abreu et al., 2008).

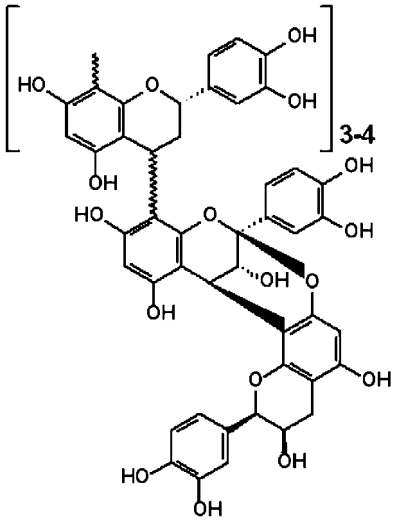

Figure. 1. Cranberry A-type proanthocyanidin.

It is of particular interest a trimeric type A proanthocyanidins (PACs) characterized in cranberry fruit by Foo et al. (2000 a,b) (Figure 1), since authors demonstrated it is the active compound in UPEC P fimbriae interference.

Pinzón-Arango et al. (2009) found also that bacterial adhesion force of UPEC was decreased by cranberry PACs. Proanthocyanidins (condensed tannins) are oligomeric and polymeric end products of the flavonoid biosynthetic pathway, A-type proanthocyanidins is not usual since a B-type linkage is more common in plant kingdom (Miranda and Cuéllar, 2001; Dixon et al., 2005).

Toxicity

Quantities consumed of cranberry as food are nontoxic and safe in pregnancy in lactation (Yarnell and Abascal, 2008), ingestion of great amounts can produce gastrointestinal disorders and diarrhoea. Dugoua et al. (2008) review safety and efficacy of cranberry during pregnancy and lactation, concluding that evidence supports the use of cranberry for UTI as a valuable therapeutic choice in the treatment of UTI during pregnancy.

Terris et al. (2001) cited by Yarnell and Abascal (2008), in a tiny study (n=5) in healthy volunteers, founded that cranberry tablets could raise urinary oxalate levels and calcium oxalate super saturation significantly, yet also elevated urinary magnesium and potassium levels significantly, suggesting that people at risk of urolithiasis should be counseled to avoid cranberry.

Drug interactions

Potential interaction, but not conclusive, have been suggested between cranberry juice and warfarin, thus, patients taking warfarin with cranberry juice should be cautioned about the potential interaction and monitored closely (Pham and Pham, 2007).

In a clinical trial to determine the effects of hypochlorhydria and acidic drink ingestion on protein-bound vitamin B_{12} absorption in elderly subjects, omeprazole causes protein-bound vitamin B_{12} malabsorption, but with cranberry juice ingestion, the omeprazole-treated group showed an increase in absorbed protein-bound vitamin B_{12} (Saltsman et al., 1994). In other clinical trial to determine possible additive effect to triple therapy with omeprazole, amoxicillin and clarithromycin (OAC) with cranberry anti-adhesion activity on *H. pylori in vitro*, Shmuely et al. (2007), concludes that the addition of cranberry to (OAC) improves the rate of *H. pylori* eradication; by gender, more significant in female subjects.

South American *Vaccinium* of interest

Even abundance of *Vaccinium* spp. in Neotropics high lands, only *V. floribundum* and *V. meridionale* seems to have documented traditional uses by Andean people as food and/or medicine. This both species has gained academic and commercial attention. Sustainable harvesting and farming of this Ericads can lead in an economical sources and employment for the mountain inhabitants living where this plant grows. Taking into account the under investigated *Vaccinium* spp. in Latin-America and the bulk of pharmacological investigations underwent on the Old World and North-America *Vaccinium* spp., bioprospection of this genus in the area is needed to develop herbal medicine or dietary supplement.

V. floribundum

In Ecuador "Mortiño" fruits are used basically fresh and are made into jams, drinks, and occasionally pies. "Mortiño" in Ecuador is use to prepare a traditional beverage: "Colada Morada", at the time of "Día de los Difuntos" (Servicio de Información y Censo Agropecuario del Ministerio de Agricultura y Ganadería del Ecuador, 2004; Lutein, 2007) and it is marketed too as herbal medicine or functional food (Mortiño, 2004; Concentrado de Mortiño, 2004). *V. floribundum* it is known in other part of South America as: frijundilla (Santa Cruz, Bolivia), macha macha (Perú), manzanilla del cerro (Ecuador), mortiño amargo (Nariño, Colombia) (Lutein, 2007).

From *V. floribundum* berries have been isolated epicatechin polymers, flavonols (quercetin and myricetin glycosides), the major phenolic compound were anthocyanin glycosides (cyanidin and delphinidin) (Vasco et al., 2010). "Mortiño" proanthocyanidin-enriched fraction were evaluated *in vitro* by Schreckinger et al. (2010), demonstrating antioxidant activity and, also determined that can inhibits adipogenesis and inflammation; in other research (Schreckinger et al., 2012), possible antidiabetic effects is suggested by the ability to inhibit both, α-glucosidase and α-amylase.

V. meridionale

Berries of "Agraz" from Colombia, also known as "mortiño" and "vichachá", have been investigated thoroughly in recent years since the phytochemical, pharmacological, agronomical, biotechnological and food science point of view. As other edible *Vaccinium* spp., it is consumed fresh and are prepare too as jams, juices, custard, pastries, ice creams and wines; as medicinal, it is use in diabetes and digestive disorders (Ávila et al., 2007; Rache and Pacheco, 2010).

Phenolic compounds, mainly anthocyanin isolated from Andean blueberry fruits also have high antioxidant activity (Rojano et al., 2009; Gaviria et al., 2009; Gaviria et al., 2012), in this

berries cyanidin 3-galactoside is the major anthocyanin compound, chlorogenic acid is major non-anthocyanin phenolic compound (Garzón et al., 2010). Beside antioxidant effect of "Agraz" fruit extracts, Gaviria et al. (2009) also found protective effect over lipidic peroxidación of corn oil and, and determined total phenolic content: 609 ± 39 (as mg eq of galic acid / 100 g fresh fruit) and anthocyanic contents 201 ± 10 (mg eq of cyanidin glicosode / 100 g fresh fruit), values comparable to other *Vaccinium* spp.

Organic acid, sugars, vitamins B and vitamin C, minerals (K, Ca, P) and fiber are content in *V. meridionale* (Avila et al., 2007; Rache and Pacheco, 2010), different studies related food science have been carried out on "Agraz" related: changes of phenolic compounds and antioxidant activity during maturation and ripening (Gaviria et al., 2012), anthocyanin stability of juice and concentrate (Martínez et al., 2011) and physiochemical and organoleptic characterization of fruits stored at 2ºC (Avila et al., 2007).

However, *V. meridionale* is a an endangered species since it has been affected by deforestation, ecosystem fragmentation, and overexploitation of fruit, and branches for floristry; in addition, it has difficult in sexual and asexual reproduction (Rache and Pacheco, 2010), thus, researches has been underwent to overcome this problem: phenotypic wild populations (Ligarreto et al., 2011), germination and dormancy (Hernández et al., 2009; Castro et al., 2012), rooting (Castrillón et al., 2008) and micropropagation (Rache and Pacheco, 2010).

Acknowledgements

Authors are grateful to NAPRALERT Database for providing free information.

References

Abreu, O. and G. Barreto. 2012. Antiadhesive Antibacterial Effect of Plant Compounds. In: Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health. V. Rao (Ed.) InTech. Available from: http://www.intechopen.com/books/phytochemicals-as-nutraceuticals-global-approaches-to-their-role-in-nutrition-and-health/antiadhesive-antibacterial-effect-of-plant-compounds.

Abreu O. A. and A. Cuéllar. 2008. Criterios de selección de plantas medicinales para ser investigadas. Rev. Cubana Plantas Med. 13(3).

Abreu, O. A., A. Cuéllar and S. Prieto. 2008. Fitoquímica del género *Vaccinium* (*Ericaceae*). Rev. Cuban Plantas Med. 13(3).

Ahmet I., E. Spangler, B. Shukitt-Hale, M. Juhaszova, S. J. Sollott, J. A. Joseph, D. K. Ingram and M. Talan. 2009. Blueberry-Enriched Diet Protects Rat Heart from Ischemic Damage. PLoS ONE 4(6):5954.

Ahuja, S., B. Kaack and A. Roberts. 1998. Loss of fimbrial adhesion with the addition of *Vaccinum macrocarpon* to the growth medium of P-fimbriated *Escherichia coli.* J. Urol. 159(2):559-562.

Allen, F. M. 1927a. Blueberry leaf extracts physiologic and clinical properties in relation to carbohydrate metabolism. J. Am. Med. Assoc. 89:1577-1581.

Allen, F. M. 1927b. Blueberry leaf extract. Can Med. Assoc. J. 17:1539-1540.

Arándano. 2004. En: http://agronomia.uchile.cl/webcursos/cmd/22003/pzavala/arandano.html

Ávila, H., J. Cuspoca, G. Fischer, G. Ligarreto and M. Quicazán. 2007. Caracterización fisicoquímica y organoléptica del fruto de agraz (*Vaccinium meridionale* Sw) almacenado a 2ºC. Rev. Facultad Nacional Agron. 60(2):4179-4193.

Avorn J., M. Monane, J. H. Gurwitz, R. J. Glynn, I. Choodnovskiy and L. A. Lipsitz. 1994. Reduction of bacteriuria and pyuria after ingestion of cranberry juice. JAMA 271:751-754.

Barreto, G., A. C. Campal, O. Abreu and B. Velázquez. 2001. El bloqueo de la adhesión fimbrial como opción terapéutica. Rev. Prod. Anim. 13(1):71-82.

Berazaín, R. 1992. *Ericaceae*. In: Flora de la República de Cuba. Fontqueria XXXV. Madrid, 21-77.

Blatherwick, N. D. and M. L. Long. 1923. Studies of urinary acidity - The increased acidity by eating prunes and cranberries. J. Biol. Chem. 57:815-818.

Blue and John Crow Mountains. 2004. http://www.nmnh.si.edu/botany/projects/cpd/ma/cb10.htm

Bonifait, L. and D. Grenier. 2010. Cranberry polyphenols: Potential benefits for dental

caries and periodontal disease. J. Can. Dent. Assoc. 76(1).

Bononi, M. and F. Tateo. 2007. Stabilization of cranberry anthocyanins in nutraceutical capsules. Internat. J. Food Sci. Nutr. 58(2):142-149.

Brantner, A. and E. J. Grein. 1994. Antibacterial activity of plant extracts used externally in traditional medicine. J. Ethnopharmacol. 44(1):35-40.

Burger, O., I. Ofek, M. Tabak, E. I. Weiss, N. Sharon and I. Neeman. 2000. A high molecular mass constituent of cranberry juice inhibits *Helicobacter pylori* adhesion to human gastric mucus. FEMS Immunol. Med. Microbiol. 29:295–301.

Castrillón, J. C., E. Carvajal, G. Ligarreto and S. Magnitskiy. 2008. El efecto de auxinas sobre el enraizamiento de las estacas de agraz (*Vaccinium meridionale* Swartz) en diferentes sustratos. Agron. Colomb. 26(1):16-22.

Castro, C., Y. Olarte, L. Rache and J. Pacheco. 2012. Development of a germination protocol for blueberry seeds (*Vaccinium meridionale* Swartz). Agron. Colom. 30.

Cignarella, A., M. Nastasi, E. Cavalli and L. Puglisi. 1996. Novel lipid-lowering properties of *Vaccinium myrtillus* L. leaves, a traditional antidiabetic treatment, in several models of rat dyslipidaemia: a comparison with ciprofibrate. Thrombosis Res. 84(5):311-322.

Cipollini, M. L. and E. W. Stiles. 1992. Antifungal activity of ripe ericaceous fruits: phenolicacid interactions and palatability for dispersers. Biochem. Syst. Ecol. 20(6):501-514.

Costantino, L., G. Rastelli, T. Rossi, M. Bertoldi and A. Albasini. 1994. Composition, superoxide radicals scavenging and antilipoperoxidant activity of some edible fruits. Fitoterapia 65(1):44-47.

Croxen, M. A. and B. B. Finlay. 2010. Molecular mechanisms of *Escherichia coli* pathogenicity. Nat. Rev. Microbiol. 8:26-38.

Debnath, S. and K. McRae. 2001. An efficient *in vitro* shoot propagation of cranberry (*Vaccinium macrocarpon* ait.) by axillary bud proliferation. In Vitro Cell. Dev. Biol. Plant 37(2):243-249.

Debnatha, S. C. 2007. Propagation of *Vaccinium* in vitro: A Review. Internat. J. Fruit Sci. 6(2):47-71.

Dhawan, B. N., G. K. Patnaik, R. P. Rastogi, K. K. Singh and J. S. Tandon. 1977. Screening of Indian plants for biological activity. VI. Indian J. Exp. Biol. 15:208-219.

Dixon, R. A., D. Y. Xie and S. B. Sharma. 2005. Proanthocyanidins – a final frontier in flavonoid research? New Phytol. 165:9–28.

Dorvault, P. 1875. Botica. La Oficina de Farmacia. 9na ed. Paris: Casa Editorial Bailly-Bailliere, p. 328.

Dugoua, J. J., D. Seely, D. Perri, E. Mills and G. Koren. 2008. Safety and efficacy of cranberry (*Vaccinium macrocarpon*) during pregnancy and lactation. Can. J. Clin. Pharmacol. 15(1):80-6.

Duke, J. 2008. *Vaccinium.* In: Phytochemical & Ethnobotanical Database, Available from: http://www.ars-grin.gov/duke/ Accessed on: March 2012.

Enache, E. and Y. Chen. 2007. Survival of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in cranberry juice concentrates at different Brix levels. J. Food Prot. 70(9):2072-2077.

Farnsworth, N. R. (Ed.). 2003. *Vaccinium*. In: The NAPRALERT Database. The Board of Trustees at the University of Illinois at Chicago. (Available from: http://pcog8.pmmp.uic.edu/mcp/MCP.html.

Feldman, M., E. I. Weiss, I. Ofek and D. Steinberg. 2009. Interference of Cranberry constituents in cell–cell signaling system of *Vibrio harveyi*. Curr. Microbiol. 59:469–474.

Fellers, C. R., B. C. Redmon and E. M. Parrott. 1933. Effects of cranberries on urinary acidity and blood alkali reserve. J. Nutr. 6:455-463.

Finn, C. 1999. Temperate Berry Crops. In: Perspectives on new crops and new uses. J. Janick (Ed.), ASHS Press, Alexandria, VA.

Foo, L. Y., Y. Lu, A. B. Howell and D. N. Vorsa. 2000a. A-Type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. J. Nat. Prod. 63(9):1225-1228.

Foo, L. Y., Y. Lu, A. B. Howell and D. N. Vorsa. 2000b. The structure of cranberry

proanthocyanidins which inhibit adherence of uropathogenic p-fimbriated *Escherichia coli* in vitro. Phytochemistry 54(2):173-181.

Fuentes V. R. and A. Expósito. 1995. Las encuestas botánicas sobre plantas medicinales en Cuba. Revista del Jardín Botánico Nacional. XVI:77-145.

Gal-Mor, O. and B. B. Finlay. 2006. Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell. Microbiol. 8(11):1707–1719.

Garzón, G. A., C. E. Narváez, K. M. Riedl and S. J. Schwartz. 2010. Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (*Vaccinium meridionale* Swartz) from Colombia. Food Chem. 122(4):980-986.

Gato, M. and J. M. Calleja. 1982. Preliminary study on the hypoglycemic activity of *Vaccinium myrtillus*. Trab. Compostelanos Biol. 9:5-14.

Gaviria, C., C. Ochoa, N. Sánchez, C. I. Medina, M. Lobo, P. Galeano, A. Mosquera, A. Tamayo, Y. Lopera and B. A. Rojano. 2009. Actividad antioxidante e inhibición de la peroxidación lipídica de extractos de frutos de mortiño (*Vaccinium meridionale SW*). Bol. Latinoam. Caribe Plant. Med. Aromát. 8(6):519-528.

Gaviria, C., J. D. Hernández, M. Lobo, C. I. Medina and B. A. Rojano. 2012. Cambios en la actividad antioxidante en frutos de mortiño (*Vaccinium meridionale* Sw.) durante su desarrollo y maduración. Rev. Fac. Nal. Agr. Medellín 65(1).

Hamilton-Miller, J. M. T. 1994. Reduction of bacteriuria and pyuria using cranberry juice. J. Am. Med. Ass. 272(8):588-590.

Helmstädter, A. and N. Schuster. 2010. *Vaccinium myrtillus* as an antidiabetic medicinal plant-research through the ages. 65(5): 315-321.

Hernández, M. I., M. Lobo, C. I. Medina, J. R. Cartagena and O. A. Delgado. 2009. Comportamiento de la germinación y categorización de la latencia en semillas de mortiño (*Vaccinium meridionale* Swartz). Agron. Colomb. 27(1):15-23.

Howell, A. B., J. D. Reed, C. G. Krueger, R. Winterbottom, D. G. Cunningham and M. Leahy. 2005. A-type cranberry proanthocyanidins and uropathogenic bacterial antiadhesion activity. Phytochemistry 66:2281-2291.

Jepson, R. G. and J. C. Craig. 2008. Cranberries for preventing urinary tract infections. Cochrane Database of Systematic Reviews. In: The Cochrane Library, Issue 04, Art. No. CD001321.

Jepson, R. G., G. Williams and J. C. Craig. 2012. Cranberries for preventing urinary tract infections. Cochrane Database of Systematic Reviews. In: The Cochrane Library, Issue 12, Art. No. CD001321.

Johnson, J. R. 2003. Microbial virulence determinants and the pathogenesis of urinary tract infection. Infect Dis. Clin. North Am. 17:261-78.

Kaper, J. B., J. P. Nataro and H. L. T. Mobley. 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. 2:123-140.

Kit, S. M., R. V. Boichuk, L. I. Khananayev and T. T. Ozarkiv. 1972. Experimental study of the hypoglycemic effect of some plants found near carpathia. Farm Zh. (Kiev) 27(4):61.

Konowalchuk, J. and J. I. Speirs. 1978. Antiviral effect of commercial juices and beverages. Appl. Environ. Microbiol. 35:1219.

Koo, H., S. Duarte, R. M. Murata, K. Scott-Anne, S. Gregoire, G. E. Watson, A. P. Singh and N. Vorsa. 2010. Influence of cranberry proanthocyanidins on formation of biofilms by *Streptococcus mutans* on saliva-coated apatitic surface and on dental caries development *in vivo*. Caries Res. 44(2):116-126.

La, V. D., A. B. Howell and D. Grenier. 2009. Cranberry proanthocyanidins inhibit mmp production and activity. JDR 88(7):627-632.

Labrecque, J., C. Bodet, F. Chandad and D. Grenier. 2006. Effects of a high-molecular-weight cranberry fraction on growth, biofilm formation and adherence of *Porphyromonas gingivalis*. J. Antimicrob. Chemother. 58(2):439-443.

Lee, Y. L., J. Owens, I. Thrupp and T. C. Cesario. 2000. Does cranberry juice have antibacterial activity? J. Am. Med. Ass. 28(13):1691.

Leitão, D. P. S., A. C. M. Polizello, I. Y. Ito and A. C. C. Spadaro. 2005. Antibacterial screening of anthocyanic and proanthocyanic fractions

from cranberry juice. J. Med. Food. 8(1):36–40.

Leshem, R., I. Maharshak, E. B.Jacob, I. Ofek and I. Kremer. 2011. The Effect of nondialyzable material (NDM) cranberry extract on formation of contact lens biofilm by *Staphylococcus epidermidis*. Invest. Ophthalmol. Vis. Sci. 52(7):4929-4934.

Ligarreto, G. A., M. P. Patiño and V. S. Magnitskiy. 2011. Phenotypic plasticity of *Vaccinium meridionale* (*Ericaceae*) in wild populations of mountain forests in Colombia. Rev. Biol. Trop 59(2):569-583.

Lutein, J. 2007. New York Botanical Garden. *Ericaceae*- Ethnobotanical Studies. Available from: http://www.nybg.org/bsci/res/lut2/name-use.htm Accessed december 2012.

Magariños, H. L. E., C. Sahr, S. D. C. Selaive, M. E. Costa, F. E. Figuerola and O. A. Pizarro. 2008. *In vitro* inhibitory effect of cranberry (*Vaccinium macrocarpum* Ait.) juice on pathogenic microorganisms. App. Biochem. Microbiol. 44(3):300-304.

Magnitskiy, S., G. Ligarreto and H. Lancheros. 2011. Enraizamiento de dos tipos de estacas de los frutales *Vaccinium floribundum* Kunth y *Disterigma alaternoide*s (Kunth) Niedenzu (Ericaceae). Agron. Colomb. 29(2):390-396.

Marcusson, L. L., N. Frimodt-Møller and D. Hughes. 2009. Interplay in the selection of fluoroquinolone resistance and bacterial fitness. PLoS Pathog. 5(8).

Mark's Fruit Crops. 2002. Cranberry-Vaccinium macrocarpon Ait. En: Fruit Crops Encyclopedia http://www.uga.edu/fruit/cranberi.htm Accessed September 2003.

Martin-Aragon, S., B. Basabe, J. M. Benedi and A. M. Villar. 1999. *In vitro* and *in vivo* antioxidant properties of *Vaccinium myrtillus*. Pharm. Biol. 37(2):109-113.

Martínez, J. B., H. A. Rojas, G. C. Borda, A. N. Hastamorir and M. F. Medina. 2011. Estabilidad de antocianinas en jugo y concentrado de agraz (*Vaccinium meridionale* Sw. Rev. Fac. Nal. Agr. Medellín 64(1):6015-6022.

Martz, F., L. Jaakola, R. Julkunen-Tiitto and S. Stark. 2010. Phenolic composition and antioxidant capacity of bilberry (*Vaccinium myrtillus*) leaves in Northern Europe following foliar development and along environmental gradients. J. Chem. Ecol. 36(9):1017-1028.

Marwan, A. and C. M. Nagel. 1986. Microbial inhibitors of cranberries. J. Food Sci. 51(4):1009-1013.

Matsunaga, N., Y. Chikaraishi, M., Shimazawa, S. Yokota and H. Hara. 2010. *Vaccinium myrtillus* (Bilberry) extracts reduce angiogenesis *in vitro* and *in vivo*. Evid. Based Comp. Alternat. Med. 7(1):47-56.

McKay, D. L. and J. B. Blumberg. 2007. Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. Nutr. Rev. 65(11):490-502.

Mediavilla, A., J. Florez and J. M. García_Lobo. 2005. Farmacología de las enfermedades infecciosas: principios generales, selección y asociación de antibióticos. In: J. Florez, J. A. Armijo and A. Mediavilla (Eds.), pp. 1084-1086. Farmacología Humana, Masson S.A., Barcelona.

Meiners, J., M. Schwab and I. Szankowski. 2007. Efficient *in vitro* regeneration systems for *Vaccinium species*. Plant Cell Tiss. Organ Cult. 89(2-3):169-176.

Miranda, M. and A. Cuéllar. 2001. Farmacognosia y Química de los Productos Naturales. Ed. Félix Varela. La Habana, pp. 173-368.

Moen, D. V. 1962. Observations on the effectiveness of cranberry juice in urinary infections. Wisconsin Med. J. 61:282-283.

Moerman, D. (Ed.) Native American Ethnobotany Database. University of Michigan. Available from: http://herb.umd.umich.edu/ Accesed november 2012.

Morozova, O. V. 2007. The Prospects for Using *Vaccinium uliginosum* L. × *Vaccinium vitis-idaea* L. Hybrid in Breeding. Internat. J. Fruit Sci. 6(4):43-56.

Mortiño, 2005. Biolcom: http://www.biolcom.com/biob2/Frutas.

Neef, H., P. Declercq and G. Laekeman. 1995. Hypoglycaemic activity of selected european plants. Phytother. Res. 9(1):45-48.

Nickey, K. E. 1975. Urine pH: effect of prescribed regimen of cranberry juice and ascorbic acid. Arch. Phys. Med. Rehab. 56:556.

Niemann, H. H., W-D. Schubert and D. W. Heinz. 2004. Adhesins and invasins of pathogenic

bacteria: a structural view. Microbes Infect. 6:101–112.

Nowack, R. and W. Schmitt. 2008. Cranberry juice for prophylaxis of urinary tract infections – Conclusions from clinical experience and research. Phytomedicine 15:653–667.

Ofek, I., J. Goldhar and N. Sharon. 1996. Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. Adv. Exp. Med. Biol. 408:179-183.

Paal, T. 1993. The Investigation and Cultivation of *Vaccinium* Species in the Soviet Union. J. Small Fruit Viticult. 1(2):3-8.

Patel, K. D., F. J. Scarano, M. Kondo, R. A. Hurta and C. C. Neto. 2011. Proanthocyanidin-rich extracts from cranberry fruit (*Vaccinium macrocarpon* Ait.) selectively inhibit the growth of human pathogenic fungi *Candida* spp. and *Cryptococcus neoformans*. J. Agric. Food Chem. 59(24):12864-12873.

Peris, J. B, Stübing, G. and B. Vanaclocha. 1995. Fitoterapia Aplicada. Valencia, España: 1st Edition. M.I.C.O.F, 151-153.

Pham, D. Q. and A. Q. Pham. 2007. Interaction potential between cranberry juice and warfarin. Am. J. Health-System Pharm. 64(5):490-494.

Pietta, P., P. Simonetti and P. Mauri. 1998. Antioxidant activity of selected medicinal plants. J. Agric. Food Chem. 46(11):4487-4490.

Pinzón-Arango, P. A., Y. Liu and T. A. Camesano. 2009. Role of cranberry on bacterial adhesion forces and implications for *Escherichia coli*–uroepithelial cell attachment. J. Med. Food. 12(2):259–270.

Prior, R. L., G. Cao, A. Marint, E. Sofic, J. McEwen, C. O'brien, N. Lischner, M. Ethlenfeld, W. Kalt, G. Krewer and C. M. Mainland. 1998. Antioxidant capacity, as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. J. Agric. Food Chem. 46(7):2686-2693.

Puupponen-Pimiä, R., L. Nohynek, C. Meier, M. Kähkönen, M. Heinonen, A. Hopia and K. M. Oksman-Caldentey. 2001. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. 90:494.

Rache, L. Y. and J. C. Pacheco. 2010. Propagación in vitro de plantas adultas de *Vaccinium meridionale* (Ericaceae). Acta Bot. Bras. 24(4):1086-1095.

Reed, J. D. and A. B. Howell. 2008. Biological Activity of cranberry proanthocyanidins: effects on oxidation, microbial adhesion, inflammation, and health. In: Botanical Medicine: From Bench to Bedside. Edited by R. Cooper & F. Kronenberg.

Riihinen, K., A. Ryynänen, M. Toivanen, E. Könönen, R. Törrönen and C. Tikkanen-Kaukanen. 2011. Antiaggregation potential of berry fractions against pairs of *Streptococcus mutans* with *Fusobacterium nucleatum* or *Actinomyces naeslundii*. Phytother. Res. 25:81–87.

Roig, J. T. 1975. Plantas medicinales, aromáticas o venenosas de Cuba. La Habana: Ed. Ciencia y Técn, p. 949.

Roig, J. T. and J. Acuña. 1957. *Ericaceae*. In: Flora de Cuba. IV. Contr. Ocas. Mus. Hist. Nat. Colegio "De la Salle". 16:91-94.

Rojano, B., C. Ochoa, N. Sánchez, C. Medina, M. Lobo, P. Galeano, A. Mosquera, A. Tamayo, Y. Lopera and C. Gaviria. 2009. Capítulo 5: Propiedades antioxidantes de los frutos de agraz o mortiño (*Vaccinium meridionale* Swartz), In: G. Ligarreto (Ed.), pp. 95-112. Perspectivas de cultivo de agraz o mortiño (*Vaccinium meridionale* Swartz) en la zona altoandina de Colombia. 1st Ed. Editorial Gente Nueva, Bogotá. p. 134.

Saltzman, J. R., J. A. Kemp, B. B. Golner, M. C. Pedrosa, G. E. Dallal and R. M. Russell. 1994. Effect of hypochlorhydria due to omeprazole treatment or atrophic gastritis on protein-bound vitamin B_{12} absorption. J. Am. Coll. Nutr. 13(6):584-91.

Schmidt, D. R. and A. E. Sobota. 1989. An examination of the anti-adherence activity of cranberry juice on urinary and non-urinary bacterial isolates. Microbios. 55:173-181.

Schreckinger, M. E., J. Wang, G. Yousef, M. A. Lila and E. González de Mejia. 2010. Antioxidant capacity and *in vitro* inhibition of adipogenesis and inflammation by phenolic extracts of *Vaccinium floribundum* and *Aristotelia chilensis*. J. Agric. Food Chem. 58(16):8966–8976.

Schreckinger, M. E., M. A. Lila, G. Yousef and E. González de Mejia. 2012. Inhibition of α-Glucosidase and α-Amylase by *Vaccinium floribundum* and *Aristotelia chilensis* Proanthocyanidins. In: Hispanic Foods: Chemistry and Bioactive Compounds. ACS Symposium Series 1109:71–82.

Seoane, J. 1984. El folclor médico de Cuba. Ed. Ciencias Sociales, La Habana, pp. 1237.

Servicio de Información y Censo Agropecuario del Ministerio de Agricultura y Ganadería del Ecuador. 2004. Available from: www.sica.gov.ec

Siciliano, A. A. 1998. Cranberry. The Journal of the American Botanical Council and the Herb Research Foundation. 38.

Singh, A. P., R. K. Singh, K. K. Kim, K. S. Satyan, R. Nussbaum, M. Torres, L. Brard and N. Vorsa 2009. Cranberry proanthocyanidins are cytotoxic to human cancer cells and sensitize platinum- resistant ovarian cancer cells to paraplatin. Phytother. Res. 23:1066–1074.

Shmuely, H., J. Yahav, Z. Samra, G. Chodick, R. Koren, Y. Niv and I. Ofek. 2007. Effect of cranberry juice on eradication of *Helicobacter pylori* in patients treated with antibiotics and a proton pump inhibitor. Mol. Nutr. Food Res. 51:746–751.

Sobota, A. E. 1984. Inhibition of bacterial adherence by cranberrry juice: potential use for the treatment of urinary tract infection. J Urol. 131:1013-6.

Song, G. Q and J. F. Hancock. 2012. Recent Advances in Blueberry Transformation. Internat. J. Fruit Sci. 12(1-3):316-332.

Stothers, L. A. 2002. Randomized trial to evaluate effectiveness and cost effectiveness of naturopathic cranberry products as prophylaxis against urinary tract infection in women. Can. J. Urol. 9(3):1558-2.

Su, M. S. and J. L. Silva. 2006. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) byproducts as affected by fermentation. 2007. Food Chem. 97:447-45.

Talavéra, S., C. Felgines, O. Texier, C. Besson, A. Mazur, J-L. Lamaison and C. Rémésy. 2006. Bioavailability of a bilberry anthocyanin extract and its impact on plasma antioxidant capacity in rats. J. Sci. Food Agric. 86(1):90-97.

Terris, M. K., M. M. Issa and J. R. Tacker. 2001. Dietary supplementation with cranberry concentrate tablets may increase the risk of nephrolithiasis. Urology 57:26-29.

Toivanen, M., S. Huttunen, J. Duricová, P. Soininen, R. Laatikainen, V. Loimaranta, S. Haataja, J. Finne, S. Lapinjoki and C. Tikkanen-Kaukanen. 2010. Screening of binding activity of *Streptococcus pneumoniae*, *Streptococcus agalactiae* and *Streptococcus suis* to berries and juices. Phytother. Res. 24:S95–S101.

Vasco, C., K. Riihinen, J. Ruales and A. Kamal-Eldin. 2009. Chemical composition and phenolic compound profile of mortiño (*Vaccinium floribundum* Kunth). J. Agric. Food Chem. 57(18):8274-81.

Vattem, D. A., K. Mihalik, S. H. Crixell and R. J. C. McLean. 2007. Dietary phytochemicals as quorum sensing inhibitors. Fitotrapia 78(4):302-310.

Viskelis, P., M. Rubinskienė, I. Jasutienė, A. Šarkinas, R. Daubaras and L. Česonienė. 2009. Anthocyanins, antioxidative, and antimicrobial properties of american cranberry (*Vaccinium macrocarpon* Ait.) and their press cakes. J. Food Sci. 74(2):C157-C161.

Wang, S. Y., H. Chen, and M. K. Ehlenfeldt. 2011. Antioxidant capacities vary substantially among cultivars of rabbit eye blueberry. Internat. J. Food Sci. Technol. 46(12):2482-2490.

Wang, S. Y., H. Chen, M. J. Camp and K. M. Ehlenfeldt. 2012-a. Genotype and growing season influence blueberry antioxidant capacity and other quality attributes. Internat. J. Food Sci. Technol. 47(7):1540-1549.

Wang, C. H., C. C. Fang, N. C. Chen, S. S. Liu, P. H. Yu, T. Y. Wu, W. T. Chen, C. C. Lee and S. C. Chen. 2012-b. Cranberry-containing products for prevention of urinary tract infections in susceptible populations. Arch. Intern. Med. 172(13):988-996.

Weiss, E. I., Y. Houri-Haddad, E. Greenbaum, N. Hochman, I. Ofek and Z. Zakay-Rones. 2005. Cranberry juice constituents affect influenza virus adhesion and infectivity. Antiviral Res. 66(1):9–12.

Westerlund-Wikström, B. and T. K. Korhonen. 2005. Molecular structure of adhesin domains in *Escherichia coli* fimbriae. Int. J. Med. Microbiol. 295:479–486.

Wiles, T. J., R. R. Kulesus and M. A. Mulvey. 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp. Mol. Pathol. 85:11–19.

Wilson, T., J. L. Luebke, E. F. Morcomb, E. J. Carrell, M. C. Leveranz, L. Kobs, T. P. Schmidt, P. J. Limburg, N. Vorsa and A. P. Singh. 2010. Glycemic responses to sweetened dried and raw cranberries in humans with type 2 diabetes. J. Food Sci. 75(8):H218-H223.

Wilson, T., J. P. Porcari and D. Harbin. 1998. Harbin Cranberry extract inhibits low density lipoprotein oxidation. Life Sci. 62(24):l381-l386.

Yan, X. J., B. T. Murphy, G. B. Hammond, J. A. Vinson and C. C. Neto. 2002. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). J. Agric. Food Chem. 50(21):5844-5849.

Yarnell, E. and K. Abascal. 2008. Antiadhesion herbs. Alternat. Comp. Therap. 14(3):139-144.

Yelioglu, Y. S., G. Mazza, L. Gao and B. D. Oomah. 1996. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46(10):4113-4117.

Zafra-Stone, S., T. Yasmin, M. Bagchi, A. Chatterjee, J. A. Vinson and D. Bagchi. 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol. Nutr. Food Res. 51:675-683.

Zafriri, D., I. Ofek, R. Adar, M. Pocino and N. Sharon. 1989. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated *Escherichia coli* to eucaryotic cells. Antimicrob. Agents Chemother. 33:92-98.

Zozulya, R.N., L.D. Musaeva and R. A. Kuzmina. 1975. Biological activity of raw material from the above-ground part of bilberry. Rast. Resur. 11:87.

Emir. J. Food Agric. 2014. 26 (7): 592-601
doi: 10.9755/ejfa.v26i7.18186
http://www.ejfa.info/

REGULAR ARTICLE

Determination of mangiferin in *Mangifera indica* L. stem bark extract (Vimang®) and pharmaceuticals by liquid chromatography

Eddy Castellanos Gil[1], Rolando González Hernández[2], Lauro Nuevas Paz[3*] and Jos Hoogmartens[4]

[1]*Université de Sherbrooke, Department of Chemical & Biotechnological Engineering, Pfizer Industrial Research Chair on PAT in Pharmaceutical Engineering, Sherbrooke, Québec, Canada*
[2]*Oficina Cubana de Propiedad Intelectual (OCPI), Habana Vieja, La Habana, Cuba*
[3]*Centro Nacional de Genética Médica. Ave, 31 y 146, Rpto Cubanacán, Playa, La Habana, Cuba*
[4]*E. van Evenstraat 4, Laboratorium voor Farmaceutische Chemie en Analyse van Geneesmiddelen, Faculteit Farmaceutische Wetenschappen, KUL, Leuven, B-3000, Belgium*

Abstract

A new liquid chromatography method for the analysis of mangiferin (I) from *Mangifera indica* L. stem bark extract (Vimang®), and pharmaceuticals is described. Screening experiments were performed by an experimental design to find the influence of some important chromatographic variables (methanol, column temperature and acetic acid) on the retention times and resolution between critical pairs in the separation. This design also permitted to estimate method robustness and optimal conditions to achieve the best resolution. The best separation was found using a LiChrospher RP 18, 5 µm, 250 x 4.6 mm I.D. column maintained at 25°C, a mobile phase comprising methanol-2.5% v/v aqueous solution of acetic acid (280:720, v/v) at a flow rate of 1.0 ml/min, and detection by UV at 254 nm. The method resolves I, the major component, from other components of the extract. The method showed good selectivity, repeatability (RSD < 2%) and linearity (r = 0.9998). The limits of detection and quantitation were 0.008% (0.9 ng) and 0.05% (6.2 ng), respectively, relative to a 0.6 mg/ml standard solution, injection volume 20 µl. This method was used to quantify I in some aqueous extracts from the natural product and pharmaceuticals.

Key words: *Mangifera indica*, Mangiferin, Vimang®, Pharmaceuticals, HPLC

Introduction

A new bioactive product of natural origin has been developed from the folk knowledge in Cuban ethnic medicine and it is used at present as antioxidant nutritional supplement (Guevara et al., 2004). The active ingredient of the developed pharmaceutical formulations (tablet, capsule, cream and syrup) is an extract of the *Mangifera índica* L. (mango) stem bark (Vimang®), obtained by decoction of some varieties grown in Cuba (Núñez Sellés et al., 2002; Acosta-Equijarosa et al., 2009). It is a fine brown powder, which has proven to be useful in the treatment of a large population sample presenting physical stress due to age or deteriorated physiological conditions caused by chronic lengthened diseases such as cancer, diabetes or cardiovascular disorders (Núñez-Sellés, 2005). Studies have shown that treatment with the extract provided significant protection against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative damage, and the former lead to better protection when compared with other antioxidants (Vitamin C, E and beta–carotene) (Sánchez et al., 2000). Furthermore, the results indicate that Vimang® is bioavailable for some vital target organs, including liver and brain tissues, peritoneal cell exudates and serum. Therefore, it was concluded that it could be useful to prevent the production of reactive oxygen species (ROS) and the oxidative tissue damages in vivo (Sánchez et al., 2000). All these effects are likely due to the synergic action of several compounds such as polyphenols, terpenoids, steroids, fatty acids and microelements, which have been reported to be present in the extract (Núñez-Sellés et al., 2002a; Núñez-Sellés et al., 2007; Núñez-Sellés and Rastrelli, 2010).

Mangiferin (1,3,6,7-tetrahydroxyxanthone-2-C-ß-D-glucopyranoside), a C-glucosylxanthone, which was first isolated from the bark, branches and leaves of *Mangifera indica* L. (Bhatia, 1967; Nott, 1968),

Received 05 October 2013; Revised 15 February 2014; Accepted 20 February 2014; Published Online 18 May 2014

*Corresponding Author

Lauro Nuevas Paz
Centro Nacional de Genética Médica. Ave, 31 y 146, Rpto Cubanacán, Playa, La Habana, Cuba

Email: lauro@cngen.sld.cu

has been found to be the major component of this extract. It is known that mangiferin (**I**) (Figure 1) is potentially a naturally-occurring chemopreventive agent in rat colon carcinogenesis (Yoshimi et al., 2001), exerts antidiabetic activity by increasing insulin sensitivity (Ichiki et al., 1998; Sellamuthu et al., 2009), appears to act as a potential biological response modifier with antitumor, immune modulatory and anti-HIV effect (Guha et al., 1996), is useful as an analgesic without adverse effects (Makare et al., 2001), and inhibits the late event in Herpes Simplex/Virus-2 replication (Zhu et al., 1995).

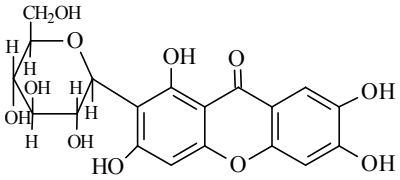

Figure 1. Structure of mangiferin (I).

A thin layer chromatography (TLC) densitometric method was developed for the quantitative determination of **I** in leaves of *Cratoxylum pruniflorum* (Nedialkov et al., 1998). On the other hand a high performance thin layer chromatographic (HPTLC) method was developed and validated as per ICH (International conferences on harmonization) guidelines for simultaneous quantification of mangiferin in *Salacia chinensis* roots (Nadagouda et al., 2010). Others liquid chromatography methods (LC) has been reported before for the quantitative determination of **I** and several other metabolites in *Mangifera indica* L. extracts or parts of the tree.

Several methods of LC for the determination of I simultaneous determination of several components including **I** in Chinese traditional pharmaceutical preparations (Ronghua et al., 2004) and determination of **I** in rat plasma and urine (Wang et al., 2006) are available. Recently report assessing the amount of mangiferin allowed into the eye (Hou et al., 2010). Therefore, a new LC method for the routine quantitative analysis of **I** in presence of other metabolites from *Mangifera indica* L. extracts and its pharmaceuticals had to be developed.

Materials and Methods

Reagents

Water was distilled twice from glass apparatus. Methanol, HPLC grade, and glacial acetic acid, analytical grade, were from Merck (Darmstadt, Germany).

Reference standards

The reference standard (RS) of mangiferin was a house standard with 94.8% of purity. The preparation chemical characterization of this reference standard was previously described (Sordo et al., 2010).

Natural product samples (Vimang®)

Natural product sample (Vimang®) was obtained by aqueous decoction of *Mangifera índica* L. stem bark grown in Cuba. The aqueous extract was dried by atomization in a spray dryer until a brown solid was achieved, which melts with decomposition between 215 and 218°C (Lot 601, water content (K. Fischer), 10.5% (RSD = 0.9%)) (Acosta Esquijarosa et al., 2009). Tablets and capsules conformed under wet granulation process (Vimang®, 300 mg/ units) were used for the applications.

Natural product sample preparation

Natural product samples of 50 mg were accurately weighed into 100 ml conical flasks. 25.0 mL of 85% v/v methanol were added, the flasks were sealed and shaken on a magnetic shaker for 20 min. Approximately 10 mL were centrifuged at 3000 rpm for 2 min. 5.0 ml of the clear supernatant were diluted to 10.0 mL with methanol 85%.

For pharmaceutical samples, 20 units were weighed and finely powdered. An accurately weighed amount of the analyte powder equivalent to the content of a unit was transferred into a 1000 mL conical flask. 250 mL of 85% v/v were added, the flask was sealed and shaken on a magnetic shaker for 20 min. Approximately 10 mL were centrifuged at 3000 rpm for 2 min. 5.0 mL of the clear supernatant were diluted to 10.0 mL with methanol 85%.

Recovery test

Four 50 mg amounts of natural product sample (one as a control) were weighed accurately and each portion (except the control) was spiked with known quantities of mangiferin house RS (1.26, 2.52, and 4.06 mg). All samples were extracted using the same procedure as indicated under 2.4 and the obtained solutions were injected for LC analysis to calculate the recovery. For pharmaceuticals, the same procedure was performed, but four amounts equivalent to a capsule and/or tablet were weighed accurately and each portion (except the control) was spiked with known quantities of mangiferin house RS (12.6, 25.2, and 40.6 mg). All samples were extracted using the same procedure as indicated under 2.4 and the obtained solutions were injected for LC analysis to calculate the recovery.

LC apparatus

The equipment consisted of an intelligent pump L-6200 (Merck-Hitachi, Darmstadt, Germany), a Model Rheodyne 7125 injector (Cotati, California, USA), with a 20 µL loop, a model L - 4250 UV detector (Merck-Hitachi). A PC was used to register the chromatograms using the software BioChrom 2.1 (CIGB, Havana, Cuba).

Experimental design

Screening and optimization of the selectivity were performed by experimental design and multivariate analysis. The screening experiment was carried out as a full-fraction factorial design at two levels. Response surface modeling (RSM) was used to optimize the significant variable factors. Three chromatographic variables that governed the separation most: quantity of methanol (mL) and acetic acid (%) in the mobile phase, and the column temperature were selected. This involves $2^3 = 8$ different experimental measurements. Considering the inclusion of the central point combination in the design (set on the preferred conditions found in the method development), as well as duplicate experiments, 18 measurements had to be performed. Robustness study for the optimized method conditions was performed using a Central Composite design Face Centered (CCF) for response surface modeling (RSM) investigations (17 measurements). The setup of the applied designs, the randomization of runs, the analysis of the measured responses, and the multivariate regression calculations, were performed using the statistical software Modde version 4.0 (Umetri, AB, Umeå, Sweden). One injection was done for each experiment and experiments were duplicated using the same mobile phase. The influence of the chromatographic variables and the interactions between two of them were considered in the analysis of response variables.

Results and Discussion

Method development

A 1 mg/ml reference standard solution of **I** in 85% methanol was initially chromatographed on a LiChrospher RP 18 column, 5 µm (250 x 4.6 mm I.D.) using water - methanol (70:30, v/v). To improve the symmetry factor of the observed peaks, a 3% v/v aqueous solution of acetic acid was used instead of water. Originally, a column temperature of 30°C was used but it was observed that by lowering the temperature to 25°C, the separation around the major peak was much improved, whereas the analysis time increased by only a few minutes.

Figure 2A shows a LC chromatogram of a 1 mg/mL preparation of a natural product sample from *Mangifera indica* L. In this chromatogram, peak **2** was assigned to **I**, and peak **3** to **II**. Peak **1** remained unidentified. UV spectra of peaks **2** and **3** were identical and showed four characteristic maximums of xanthones at 240, 258, 320 and 368 nm (Berardini et al.,2005; Barreto et al., 2008), whereas the one corresponding to peak **1** showed only two maxima at 224 and 274 nm. The three chromatographic peaks were homogeneous (UV spectra, which were recorded stopping the pump and using a facility of the UV detector, were the same at the beginning, in the maximum and at the end of each peak).

Optimization and Robustness

Optimization testing was performed using a full-fraction factorial experimental design including a low number of experiments, which allows estimating the effect of chromatographic parameters and their interactions. The estimated response surface plot for **R1** (resolution between 1 and 2) **R2** (resolution between 2 and 3) revealed that the best resolution within the studied range is achieved when the chromatographic variables were at their lower values (Figure 3). Due to the Cuban tropical climate it was preferred not to choose for temperatures below 25°C. Figure 2B shows a chromatogram obtained using the optimized conditions.

Robustness of the method was tested using a Central Composite design Face Centered (CCF). Table 1 shows the three chromatographic variables to be examined and the corresponding values for the design. As response variables in the factorial design, retention times of compounds were measured and selectivity between critical pairs was calculated.

The data collected were used to estimate the coefficients of the model, which represent the relationship between the response (Y) and the factors (X_n). Multiple linear regression (MLR) was used to estimate the coefficients of the terms in the model that are computed to minimize the sum of squares of the residuals, i.e. the sum of squared deviations between the observed and fitted values of each response. The least square regression method yields small variances for the coefficients and small prediction errors. It is important to note that MLR separately fits one response at a time and hence assumes them to be independent. One may review the fitted model by: examining the summary of the fit, R^2 and Q^2 for every response, examining the coefficients and their 95% confidence intervals, and examining the analysis of variance (ANOVA) table.

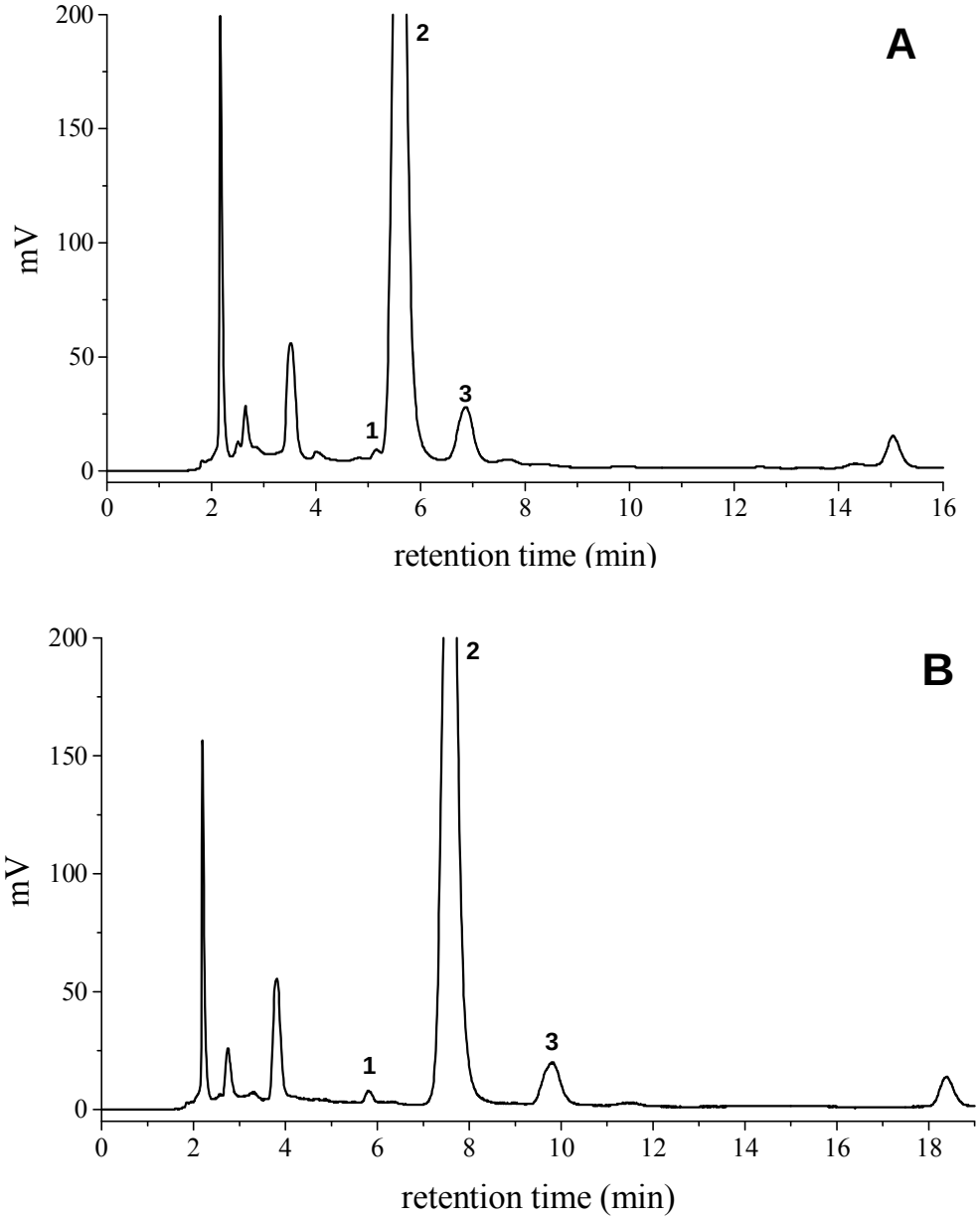

Figure 2. LC chromatogram of an extract of *Mangifera indica* L. (A) Stationary phase, LiChrospher RP 18, 5 µm (250 x 4.6 mm I.D.); column temperature, 30°C; mobile phase, 3% acetic acid aqueous solution – methanol (700:300, v/v); flow rate, 1.0 mL/min; sample concentration, 1 mg/mL; injection volume, 20 µl; detection, UV at 254 nm. Peaks: 1 = unknown, 2 = I, and 3 = compound structurally related to I. (B) as A, but column temperature, 25°C; mobile phase, 2.5% acetic acid aqueous solution – methanol (720:280, v/v).

Table 1. Values corresponding to –1, 0, and +1 levels.

Chromatographic variable	Low value (-1)	Central value (0)	High value (+1)
(A) methanol (mL)	260	280	300
(B) temperature (°C)	23	25	27
(C) acetic acid (%, v/v)	2.0	2.5	3.0

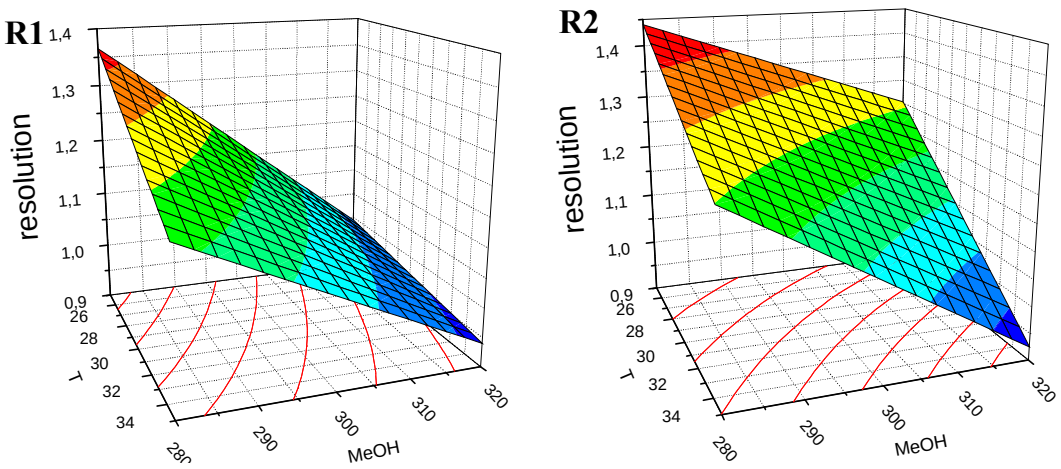

Figure 3. Response surface plot showing the resolution as a function of significant chromatographic variables (MeOH – methanol; T – column temperature). R1 – resolution between peaks 1-2; R2 – resolution between peaks 2-3. Percent of acetic acid was constant at 3 %.

The percent of the variation of the response explained by the model (R^2) and the predictive power of the model (Q^2) were over 0.97 and 0.7, respectively, implying that the data fitted well with the model. R^2 overestimates the good quality of fit, whereas Q^2 underestimates the veracity of fit. R^2 and Q^2 values close to 1 indicate an excellent model. A Q^2 larger than zero indicates that the dimension is significant (predictive). Large Q^2, 0.7 or larger, indicates that the model has good predictive ability and will have small prediction errors. The probability values for lack of fit in the ANOVA table were greater than 0.10 for every response variable. For this reason, the model appeared to be adequate for the observed data.

Evaluation of the coefficients calculated by the model allows obtaining the effect plot for each response variable. This plot displays the values of the effects (twice the coefficients) sorted (in absolute value) in descending order. The ± 95% confidence interval is drawn as an upper and lower line. For chromatographic variables the plot displays the predicted change in the response when the factor varies from its low to its high level, all other factors in the design being set on their average. When one selects a 2 factor interaction, the predicted change in the response when one factor varies from its low to its high level is plotted for both levels of the other factor, all remaining factors in the design being set on their average.

Peaks **1**, **2** and **3** were selected to review the effects of chromatographic variables and their interaction on the retention times and selectivity.

Figure 4 shows that two parameters, methanol and column temperature, have significant negative effects on the retention times of all compounds. This means that an increase of these variables provokes a decrease of the retention times. It can be noted that these effects are larger for peaks **2** and **3**. Interaction variables (column temperature and per cent of acetic acid) had only significant positive effect on the retention time for peak **1**. Within the range examined only methanol has significant negative effect on the selectivity between peaks **1-2** and **2-3**, denoting a decrease of the selectivity with an increase of methanol. Nevertheless, the peaks never overlapped, indicating the robustness of the method.

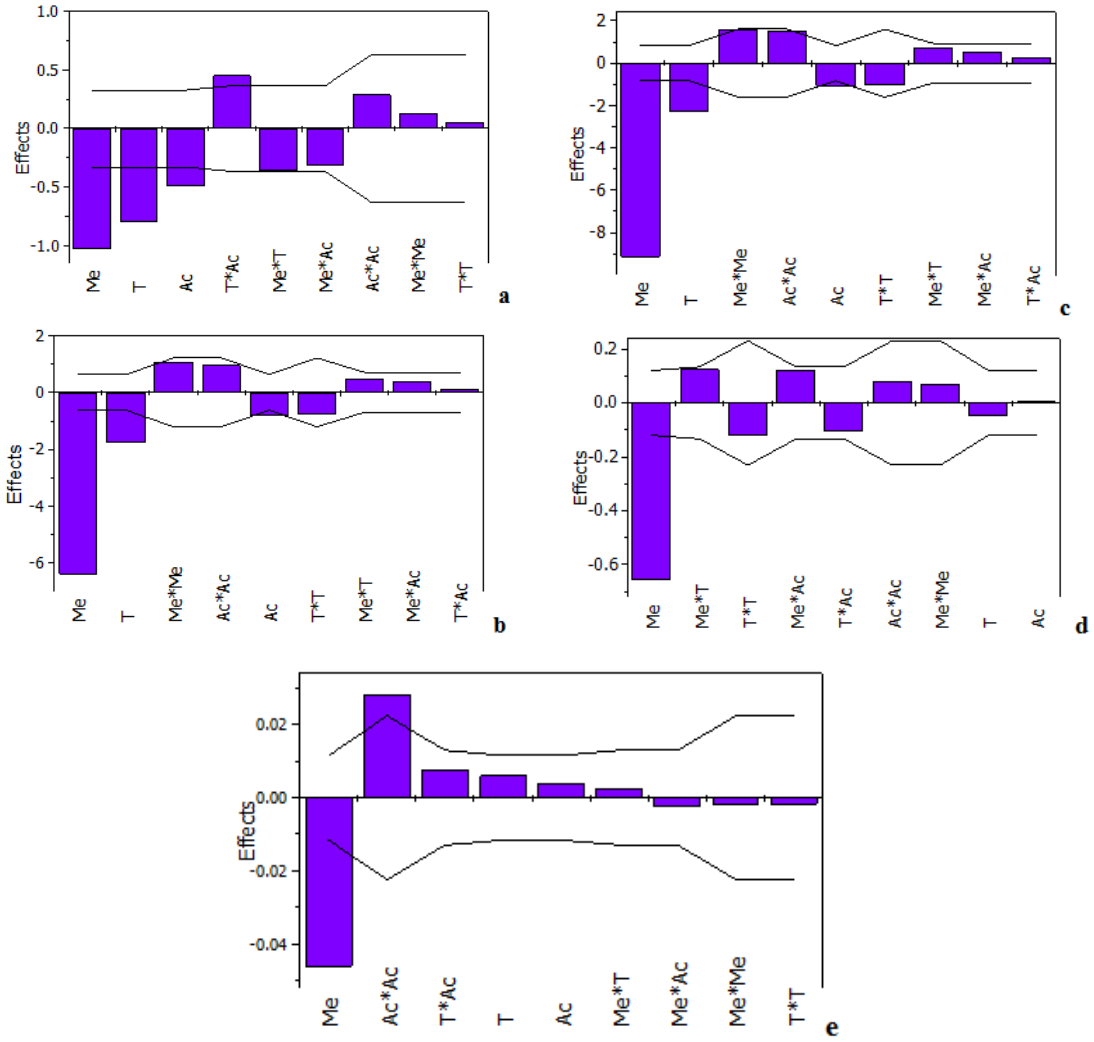

Figure 4. Effect plot for the response variables: a, b and c – retention times of peaks 1, 2 and 3, respectively; d and e – selectivity between peaks 1-2 and 2-3, respectively. Me – quantity of methanol (mL); Ac – percent of acetic acid; T – temperature.

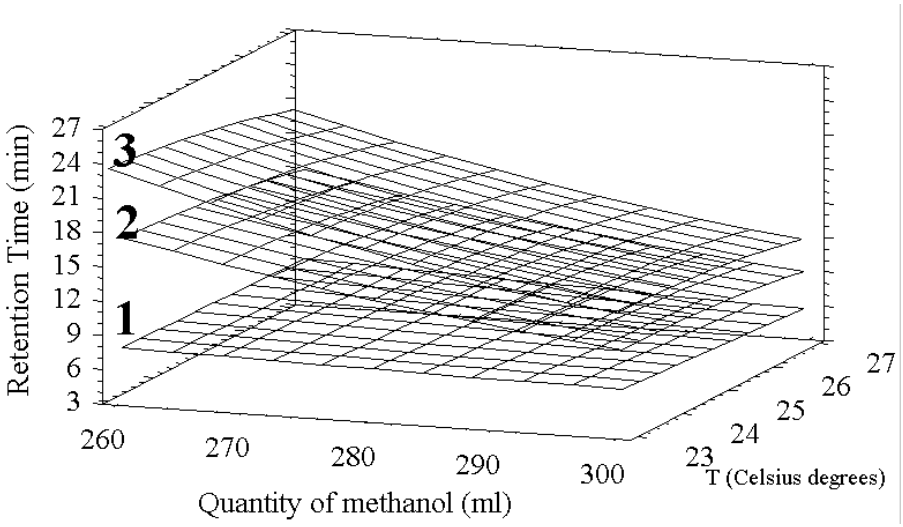

Figure 5. Estimated response surface plots constructed with retention times (tr) as a function of the quantity of methanol and the temperature of the column. The percent of acetic acid was kept at 3%.

Table 2. Relative recoveries of mangiferin in *Mangifera indica* L.

Added (mg)	Mean found (n = 3)	Relative Recovery (%) ± SD (n = 3)	Mean ± SD	RSD (%)
1.26	1.26	100.0 ± 3.2		
2.52	2.54	100.8 ± 2.2	100.9 ± 2.3	2.3
4.06	4.12	101.3 ± 1.8		

Response surface plots (Figure 5) constructed with retention time, as a function of the most significant chromatographic variables (methanol and column temperature) shows no overlapping, thus confirming the robustness of the method.

Quantitative analysis of mangiferin

Validation parameters such as precision, linearity, accuracy, selectivity and limits of detection (LOD) and quantitation (LOQ) were determined for mangiferin. Precision (repeatability) was checked with a solution of natural product sample having a 0.4 mg/mL concentration, equivalent to mangiferin. The relative standard deviation (RSD) was 1.6% (one analyst, n = 6) and 1.7% (two analysts, n = 12) for within-day and day-to-day repeatability, respectively. The calibration curve obtained by replicated analysis (n = 3) of a series of analyte concentrations corresponding to 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL of mangiferin reference standard was subjected to linear regression analysis: y = -0.05 + 474.6 x, where x = mangiferin concentration in mg/ml, y = peak area; correlation coefficient r = 0.9998, standard error of the estimate $S_{y,x}$ = 1.8. The smallest difference of analyte concentration that can be recorded with a 95% probability is 0.006 mg/mL (1%).

The relative recoveries of **I** are shown in Table 2. 100.0% of **I** was recovered in presence of natural product sample with 85% methanol. The extraction recoveries of **I** from the related pharmaceuticals with three spiked levels were above 98% in all cases.

Homogeneous chromatographic peaks for **I** were obtained from 1 mg/ml solutions of natural product sample dissolved in acid (0.1 M HCl), aqueous and basic media (0.1 M NaOH), respectively, which were submitted to 1 h reflux, confirming the selectivity. The content of **I** was diminished only under basic media (51.0% of the original remained after degradation), whereas under other media no degradation was observed.

In the determination of LOD and LOQ, a solution of **I** reference standard was diluted gradually. The LOQ with signal-to-noise ratio of 10

was 0.05% of 0.6 mg/mL, i.e. 6.2 ng injected mass (n = 6; RSD = 7.6%). The LOD with signal-to-noise ratio of 3 was 0.016%, i.e. 1.98 ng injected mass.

Applications

The application of this method to the quality control of 16 batches of Vimang® active ingredient, obtained from different provinces of the country, and its pharmaceuticals were investigated and demonstrated in Table 3. Each sample was analyzed in triplicate and the average value was listed. All of the assay results fell between 100~300 µg of mangiferin per mg of Vimang ® powder, but samples No. 12 to No. 16 and they were rejected. The differences found are probably due to the fact that the mangiferin content in the plant varies with the season of the year and the zone in which it was grown. The claimed contents of this natural product required by ours producer are 85-115% for tablets and capsules. A typical chromatogram for the analysis of pharmaceuticals is shown in Figure 6. We established a simple and selective HPLC method for the assay of Vimang®.

Table 3. Content of mangiferin (µg/mg) in natural product samples from *Mangifera indica* L. and assay result of pharmaceuticals.

Sample	Content, µg/mg (RSD, %)	Sample	Content, µg/mg (RSD, %)
No.1 (batch 901)	254 (0.7)	No.9 (batch 0201)	125 (0.1)
No.2 (batch 903)	195 (2.0)	No.10 (batch 0202)	109 (1.5)
No.3 (batch E-923)	187 (1.6)	No.11 (batch 0203)	116 (1.2)
No.4 (batch E-924)	180 (1.4)	No.12 (batch 0204)	79 (2.4)*
No.5 (batch E-032)	206 (1.6)	No.13 (batch 0205)	56 (0.5)*
No.6 (batch 0103)	149 (5.7)	No.14 (batch 0206)	55 (2.3)*
No.7 (batch 0104)	162 (0.4)	No.15 (batch 0207)	66 (1.8)*
No.8 (batch 0112)	159 (0.3)	No.16 (batch 0208)	49 (0.2)*
Pharmaceuticals	Amount of Vimang® (mg)	Percentage of claimed content %, (RSD, %)	
Tablets (batch 1)	299.91	99.97 (5.47)	
Tablets (batch 2)	291.36	97.12 (3.14)	
Tablets (batch 3)	310.51	103.51 (1.42)	
Capsules (batch 1)	309.03	103.01 (1.66)	
Capsules (batch 2)	307.83	102.61(1.78)	
Capsules (batch 3)	313.89	104.63 (1.18)	

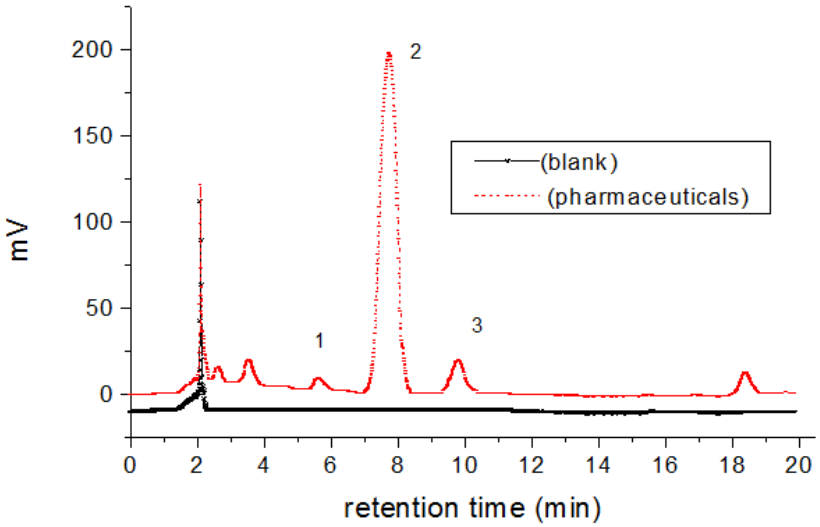

Figure 6. LC chromatograms of blank and Vimang® pharmaceuticals (tablets and capsules). Stationary phase, LiChrospher RP 18, 5 µm (250 x 4.6 mm I.D.); column temperature, 25°C; mobile phase, 2.5 % acetic acid aqueous solution – methanol (720:280, v/v); flow rate, 1.0 mL/min; injection volume, 20 µL; detection, UV at 254 nm. Peaks: 1 = unknown, 2 = I, and 3 = compound structurally related to I.

Conclusion

A LC method was developed for the quantitative determination of **I** as the major component of *Mangifera indica* L. extracts. A full-factorial design indicated that the three studied chromatographic variables (methanol, acetic acid and column temperature) have a significant effect on the retention times of all compounds, whereas the influence on the resolution among them is not significant, showing that the method was robust. It also pointed out the optimal conditions needed to achieve the best resolution. Validation of the method has shown its usefulness in the quantitative routine analysis of **I** in extracts and pharmaceuticals of the natural product under study.

References

Acosta-Esquijarosa, J., U. Jáuregui-Haza, D. Amaro-González and L. Sordo-Martínez. 2009. Spray drying of aqueous extract of *Mangifera indica* L. (Vimang®): Scale up for the process. World Appl. Sci. J. 6(3):408-412.

Barreto, J. C., M. T. Trevisan, W. E. Hull, G. Erben, E. S. de Brito, B. Pfundstein, G. Wurtele, B. Spiegelhalder and R. W. Owen. 2008. Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). J. Agric. Food Chem. 56:5599-5610.

Berardini, N., R. Fezer, J. Conrad, U. Beifuss, R. Carle and A. Schieber. 2005. Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol O- and xanthone C-glycosides, anthocyanins, and pectin. J. Agric. Food Chem. 53:1563-1570.

Bhatia, V. K., J. D. Ramnathan and T. R. Sbshadri. 1967. Constitution of mangiferin. Tetrahedron Lett. 23:1363-1368.

Guevara, G. M., A. Riaño-Montalvo, A. Alvarez-León, G. Garrido-Garrido, E. Paéz-Betancourt and R. Delgado-Hernández. 2004. Uso etnomédico de la corteza de Mangifera indica L. en Cuba. Rev. Cub. Plant Med 9:1-9.

Guha, S., S. Ghosal and U. Chattopadhyay. 1996. Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. Chemotherapy 42:443-451.

Makare, N., S. Bodhankar and V. Rangari. 2001. Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. J. Ethnopharmacol. 78:133-137.

Nadagouda, S. G., A. A. Karigar, V. G. Joshi and M. S. Sikarwar. 2010, Validated HPTLC method for mangiferin in *Salacia chinensis*. J. Pharm. Res. 3(5):1107-1109.

Nedialkov, P., G. Kitanov and J. Tencheva. 1998. Densitometric determination of the xanthone isomers mangiferin and isomangiferin in plant materials. Acta Pharm. 48:211-214.

Nott, P. E. and J. C. Roberts. 1968. The structure of mangiferin. Phytochem. 6:741-747.

Núñez-Sellés, A. J., E. Paéz, D. Amaro, J. Acosta, J. Agüero, R. Capote, M. R. Gárcia, I. G. Morales, O. García, G. Garrido, G. Martínez and M. A. Morales. 2002. Composiciones farmacéuticas a partir del extracto de *Mangifera indica* L. Patente No. 1814, Octubre, Oficina Cubana de la Propiedad Industrial.

Núñez-Sellés, A. J., M. D. Rodriguez, E. R. Balseiro, L. N. Gonzalez, V. Nicolais and L. Rastrelli. 2007. Comparison of major and trace element concentrations in 16 varieties of Cuban mango stem bark (*Mangifera indica* L.). J. Agric. Food Chem. 55:2176-2181.

Núñez-Sellés, A. J. 2005. Antioxidant Therapy: Myth or Reality? J. Braz. Chem. Soc. 16:699-710.

Núñez-Sellés, A. J. and L. Rastrelli. 2010. Chemical composition of the mango stem bark extract (*Mangifera indica* L.), In: V. K. Gupta (Ed.), Comprehensive Bioactive Natural Products. Stadium Press, Houston, USA. Vol. 6(15):433-456.

Núñez-Sellés, A. J., H. T. Velez-Castro, J. Aguero-Aguero, J. Gonzalez-Gonzalez, F. Naddeo, F. De Simone and L. Rastrelli. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J. Agric. Food Chem. 50:762-766.

Dai, R., J. Gao and K. Bi. 2004. High-performance liquid chromatographic method for the determination and pharmacokinetic study of mangiferin in plasma of rats having taken the traditional Chinese medicinal preparation Zi-Shen pill. J. Chromatogr. Sci. 42:88-90.

Sanchez, G. M., L. Re, A. Giuliani, A. J. Núñez-Sellés, G. P. Davison and O. S. Leon-Fernandez. 2000. Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. Pharmacol. Res. 42:565-573.

Sellamuthu, P. S., B. P. Muniappan, S. M. Perumal and M. Kandasamy. 2009. Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rats. J. Health Sci. 55:206–214.

Sordo-Martínez, L., J. Lora-García, L. Nuevas-Paz, F. Concepción-Martínez, H. Curiel-Hernández, A. Fernández-Villalobo, H. Vélez-Castro, M. I. Reyes-Naranjo and D. Amaro-González. 2010. Desarrollo del material de referencia químico nacional de mangiferina. Rev. Cub. Farm. Suplemento especial.

Wang, H., G. Ye, Y. H. Tang, H. Y. Zhu, R. R. Ma, Z. L. Sun and C. G. Huang. 2006. High-performance liquid chromatographic method for the determination of mangiferin in rat plasma and urine. Biomed. Chromatogr. 20:1304-1308.

Yoshimi, N., K. Matsunaga, M. Katayama, Y. Yamada, T. Kuno, Z. Qiao, A. Hara, J. Yamahara and H. Mori. 2001. The inhibitory effects of mangiferin, a naturally occurring glucosylxanthone, in bowel carcinogenesis of male F344 rats. Cancer Lett. 163:163-170.

Hou, Y., S. Fan, H. Zhang, Y. Gu, X. Yu and B. Li. 2010. Pharmacokinetic study of mangiferin in rat plasma and retina using high-performance liquid chromatography. Mol. Vis. 16:1659-1668.

Zhu, X. M., J. X. Song, Z. Z. Huang, Y. M. Wu, and M. J. Yu. 1993. Antiviral activity of mangiferin against herpes simplex virus type 2 *in vitro*. Zhongguo Yao Li Xue Bao 14:452-454.

Emir. J. Food Agric. 2014. 26 (7): 602-608
doi: 10.9755/ejfa.v26i7.17513
http://www.ejfa.info/

REGULAR ARTICLE

Chemical compositions of the Tunisian *Ziziphus jujuba* oil

M. Elaloui[1*], A. Laamouri[1], A. Albouchi[1], M. Cerny[2], C. Mathieu[2], G. Vilarem[2] and B. Hasnaoui[3]

[1]*Institut National des Recherches en Génie Rural, Eau, Forêts (INRGREF), rue Hedi Karray, Elmenzeh IV, BP 10, 2080, Ariana, Tunisie*
[2]*Laboratoire de Chimie Agro-Industrielle (LCAI), UMR 1010 INRA-INPT/ENSIACET 4 allée Emile Monso 31030, Toulouse cedex, 4, France*
[3]*Institut Sylvo-pastoral de Tabarka, 8110, Tabarka, Tunisie*

Abstract

Thirteen fatty acids were identified from the pulps of four Tunisian *Ziziphus jujuba* ecotypes (Sfax, Choutrana, Mahres and Mahdia), using capillary gaseous chromatography method. These oils presented 8.31% to 12.35% of dry weights. Compared to the other ecotypes, Mahres and Choutrana were the richest of the oleic acid. The palmitic acid was the most important compound of the Sfax ecotype. Unsaturated fatty acids ranged from 62.63% to 72.40% of the total fatty acids of each ecotype. So a ratio of the unsaturated/saturated (U/S) varied from 1.68 to 2.37. The β-sitosterol and the Stigmasterol were identified as major sterols. The β-sitosterol was the prominent component in the all ecotypes and the highest level (10.65 mg/100g) was noted in Choutrana ecotype. Stigmasterol was more abundant in Sfax (16.12 mg/100g) and Choutrana (4.67 mg/100g) ecotypes. The campesterol sterol was identified only in Sfax and Choutrana ecotypes being more important (2.4 mg/100g) in Choutrana pulps.

Key words: Fatty acids, Linoleic acid, Oleic acid, β-sitosterol, *Ziziphus*

Introduction

Jujube (*Zizyphus* spp.), are cultivated economically in several countries. It is a species of about 40 species and grows mainly in tropical and subtropical parts of the world (Mukhtar et al., 2004; Laamouri, 2009). Three species are known in Tunisia: *Z. lotus*, Z. *spina-christi* and *Z. jujuba*. The *Z. jujuba* also known as "anneb" is the most popular specie. This tree, with 10 cm of length and 50 cm of diameter, offer a delicious read fruit (jujube) that was consumed fresh, dried and processed (jams, loaf, cakes, etc.). This jujube has many medicinal and pharmaceutical fields. In fact, it was widely used to treat chronic hepatitis or distress and fullness in the chest (Yamaoka et al., 1996; Sheng et al., 2009). Oil extracted from different *Z. jujuba* organs has been widely used to treat immunity deficiency (Zhao et al., 2006; Goncharova et al., 1990; Peng et al., 2000). The fatty acid profiles of jujube were influenced by their developmental stage (Guil-Guerrero et al., 2004). The oleic and the palmitic acids are defined as the major compound of oil pulps extracts (Guil-Guerrero et al., 2004). San et al. (2010) added the linoleic and the palmitolic acids to this composition.

Z. jujuba oil was also rich on unsaturated fatty acids that constitute the important cell membranes components and provide protection effects against heart disease, diabetes, certain types of cancer and some other diseases (Lunn and Theobald, 2006).

Phytosterols had been also detected in different part of *Z. jujuba*. They had been used to lower plasma total cholesterol and lipoproteins (Aioi et al., 1995; Owen et al., 2000; Das et al., 2003). In fact, and according to Bonanome et al. (1988), the sterols ration had been envisaged as cosmetic ingredient. However, there is a little information detailed fatty acids, and sterols compositions of *Z. jujuba.*

In Tunisia, *Z. jujuba* is located especially in the southern country (Mahdia, Gafsa Kébili, Sfax, Mahres). In the north, some trees are present in private gardens (Ariana, Choutrana). In the previous study, four *Z. jujuba* ecotypes: Sfax, Choutrana, Mahres and Mahdia were selected (Elaloui, 2013). The objectives of the present study

Received 26 November 2013; Revised 20 February 2014; Accepted 27 February 2014; Published Online 18 May 2014

*Corresponding Author

M. Elaloui
Institut National des Recherches en Génie Rural, Eau, Forêts (INRGREF), rue Hedi Karray, Elmenzeh IV, BP 10, 2080, Ariana, Tunisie

Email: maryoumaa2000@yahoo.fr

were to characterize and to compare the chemical composition of fatty acids and sterols in the pulp oils.

Materials and Methods

Plant material

Pulps were collected from plants cultivated in the tunisian experimental station of "Rouhia" (northwestern Tunisia; 35° 40'-15.39" N; longitude 9° 0.3 - 15.29 E; altitude 636 m). The fruits (Figure 1) of *Ziziphus zizyphus* were collected in September 2009 and identified by (Laamouri, 2009). A voucher sample is deposited at the Herbario of National Institute for Research in Rural Engineering, Water and Forests (INRGREF) in Tunisia.

Figure 1. Fruits of *Ziziphus jujuba* (experimental station-Rouhia).

Pulps, hand removed from fruits, were grounded by a mill equipped with a grid whose holes are 1.00 mm in diameter.

Reagents and standards

All solvents used in our experiments: Tert-Butyl-Methyl Ether (TBME), cyclohexane; KOH; N-methyl-N-trimethylethylsilyl-heptafluoro butyramide (MSHFBA); dihydrocholesterol; chloroform the homologous fatty acids and sterol standards were purchased from Sigma Aldrich (Steinheim, Germany).

Lipids extractions

Ground *Ziziphus* pulps were extracted with cylohexane in a soxhlet apparatus for 6 h. The extract was concentrated under reduced pressure using a rotary evaporator at 60°C. The extracted oil was kept in obscurity at 4°C in waiting for analysis. The extractions were carried out in duplicate.

Fatty acids extraction

For FAs extractions, the procedure of Macherey Nagel was adopted. We dissolve 20 mg of oil with 1 ml of a suitable solvent (TBME: Tert Butyl Metyl Ether). Then, we Add 50 µL reagents to 100 µL of this solution. A methylation with TMSH is recommended for free acids, chlorophenoxycarboxylic acids, their salts and derivatives as well as for phenols and chlorophenols (Butte, 1983). One great advantage is the simplification of the sample preparation. Lipids or triglycerides can be converted to the corresponding fatty acid methyl esters (FAMEs) by a simple transesterification. This reaction is very elegant and convenient, because it is just necessary to add the reagent (0.2 M in methanol) to the sample solution. Removal of excess reagent is not required, since in the injector of the gas chromatograph at 250°C pyrolysis to volatile methanol and dimethylsulfide will occur. Due to the high reactivity, complete derivatization is often obtained at ambient temperature. However, heating (10 min at 100°C) in a closed sample vial may be necessary.

Sterols extraction

Unsaponifiables and sterols extractions were carried out according to (Sriti et al., 2009). A mixture was prepared by adding 100 µg of dihydrocholesterol (internal standard dissolved in chloroform) to 140 mg of oil and mixed to 3 ml of a solution of KOH (1 M in ethanol). After heating the mixture at 75°C for 30mn, 1ml of distilled water and 6 ml isohexane were added. The isohexane phase was allowed to isolate unsaponifiable fraction which was analyzed by GC (Sriti et al, 2011).

For silylation, 160 µl of the organic phase containing the sample was added to 40 µl of silylation reagent (1 ml N-methyl-N-trimethylsilyl-heptafluorobutyramide (MSHFBA)) mixed with 50 µl of 1-methylimidazole and heated for 5 min at 103°C before GC analysis.

Analysis conditions

FAs analyses were done on a capillary gaseous chromatography Varian (CPG). The mixture is injected directly. The temperature of the injector must be at least 250°C. The column used was Select CB for FAME fused silica WCOT (50 m x 0.25 mm; film thickness 0.25 mm). The temperature gradient was185°C for 40 min, then at 15°C/min to 250°C, and 250°C for 10 min. The

analysis time was 55 min. The detector FID was set up at 250°C. The helium was the carrier gas at 1.2 mL/min.

Sterols analyses were performed by GC using a flame ionization detector (FID) Perkin Elmer (Waltham, MA, USA) chromatograph. A CP-SIL 8 CB capillary column (30 m; 0.25 mm; 0.25 µm) was used. Chromatograph worked under suitable programs: in the first time 160°C during 0.5 min, then increased from 160°C to 260°C at a rate of 20°C min^{-1}, 2°C min^{-1} to 300°C and 45°C min^{-1} to 350°C respectively. The carrier gas was helium with a flow rate of 1 ml min^{-1} (on column injection was used). The detector was set up at 360°C. The injection volume was 1 µL. The identification of the compounds was done by comparison to the commercial standards. Sterols quantification was done by internal calibration with the addition of cholestanol. It is estimated that analyzed sterols and cholestanol reply the same way.

Statistical analysis

Data were subjected to a statistical analysis using the program package STATISTICA. Total volatile compounds are means ± SD of three experiments. The one-way analysis of variance (ANOVA) followed by Duncan multiple range test was employed.

Results and Discussion

Oil yields

Yields of oils obtained from *Z. jujuba* pulps is shown in Figure 2. Oils yield varied from an ecotype to another and flow from 8.31% to 12.35% based on the dry weight of each ecotype.

The pulps of ecotype Sfax had the highest oil content (12.35%) followed by Choutrana and Mahres (10%) whereas the ecotype Mahdia presented the lowest oil yield (8.31%).

Fatty acid profile

In this section, we focused on the analysis of fatty acids (FA) extracted from *Z. jujuba* pulps oils grown in an experimental station. The typical GC profile (Figure 3) showed the existence of large variation between the four ecotypes (Sfax, Choutrana, Mahres and Mahdia).

The major FA observed was the oleic acid (omega-9) at a level of 50.68% and 42.82% of the total oil in Mahres and Choutrana ecotype, respectively. Then its composition decreased slightly to reach the proportions of 38.67 and 32.37% in pulps from ecotype Sfax and Mahdia respectively.

On the other hand and as shown in table 1, palmitic acid (C16:0) was a major compound of the Sfax subjects (18.67%). The linoléic acid (C 18: 2n6) was present in all ecotype at 18.13% and 10.88% in Choutrana and Sfax ecotype respectively. The pulps oil were also rich on C18:0 FAs ranging from 7.29% in Choutrana to 8.43% in Sfax. The *Ziziphus* pulps composition was more important than those confirmed in *Z. mauritiana* pulps (Guil-Guerrero et al., 2004). In fact, they adopt this composition C12 : 0 (18,3%), C10 : 0 (12,5 %), C18 : 2 (9,27%), C16 : 1 (8,50%), C16 : 0 (7,25%), et C18 : 1 (5,34%). San et al. (2010) have qualified *Ziziphus* oil as a mixture of linoleic, oleic, palmitic and palmitoliec acid.

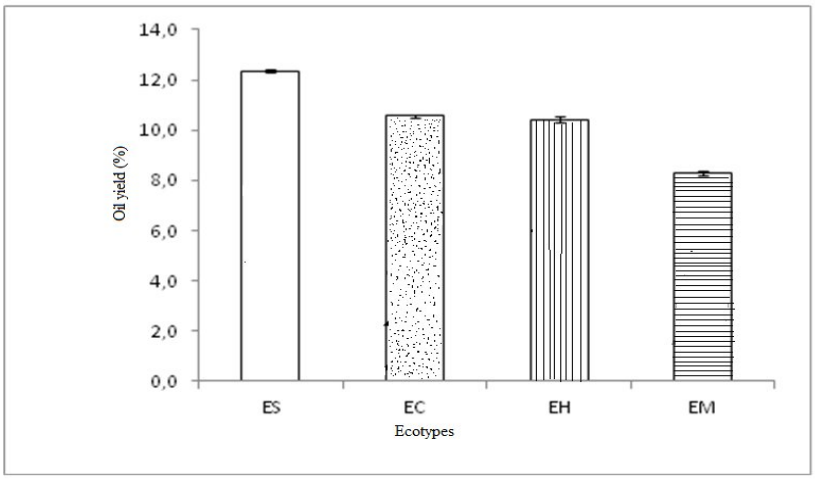

Figure 2. Oil yield (%) of four tunisian *Ziziphus zizyphus* ecotypes (Sfax, Choutrana, Mahres and Mahdia). The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

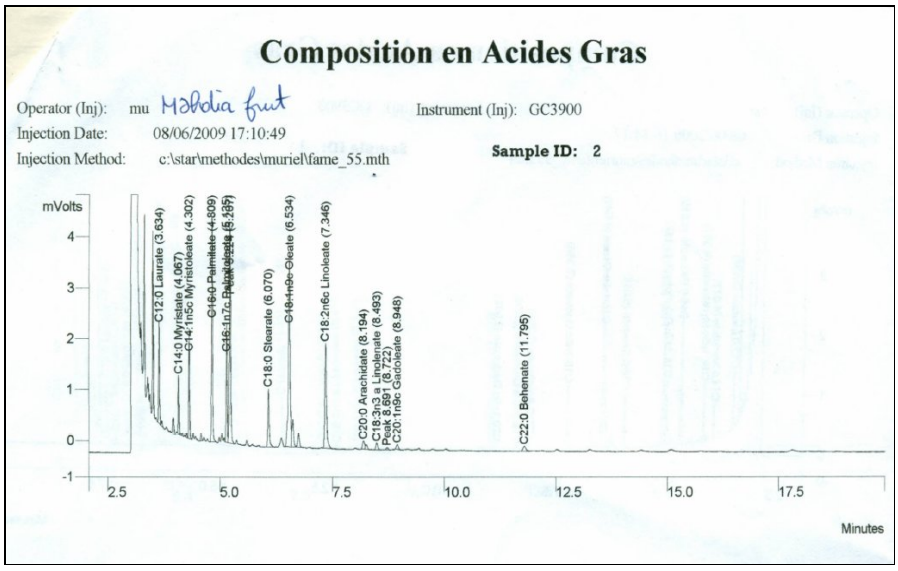

Figure 3. Fatty acids compositions of *Ziziphus jujuba* pulps.

On the other hand, the results showed an assessment of saturated, monounsaturated and polyunsaturated fatty acids in the pulp of *Ziziphus* values 29.62% to 37.3%; 48.17% to 59.89% and 12.51% to 15.05% the most important saturated fatty acids were palmitic, stearic, behenic and arachidic acid. Oleic and gadoleic acids were the major monunsaturated fatty acids. But the polyunsaturated acids are formed essentially by linolic and linolenic acid. This abundance of unsaturated fatty acids (68.5% to 72.4%) was also noticed in jujube fruits and 26.4 to 30.2% for saturated fatty acid (San et al., 2010).This composition can give preventive action against cardiovascular disease by lowering levels of cholesterol (Watkins et al., 2003; Tapiero et al., 2002; Hachicha et al., 2006).

Table 1. Fatty acids compositions of the pulp of four Tunisian *Ziziphus jujuba* ecotypes (Sfax, Chautrana, Mahres and Mahdia).

Fatty acids	Saturation	Percentages of fatty acids			
		Sfax	Choutrana	Mahres	Mahdia
Lauric	C 12 : 0	4.68 ± 0.13b	3.19 ± 01c	3.29 ± 0.02c	5.76 ± 0.99a
Myristic	C14 : 0	2.91 ± 0.37a	1.23 ± 0.02b	1.31 ± 0.01b	3.29 ± 0.41a
Myristoleic	C14:1n5	8.53 ± 0.79a	1.52 ± 0.14c	1.3 ± 0.01c	6.45 ± 1.19b
Palmitic	C16:0	18.67± 0.08a	18.59 ± 0.16a	15.58 ± 0.2b	18.36 ± 0.31a
Palmitoleic	C 16 :1n7	8.69 ± 0.79a	2.91 ± 0.08b	2.59 ± 0.01b	8.45 ± 1.6a
Stearic	C18 :0	8.43 ± 0.58a	7.29 ± 0.13b	8.13 ± 0.08ab	7.66 ± 0.72ab
Elaidic	C 18 : 1n9t	2.7 ± 0.01a	*	*	*
Oleic	C 18 : 1n9	38.67 ± 1.59b	42.82 ± 0.28b	50.68 ± 0.28a	32.37 ± 6.33c
Linoleic (oméga-6)	C 18 : 2n6	10.88 ± 0.89c	18.13 ± 0.51a	13.29 ± 0.04b	13.6 ± 1.76b
Arachidic	C20 : 0	1.59 ± 0.55a	1.06 ± 0.05b	0.73 ± 0.02b	1.64 ± 0.06a
Linolenic (oméga-3)	C18: 3 n3a	1.63 ± 0.07a	1.33 ± 0.05b	1.76 ± 0.04a	0.86 ± 0.29c
Gadoleic	C20 :1c	1.3 ± 0.15a	1.08 ± 0.08a	0.77 ± 0.04c	0.9 ± 0.13bc
Behenic	C22 : 0	0.56 ± 0.92a	0.85 ± 0.05a	0.58 ± 0.01a	0.67 ± 0.67a
Omega-6/Omega-3		6.67	13.63	7.55	15.81
∑polyunsat.		12.51	19.46	15.05	14.46
∑monunsat.		59.89	48.33	55.34	48.17
∑sat.		36.84	32.21	29.62	37.38
U/S		1.97	2.1	2.37	1.68

The first number indicates the length of the fatty acid chain and the second the number of double bonds (all *cis*) with signifying the location of the double bond(s). Saturated = 14:0 + 16:0 + 18:0 + 20:0 + 22:0. Monunsaturated = 16:1 + 18:1 + 20:1 Poly-unsaturated = 18:2 + 18:3. The data are means values of three measurement ± SE. for each column, values with the same letter indicate no-significant differences at 5%.

The ratio unsaturated/saturated (U/S) gives 1.68; 1.97; 2.1 and 2.37 in ecotypes Mahdia, Sfax Choutrana and Mahres respectively. This ratio is higher than other cited who had noticed equality between les mono and polyunsaturated acids.

This richness of unsaturated fatty acids can give preventive action against coronary, heart disease, diabetes and certain types of cancer (Berra et al., 1998; Jacot, 2001, Lunn and Theobald, 2006). Finally the ratio omega-6/omega-3 gives a large 5/1 (ES) to 14/1 (EM).in fact, that food enriched by jujubes can protect body against cancer (Jacot, 2001).

Sterols compositions

Oil extracted from pulps (dry matter) contains almost 14 mg/100g of total sterols (TSs). This quantitative richness differed from ecotype to another as (Figure 4).

As illustrate in Figure 4, all ecotypes (Sfax, Choutrana, Mahdia and Mahres) showed similar sterols constituents. In fact, all pulps oils were dominant on β-sitosterol and campesterol which β-sitosterol was the most prominent in these ecotypes.

Thus, the highest percentage (10.65 mg/100 g) was observed on Choutrana pulps. In contrast, the lowest level (3.02) was estimated in the pulp oil from Mahdia. However, the β-sitosterol level remained high for the species from Sfax (9.69 mg/100 g) of TSs (Table 2). Actually, this compound is the most intensively investigated with respect to its beneficial and physiological effects on health. The analyses of *Ziziphus* sterols pulps showed also the high level of the stigmaterol. In fact, pulps from Sfax were the most rich of this sterol with the rate of 16.12 mg/100 g oil, flowed by Choutrana pulps with the rate of 4.69 mg/100 g. The ecotype of Mahdia contains 1.81 mg/100 g. However the pulps of Mahres showed the poorer fraction of this sterol with only 1.35 mg/100 g oil. Campesterol was the third common sterol and exist only in Sfax and Choutrana selections. In fact, it was between 1.37 mg/100 g (Sfax) and 2.4 mg/100 g (Choutrana).

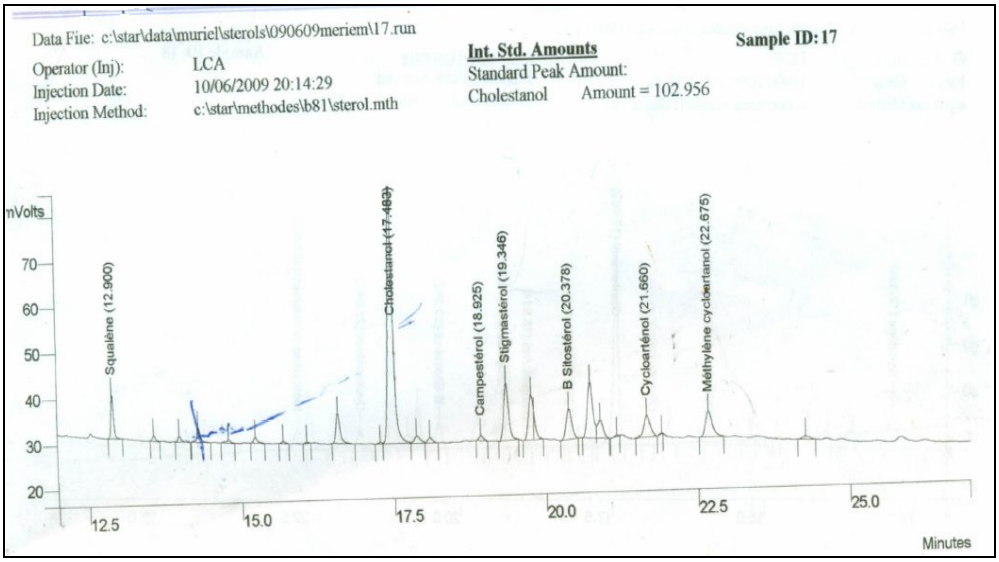

Figure 4. Sterols compositions of *Ziziphus jujuba* pulps.

Table 2. Sterols compositions (mg/100g) of four Tunisian jujube selections (Sfax,Choutrana, Mahres and Mahdia).

Ecotyps/ Sterols	Sfax	Choutrana	Mahres	Mahdia
Campesterol	1,37 ± 0,11b	2,4 ± 0,64a	*	*
Stigmasterol	16,12 ±0,87a	4,69 ± 1,18b	1,35 ± 0,9c	1,81 ± 0,16c
β-Sitosterol	9,69 ± 0,45a	10,65 ± 2,86a	4,14 ± 0,91b	3,02 ± 0,09b
d- 7- Stigmastenol	*	0,82 ± 0,01a	*	*

*Not identified.

The data are means values of three measurement ± SE. for each column, values with the same letter indicate no-significant differences at 5%.

As we can see in this Table, *Ziziphus* pulps were a big source of stigmasterol which is an excellent material for the synthesis of the progesterone hormone. This propriety gives a great importance to *Ziziphus* oil. Hamid et al. (2006) and Feng et al. (2011) adopted the importance of the β-sitosterol in olive oil, cannabis seed, in soja oil and *Z. spinosus* seeds respectively. The same result was observed on *Lens esculentus* (79.7%) and in *Arachis hypogaea* (72.0%) (Emile et al., 1983).

Conclusion

According to the described results, the *Z. jujuba* oil composition was dominated by oleic and palmitic acid with 32.4 - 50.7% and 15.6 - 18.7% respectively. The lipid of the jujube pulps was especially rich on omega-6, that human body is not capable of producing. Total sterol contents were 14 mg/100 g oil. The β-sitosterol was the prominent component in Sfax ecotype (16.12 g kg^{-1}) of the total sterols. The high rate of extraction insists on the importance of exploitation of this oil in cosmetic and pharmaceutics industries. Therefore jujube fruits can be recommended by nutritionists to be part of the diet.

References

Aioi, A., T. Shimizu and K. Kuriyama. 1995. Effect of squalene on superoxide anion generation induced by a skin irritant, lauroylsarcosine. Intl. J. Pharm. 113:159-164.

Berra, B. 1998. Les composants mineurs de l'huile d'olive: aspect biochimiques et nutritionnels. Olivae. 73:29-30.

Bonanome, A. and S. M. Grundy. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Engl. J. Med. 318:1244-1248.

Butte, W. 1983. Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulphonium hydroxide for transesterification. J. Chromatogr. 261:142-145.

Das, B., H. Yeger and H. Baruchel. 2003. *In vitro* cytoprotective activity of squalene on a bone marrow versus neuroblastoma model of cisplatin-induced toxicity: Implications in cancer chemotherapy. Eur. J. Cancer. 39:2556-2565.

Elaloui, M. 2013. Suivi de la phénologie et caractéristion morpho-chimique comparés de quatre écotypes de *Ziziphus jujuba* (Miller) dans la station expérimentale de Rouhia (Tunisie) (semi-aride supérieur). Ph.D., National Agronomic Institute. Tunisia.

Emile, M. G., J. P. Bianchini and J. V. Ratovohery. 1983. Triterpene alcohols, methyl sterols, sterols, and fatty acids in five malagasy legume seed oils. J. Agric. Food Chem. 31:0833-836.

Feng, W., L. Xuesong, Ch. Yong and W. Longhu. 2011. Characterization of Fatty Oil of *Zizyphi spinosi* semen obtained by Supercritical Fluid Extraction. J. Am. Oil Chem. 88:476-472.

Goncharova, N. P., A. S. H. Isamukhamedov and A.I. Glushenkova. 1990. Lipids of *Ziziphus jujuba*. Chem. Nat. Comp. 26:16-18.

Guil-Guerrero, J. L., A. DiAz Delgado, M. C. Matallana Gonza´ Lez and M. E. Torija Isasa. 2004. Fatty Acids and Carotenes in Some Ber (*Ziziphus jujuba Mill*) Varieties. Plant Foods Hum. Nut. 59:23-27.

Hachicha, S. F., S. Barrek, T.Z. Skanji, G. Ghrabi and H. Zarrouk. 2006. Composition chimique de l'huile des graines d'*onopordon nervosum* subsp. *platylepis*murb (astéracées). J. Soc. Chim. Tun. 9:23-28.

Hamid, S., E. Aziz, B. Taoufik and B. Ahmed. 2006. Seeds oil characterization of *cannabis stiva l*. cultivated in Northen Morocco. Ann Toxicol Anal. 18:119-125.

Jacot, B. 2001. Intérêt nutritionnel de l'huile d'olive. Olivae. 86:27-29.

Laamouri, A. 2009. Contribution à l'étude des jujubiers en Tunisie: Identification, caractérisation, adaptation au déficit hydrique et multiplication. Ph. D., National Agronomic Institute. Tunisia, Tunisia.

Lunn, J. and H. E. Theobald. 2006. The health effects of dietary unsaturated fatty acids. Nutr. Bull. 31:178-224.

Mukhtar, H., S. Ansari, M. Ali and T. Naved. 2004. New compounds from *Zizyphus vulgaris*. Pharma. Biol. 42:508-511.

Owen, R. W., A. Giacosa and W. E. Hull. 2000. Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncol. 1:107-112.

Peng, W. H., M. T. Hsieh, Y. S. Lee, and Y.C. Lin. 2000. Anxiolytic effect of seed of *Ziziphus*

jujuba in mouse models of anxiety. J. Ethnopharmacol. 72:435-441.

San, B. and A. N. Yildirim. 2010. Phenolic, alpha-tocophérol, beta-carotène and fatty acid composition for four promising jujube (*Ziziphus jujuba miller*) selections. J. Food Comp. Anal. 23:706-710.

Sheng, G., T. Yu Ping, A. O. D Jin, S. Shu Lan and D. An Wei. 2009. Two new terpenoids from fruits of *Ziziphus jujube*. Chin. Chem. Lett. 20:197-200.

Sriti, J., T. Talou, A. wannes, M. Cerny and B. Marzouk. 2009. Essential oil, fatty acid and sterol composition of Tunisian coriander fruit different. J. Sci. Food Agric. 89:1659-1664.

Sriti, J., T. Talou, M. Faye, G. Vilarem and B. Marzouk. 2011. Oil extraction from coriander fruits by extrusion and comparison with solvent extraction processes. Ind. Crop. Prod. 3:659-664.

Tapiero, H., G. N. Ba, P. Couvreur and K. D. Tew. 2002. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomed. Pharmacother. 56:215-222.

Watkins, B. A., Y. Li, H. E. Lippman and S. Feng. 2003. Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast functions and bone metabolism. Prostaglandins, Leukot. Essent. Fatty Acids. 68:387-398.

Yamaoka, Y., T. Kawakita, M. Kaneko and K. Nomoto. 1996. A polysaccharide fraction of *Zizyphi Fructus* in augmenting natural killer activity by oral administration. Biol. Pharm. Bull. 19:936-939.

Zhao, J., S. P. Li, F. Q. Yang, P. Li and Y. T. Wang. 2006. Simultaneous determination of saponins and fatty acids in *Ziziphus jujube* (Suanzaoren) by high performance liquid chromatography-evaporative light scattering detection and pressurized liquid extraction. J. Choromatogr. A. 1108:188-194.

Emir. J. Food Agric. 2014. 26 (7): 609-615
doi: 10.9755/ejfa.v26i7.18187
http://www.ejfa.info/

REGULAR ARTICLE

Chemical and nutritional characterization of *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds

Diana Yamile Gallego Villa[1*], Luigi Russo[2], Khawla Kerbab[3] Maddalena Landi[2] and Luca Rastrelli[2]

[1]*Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Cra 30 Nº, 45-03, Bogotá, Colombia*
[2]*Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II, 84084 Fisciano (SA), Italy*
[3]*Laboratoire de Phytochimie et Analyses Physico-Chimiques et Biologiques, Departement de Chimie, University of Mentouri, Constantine, Route de Ain El Bey, 25000 Constantine, Algerie*

Abstract

Quinoa (*Chenopodium quinoa* Willd.) and cañihua (*Chenopodium pallidicaule* Allen) are native Andean food plants of high nutritional value used as food by the Incas and previous cultures. An extensive analytical study was done on three samples for each species for all amino acids, sterols, fatty acids and mineral determination. The aim was to evaluate the chemical and nutritional characterization of cañihua and quinoa in relationship with wheat, corn, rice, rye, as sources of dietary fiber and other bioactive compounds in human and animal. *C. quinoa* and pallidicaule present an excellent nutritional value with high (14-18%) protein content, balanced amino acid composition, trace elements and vitamins and contain no gluten. This food species presented rich flavonol and triterpene glycosides fractions that include different compounds. *C. quinoa* and pallidicaule are an excellent example of functional foods that aims to prevent the risk of various diseases.

Key words: *Chenopodium quinoa*, *Chenopodium pallidicaule*, Chenopodiaceae, South-American crop, Nutritional value

Introduction

Quinoa (*Chenopodium quinoa* Willd.) and cañihua (*Chenopodium pallidicaule* Allen) are native food plants of high nutritional value grown in the Andean region and used as food by the Incas and previous cultures.

Cañihua has long been considered a variety of quinoa, cañihua in 1929 was ranked as distinct species, are annual herbaceous plants, differ in height between 20 and 60 cm for the cañihua and 2 m for quinoa, significant differences are also found in inflorescences and flowers. They are pseudograin that formed a major part of the diet of the Incas. Unlike other Andean crops such as beans, maize, and potato, quinoa and cañihua have not been cultivated in recent years on a wide scale in other countries. Recently, there has been a renewed interest in these crops. This interest is due partly because of its high (14-18%) protein content and balanced amino acid composition and partly because of the tolerance of the plant to a wide range of unfavorable climatic conditions (Rastrelli et al., 1998). These crops have a remarkable adaptability to different agro-ecological regions. They can grow at relative humidity from 40% to 88%, and withstands temperatures from -4°C to 38°C. Is tolerant and resistant to lack of soil moisture, and produces acceptable yields with rainfall of 100 to 200 mm (FAO/WHO, 2011).

Because of the execellent nutritional value, quinoa and cañihua served as a substitute for scarce animal proteins and are still one of the principal protein sources of the region. They contain all the essential amino acids, trace elements and vitamins and contain no gluten. The importance of these proteins is based on their quality, with a balanced composition of essential amino acids similar to the composition of casein, the protein of milk. They are high in lysine, considered to be deficient in most cereal grains, making their protein profile incomplete (Jacobsen et al., 1997; Valencia et al., 2009).

Andean cereals may be promising food as regard to their content of phenolic secondary

Received 05 August 2013; Revised 15 February 2014; Accepted 20 February 2014; Published Online 18 May 2014

*Corresponding Author

Diana Yamile Gallego Villa
Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Cra 30 Nº, 45-03, Bogotá, Colombia

Email: dianvet@gmail.com

metabolites such as polyphenols, synthesized to counteract adverse climatic and growing conditions. These metabolites are linked to the reduction of the risk of major chronic diseases when consumed via diet (Liu, 2004). For example, plant polyphenols, natural antioxidants, are ideal candidates linked to the protective effects of vegetables and fruits against cancer and cardiovascular diseases (Arts and Hollman, 2005).

The nutritive and antioxidant properties of cañihua and quinoa could be extended to animal nutrition field where several experimental studies have been conducted on the use of quinoa in feed for chickens (Jacobsen et al., 1997; Improta et al., 2001; Munoz, 1980) and pigs (Cardozo et al., 1979; Van der Peet-Schwering et al., 1993), yielding excellent results. Cardozo et al. (1979) mentioned that although the main objective in the cultivation of quinoa is the production of grain for human consumption, it has been considered a second-class grain from the by-products of a crop that can be used in feeding poultry, pigs and cattle in special conditions (Jacobsen, 2003; Rosero et al., 2010). Nevertheless, it has been reported the low uptake of quinoa by-products for livestock feed among local farming communities (Rosero et al., 2010). In turn, cañihua is mainly grown for families by their own consumption in instance of its high nutritive value (Valencia et al., 2009). Also cañihua by-products are used as forage in the Altiplano areas where the agriculture is limited, moreover, these are used as a supplement for fattening poultry such as chickens and ducks (Rosero et al., 2010).

The main producers are Bolivia, Peru and the United States, quinoa production is expanding to other continents and it is currently being cultivated in several countries in Europe and Asia with interesting yields. Quinoa and cañihua could have a significant potential in the world as a new species of cultivation imported from South America. The main uses of this Chenopodiaceae are for cooking, products for people allergic to gluten; animal feed, green fodder, and pellets; modified food products such as breakfast cereals, pasta, and cookies; industrial use of starch, protein, and saponin; and as a game-cover crop. In developing countries of Africa and Asia, quinoa may be a crop able to provide highly nutritious food in extreme conditions.

This paper deals with the relative amounts of nutrient such as carbohydrates, lipids and proteins and also the amino acid, fatty acid and sterol composition of quinoa and cañihua in relationship with major crops like wheat, corn, rice and rye.

Material and Methods

Material

C. quinoa and *pallidicaule* were supplied by Central Peruviane de Servicios and was collected in Ayavaca (D. of Piura), Peru, in 2009. A voucher specimen is deposited in the Herbario del Museo de Historia Natural "J. Prado" Un. H. S. Lima (Peru).

Methods

Moisture content was determined by slicing and powdering the seeds in a blender and drying them in an oven at 130°C to constant weight by the AOAC 934.06 method (AOAC, 2000).

Fat was determined by weighing the dichloromethane extracts. The nitrogen content was established in a Kjeldahl apparatus, following the 920.87 AOAC method; the factor N × 6.25 was used to convert nitrogen into crude protein. Amino acid contents were determined after hydrolysis with 6N HCI for 4 h at 145°C in vacuum hydrolysis tubes from Pierce (Product N. 29560) (Dini et al., 1994).

The analyses were done by reverse-phase HPLC using a system of Hewlett-Packard HP 1050 series modules with quaternary pump, an autosampler provided with injector programme, a variable wavelength detector, an HP 3396A integrator and a Spherisorb ODS-2, 5 /zm 250 mm x 4 mm column. The derivatization procedure was automated, withdrawing from different vials suitable amounts of sample and reagents OPA from Sigma (50 mg) in CH_3OH (1 ml), FMOC from Aldrich and buffer, mixing for a minute and injecting into the column [Dini et al., 1994]. The eluents were MeOH(A) and a 50 mM AcONa solution (B) (flow rate 1.0ml/min; Gradients: 18-23% A (10 min linear); 23-27.2% A (12 min linear) held at 27-2% A for 4 min; 27-2-50% A (12 min linear), held at 50% A for 10 min: 50-80% A (10 min linear), held at 80% A for 10 min).

Fatty acids were analysed as methyl esters after hydrolysis in 2N KOH by using a Hewlett-Packard 5890 apparatus and an HP-5 column, a gas chromatograph fitted with an HP 5970B mass detector (helium was used as a carrier gas, flow 6-845 kPa (10 psi) and an HP 59970 MS Chemstation. Conditions here as in Table 3.

Sterols were examined after purification on a silicagel column (eluent: CH_2C1_2) of the insaponifiable matter by gas chromatography using the same apparatus as the methyl ester; conditions as in Table 4.

Mineral ions were examined using a Varian AA-475 flame photometer and a Varian AA-475 atomic adsorption spectrophotometer.

Starch and hydrolysable carbohydrates were examined by the AOAC 948.02 method (AOAC, 1990). Ash was determined by the AOAC 923.03 method (AOAC, 1990), and the crude fiber content by the Bellucci method (Bellucci, 1932) 3 g of powder were boiled for 25 min with 50 ml of AcOH (80%) and conc. HNO_3 (45/5, v/v), filtered and, after a wash with boiling water (10 ml), ethanol (20 ml), ethyl ether (20) and boiling water to neutralize, the precipitate was dried in an oven for 3 h at 105°C, weighed, burned on flame in a crucible and re-weighed.

Statistical analysis

Three independent analyses were done on three sample for each species for all amino acids, sterols, fatty acids and mineral determination. Statistical analysis was performed by Mann - Whitney U Test.

Results and Discussion

C. quinoa and *C. pallidicaule* (quinoa and cañihua) have been cultivated as a food crop for centuries in Latin America. Still today, descendants of the Inca Empire still use its seeds as an important component in their diet, and by-products are partially used in animal nutrition. The chemical analysis showed a chemical composition in protein, lipids and carbohydrates comparable to that of the most common cereals. The carbohydrates constitute 59.9% in cañihua and 55.3% in quinoa, the lipids 7% (cañihua) and 12.4 % (quinoa), the proteins 12.8% (cañihua) and 11.7% (quinoa) (Table 1). The seeds showed high protein content and average 12-18% on a fresh basis. Moreover, this protein are of an exceptionally high quality and are particularly rich in essential amino acids, such as sulfur amino acids, lysine and aromatic amino acids, higher than those recommended by the FAO-WHO (Table 2) and which are deficient in most grain crops but necessary for proper nutrition in humans. This fact results in protein content comparable to that of whole dry milk. The seeds are nutritionally very interesting also in comparison with other cereals such as wheat, corn, rice and rye. Furthermore, the nutritive value in quinoa and cañihua by-products could provide advantage in animal performance through diet supplementation, encouraging animal health and production cualities.

The analysis of the mixture of fatty acids after saponification showed a high content of unsaturated fatty acids (71.4% for cañihua and 72.5% for quinoa), with high concentrations of linoleic acid (39.2% for cañihua and 38.9% for quinoa), the most distinctive polyunsaturated fatty acid of both seeds studied and oleic (28.6% for cañihua and 27.7% for quinoa) (Table 3). For both species polyunsaturated fatty acids were the highest, followed by monounsaturated and saturated. Some authors was also mentioned the importance of a proper relationship between saturated and unsaturated fatty acids feeding; in fact, while a high saturation may promote the onset of hepatic steatosis and were hypercholesterolemic and atherosclerotic excess unsaturation may result in harmful consequences such as liver necrosis and nutritional encephalomalacia.

The sterol fraction, which is very useful in characterising the source of vegetable oils, shows Δ7-stigmasterol (46.6% in cañihua), (43.9% in quinoa) as the main component, followed by $\Delta^{7,22}$-stigmastedienol acetate (29.4% in cañihua), β-sitosterol acetate (10.7 in cañihua) and (15.0 in quinoa) (table 4). $\Delta^{5,22(28)}$-Avenasterol was present in quinoa but not in cañihua whereas Δ^{7}-campesterol and $\Delta^{7,22}$-stigmastedienol acetate were found only in cañihua. In addition, quinoa and cañihua seeds are rich in Ca, Fe, K, (Table 5).

Table 1. Analytical Composition of *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (quinoa) seeds and other cereals*.

%	Cañihua	Quinoa	Wheat**	Corn**	Rice**	Rye**
Water	10.8 ± 0.2	14.7 ± 0.3	12.0	12.0	12.0	12.0
Proteins	12.8 ± 0.3	11.7 ± 0.2	12.2	9.2	7.4	11.1
Lipids	7.0 ± 0.2	12.4 ± 0.1	2.3	3.9	0.5	1.9
Hydrolyzable carbohydrates	59.9 ± 1.7	55.3 ± 0.6	71.8	73.7	80.0	73.1
Whole fibre	6.3 ± 0.1	2.2 ± 0.1	2.1	1.6	0.4	-
Ash	3.1 ± 0.1	3.0 ± 0.1	-	-	-	-

* Mean ± SD of three determinations; **from Documenta Geigy

Table 2. Aminoacid composition of *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (Quinoa) seeds*

Rt	Aminoacid	mg/g protein in Cañihua		mg/g protein in Quinoa		Essential aminoacid pattern (FAO-WHO)	Chemical Index Cañihua	Chemical Index Quinoa
2,8	Aspartic acid	67.5		66.8		-	-	-
3,9	Glutamic acid	169.1		16 166.7		-	-	-
9,8	Serine	36.3		38.3		-	-	-
14,2	Histidine	16.7		19.9		-	-	-
15,1	Glycine	63.5		60.9		-	-	-
15,6	Threonine	37.2		34.9		40	93	87
22,4	Arginine	87.4		84.3		-	-	-
24,5	Alanine	568		57.1		-	-	-
25	Tyrosine	29.0	} 68.8	31.3	} 74.7	60	115	125
37,8	Phenylalanine	39.8		43.4				
	Cystine	20.3	} 41.7	22.1	} 44.6	35	119	127
30,8	Methionine	21.4		22.5				
31,1	Valine	48.2		60.0		50	96	120
35,7	Isoleucine	37,5		743		41	94	81
37,8	Leucine	67.2		75.0		70	96	107
46,9	Lysine	58.3		45.8		55	106	83
	Tryptophan	n.d.		n.d.		10	n.d.	n.d.
42,9	Proline	18.4		22.6		-	-	-
Chemical score 93.3 Threonine limiting								

*Data are the means of five experiments performed in triplicate. Standard deviations were below 10 %

Quinoa is an excellent example of 'functional food', it give a significant contribution to human nutrition, protecting cell membranes, with proven good results in brain neuronal functions. In our study C. *quinoa* and *pallidicaule* presented a rich flavonol glycosides fraction that includes different compounds. They contain the aglycons of quercetin, isorhamnetin, and kaempferol and oligosaccharide moieties as disaccharides and trisaccharides linked at the C-3 position. The quantitative content (1.09 and 1.33 g/kg, respectively in quinoa and cañihua) as well as the structural variability appears to be very interesting for the alimentary and taxonomic properties ascribed to flavonol glycosides. Moreover, flavonol apiosides appears to occur frequently in glycosides of the *Chenopodiaceae* (Rastrelli et al., 1995; De Simone et al., 1990).

One of the problems associated with the use of quinoa and cañihua for production of food products is the bitter taste due to the presence of saponins, are a vast group of glycosides, the surfactant properties are what distinguishes these compounds from other glycosides.

Most saponins have haemolytic properties and are toxic to most cold-blooded animals, have pharmacological properties and used in phytotherapy, cosmetic industry and folk medicines. Animal nutritionists have generally considered saponins to be deleterious compounds. In ruminants, some saponins are considered to have detrimental effects on protozoa through their binding with sterols present on the protozoal surface; furthermore, in other domestic animals the dietary saponins have significant effects on all phases of metabolism, from the ingestion of feed to the excretion of wastes (Francis et al., 2002). Saponins can have effects on animal growth and feed intake. In turn, chickens feeded with higher levels of bitter quinoa (with saponin) have been reported a deficiency of vitamin A (Ward, 2000).

Table 3. Fatty acids as methyl ester derivatives, present in *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds.

Carbon	Fatty acids	% of methyl ester mixture in Cañihua	% of methyl ester mixture in Quinoa	Rt (min)
$C_{12:0}$	dodecanoic (lauric)	1.3		16.8
$C_{14:0}$	tetradecanoic (myristic)	1.5	1.3	17.6
$C_{15:0}$	pentadecanoic	1.2	0.4	21.4
$C_{16:1}$	9-esadecenoic (palmitoleic)	0.9	1.1	22.0
$C_{16:0}$	esadecanoic (palmitic)	22.8	24.3	22.2
$C_{18:3}$	linolenic	1.2		23.7
$C_{18:2}$	9, 12-octadecadienoic (linoleic)	39.2	38.9	24.6
$C_{18:1}$	9-octadecenoic (oleic)	29.8	27.7	24.8
$C_{18:0}$	octadecanoic (stearic)	0.6	0.8	25.2
$C_{19:0}$	nonadecanoic		0.6	26.3
$C_{19:1}$	11-nonadecenoic	0.3	0.3	26.1
$C_{20:0}$	eicosanoic (arachidic)	0.9	1.0	27.3
$C_{20:1}$	15-eicosenoic		trace	28.4
$C_{22:0}$	docosanoic (behenic)	0.3	0.7	29.1
$C_{24:0}$	tetracosanoic (lignoceric)		trace	31.5
Fatty acids:	Saturated	28.6	22.7	
	Unsaturated	71.4	72.5	
Saturated/Unsaturated ratio		0.4	0.31	

Column HP-5; 25 m × 0.2 mm; i.d., 0.33 pm film; temperature 180°C for 3 min, then to 290°C at 6°C/rain; injection temperature, 290°C; transfer line temperature 290°C; carrier gas He (6.845 kPa (10 psi)). The compounds were characterized by comparison with retention times of a reference mixture and the MS-spectra. FID area % were corrected to wt % according to total weight. Data are the means of three experiments performed in triplicate. Standard deviations were below 10%.

Table 4. Sterols as steryl acetate derivatives in *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (Quinoa) seeds.

Sterol	% of sterol mixture in Cañihua	% of sterol mixture in Quinoa	Rt (min)
Δ^5-Campesterol acetate	4.3	2.3	24.8
$\Delta^{5,22}$-Stigmasterol acetate	5.1	5.5	25.0
Δ^7-Campesterol acetate	3.8	---	28,1
$\Delta^{7,22}$-Stigmastedienol acetate	29.4	---	28.8
β- Sitosterol acetate	10.7	15.0	29.2
Δ^7-stigmasterol	46.6	43.9	30.9
$\Delta^{5,22(28)}$-Avenasterol acetate	---	21.7	

Column: HP-5, 25m x 0-2 mm; i.d., 0.33 /zm film; temperature, 290°C; injection temperature, 290°C; transfer line temperature, 290°C; carrier gas, He (6.845 kPa (10 psi)). FID area percents were corrected to wt % according to total weight. Data are the means of three experiments performed in triplicate. Standard deviations were below 10 %.

Table 5. Mineral composition of *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds (mg/Kg dry wt).

Mineral	Quinoa	Cañihua	Wheat	Corn	Rice	Rye
Fe	26.1	24.7	26.4	27.2	10.1	54.3
Mg	39.2	33.6	49.0	-	-	35.1
Cu	2.1	2.6	5.2	-	-	7.2
Zn	27.0	28.3	-	-	-	-
P	4244,1	4189.2	4375.1	2981.1	1136.2	4192.1
Na	313.1	305.3	-	227.0	-	693.1
K	75.6	74.7	368.0	1363.4	897.5	5112.2
Ca	675.5	664.5	431.1	114.3	114.3	693.1

* Data are the means of three experiments performed in triplicate. Standard deviations were below 10 %

Quinoa and cañihua saponins can be divided into three different saponin groups; namely, groups containing either oleanolic acid, hederagenin, or phytolaccagenic acid as the aglycon. Previous studies (Dini et al., 2001; Woldemichael et al., 2001; Zhu et al., 2002) had determined the existence of four monodesmosidic and 22 bidesmosidic triterpene quinoa saponins based on four different aglycones (e.g., oleanolic acid, hederagenin, phytolaccagenic acid and serjanic acid). However, a recent analysis based on nano-HPLC electrospray ionization (ESI) multi-stage mass spectrometry revealed the existence of 87 triterpene saponins, comprising 19 reported and 68 novel components (Madl et al., 2006).

Quinoa and cañihua saponins concentrate in the outer husk of the grain, (8-12% w/w of the grain) which is removed before consumption to reduce the bitter taste of saponins, are considered a by-product with no commercial value. Investigations on the biological and pharmacological activities of *C. quinoa* saponins, inhibition of fungus growth, effects against viral diseases, cholesterol lowering effects, and an enhancing of mucosal drug absorption for this reason, saponin extracts of *C. quinoa* are used in agriculture for treatment, control, and prevention of fungal and viral diseases (San Martin et al., 2008).

References

AOAC. 1990, in Official Methods of Analysis of AOAC International. Washington, DC.

AOAC. 2000, in Official Methods of Analysis of AOAC International. Washington, DC.

Arts, I. and P. Hollman. 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr. 81(1 Suppl):317S–325S.

Bellucci, C. 1932. La determinazione della cellulosa nelle farine di frumento e dei sottoprodotti. Ann. Chim. Appl. 22:25.

Cardozo, A. and M. Tapia. 1979. Valor nutritivo. In: M. Tapia, H. Gandarillas, S. Alandia, A. Cardozo and A. Mujica (Eds.). pp. 149–192, Quinua y Kañiwa, Cultivos Andinos. Bogotá CIID, Oficina Regional para la América Latina, ISBN: 0-88936-200-9.

De Simone, F., A. Dini, C. Pizza, P. Saturnino and O. Schettino. 1990. Two flavonol glycosides from *Chenopodium quinoa*. Phytochemistry 29:3690-3692.

Dini, A., G. Migliuolo, L. Rastrelli, P. Saturnino and O. Schettino. 1994. Chemical composition of *Lepidium meyenii*. Food Chem. 49:347-349.

Dini, I., O. Schettino, T. Simioli and A. Dini. 2001. Studies on the constituents of *Chenopodium quinoa* seeds: isolation and characterization of new triterpene saponins. J. Agric. Food Chem. 49:741–746.

FAO/WHO. 2011. Quinoa: An ancient crop to contribute to world food security. Regional Office for Latin America and the Caribbean.

Francis, G., Z. Kerem and K. Becker. 2002. The biological action of saponins in animal systems: a review. British J. Nutr. 88:587–605.

Jacobsen, E., B. Skadhauge and S. Jacobsen. 1997. Effect of dietary inclusion of quinoa on broiler growth performance. Anim Feed Sci Tech, 65:5–14.

Jacobsen, S. 2003. The worldwide potential for Quinoa (*Chenopodium quinoa* Willd). Food Rev. Internat. 19(1-2):167–177.

Liu, R. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutr. 134(12 Suppl):3479S–3485S.

Improta, F. and R. Kellems. 2001. Comparison of raw washed and polished quinoa (*Chenopodium quinoa* Willd) to wheat, sorghum or maize based diets on growth and survival of broiler chicks. Livestock Res. Rural Dev. 13(1):10.

Madl, T., H. Sterk, M. Mittelbach and G. Rechberger. 2006. Tandem mass spectrometric analysis of a complex triterpene saponin mixture of *Chenopodium quinoa*. J. Am. Soc. Mass Spect. 17:795–806.

Munoz, V. 1980. Effect of 5 levels of *Chenopodium quinoa* (Quinoa) as a partial substitute of soybean cake in the broilers chickens feeding. Thesis Zootechnician Degree, Universidad de Nariño, Pasto, Colombia.

Rastrelli, L., R. Aquino, S. Abdo, M. Proto, F. De Simone and De N. Tommasi. 1998. Studies on the constituents of *Amaranthus caudatus* leaves: Isolation and structure elucidation of new triterpenoid saponins and ionol-derived glycosides. J. Agric. Food Chem. 46:1797-1804.

Rastrelli, L., P. Saturnino, O. Schettino and A. Dini. 1995. Studies on the constituents of *Chenopodium pallidicaule* (Canihua) sedes: Isolation and characterization of two new flavonol glycosides. J. Agric. Food Chem. 43:2020-2024.

Valencia, R. C., A. Acevedo de La Cruz, J. C. Icochea Alvarez and H. Kallio. 2009. Chemical and functional characterization of Kañiwa (*Chenopodium pallidicaule*) grain, extrudate and bran. Plant Foods Hum. Nutr. 64:94–101.

Rosero, O., D. Rosero and D. Lukešová. 2010. Determination of the capacities of farmers to adopt quinoa grain (*Chenopodium quinoa willd*) as potential feedstuff. Agric. Trop. Subtrop. 43(4):308–315.

San Martin, R., K. Ndjoko and K. Hostettmann. 2008. Novel molluscicide against *Pomacea canaliculata* based on quinoa (*Chenopodium quinoa*) saponins. Crop Prot. 27:310–319.

Van der Peet-schwering C., M. Van Krimpen, P. Kemme, G. Binnendijk, J. Van Diepen, A. Jongbloed, G. Henniphof-Schlick and D. Bubenheim. 1993. Quinoa: An Emerging "New" crop with potential for CELSS. NASA Technical Paper 3422.

Ward, S. 2000. Response to selection for reduced grain saponin content in quinoa (*Chenopodium quinoa* Willd). Field Crops Res. 68(2):157–163.

Woldemichael, G., and Wink, M. 2001. Identification and biological activities of triterpenoid saponins from *Chenopodium quinoa*. J. Agric. Food Chem. 49:2327–2332.

Zhu, N., S. Sheng, S. Sang, J. W. Jhoo, N. Bai, M. Karwe, R. Rosen and Ch. T. Ho. 2002. Triterpene saponins from debittered Quinoa (*Chenopodium quinoa*) seeds. J. Agric. Food Chem. 50:865–867.

Emir. J. Food Agric. 2014. 26 (7): 616-622
doi: 10.9755/ejfa.v26i7.18188
http://www.ejfa.info/

REGULAR ARTICLE

Extraction of mangiferin from *Mangifera indica* L. leaves using microwave-assisted technique

Suslebys Salomon[1], Iliana Sevilla[2], Rafael Betancourt[2], Aylema Romero[1], Lauro Nuevas-Paz[3] and Jhoany Acosta-Esquijarosa[2*]

[1]Centro de Investigación y Desarrollo de Medicamentos (CIDEM), 26 y 51, Plaza, La Habana, Cuba
[2]Centro de Ingeniería de Procesos (CIPRO), Facultad de Ingeniería Química, Instituto Superior Politécnico José A. Echeverría, Calle 114 No. 11901 e/ 119 y 127, Marianao, La Habana, Cuba
[3]Centro Nacional de Genética Médica, Ave, 31 y 146, Rpto Cubanacán, Playa, La Habana, Cuba

Abstract

Mangiferin, a bioactive metabolite having potent antioxidant and pharmacological properties has been obtained in this work by microwave assisted extraction (MAE) technique, from *Mangifera indica* leaves. A Box–Behnken design was used to evaluate the effect of three parameters: microwave power, the time of extraction, and the ratio of solvent to raw material (mL/g) over the mangiferin yield. Irradiation time was the most important parameter on the recovery of mangiferin. The results indicated that the optimal conditions should be as follows: power of microwave 900 W, extraction time of 5 min and a solvent volume / vegetal material relation of 10. With these parameters the maximum mangiferin yield was 63.22%. The MAE process was compared with the conventional extraction in stirred tank. No significant statistical differences were found (p= 0.5639) between both extraction methods of mangiferin. The presence of the metabolite was confirmed using high performance liquid chromatography. The method was validated with the guidelines set on the International Conference on Harmonization (ICH), for the validation of analytical procedures.

Key words: Mangiferin, *Mangifera indica*, Microwave assisted extraction (MAE), HPLC

Introduction

Mango, (*Mangifera indica* L.), belongs to the family of Anacardiaceae, and is frequently found in tropical and sub-tropical regions. It is one of the best popular comestible fruits in the world. The chemical composition of this plant has been studied widely in the past and was reported that the extracts contain different chemical family such as triterpenes, phytosterols, flavonoids and polyphenols (Núñez et al., 2002; Núñez et al., 2002a; Núñez and Rastrelli, 2010; Núñez et al., 2007).

Mangiferin is a significant bioactive constituent of mango containing xanthone-C-glycoside, which has many pharmacological properties and is very important as phytochemical. It has antioxidant, immunomodulatory, antidiabetic, antitumour properties and is convenient in the treatment of asthma and inflammation (Jatoi et al., 2007). Some reports show the development of method for determination of mangiferin using a thin layer chromatography (TLC) and a high performance thin layer chromatographic (HPTLC) (Nedialkov et al., 1998). However, Liquid Chromatography (LC) method has not been reported before for the quantitative determination of mangiferin in extracts obtained from *Mangifera indica* L. leaves. Only a few reports on LC simultaneous determination of several components including mangiferin in Chinese traditional pharmaceutical preparations (Ronghua et al., 2004) and recently report measuring the amount of mangiferin permissible into the eye (Hou et al., 2010).

Extraction is considering the first basic stage in medicinal plant research due to the preparation of extracts from raw materials is the starting point for the separation and purification of chemical constituents (Romanik et al., 2007). Generally, the conventional methods for obtaining of natural products are mainly maceration, heat reflux and Soxhlet extraction. These processes are of easy operation; however, in most cases, they are arduous and highly time consuming with small yields and is

Received 05 October 2013; Revised 13 February 2014; Accepted 22 February 2014; Published Online 18 May 2014

*Corresponding Author

Jhoany Acosta-Esquijarosa
Centro de Ingeniería de Procesos (CIPRO), Facultad de Ingeniería Química, Instituto Superior Politécnico José A. Echeverría, Calle 114 No. 11901 e/ 119 y 127, Marianao, La Habana, Cuba

Email: jhoany.acosta@quimica.cujae.edu.cu

possible to a severe risk of thermal degradation for most of the phytoconstituents. The traditional techniques of solvent extraction of plant materials are typically based on the correct choice of solvents of extractions and the use of methods (heat or agitation) with the objective to increase the solubility of the chosen metabolite and increase the mass transfer. The isolation of different constituents present in raw material is limited by the extraction stage (Mandal, 2007). In recent years, the use of microwave for obtain of constituents from plant material has shown great interest. The practice of microwaves in analytical sciences is not new; the first reported was in 1986 for the extraction of organic compound (Dean, 2010). Actually, MAE has attracted growing interest due to permit a rapid extraction of solutes containing into solid matrices, with extraction efficiency similar or superior to that of the conventional techniques (Camel, 2000). Heating occurs in a targeted and selective way in MAE with practically no heat being lost to the environment, and the apparatus can significantly diminished the extraction time (Huie, 2002). Different are the factors that affecting MAE, such as solvent type and volume, microwave power, extraction time, temperature and matrix characteristics (Mandal, 2007; Chan et al., 2011).

The feasibility of obtain mangiferin via MAE was reported by (Venkatesh et al., 2010). Longer time and moderate power were used, ethanol was employed by extraction solvent. In previous study MAE of mangiferin isolation from *Curcuma amada* was studied evaluating two independent parameters, microwave power and extraction (irradiation) time (Padmapriya et al., 2012). More recently, the influence of some variable such as microwave power, ethanol concentration, extraction (irradiation) time and pre-leaching time has been studied (Kullu et al., 2013).

Considering that other factor the importance in the extraction stage such as the type of the solvent and the relation vegetal material/solvent volume have been not evaluated, in the present paper, a more rigorous studied has been applied to know the influence of these independent variable on mangiferin extraction. The existence of metabolite in the final extract from *Mangifera indica* leaves was confirmed using high performance liquid chromatography (HPLC), employed mangiferin as standard.

Materials and Methods

Plant material

The leaves of *Mangifera indica* L. were collected from plants grown in a fruit farm in San Antonio de los Baños, Mayabeque, Cuba and successively dried and milled to obtain particles of around 2-3 mm. The content of mangiferin and the water is 3.23% and 6.5%, respectively.

Chemicals and Reagents

Acetic acid and methanol were chromatography grade from Merck (Darmstadt, Germany) and used without further purification. The reference standard (RS) of mangiferin was a house standard with 94.8% of purity determined by NMR, HPLC and DSC. Water, HPLC grade, was obtained using a Millipore Milli Q plus purification system (Billerica, MA, USA).

Quantification of mangiferin in the extracts from *Mangifera indica* leaves by HPLC

The determination of mangiferin was achieved by high performance liquid chromatography (HPLC). An HPLC system (Shimadzu LC20 model), equipped with two solvent delivery pump (LC20AD), UV/VIS detector (SPD20A), autosampler (SIL20AC) and a column oven (CTO20A) was used for the quantification of mangiferin in the extracts. The chromatographic separation was made on a RP18 column (Superspher 100, 250 mm x 4.6 mm; 5 μm) using methanol and acid acetic 2.5% (28/72 v/v) as the mobile phase at 1 mL min^{-1} and room temperature. The injection volume was 20 μL and detection was achieved at 254 nm. The chromatogram were acquired and processed by LCMS Solution software (Shimadzu). The guidelines set on the International Conference on Harmonization (ICH) for the validation of analytical procedures was using to validate of analytical method [ICH, 1995]. With the objective to validate the method of analysis were used the parameters linearity, accuracy, range, precision, limit of detection (LOD), limit of quantitation (LOQ) and specificity.

Equipment for the microwave-assisted extraction (MAE)

Microwave-assisted extraction was realized using a microwave apparatus (*SANYO* EM-T109SS) in a closed vessel system, with operation frequency of 2 450 MHz. It was equipped with one 1000 mL container collocated in the space with mayor radiation, determined previously (Pérez, 2012). Around 10.0 g of leaves powder was put into extraction vessel and processed under different MAE situations. The ranges of the variables considered are showed in Table 1.

Table 1. Level of independent variable chosen for BBD.

Variables	(-1)	(-1)	Unit
Time	1.0	5.0	min
Power	540.0	900.0	W
Solvent volume /vegetal material rate	10.0	20.0	mL/g

Reflux extraction (RE)

Powdered *Mangifera indica* leaves (10.0 g) was refluxed with 200 mL of water (Rusakova et al., 1985). Subsequently filtration through the filter paper, the end extracts was analyzed by HPLC.

Experimental procedure

Box–Behnken design was used to optimize the extraction variable and evaluate some statistical parameters as main effects, interaction effects and quadratic effects over the extraction yields of mangiferin that is the response variable. Were taken as the factors the microwave power, extraction time, and raw ratio of solvent to raw material, which have strong effects on the yields. A total of 15-run experiment were realized. Three assays were carried out in the plane center. After extraction the liquor was separate from material vegetal waste and filter by reduce pressure. Finally was determined the mangiferin content by HPLC inside liquor. The yield of drying was calculated to take account the initial content of mangiferin present in the mango leaves and the content of mangiferin existent in the liquor at the end of the experiments.

For calculating the optimal point, a second-order polynomial model was fitted to correlate link between independent variables and response (mangiferin yield). Analysis of the experimental design results were carried out using a statistical program. The regression analysis (ANOVA) was effected, and the fitness of the polynomial equation was evaluated by the coefficient of determination R^2. Both model and regression coefficients were considered statically significant when the p-values were lower than 0.05.

Result and Discussion

HPLC method validation

Different parameters were determined such as linearity, accuracy, precision, specificity and selectivity. The calibration curve in the rage of 5 and 100 µg/mL was found to be linear with good correlation coefficient 0.998-0.999. The limit of detection and quantification estimated from the calibration curve were 0.1 and 0.62 µg/mL respectively. For repeatability six samples at three different concentrations were prepared and analyzed by the HPLC method to determine variation arising from method and expressed as % RSD. The relative standard deviation (RSD) values were found to be below 5%. The results show that the average recovery at each level between 98 and 102%. A study of peak purity of the chromatographic peak mangiferin was made by the comparison of the UV spectrum at different parts of the peak. No differences were observed in the UV spectra obtained for mangiferin peak, so we can conclude that no exist interferences in the determination of mangiferin in the extracts under the analysis conditions used (Figure 1).

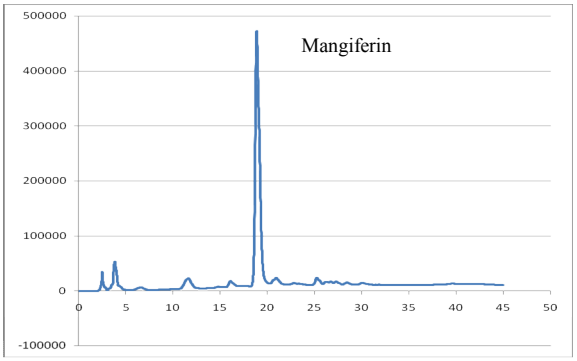

Figure 1. LC chromatogram of an extract of *Mangifera indica* L. leaves. Mangiferin a majority compound.

Optimization of MAE conditions by BBD

A precise choice of solvent is necessary for obtaining an optimal extraction procedure. The best solvent employed for MAE is establishment by the solubility of the chemical market, by the interaction that is creation between solvent and plant matrix, and the end by the microwave absorbing properties of the solvent. 10 g of *Mangifera indica* leaves were extracted with different volume of solvent at different microwave power. The solvent choice was water, as the polar solvent was found to increase mangiferin extraction (unpublished values of results). The influence of different factors such as the power, the time and the solvent volume/ vegetal material over the yield of mangiferin extraction in a MAE process was evaluated. The results of the experimental design appear in the Table 2.

The yield ranged from 34.11% (100.63 mg of mangiferin) to 62.16% (183.38 mg of mangiferin). The major result of yield achieved is near to 63% and is superior to the report where the yield of mangiferin is 31.1% (Venkatesh et al., 2010).

Table 2. Results obtained from the MAE tests. Experimental Box-Behnken response surface design.

Exp	Time (min)	Rate (solv vol / mat veg) (mL/g)	Power (W)	Mass (mg)	Yield (%)
1	1	15	540	105.98	35.92
2	3	20	540	183.38	62.16
3	1	20	720	109.89	37.25
4	1	15	900	100.63	34.11
5	3	10	540	119.07	40.36
6	5	15	540	121.10	41.05
7	1	10	720	103.14	34.96
8	5	10	720	157.85	53.51
9	3	10	900	156.32	52.99
10	5	20	720	157.28	53.32
11	3	20	900	149.58	50.70
12	5	15	900	179.27	60.77
13-15	3	15	720	140.69 ±15.29	47.69 ±5.19

* average of three experiment expressed as X±SD.

It is known that the microwave power and exposure time are two factors, which eject effects of considerable magnitude. A mixture of low or little power with extensive exposure time may be a sensible approach. The concentration of mangiferin and the extraction efficiency were found to increase when the microwave power improve until 900 W. In the experiments when was necessary elevated the microwave power from 540 to 900 W, electromagnetic energy was transmitted to the extraction vessel quickly and this enhanced the extraction efficiency of mangiferin.

Also is considering a critical factor the volume of the extracting solvent. The quantity of the solvent volume must be enough to ensure that the vegetal material is always completely absorbed in the solvent during the entire extraction time. In the literature is referred many reports concerning the volume of solvent to be used with respect to the amount of vegetal material. In this case, the diminished of solvent volume/ vegetal material rate increased the extraction of mangiferin. This behavior has been reported previously in the literature by other authors (Wang and Weller, 2006). Generally, a higher relation of solvent volume to vegetal material may be very effective in conventional extraction methods such as agitated tank. Conversely, in MAE a higher ratio may diminish the yield of recovery. This behavior is probably due to an inadequate stirring and mixture of the solvent into the microwaves (Wang and Weller, 2006).

The time, as in other extraction process, is other parameter whose influence needs to be taken into account. Normally, if the extraction time is increased, the mass or quantity of metabolites extracted is increased, although in sometimes there is the risk that chemical degradation may take place. In our investigation the concentration of mangiferin first increased for time near to 4.5 min and then decreased at 5 min. Analogous results were observed in the withdrawal of flavonoid from *Radix astragali* (Xiao et al., 2008). In this work, during smaller irradiation time, the mangiferin content increased with microwave power. Nevertheless, when the vegetal material and the water (as the solvent) were heated beyond 20 s, the mangiferin content diminished. This behavior was observed for all microwave powers studied. Is possible that, due to the high temperature involved in the extraction process, the degradation of mangiferin can be occurs (Yan et al., 2010). Therefore, very high irradiation time is not appropriate for mangiferin extraction using microwave technique.

In Table 3 are given the results of analysis of variance (ANOVA) realized. The time is the parameter with significant influence in the process ($p < 0.05\%$) to take account the statistical results. Not significant effects were achieved for the rest the variables (solvent volume/vegetal material rate and radiation power) respect to the mangiferin concentration. The ANOVA of the regression equation showed that the values of the determination coefficient (R^2) were 0.8869. This result suggested that has an elevated high degree of correlation between the observed and predicted values. Additionally, a low value of coefficient of the variation (CV 5.19%) showed an extraordinary degree of precision and a good reproducibility of the experimental values. The coefficients of the regression models together the response to the operating variables and their interactions are showed in Table 4.

Table 3. Results of ANOVA showing the significance of the sources on the dependent variable (mangiferin yield).

Source	Sum of squares	Degree freedom	Mean square	F value	Significance
A:time	551.286	1	551.286	32.87	0.0004
B:rate	58.374	1	58.374	3.48	0.0991
C:power	45.5058	1	45.5058	2.71	0.1381
AA	136.303	1	136.303	8.13	0.0214
AC	115.885	1	115.885	6.91	0.0302
BC	145.082	1	145.082	8.65	0.0187
Error	134.159	8	16.7699		
Corrected Total	1186.59	14			

Table 4. Coefficients of the regression equation.

	Yield of mangiferin
constant	-33.7623
A: time	2.44911
B: rate	5.35825
C: power	0.08771
AA	-1.51058
AC	0.01495
BC	-0.00669

R^2 = 88.6938 %

The p values were used to check the significance of each coefficient, which in turn may indicate the pattern of the interaction between the variables. The Pareto□s graphic show that time was the most significant single factor, which affect the mangiferin yield (Figure 2). To take account the graphic of principle interactions, the content of mangiferin increased until a maxim value and then diminished. Probably due to the elevated value of the temperature obtaining in the extraction process, which might will be caused damage of mangiferin. Water has the maximum value of the dielectric constant of common solvents used in the pharmaceutical industry. For these reason the dissipation factor is significantly inferior to other solvents. So, the velocity at which water absorbs the microwave energy is more elevated respect to the rate at which the system can dissipated the heat. This phenomena explain the "superheating" effects, which occur when water existing in the vegetal material. This phenomenon (localized superheating) can have positive or negative repercussion, depending on the vegetal matrix. In some circumstances it can increase the diffusivity of analyte in the raw vegetal material. In other cases, the powerful heating can origin the degradation for the thermo degradable compound (Wang and Weller, 2006; Mandal et al., 2007; Jain et al., 2009).

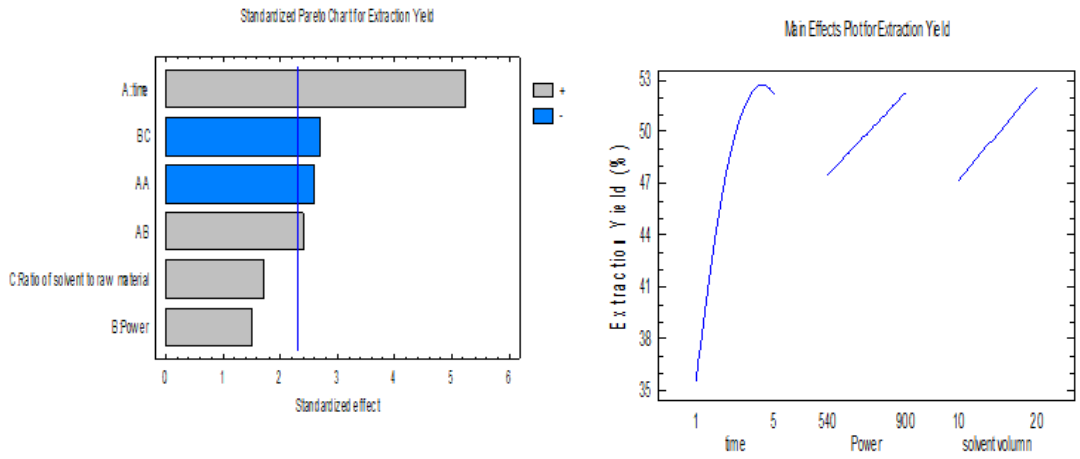

Figure 2. Pareto□s graphic and principle effects from yield of mangiferin.

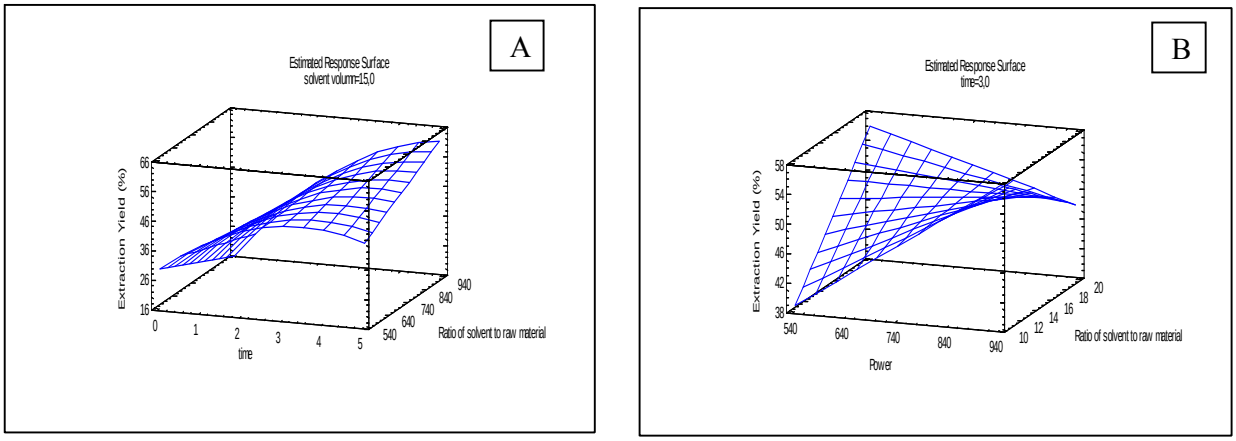

Figure 3. Estimated response surfaces for yield variable. A) Volume solvent/vegetal material rate 15 mL/g, B) Radiation time 3 min.

Two independent response surface plots are shown in Figure 3. The shapes of the plots indicated whether the common interactions between the independent variables were significant statically or not. It permit concluded that the optimal MAE conditions of mangiferin from *Mangifera indica* leaves were as: microwave power 900 W, irradiation time 5 min., and relation of solvent to raw material 10, for to obtain a 63.22% of yield of mangiferin. This value is superior to achieve in other previous report (Venkatesh et al., 2010). However successive stage will be evaluated if is necessary to extract all the mangiferin present in the vegetal matrix.

Comparison with conventional extraction method

The conventional isolation of mangiferin from *Mangifera indica* L. involves reflux extraction, using two times the amount of solvent (water). The best experimental conditions for MAE were compared to RE parameters. The MAE yield obtain was similar respect to RE which 63.22 ± were 2.1% and 64.18 ± 1.29% respectively. Moreover, significant advantages due to the low consumption of solvent and extraction time show that MAE provides a very good and consistent extraction method of bioactive metabolite from natural plants (Jain et al., 2009; Mandal et al., 2007; Chan et al., 2011).

Conclusion

An efficient method of MAE was evaluated for the extraction of mangiferin from *Mangifera indica* L. leaves with enhanced yield. A BBD design was satisfactorily employed to optimize extraction parameters, in this work. Results showed that microwave power 900 W, extraction time 5 min., and ratio of solvent to raw material 10:1 were the best conditions to extract mangiferin from mango leaves. The best value of yield achieved was 63.22%. This value is superior to obtain in other previous report. Successive stage will be evaluated if is necessary to extract all the mangiferin present in the vegetal matrix. When compared to the conventional extraction method (RE) the MAE is more efficient method with both lower solvent consumption and time. The yield obtained in both methods are similar, to take values near to 64%. No significant statistical differences were found (p= 0.5639) between conventional and non-conventional extraction methods.

References

Camel, V. 2000. Microwave-assisted solvent extraction of environ- mental samples. TrAC Trends Anal. Chem. 19:229–248.

Chan, C. H., R. Yusoff, G. Ngoh and F. W. L. Kung. 2011, Microwave-assisted extractions of active ingredients from plants. J. Chromatogr. A. 1218:6213–6225.

Dean, J. R. 2010. Extraction Techniques in Analytical Sciences. Wiley, New York, pp. 167–183.

Hou, Y., S. Fan, H. Zhang, Y. Gu, X. Yu and B. Li. 2010. Pharmacokinetic study of mangiferin in rat plasma and retina using high-performance liquid chromatography. Mol. Vis. 16:1659-1668.

Huie, C. W. 2002. A review of modern sample preparation techniques for the extraction and

analysis of medicinal plants. Anal. Bioanal. Chem. 373:23–30.

International Conference on Harmonization (ICH). Text on Validation of Analytical Procedures. 1995.

Jain, T., V. Jain, R. Pandey, A. Vyas and S. S. Shukla. 2009. Microwave assisted extraction for phytoconstituents - An overview. Asian J. Res. Chem. 2:19-25.

Jatoi, S. A., A. Kikuchi, S. A. Gilani, K. N. Watanabe. 2007. Phytochemical, pharmacological and ethnobotanical studies in mango ginger (*Curcuma amada* Roxb. Zingiberaceae). Phytother. Res. 21:507–516.

Kullu, J., A. Dutta, D. Constales, S. Chaurari and D. Dutta. 2013. Experimental and modeling studies on microwave-assisted extraction of mangiferin from *Curcuma amada*. 3 Biotech 4:107-120.

Mandal, V., Y. Mohan and S. Hemalatha. 2007. Microwave assisted extraction – an innovative and promising extraction tool for medicinal plant research. Pharmacog. Rev. 1(1):7-18.

Núñez, A. J. and L. Rastrelli. 2010. Chemical composition of the mango stem bark extract (*Mangifera indica* L.), In: V. K. Gupta (Ed.), pp. 433-56. Comprehensive Bioactive Natural Products, Stadium Press, Houston, USA.

Núñez, A. J., H. T. Velez-Castro, J. Aguero-Aguero, J. Gonzalez-Gonzalez, F. Naddeo, F. De Simone and L. Rastrelli. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J. Agric. Food Chem. 50:762-766.

Núñez, A. J., E. Paéz, D. Amaro, J. Acosta, J. Agüero, R. Capote, M. R. Gárcia, I. G. Morales, O. García, G. Garrido, G. Martínez and M. A. Morales. 2002a. Composiciones farmacéuticas a partir del extracto de *Mangifera indica* L. Patente No. 1814, Octubre, Oficina Cubana de la Propiedad Industrial.

Núñez, A. J., M. D. Durruthy Rodríguez, E. Rodríguez Balseiro, L. Nieto González, V. Nicolais and L. Rastrelli. 2007. Comparison of major and trace element concentrations in 16 varieties of Cuban mango stem bark (*Mangifera indica* L.). J. Agric. Food Chem. 55(6):2176-2181.

Padmapriya, K., A. Dutta, S. Chaudhuri and D. Dutta. 2012. Microwave assisted extraction of mangiferin from *Curcuma amada*. 3 Biotech 2:27-30.

Pérez, J. C. 2012. Extracción asistida por microondas de los lípidos de las semillas de *Cucurbita pepo* L., Tesis en opción al título de Licenciado en Química, Universidad de La Habana, Facultad de Química.

Romanik, G., E. Gilgenast, A. Przyjazny and M. Kaminski. 2007. Techniques of preparing plant material for chromatographic separation and analysis. J. Biochem. Biophys. Methods 70:253-261.

Ronghua, D., G. Jun and B. Kaishun. 2004. High-performance liquid chromatographic method for the determination and pharmacokinetic study of mangiferin in plasma of rats having taken the traditional chinese medicinal preparation Zi-Shen Pill. J. Chromat. Sci. 42:February.

Rusakova, S., V. I. Glyzin, S. I. Kocherga, L. F. Libizova and A. A. Ananieva. 1985. Process for preparing mangiferin. Patent No FR2486941.

Venkatesh, P., V. Soumya, K. Hariprasath and P. Hari. 2010. Microwave assisted extraction of Mangiferin from *Mangifera indica* L. IRJP. 1:209-212.

Wang, L. and C. L. Weller. 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. 17:300-312.

Xiao, W, L. Han and B. Shi. 2008. Microwave-assisted extraction of flavonoids from *Radix Astragali*. Sep. Purif. Tech. 62:616–620.

Xue-Lian, B., Y. Tian-Li, Y. Ya-Hong and Z. Hua-Wei. 2010. Optimization of microwave-assisted extraction of polyphenols from apple pomace using response surface methodology and HPLC analysis. J. Sep. Sci. 33:3751–3758.

Yan, M. M., W. Liu, Y. J. Fu and Y. G. Zu. 2010, Optimisation of the microwave-assisted extraction process for four main astragalosides in *Radix astragali*. Food Chem. 119:1663–1670.

Emir. J. Food Agric. 2014. 26 (7): 623-629
doi: 10.9755/ejfa.v26i7.18193
http://www.ejfa.info/

REGULAR ARTICLE

A study of hypoglycemic effects of *Azadirachta indica* (Neem) in human blood cells

Nubilde Martínez[1,2*], Yamileth Rodríguez[2], Omar Salguero[2], Dayana Requena[2,3], Ledia Triana[2,4] and Luis Pérez-Ybarra[4]

[1]*Departamento de Farmacología y Fisiopatología, Escuela de Medicina, Universidad de Carabobo, Sede Aragua, Venezuela*
[2]*Instituto de Investigaciones Biomédicas "Dr. Francisco J. Triana –Alonso", Universidad de Carabobo (Biomed – UC), Venezuela*
[3]*Departamento Clínico Integral, Escuela de Bioanálisis. Universidad de Carabobo, Sede Aragua, Venezuela*
[4]*Departamento de Ciencias Básicas, Escuela de Bioanálisis, Universidad de Carabobo, Sede Aragua, Venezuela*

Abstract

The present investigation was conducted with an objective to validate the effect of the plant *Azadirachta indica* (neem) in human blood cells in a normoglycemic medium. This hypoglycemic effect had been attributed by the communities to this plant. In this study, aqueous extract of the plant was used in a concentration of 6.4% m/v and from this, following doses were prepared: 0.01 mg; 0.05 mg (low doses); 0.1 mg; 0.175 mg (medium doses); 0.7 mg and 1.4 mg (high doses); also it was used insulin Humulin R® in different concentrations (0.1, 1, 10 nM) in order to compare both effects in a normoglycemic medium with human blood cells. The results demonstrated a hypoglycemic effect when the levels of glucose concentration went down in the normoglycemic medium in relation to the control. There was a significant effect ($p<0.001$) for the high doses group. These findings validate the hypoglycemic effect of this plant attributed by the communities.

Key words: Aqueous extract, Human blood cells, Hypoglycemic effect, Neem, Normoglycemic medium

Introduction

Azadirachta indica, also called neem, is an Asiatic origin arborous plant, specifically from the region from India to New Guinea; it belongs to the meliaceae family, its height varies from 6 to 25 m, and the trunk diameter ranges from 1 to 1.6 m. This plant was introduced in Venezuela in 90's, and it's widely spread along the country (Reyes et al., 2003). In Aragua state is very abundant and used as an ornamental plant.

Popular belief attributes to this plant, *A. indica*, with a lot of pharmacologic effects such as fungicide (Viveros and Castaño, 2006), insecticide (Reyes et al., 2003; Iannacone et al., 2005), antimicrobial (López et al., 2007), antihelmintic (Avello et al., 2006), as well as hypoglycemic effects (Urdaneta, 2001). It's used frequently by diabetic patients who are looking for therapeutic alternatives instead the conventional pharmacologic treatment (Urdaneta, 2001).

Several surveys about the neem hypoglycemic effect in animals had been carried out around the world, usually by using rats and mice, all the findings had shown significant hypoglycemic effect (Akinola et al., 2010; González et al., 2010; Isea et al., 2011; Manish et al., 2010), besides, Akinola et al. (2010), also found that lesions in pancreatic islet cells had improved in the neem treated experimental group.

In this research, the aim is to validate the hypoglycemic effect of this plant in human blood cells in normoglycemic medium, and verify the pharmacologic effects attributed by popular belief and surveys carried out in animals. Depending on this, there will be more reliable therapeutic alternatives that contribute to the diabetes mellitus treatment.

Materials and Methods

Plant sample collection

The botanical sample used in this studio was collected from the leaves taken from one single neem tree located in the garden of the University of

Received 04 September 2013; Revised 15 January 2014; Accepted 24 February 2014; Published Online 18 May 2014

*Corresponding Author

Nubilde Martínez
Departamento de Farmacología y Fisiopatología, Escuela de Medicina, Universidad de Carabobo, Sede Aragua, Venezuela

Email: nubildemartinez1@hotmail.com

Carabobo (Universidad de Carabobo) campus Aragua. It was identified by the "Victor M. Badillo" herbarium of the Central University of Venezuela (Universidad Central de Venezuela) campus Aragua.

Human blood samples

The blood samples were taken from adults in good health conditions under their approval.

Vegetal sample process

Once the sample was collected, the leaves were washed, dried at ambient temperature, and then were dried again at 45°C for 24 hours. After this, the leaves were triturated manually by using a ball mill.

Preparation of the aqueous extract

32 g of the pulverized vegetal material were dissolved in 200 ml of saline solution at 0.9%, and then cooking for 30 minutes in a heating iron at 150°C. It was keeping at ambient temperature to get colder, and then the sample was separated in small fractions, it was centrifuged for 10 minutes at 3000 rpm. The supernatant was filtered with Whatman paper N° 2 and then was stored and preserved at -20°C. The concentration of the extract was 6.4% m/v, from which were prepared the following doses: 0.01, 0.05, 0.175, 0.5, 0.7 and 1.4 mg (Martínez et al., 2010).

Leucocytes separation by centrifugation

To carry out the human blood cells separation, were taken 5 ml of blood from donors forearm vein in good health conditions, this samples were placed in a tube with ethylene diamine tetra acetic anticoagulant (EDTA), 4 ml of each sample were used for leukocytes preparation, and the remaining 1 ml was taken as a control and was not centrifuged. The process to obtain leukocytes was started with the centrifugation of 4 ml of blood for 5 minutes at 2700 rpm (Beckman® rotor JA-20), the plasma was dismissed and then it was taken 1 ml from the first layer of cells, in which there are the biggest quantity of leukocytes, it was divided in two 500 µl aliquots (A and B) which were washed with 600 ml of saline phosphate buffered to dismiss the plasma remainder. The count of cells was made with a Mindray BC-2300 hematological analyzer. To carry out the glucose consume test, was selected the aliquot (A or B) in which was obtained the highest leukocytes concentration.

Tests of glucose consume

Some tests of glucose consume were carried out using different quantities of *A. indica* aqueous extract, insulin was used as a control, because is the most powerful endogenous hypoglycemic.

Some samples of 1×10^6 leukocytes, at 37°C were incubated for a range of time between 0 and 10 minutes, in 1 ml of a suitable ionic medium (Hepes-KOH 20 mM pH 7.5; buffered phosphate 5 mM, pH 7.5; KCl 2.5 mM, NaCl 70 mM and Mg_2SO_4 2.5 mM) with normoglycemic conditions (100 mg/dL) and an additive that could be insulin Humulin R® in different concentrations (0.1; 1 y 10 nM) or aqueous solution of the phytomedicine (3, 9, 18, 36 y 72 µg). Once the incubation started at 37°C, aliquots of 40 µl were taken in each essay at increasing times from 0 minutes until 10 minutes (0, 1.5, 3.5, 5 y 10 minutes), these aliquots were centrifuged at 4000 rpm for 2 minutes with a micro centrifugation machine Eppendorf® 5415. 5 µl of supernatant were taken and mixed with 500 µl of the glucose reactive (Bioscience®) following the manufacturer instructions, absorbency of the different media were registered by using a spectrophotometer Beckman DU 650.

Determination of the glucose concentration

The quantification of glucose was made using the method of Trinder (Braham and Trinder, 1972).

Data analysis

The spectrophotometric readings, to determine the glucose levels in the medium treated with insulin and aqueous extract of the plant *A. indica*, were tabulated using MS Excel software, and some comparative bar and curve graphics were done to show the glucose value obtained as well as the percentage of the consume of the insulin and the plant extract.

The test carried out corresponds to an experiment design with eight treatments, seven doses and a control, under a random blocks experimental design, the people sampled represents the blocks, repeated observations were taken at some times (0, 1.5, 3, 5 and 10 minutes). One strategy to analyze this experiment is the split-plot design analysis of variance, where the main plot is referred to the treatments and the secondary plot to the repeated observations along the time. The multiple comparisons of means were carried out using the Tukey's test. These analyses were carried out by using the statistical software Statistix 8.0 and Minitab 14.0.

Results

Hypoglycemic effect of the *A. indica* aqueous extract

In these tests the insulin was used as an additive in the normoglycemic medium, to verify

the efficiency of the test designed to evaluate the glucose consume. In the figure 1A it can be noted a progressive increase of the glucose consume in the medium, when it was added the insulin in a concentration of 10 nM since 1.5 until 5 minutes, in which is observed a quick glucose consume. Since the minute 5 until 10 the glucose consume becomes constant. The figure 1B shows at 10 nM of insulin, the glucose consume is 27.7% at 3.5 min, whereas after 10 min the consume reach 40%.

In the Figure 2, a mild hypoglycemic effect of the aqueous extract de *A. indica* at different doses after 10 min of reaction is observed. The hypoglycemic effect of the extract started to be observed at the dose 0.7 mg, but the biggest effect was obtained at 1.4 mg.

In the Figure 3, the action of the high doses of the aqueous extract at different times of reaction is observed, the consume with 0.7 mg of extract starts since the 3 minutes, decreasing the concentration of glucose at 89.8 mg/dL at 10 minutes, to 1.4 mg of the aqueous extract the consume starts at the minute 1.5, reaching 65.7 mg/dL at 10 minutes.

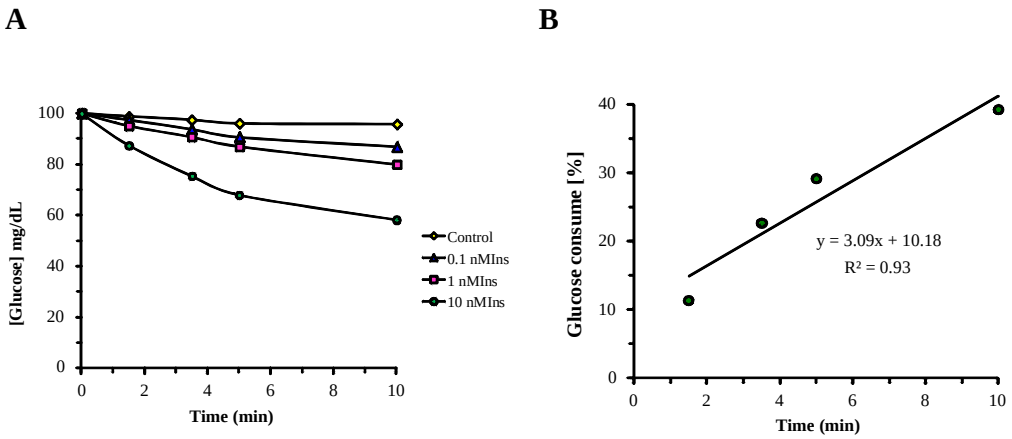

Figure 1. A) Glucose consume with different concentrations of insulin in relation to the control. B) Percentage of glucose consume with 10nM of insulin.

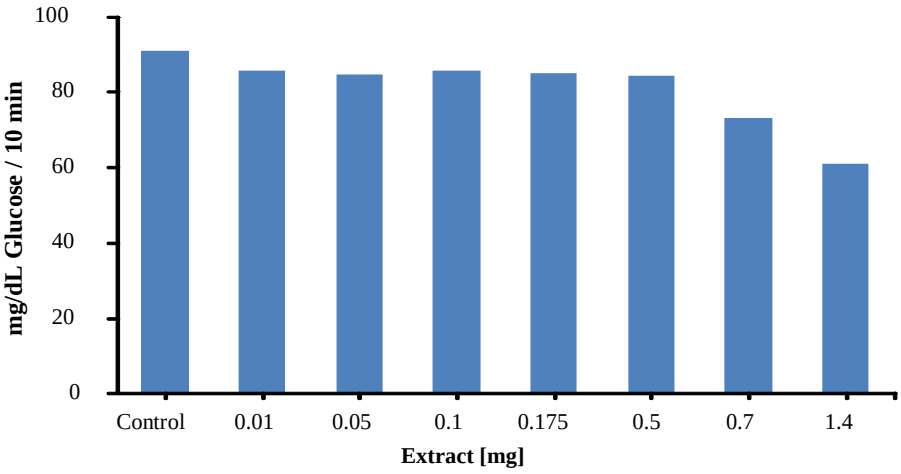

Figure 2. Effect of different quantities of *A. indica* aqueous extract at 10 min of reaction.

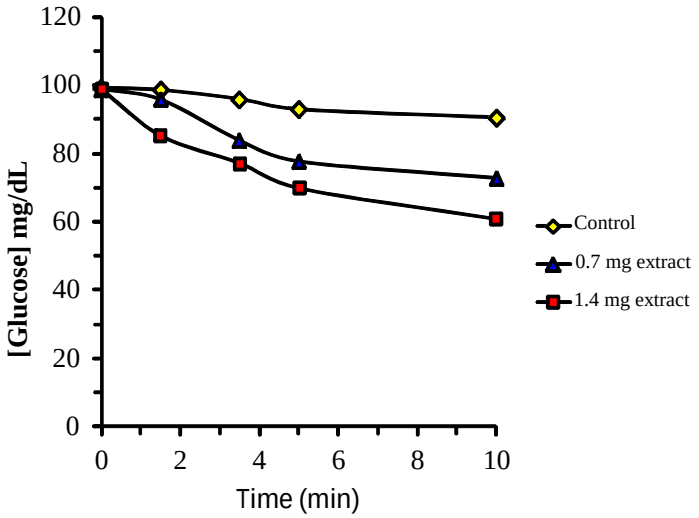

Figure 3. Hypoglycemic effect at 0.7 mg and 1.4 mg of *A. indica* extract.

This reduction in the concentration of glucose in the medium by the effect of the extract is represented as the consumed percentage of glucose in the figure 4, in which is observed 26.6% of the consume for the doses of 1.4 mg of the aqueous extract of neem at 10 min, which is an analogous result to the percentage of insulin consume, 27.7% at the minute 3.5 and 29.2% at 10 min, seen in figure 1B. Once it was added the doses of 0.7 mg of the extract, it was obtained a consumed percentage of glucose of 15.4% at 10 min as it is shown on the Figure 5.

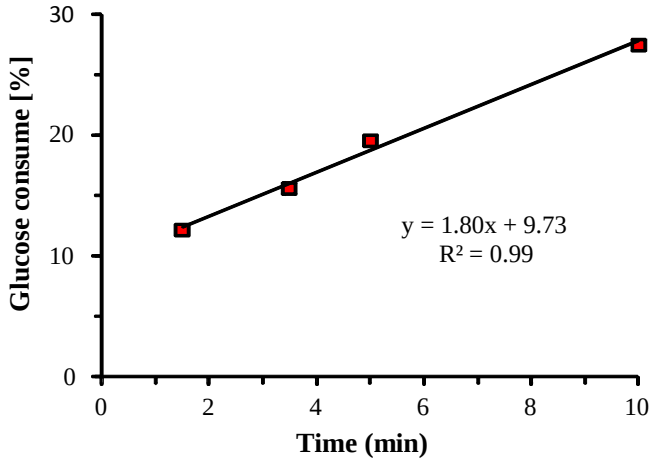

Figure 4. Percentage of glucose consume with 1.4 mg of *A. indica* aqueous extract.

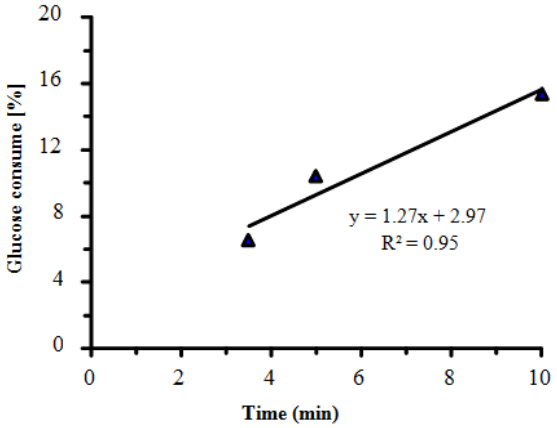

Figure 5. Percentage of glucose consume with 0.7 mg of *A. indica* aqueous extract.

The analysis of variance for the glycaemia concentration showed significant differences between the doses ($p<0.0001$) which indicates that the glucose reduction was not homogeneous for the different treatments. The results of the means comparison with Tukey's test for the treatments are shown in the Table 1.

Table 1. Tukey's test comparisons for the treatments.

Doses (mg)	Average	Homogeneous group
0	95.180	A
0.01	94.840	A
0.1	94.400	A
0.05	94.360	AB
0.175	94.307	AB
0.5	93.493	B
0.7	88.860	C
1.4	81.220	D

Note: Treatments with the same letter are statistically homogeneous.

The first five doses 0, 0.01, 0.1, 0.05 and 0.175 mg, were statistically equals to the control sample, the three remaining samples, 0.5, 0.7 and 1.4 mg, showed a middle reduction of glucose, also it was found differences statistically significant for the different times of reaction ($p<0.0001$), which indicates that the decrease of glucose levels resulted modified by the time factor, see Table 2.

Table 2. Tukey's test comparisons for time factor.

Time (minutes)	Average	Homogeneous group
0	100.03	A
1.5	96.29	B
3	91.73	C
5	88.14	D
10	84.22	E

Note: Treatments with the same letter are statistically homogeneous.

The average content of glucose reduction decreased along the time and a minimum apparently was not reached. The analysis of variance showed significant differences for the interaction doses-time ($p<0.0001$), which indicates that the treatments were not homogeneous at different times and the behavior was not the same for the different concentrations of aqueous extract of the plant.

The Figure 6 shows that the treatments were homogenous until the doses 0.5 mg approximately. Only with the doses 0.7 and 1.4 mg it can be observed a significant change in the angle of the line, which it means that these two treatments are, specifically, which produces more quickly the biggest glucose reduction in the medium.

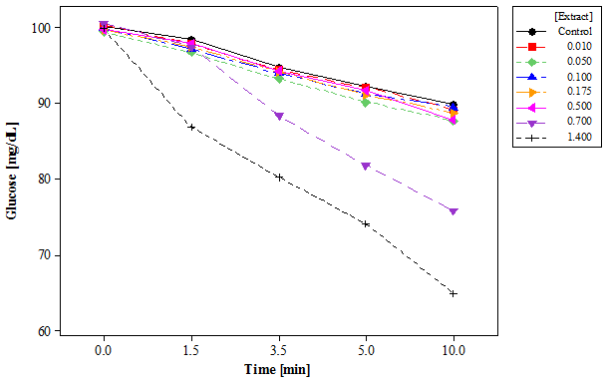

Figure 6. Interaction of glucose concentration and time.

Discussion

The test *in vitro* carried out with the aqueous extract of the plant *A. indica* in a normoglycemic medium with human blood cells evidenced a hypoglycemic effect, it was confirmed once did was observed the decrease of the concentration of glucose in relation to the control sample.

The extract doses applied, showed some differences in the glucose consume, so they were classified in groups according to the doses: small doses (0.01 and 0.05 mg), it was not observed glucose consume in the medium; middle doses (0.1, 0.175 and 0.5 mg), it was observed a non-significant decrease of the glucose levels in the medium; and as a high doses (0.7 and 1.4 mg), in this case, it was shown a continuous glucose consume, with a significant value since the five minutes until ten minutes. The concentrations 0.7 and 1.4 mg of the extract produced a hypoglycemic effect with a consume percentage of 15.4% and 26.6%, respectively in ten minutes.

As time passes, and increases the concentrations of the aqueous extract of the plant, the hypoglycemic effect is bigger. It may suggest that in high doses of the extract and in a longer time of reaction, the glucose consume will increase by the cells in the normoglycemic medium.

This results are different from the obtained in another research, in which was evaluated the hypoglycemic effect of the plant *Bixa orellana L.* in rabbits (Martínez et al., 2010). When taking the glycaemia measure, pre-test and post-test of the rabbits, it was not evidenced a significant difference; that's why it was studied the hypoglycemic effect of the plant with the realization of test *in vitro* in a hyperglycemic medium (11.1 mM glucose) in human blood cells, having as an observation a decrease in the glucose concentrations in relation to the control sample at small doses of the extract of the plant in the first minutes of reaction, whereas the doses 0.7 and 1.4 mg were considered not reliable, because they showed a decrease of the percentage of consumed glucose.

The results obtained with the aqueous extract of *A. indica* are different of other results, where the hypoglycemic effect of the extract of the plant *Petiveria alliacea L* was analyzed (Rojo et al., 2002), regarding the glucose consume of the erythrocytes in a cultivation medium, demonstrating there was not decrease in the glucose levels, due to the action of the extract of the plant, because it did not affected the glucose consume by the erythrocytes.

The hypoglycemic effect of the plant *A. indica* had been studied before in animals (Akinola et al., 2010; Ali et al., 2003; González et al., 2010; Isea et al., 2011; Manish et al., 2010), usually in rats and mice, finding statistical significant neem extract effect, but not in *in vitro* systems using human blood cells in medium with glucose, like the research done in this case, in which it was obtained a significant result, when the glucose levels decreased significantly in the medium, as wells as the obtained by the surveys carried out in animals.

Based on the results obtained in the tests with the aqueous extract of the plant *A. indica*, in a normoglycemic medium with human blood cells, it is considerate that some posterior test should be done in order to extend the research about the hypoglycemic effect of this plant, such as to use a hyperglycemic medium to evaluate the hypoglycemic effect of the aqueous extract of *A. indica* under this condition, or using concentrations and incubations with longer time intervals than the used in this research, and carry out researches that let know the action mechanism of the hypoglycemic effect of the *A. indica* aqueous extract.

Conclusions

It is concluded that the aqueous extract obtained from the leaves of the plant *A. indica*, produce a hypoglycemic effect observed through the determinations of glucose in the normoglycemic medium.

References

Akinola O. B., E. A. Caxton-Martins and L. Dini. 2010. Chronic treatment with ethanolic extract of the leaves of *Azadirachta indica* ameliorates lesions of pancreatic islets in streptozotocin diabetes. Int. J. Morphol. 28(1):291-302.

Ali, A., A. Chandra, R. Kumar. S. Shukla, L. C. Mishra and S. Ahmad. 2003. Effect of herbal hypoglycemic agents on oxidative stress and antioxidant status in diabetics rats. Indian J. Clin. Biochem. 18(2):8-15.

Avello, E., E. A. Silveira, F. I. Peña, M. C. Camacho and M. A. Arce. 2006. Actividad antihelmíntica in vitro de extractos de *Azadirachta indica* A Juss, *Momordica charantia* L. y *Chenopodium* (Teloxys) *ambrosioides* L. Weber. REDVET. 7(11):1-10.

Braham, D. and P. Trinder. 1972. An improved colour reagent for the determination of blood glucose by the oxydase system. Analyst. 97:142-145.

González, M. S., A. A. Márquez, C. E. Meléndez and A. López-Ortega. 2010. Efecto del extracto de hojas de Nim (*Azadirachta indica* A. Juss) en la diabetes mellitus inducida por estreptozotocina en ratones. Gaceta Cien. Veter. 15(2):64-71

Iannacone, J., H. Ayala and A. Román. 2005. Efectos toxicológicos de cuatro plantas sobre el gorgojo del maíz *Sitophilus zeamais* Motschulsky 1855 (Coleoptera: Curculionidae) y sobre el gorgojo de las galletas *Stegobiumpaniceum* (Linnaeus 1761) (Coleoptera: Anobiidae) en Perú. Gayana 69(2):234-240.

Isea, G. A., I. E. Rodríguez, E. E. Sánchez and M. A. Montero. 2011. Efecto hipoglicemiante de *Azadirachta indica* A. Juss., *Phyllantus niruri* L. y su combinación en ratas normales. Rev. Cubana Plantas Med. 16(2):183-189.

López, Y., M. Angulo, C. Martínez, J. Soto y C. Chaidez. 2007. Efecto antimicrobiano de extractos crudos de neem (*Azadirachta indica* A. Juss) y venadillo (*Swietenia humilis* Zucc) contra *E. coli*, *S. aureus* y el bacteriófago P22. Bioquimia. 32(4):117-125.

Manish, G., G. K. Jana and A. K. Jha. 2010. A comparative study of Aloe, Kundru and Neem as an antidiabetic agent. Drug Invent. Today 2(1):22-24.

Martínez, N., R. Jiménez, W. Moreno, L. Lamont, E. Cayama, C. Espino and D. Requena. 2010. Efecto hipoglicemiante de la planta *Bixa orellana* L. en conejos de la raza Nueva Zelanda y en medios de glucosa con células resuspendidas. In: Anuario de investigación en etnomedicina, medicina complementaria y utilización de plantas medicinales. Publisher: Universidad Autónoma Metropolitana de México-Unidad Iztapalapa. México D.F. México. pp. 45-50.

Reyes, E. C., S. W. Valero and D. A. Garay. 2003. Estudio preliminar de las propiedades físicas de la especie *Azadirachta indica* (Neem) procedente del estado Falcón (Venezuela). Revista Forest. Venez. 47(2):23-29.

Rojo, D., L. Bell, E. Cancio and R. Iglesias. 2002. Efecto del extracto hipoglicemiante de *Petiveria alliacea L* sobre el consumo de glucosa por los eritrocitos. Rev. Cubana Invest. Biomed. 21(3):161-166.

Viveros, J., and J. Castaño. 2006. Evaluación *in vitro* de extractos vegetales sobre *Mycosphaerella fijiensis* Morelet. Agron. 14(1):37-50.

Urdaneta, J. 2001. Neem: un árbol milagroso. Manual técnico. Universidad del Zulia, Facultad de Agronomía, División de Extensión Agrícola. Maracaibo. Venezuela. pp. 50.

Emir. J. Food Agric. 2014. 26 (7): 630-638
doi: 10.9755/ejfa.v26i7.18194
http://www.ejfa.info/

REGULAR ARTICLE

Control of spot blotch in barley plants with fungicide and *Bauhinia variegata* Linn. leaf extract

Erna E. Bach[1*], Kamila F. Esquerdo[2], Mila B. F. Oliveira[2], Felipe A. Reis[2], Vinicius O. Cardoso[2] and Nilsa S. Y. Wadt[1]

[1]Department of Healthy, UNINOVE, São Paulo, Brazil. R. Dr. Adolfo Pinto, 109, Barra Funda, CEP 01156-050, São Paulo, SP, Brazil
[2]Department of Healthy, IC-UNINOVE, São Paulo, Brazil. R. Dr. Adolfo Pinto, 109, Barra Funda, CEP 01156-050, São Paulo, SP, Brazil

Abstract

In barley plants (BRS-195), the disease detected as spot blotch is caused by *Bipolaris sorokiniana*, and is the most deleterious disease for the producers and the beer industry. For fungicides mediated control of this disease can cause risks to environment and human health. To eliminate these drawbacks, one of the methods considered is the use natural products. The purpose of the present study was to investigate the use of extract of leaves from *Bauhinia variegata* Link. and Opera® fungicide (active ingredient: pyraclostrobin) for controlling *Bipolaris sorokiniana* in barley plants. In Brazil there are two species of *Bauhinia* are seen, namely *Bauhinia forficata* and *B. variegate*. Extracts from *B. variegata* didn't show fungitoxic action but *B. forficata* the action exist. The barley plant showed protection of 92 to 100% in local and systemic action. The chemical TLC assays showed the presence of phenols (rutin, coumaric acid, kaempferol) that can be related to a signal for activation of the defense responses against pathogen or mechanism of salicylic acid. Treatment with fungicide Opera® gave another mechanism and have only 60% of protection.

Key words: Barley, *Bipolaris sorokiniana*, *Bauhinia variegata*, Opera®

Introduction

In Brazil Barley has been used by beer makers for malting and for food purpose. During the barley development, several fungal diseases have been detected. Spot blotch is caused by *Bipolaris sorokiniana* (asexual fungi), and is the most serious deleterious diseases for the producers which affect the ears, darkening the grains and impairing the quality of malt and beer. The disease causes considerable losses in yield that can reach up to 30% of production. The infection depends on climatic conditions and ranges from 10 to 100% infection by the fungus. Another problem is the spots enlargement as the leaf grows and spread along the entire leaf blade and can be produce brown lesions. The producers have 100% losses because infected plant cannot produce normal heads (Teng, 1987; Agrios, 1988; Minella, 2001; Castro and Bach, 2004).

To prevent these losses, Opera® fungicide (active ingredient: pyraclostrobin) has been used to control disease but have a higher cost, risk of environmental contamination and intoxication during application for human. For eliminating these threats, alternative control measures can be adopted. The alternative control can be of induction of resistance in plants or, preventing or restricting the development and multiplication of the pathogen (Kuc, 1987). Inducers or elicitors of resistance have already been evaluated in the control of several diseases of plants with natural products (Guzzo et al., 1993; Benhamou, 1996; Gatz, 1997; Bach et al., 2003; Castro and Bach, 2004).

In Brazil, there are two species of *Bauhinia* one as ornamental plant, cultivated for afforestation and the other for manufacturing of wood wool board from Rio de Janeiro to Rio Grande do Sul. The two species can be confused because the flowers are similar but leaves and action in human health are different. *Bauhinia forficata* Link leaves extract was used with water as medicine for antidiabetic action in human (Arigony, 2005). *B. variegata* leaves extract showed the laxative action and used

Received 20 November 2013; Revised 14 February 2014; Accepted 17 February 2014; Published Online 18 May 2014

*Corresponding Author

Erna E. Bach
Department of Health, UNINOVE (University Nove de Julho), São Paulo, Brazil

Email: ebach@uninove.br

against diarrhea (Asima and Satyesh, 1992). In morphology *B. forficata* has thorns, white flowers and the leaves present two lobes divided and, in adults the leaves can be reach with 7 to 12cm in length. *B. variegata* has no thorns, flowers can be white or pink and, leaves have also two lobes but reach a smaller size (Miyake et al., 1986; Fortunato, 1986). The chemical substances are also different as evidenced by Engel et al. (2008) where *B. variegata*, didn't show chemical marker as kaempferitrin but was present in *B. forficata*.

The aim of the present study was to investigate the effect of leaf extract of *B. variegata* and Opera® fungicide against *B. sorokiniana* infection in barley plants (cultivar Embrapa 195) under greenhouse conditions. For explaining the possible elicitor action, biochemical analyses were conducted in treated, untreated or infected leaves.

Materials and Methods

Suspension of pathogen

The pathogen used was *B. sorokiniana* obtained from infected barley leaves (Fundação Guarapuava-Agraria, Paraná) and kept in potato dextrose agar (PDA) medium on plates. After 10 days, conidia were removed by brushing the surface of the agar and material was suspended in 10 ml of sterile water followed by filtration through gauze. Concentration was adjusted to 10^5conidia/mL and added Tween 20 (poly-oxyethylene sorbitan monolauret, Sigma Chemical Co.) to a final concentration of 0.05%.

Biological control

Extracts from *Bauhinia* leaves were prepared with 1g of leaves in 5mL of water, filtered in whatman paper n°1. For biological control, one milliliter of each extract, in three dilutions, were incorporated in 5mL of culture medium PDA (Potato-dextrose-agar) submitted before to autoclave and after transferred in a slide of microscope and inoculated conidia of fungi (*B. sorokiniana*) and maintained in petri plates with humid and temperature of 27°C. After 5 days, the area (cm^2) occupied by the fungus was measured, and conidia were removed using 4mL of sterile distilled water and counted in a haemotocytometer. Three replicates were made for each treatment.

Extracts from *B. variegata* (elicitor)

Leaves from *B. variegata* (pink flowers) were collected in Ibiuna, Sao Paulo and transported to the UNINOVE laboratory in a cooler. For extraction, 50g of leaves were ground in a blender with 250mL of distilled water. The homogenate was incubated in a refrigerator for one hour prior to being filtered through gauze and a 0.45µm Millipore filter. The filtered solution was then stored at -4°C until biochemical analysis and treatments. The concentrations of proteins (Lowry et al., 1951) and phenols (Swain and Hillis, 1959) were quantified as part of the biochemical analysis.

Preparation of barley plants and induced local protection

Barley plants (Embrapa BRS195 – from Foundation Agraria, state of Paraná), were grown from ten seeds in clay pots (15cm diameter) filled with a fertilized soil (red soil with NPK 10:10:10 with micronutrients) and maintained in a greenhouse under 12h photoperiod (approximately 190IE/m^2/s) for around 3 weeks, until they were in stage 5 (tillering) (Large, 1954).

Groups of 10 plants were used in each treatment. Each treatment was replicated three times and data were submitted to variance analysis. Plants were arranged in a complete randomized block design and the combination of challenger and protector in each treatment. Around 10mL of the conidia suspension, or extract of elicitor or water were used in each treatment. Treatments were: (a) healthy (plants sprayed with water); (b) Inducer: plants sprayed with elicitor extract in two dilutions (0.535mg of proteins and 0.267mg of proteins); (c) pathogen inoculated (plants pulverized with conidia suspension of pathogen); (d) Inducer-pathogen and in two dilutions: inducer-treated and after 24 h inoculated with conidial suspension; e) ditto the group d, however, after 48 hours; f) ditto the group d, however, after 72 hours; g) plants pulverized with Opera® fungicide (pulverized with 2mL of a 200 fold dilution of product in water) and afterwards inoculated with a conidial suspension. Plants from groups d, e, f were initially pulverized with the elicitor, and after 24, 48 and 72 hours, at room temperature and 12-hour photoperiod (fluorescent light 7.35 W m^{-2}), the leaves were inoculated with conidia suspensions, by pulverization. During the first 24 hours after the inoculation of the pathogen, all plants were kept in a humid chamber (80% relative humidity), at room temperature and in the dark. After that, plants were transferred to the greenhouse and kept at room temperature and 12 hours of light per day (Bach et al., 2003).

Preparation of barley plants and systemic resistance

In another group of plants the systemic protection assay was performed using the same cultivar and the elicitor extract in the dilution that have a positive reaction in local protection. Three leaves were market and the first leaf were the

oldest, the second leaf intermediary and the thirty a new leaf. The second leaves of barley plants were coated with the elicitor extract (Syst L2T) and the first and thirty were coated with water. After 48 hours, all the leaves were submitted to total pulverization with suspension of the conidia. Healthy groups were pulverized using water. Protection level was evaluated 7 days after the inoculation of the pathogen, based on the number of infected leaves in ten plants (Bach et al., 2003). First, second and thirty leaves was removed, separated and submitted to extraction.

Extraction of barley leaves

Barley leaves from all experiments were performed in duplicate and analyzed by the Student's test. One gram of the leaves were ground in presence of 1mL of cold phosphate buffer (pH=7, 0.05 mol/L). After one hour incubation at 4°C, each extract was filtered through gauze and then quantified proteins and phenols. The amount of protein in sample was estimated using Lowry method (Lowry et al., 1951), based in a standard curve of standard protein solution as BSA (mg bovine serum albumin/mL- Sigma) and absorbance were determinate using a spectrophotometer Fenton with software. For phenols the method used was based in Swain and Hill (1959) with standard curve prepared with chlorogenic acid (mg/mL) (Sigma).

From plants healthy, BAU control, infected, treated with Opera®, BAU 48h and BAU 72h, one gram of leaf material was ground in methanol and 10µL of each samples was subjected to thin layer chromatography (Merck) and separated with organic phase from Butanol-acetic acid-water (BAW 4:1:5). Spots were visualized with UV light and ferric chlorite (1% in alcohol). The results was compared with standard Kaempferol (Rf=0.94), benzoic acid (Rf=0.74), *p*-coumaric acid (Rf=0.68), *o*-coumaric acid (Rf=-0.62) and rutin (Rf=0.48).

Analysis in thin layer chromatography (TLC) from *Bauhinia* extracts

Another aliquot from leaves from *B. variegata* (pink and white flowers), were dried and crushed in a mill until 20 mesh powder that being percolated in methanol at concentration of 2mg/mL (Engel et al., 2008) and performed thin layer chromatography (Merck). 10µL of the all samples were put in two plates of TLC. One plate was separated with reagent dichloromethane-methanol (DC-Meth 7:3) and the other was used organic phase from Butanol-acetic acid-water (BAW 4:1:5). Spots were developed with UV (violet ultra-light) and ferric chlorite (1% in alcohol). Areas from bands were measure in program CP Atlas. Standard samples used for DC-Meth was kaempferitrin (Rf=0.36) and for BAW was rutin (Rf=0.48), kaempferol (Rf=0.94) and *o*-coumaric acid (Rf=0.68).

Results and Discussion

Morpho-anatomical diagnosis

The preliminary investigation was morpho-anatomical diagnosis from leaves and flowers present in two species from the genus *Bauhinia*, that have peculiar bilobate leaves, which render the common name pata-de-vaca (cow`s hoff) and were used as medicinal plants. *B. variegata* presented leaves whose two lobes are sharply rounded apex, which differs from the apex acute *B. forficata*. Flowers from *B. forficata* were white and in *B. variegata* can be white and pink (Miyake et al., 1986; Fortunato, 1986; Shah et al., 2010).

Biological control

The quantification of concentration of proteins and phenols was: for *B. variegata* was present 5.35mg of proteins and 0.28mg of phenols while for *B. forficata* was 1.25mg of proteins and 3.8mg of phenols. Extract from *B. forficata* was preliminary concentrated in dialysis membrane (MM3000) against carbowax (PEG-poliglicol MM6000), maintained in refrigerator by 10 hours. The proteins were obtained eight times more concentrated and then diluted with water at same concentrations from *B. variegata* and used for test involved biological control in four concentrations.

The results in biological control above the *B. sorokiniana* showed that aqueous extract of *B. forficata* inhibited the fungal development and conidial production that can be considered as fungitoxic action and the extract of *B. variegata* showed no difference in the development and production of conidia when compared to the control slide because of lack of biological control above the fungi (Table 1).

Accordingly with Georgopoulos (1984) when worked with adaptation of fungi to fungitoxic compounds that can be concluded that some fungitoxic chemicals often fail to protect crops because the target fungi develop resistance. So, in this work we used *B. variegata* (pink flower) because the extract didn't presented fungitoxic action against fungi.

Table 1. Development and production of conidia from *B. sorokiniana* submitted to different concentrations of proteins from aqueous extracts from *B. variegata* and *B. forficata*.

Extracts	dilution of extract (concentration of proteins mg)	Number of conidia x 10^4*	Total area (cm)*
B. variegata	2.00	2.0^{a}±0.05	2.0^{a}±0.02
B. forficata	2.00	0.1^{b}±0.004	0.6^{b}±0.03
B. variegata	1.07	2.0^{a}±0.03	2.0^{a}±0.02
B. forficata	1.07	0.1^{b}±0.003	0.8^{c}±0.02
B. variegata	0.50	1.9^{a}±0.03	2.0^{a}±0.03
B. forficata	0.50	0.2^{c}±0.002	1.0^{d}±0.04
B. variegata	0.26	1.9^{a}±0.004	2.0^{a}±0.02
B. forficata	0.26	0.3^{d}±0.003	1.2^{c}±0.03
Control (*B. sorokiniana*)	X	2.0^{a}±0.08	2.0a±0.03

*Media of three repetitions with±SD. Same letters in columns was not different statistically when compared with control. Different letters in columns were different statistically compared with control (student T test).

Induced of local resistance

The percentage of local protection was evaluated at two concentrations of the inducer (aqueous extract from *B. variegata*) containing 0.535mg and 0.267mg of protein. At dilution with 0.535mg proteins the protection ranged from 92 to 100% while with 0.267mg of proteins the protection ranged from 78 to 100%.

The results were compared with those of the treatment with the fungicide with the level was 60% (Table 2). It is interesting to note that plants treated with inducers and challenge presented more protection than a plant with fungicide. In biochemical analyses, barley plants treated with elicitor at all intervals of time and plants treated with fungicide, showed higher amounts of protein, lower concentration of phenols when compared with infected plants. For example, in period of 72h inducer-challenge, the protection and concentration of protein was higher but quantity of phenols was decreased when compared with untreated plants, treated with fungicide and also infected plants. Infected plants presented more concentration of phenols and decreased protein when compared with treated plants.

Induced of systemic resistance

Systemic protection against *B. sorokiniana* was also obtained when aqueous extract from *B. variegata* (with 0.535mg of proteins) was applied 48h before challenge inoculation (Table 2).

The higher protection occurred with upward effect but a minor action also occurred in lower leaf. In 72h between inducer-challenge, in the third leaf (young leaf) presented 90%, the leaf 2 that was treated presented 99% and the leaf 1 (oldest leaf) presented 100% of protection. The same effect occurred in other periods from 24h and 48h. This demonstrates that even if the inducer is applied at the bottom of the plant, its effect will move to the upper leaves. This effect was also observed by Bach et al. (2003), Castro and Bach (2004) in work with other elicitors.

In systemic treatments, the leaf that received inducer (L2T) presented higher concentration of protein when compared with leaf 1 and 3. For induced protection, the leaf 2 presented higher protection as the leaf 3 showing action upward.

Other direction downward also presented protection but that was decreased when compared with the leaf 1 (Table 2, 3). Presence of higher concentration of proteins was associated with the action of induced resistance because the elicitors are responsible for triggering a signal in the host when occur the attack of a pathogen. These results were also in par with other authors (Hwang and Kim, 1990; Du and Wang, 1992; Anuratha et al., 1996; Benhamou, 1996; Manandhar et al., 1999; Kuc, 2001; Kombrink and Schmelzer, 2001) (Table 2, 3).

Table 2. Number of leaves with spot bloth caused by *B. sorokiniana* and percentage of protection in barley leaves (Embrapa 195), using aqueous extract of *B. variegata* (pink flower) as inducer.

Treatments	mg proteins	number of total leaves	number of infected Leaves	% protection
Healthy		20	X	X
Infectada		20	19	0 a*
Opera®		20	8	60±1 h**
BAU C	0.535			
BAU 72h	0.535	81	0	100 b,b*
BAU 48h	0.535	81	3.2	96±0.5 c, f
BAU 24h	0.535	81	7.2	92±1 d, g
BAU C	0.267			
BAU 72h	0.267	20	0	100 b,b
BAU 48h	0.267	20	3.4	83±0.8 c,h
BAU 24h	0.267	20	4.5	78±0.5 e,i
Syst BAU 72h	L1	20	2	90±1c**
	L2T	20	0.2	99±0.4 b
	L3	20	0	100 a
Syst BAU 48h	L1	20	2	90±0.4 c
	L2T	20	1	95±0.2 b
	L3	20	0.4	98±0.1 a
Syst BAU 24h	L1	20	5.4	73±0.05 c
	L2T	20	4.8	76±0.1b
	L3	20	4.4	78±0.5 a

Groups: Healthy (plants sprayed only with water); Infected (plants sprayed only with conidial); Opera® (0.1mL from product diluted in 20mL of water and used in pulverization only 2 mL/plant); BAU C (plants sprayed only with extracts of *B. variegata* in two dilutions); BAU 72h (plants sprayed with extract (two dilutions) of the inductor and 72h after inoculated with pathogen; BAU 48h (ditto with the previous range of 48 hours); BAU 24h (ditto with the previous range of 24h); Syst BAU 72h L1(oldest leaf) : coated the first leaf with 2 mL of water; L2T: coated the second leaf with 2 mL of the extract of the inductor; L3(new leaf): coated the thirty leaf with 2 mL of water. After 24, 48 and 72 were inoculated the total plant with conidia of *B. sorokiniana*. *media of percentage of protection of total of 10 plants per treatment from three experiments ±SD. Mean values with different first letter, statistically significantly different from the infected plants ($P<0.05$); and when the second letter was the same have not difference statistically between treatments but when these letters was different statistically was number different between treatments ($P<0.05$), according to the students t-test and Origin (Anova). **Media of percentage of systemic protection of total of 10 plants ± SD and compared with infected plants and with extract of *Bauhinia*. Value was different statistically from all plants submitted to systemic protection.

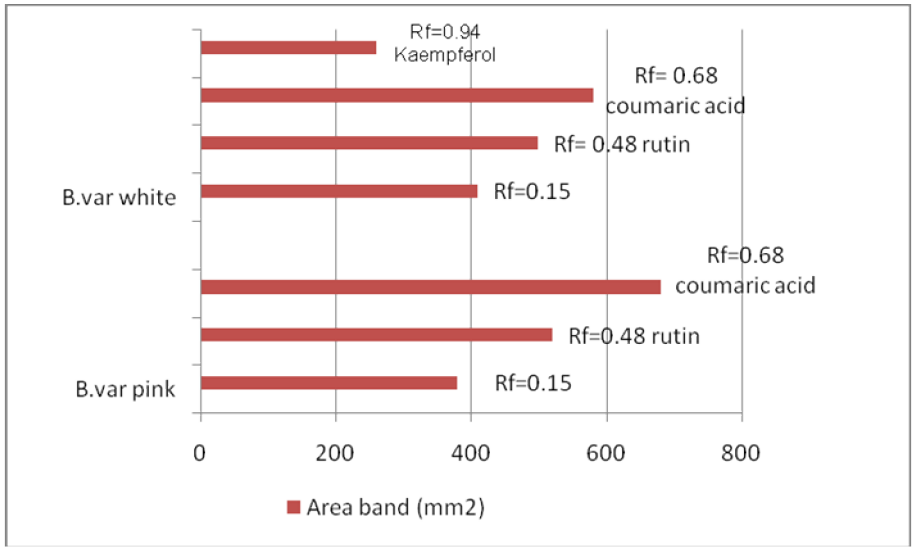

Figure 1. Rf (mobility) and band area (mm^2) from extract of *Bauhinia variegata* (pink and white flower) and *B. forficate*. Samples with 10 µL. Reagent: dichloromethane-methanol (7:3) for Kaempferitrina (Kaempf Rf=0.36). Reagent BAW (4:1:5 organic phase), standart Rutin (Rf=0.48), Kaempferol (Kaemp Rf=0.94) and coumaric acid (coumacid Rf=0.68). Spots revelated with UV and ferric chlorite 1% and area measure program CP-Atlas.

Table 3. Concentration of protein (mg SAB), and phenols (mg chlorogenic acid), present in leaf extracts of barley plants (cultivar Embrapa 195) after treatment with aqueous extract of *B. variegata,* Opera®, water against *B. sorokiniana.*

Treatments	Conc. proteins in extract	Proteins (mgBSA/mL)*	Phenols(mg clorog ac/mL)*
Healthy		0.487±0.04 b	0.10±0.02b
Infected		0.120±0.01 a	0.86±0.20a
Opera®		0.320±0.04b	0.13±0.01 b
BAU C	0.535	0.468±0.06b	0.03±0.005c
BAU 72h	0.535	0.575±0.04b	0.03±0.005c
BAU 48h	0.535	0.498 b±0.04	0.05±0.005c
BAU 24h	0.535	0.471 b±0.03	0.05±0.004c
BAU C	0.267	0.445±0.05b	0.03±0.002c
BAU 72h	0.267	0.420±0.04 b	0.03 c±0.002
BAU 48h	0.267	0.431±0.04b	0.06±0.003c
BAU 24h	0.267	0.417±0.05b	0.08 c±0.002
Syst BAU 72h	L1	0.480 b	0.05 c
	L2T	0.520 b	0.03 c
	L3	0.500 b	0.08 c
Syst BAU 48h	L1	0.385 b	0.06 c
	L2T	0.450 b	0.06 c
	L3	0.480 b	0.09 c
Syst BAU 24h	L1	0.350 b	0.08 c
	L2T	0.410 b	0.09 c
	L3	0.430 b	0.10 b

Groups: Healthy (plants sprayed with water); Infected (plants sprayed only with conidial); Opera® (0.1mL from product diluted in 20mL of water and used in pulverization only 2 mL/plant); BAU C (plants sprayed only with elicitor in two dilutions); BAU 72h (plants sprayed with elicitor 72h (with 2 dilutions) and after inoculated pathogen); BAU 48h (ditto with the previous range of 48 hours), BAU 24 (ditto with the previous range of 24); SYST: L1: Strokes the first leaves (F1) with 2 ml of water L2T: Strokes the second sheet (F2) with 2 mL elicitor; L 3: Strokes thirty leaf (F3) with 2 mL of water. After 24, 48 and 72 were inoculated with conidia of *Bipolaris sorokiniana* in all leaves. * Averages involving three replicates of each test ± SD. Means with different letters in columns differ significantly at 0.05% (Student t-test `s) when compared with infected plants. In Syst treatments don`t have SD because the numbers are very little about 0.0001 or 0.0002.

Chromatography from extracts of Bauhinia

To prove that in work was used *B. variegata*, were used a test thin layer chromatography with two reagents. Engel (2008) observed that *B. forficata* in reagent DC-Meth showed a band of Rf=0.36 and that is a marker for presence of kaempferitrina. In the results from present work was observed that *B. variegata* both with pink or white flowers did have not that band.

With reagent BAW (organic phase) *B. variegata* pink flower showed three bands with Rf 0.15; 0.48 and 0.68 but the white flower had four bands with Rf 0.15, 0.48; 0.68; 0.94. The band with Rf=0.15 is a marker for all varieties from *Bauhinia.* Extract from *B. variegata* pink and white flower present the same band with Rf=0.48 that coincide with rutin standard (Figure 1).

Ferrer et al. (2008) and Victório et al. (2009) described flavonoids are metabolites produced as part of plant defense, especially against the effects of ultraviolet radiation and their contents are in greater concentration in the leaves that is the main part of plants exposed to solar incidence. The rutin and Kaempferol in cassava increased with defense responses to diseases (Tanaka et al., 1983; Buschmann et al., 2000). The coumaric acid is related to resistance for produce salicylic acid and that can be related to inducer of resistance or mechanism of resistance.

Chromatography from extracts of Barley plants

TLC analysis of a healthy barley plant extract included three bands and infected plant with *B. sorokiniana* presented only one band. The other two bands from infected leaves perhaps were used or degraded by fungus.

With barley plant (treated only the elicitor BAU) present five bands but three were equal in area and Rf to bands obtained from healthy plants. The other two bands correlated with p-coumaric acid and rutin (Table 4). It is interestingly that p-coumaric acid and rutin is likely come from elicitor extract.

Barley plants treated with Bauhinia extract and sprayed with conidia suspension, presented three bands correlated to standards o-coumaric acid, p-coumaric acid and benzoic acid. The band of p-coumaric acid in treated plants presented an area

equal to 400mm^2 and in control plants, an area equal to 590mm^2 indicating that only some of the *p*-coumaric acid penetrated into the plant in 48 and 72h (Table 4). Area of *o*-coumaric acid increased over time and correlated to increases protection.

Table 6. Bands observed in TLC following barley treatments.

Treatments	Rf	Area *	Standard**
Healthy	0.428	82	
	0.560	4069	
	0.710	2691	
Infected	0.440	8685	
Opera (48h)	0.539	2793	
	0.586	1163	
	0.763	1186	
	0.839	1318	
	0.915	163	
	0.991	661	
BAU C	0.428	82	
	0.480	420	Rutin
	0.560	4069	
	0.680	590	p-coum acid
	0.710	2691	
BAU 48h	0.420	58	
	0.560	1852	
	0.620	1580	o-coum acid
	0.680	400	p-coum acid
	0.740	2060	benz acid
BAU 72h	0.420	38	
	0.560	1022	
	0.620	2950	o-coum acid
	0.680	400	p-coum acid
	0.740	4050	benz acid

*Area (mm2)
**Bands correlated to standard

Raskin (1992) and Ribnicky et al. (1998) explained that salicylic acid (SA) is a signal in systemic acquired resistance and as an inducer of the alternative protein in tobacco cell suspensions. The occurrence of SAR (systemic adquired resistance) in response to a pathogen requires a long-distance transport of a factor originating in the tissue expressing the hypersensitive response that moves systemically to other parts of the plant (Chong et al., 2001; Dmitriev, 2003; Sequeira, 1979, 1983). It was suggested that SA is responsible for SAR plants. In this work the results demonstrated that in *B. variegata* (pink flower) have cumaric acid, rutin and kaempferol that`s related to a signal for the activation the defense responses against pathogen or mechanism of salicylic acid.

In barley plants treated with Opera®, six bands were identified but none correlated to the standards used in work and possible the mechanism was different.

Conclusion

In conclusion, extract of *B. variegata* acted as elicitor of local and systemic resistance (ascending higher than in the downswing) in barley plants against *Bipolaris sorokiniana* upon 90% of protection. Presence of inducer was correlated with increased protein, decreased phenols and presence of *o*-coumaric acid. This suggests that the mechanism of protection involves SA biosynthesis. With barley plants treated with fungicide Opera® and later challenged with conidia from same fungi, demonstrated 60% of protection and mechanism is probably different because don't have *o*-coumaric acid.

Acknowledgements

We wish to thank Noemir Antomiazzi (Fundação Agrária dos produtores de cevada de Guarapuava) for providing barley seeds.

References

Agrios, G. N. 1988. Plant Pathology. Academic Press. p. 803.

Anuratha, C. S., K. C. Zen, K. C. Cole, T. Mew and S. Muthukrishnan. 1996. Induction of chitinases and beta-1-3-glucanase in *Rhizoctonia solani*-infected rice plants: Isolation of an infection-related chitinase cDNA clone. Physiol. Plant. 97:39-46.

Arigony, A. L. V. 2005. Determinação química e biológica de *Bauhinia forficata* Link subespécie pruinosa (pata-de-vaca-Leguminosae) Dissertação de mestrado UFRGS. p. 160.

Asima C. and C. P. Satyesh. 1992. In: The treatise of Indian medicinal plants. Vol. 2. New Delhi: Publication and information directorate CSIR. pp. 24-26.

Bach, E. E., B. C. Barros and H. Kimati. 2003. Induced resistance against Bipolaris bicolor, *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in wheat leaves by xanthan gum and heat-inactivate conidia suspension. J. Phytopathol. 151:411-418.

Benhamou, N. 1996. Elicitor-induced plant defense pathways. Trends Plant Sci. 7:233-240.

Buschmann, H., M. X. Rodrigues, J. Tohme and J. R. Beeching. 2000. Accumulation of hydroxycoumarins during post-harvest deterioration of tuberous roots of cassava

(*Manihot esculenta* Crantz). Ann. Bot. 86:1153-1160.

Castro, O. L. and E. E. Bach. 2004. Increased production of β-1,3 glucanase and proteins in *Bipolaris sorokiniana* pathosystem treated using commercial xanthan gum. Plant Physiol. Biochem. 42:165-169.

Chong J., M. Pierrel, R. Atanassova, D. Werck-Reichhart, B. Fritig and P. Saindrenan. 2001. Free and conjugated benzoic acid in tobacco plants and cell cultures. Induced accumulation upon elicitation of defense responses and role as salicylic acid precursors. Plant Physiol. 125:318-328.

Dmitriev, A. P. 2003. Signal molecules for plant defense responses to biotic stress. Russian J. Plant Physiol. 50:417–425.

Du, L. C. and J. Wang. 1992. Activities and distribution of chitinase and beta-1-3-glucanase in rice induced by *Pyricularia oryzae.* Acta Phytopathol. Sin. 18:29-36.

Engel, I. A., R. A. Ferreira, V. Cechinel-Filho and C. Meyre-Silva. 2008. Controle de qualidade de drogas vegetais a base de *Bauhinia forficata* Link (*Fabaceae*). Rev. Brasil. Farm. 18:258-264.

Ferrer, J. L., M. B. Austin, C. Stewart and J. P. Noel. 2008. Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiol. Biochem. 46:356-370.

Fortunato, R. H. 1986. Revision del genero *Bauhinia* (*Cerdideae, Calsalpinioidea, Fabacea* para la Argentina). Darwiniana 27:527-557.

Gatz, C. 1997. Chemical control of gene expression. Annu. Rev. Plant Mol. Biol. 48:89-108.

Georgopoulos, S. G. 1984. Adaptation of fungi to fungitoxic compounds. Ciba Foundation Symp. 102:190-203.

Guzzo, S. D., E. E. Bach, E. M. F. Martins and W. B. C. Moraes. 1993. Crude exopoly saccharides (EPS) from *Xanthomonas campestris pv. manihotis*, X. *campestris* pv. *campestris* and commercial xanthan gum as inducers of protection in coffee plants against *Hemileia vastatrix*. J. Phytopathol. 139:119-128.

Hwang, B. K. and Y. J. Kim. 1990. Capsidiol production in pepper plants associated with age-related resistance to *Phytophtora capsici*. Korean J. Plant Pathol. 6:193-200.

Kombrink, E. and E. Schmelzer. 2001. The hypersensitive response and role in local and systemic disease resistance. Eur. J. Plant Pathol. 107:69-78.

Kuc, J. 2001. Concepts and direction of induced systemic resistance in plants and its application. Eur. J. Plant Pathol. 107:7-12.

Kuc, J. 1987. Plant immunization and its aplicability for disease control, In: K. Chet (Ed.). pp. 255-274. Innovative approaches to plant disease control. New York, John Wiley Sons.

Large, E. C. 1954. Growth stages in cereal: Illustration of the Feekes scale. Plant Pathol. 3:129.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.

Manandhar, H. K., S. B. Mathur, V. Smedegaard-petersen and H. Thordal-christensen. 1999. Accumulation of transcripts for pathogenesis-related proteins and peroxidases in rice plants triggered by *Pyricularia oryzae*, *Bipolaris sorokiniana* and U.v. light. Physiol. Mol. Plant Pathol. 55:289-295.

Minella, E. 2001. Barley for beer: agronomic characteristics from cultivars in Sul region. Cevada cervejeira: características e desempenho agronômico das cultivares indicadas para a Região Sul. Passo Fundo: Embrapa Trigo. Circular Técnica Online. 4:1-8. www.cnpt.embrapa.br/biblio/p_ci04.htm.

Miyake, E. T., G. Akisue and M. K. Akisue. 1986. Pharmacognostic characterization of pata-de-vaca *Bauhinia forficata*. Rev. Brasil. Farmacog. 1:58-68

Raskin, I. 1992. Role of salicylic acid in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:438-463.

Ribnicky, D. M., V. Shulaev and I. Raskin. 1998. Intermediates of salicylic acid biosynthesis in tobacco. Plant Physiol. 118:565-572.

Sequeira, L. 1983. Mechanisms of induced resistance in plants. Annu. Rev. Microbiol. 37:51-79.

Sequeira, L. 1979. The acquisition of systemic resistance by prior inoculation. In: J. M. Daly and I. Uritani (Eds.) pp. 231-251. Recognition and Specificity in Plant Host-parasite Interactions.

Shah, B. R., M. R. Shah, D. C. Modi and D. Sahu. 2010. Phyto-pharmacological profile of *Bauhinia variegata*. Pharmacol. Online 2:829-837.

Swain, R. and W. E. Hillis. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric.10: 63-68.

Tanaka, Y., E. S. Data, S. Hirrose, T. Taniguchi, and I. Uritani. 1983. Biochemical changes in secondary metabolites in wounded and deterioted cassava roots. Agric. Biol. Chem. 47:693-700.

Teng, P. S. 1987. Crop Loss Assessment and Pest Management. APS Press, St. Paul, MN. p.58.

Victório, C. P., R. M. Kuster and C. L. S. Lage. 2009. Detection of flavonóides in *Alpinia purpurata* (Vieill) K. Schum. leaves by high-performance liquid chromatography. Braz. J. Med. Plants 11:147-153.

Emir. J. Food Agric. 2014. 26 (7): 639-642
doi: 10.9755/ejfa.v26i7.18198
http://www.ejfa.info/

SHORT COMMUNICATION

Chemical composition of essential oil of *Apis mellifera* propolis from Falcón State, Venezuela

Nurby Rios[1*], Carlos Yánez[2], Luis Rojas[3], Flor Mora[1], Alfredo Usubillaga[3] and Patricia Vit[4]

[1]*Departamento de Farmacognosia y Medicamentos Orgánicos, University of the Andes, Mérida, Venezuela*
[2]*Departamento de Toxicología y Farmacología, University of the Andes, Mérida, Venezuela*
[3]*Instituto de Investigaciones, Sección Productos Naturales, University of the Andes, Mérida, Venezuela*
[4]*Apiterapia y Bioactividad, Departamento de Ciencias de los Alimentos, Facultad de Farmacia y Bioanálisis, University of the Andes, Mérida, Venezuela*

Abstract

Apis mellifera bees obtain propolis by adding wax and salivary secretions to resinous, gummy or balsamic materials collected from various plant sources. Its chemical composition depends on many factors (type of bee, flora accessed, environment, management, season, vegetation and geographical area of collection). In the hive, the bees use propolis to consolidate structural components, varnish inside the cells and prevent vibrations. Propolis is known for its antibacterial, fungicidal, antiviral, anesthetic, antiulcer, immunostimulating, hypotensive, cytostatic, and antioxidant properties, the essential oil of *Apis mellifera* propolis, collected in Falcon state, was obtained by hydrodistillation using a Clevenger trap (0.06% yield). The oil was analyzed by gas chromatography coupled to mass spectrometry (GC / MS) in an HP GC-MS System Model 5973. Twenty-three compounds were identified (93.6%), of which the three major compounds were germacrene D (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%).

Key words: Essential oil, *Apis mellifera*, Propolis

Introduction

Propolis is a complex resinous material produced by bees from several plant exudates. *Apis mellifera* species obtain their propolis by addition of waxes, salivary secretions, or gummy and balsamic material collected from various plant species (De Albuquerque et al., 2008). The bees transport the propolis within the pollen racks, in the form of small and bright droplets. They use it as a filler, cement, or balsam to sold wall panels, close cracks and crevices in the hive to avoid the entry of foreign bodies such as insects, dust and water. It is also used to coat strange surfaces forming an antiseptic layer that prevents the formation or spread of any form of infection caused by insects or mice. Finally, propolis consolidates structural components and varnish inside the cells with disinfecting purposes and prevent vibrations (Oliveira et al., 2010; Mendizabal, 2005). This product beekeeping has a color ranging from brown to dark red depending on the plant or area origin, has a sweet smell, wet, bitter or strong, hard and brittle, insoluble in water, but soluble in alcohol, ether, ammonia, benzene-chloroform (Chaillou, 2004). Its chemical composition depends on many factors (type of bee, flora accessed, environment, management, season, vegetation and geographical area of collection). For this reason it does not have a specific chemical formula, some propolis analysis indicate that it contain mainly: resin (55%), wax (35%), oil (5%), organic material, minerals such as aluminum, cobalt, iron, nickel, calcium, silicon, zinc (5%), pollen and mechanical impurities. Their ratio thereof is variable and depends on the time of collection as well as the resin plants and bee (De Albuquerque et al., 2008; Lesser, 1987).

Since ancient times in many parts of the world, propolis has had various applications. It is used in the manufacture of cosmetics, varnishes and paints. In traditional medicine is used to improve and preserve health. Aristotle described that propolis cures skin diseases, heal wounds and fight infections effectively. Currently it is considered among the categories of natural therapy without toxicity and plays an important role in apitherapy.

Received 17 December 2013; Revised 20 March 2014; Accepted 24 March 2014; Published Online 18 May 2014

*Corresponding Author

Nurby Rios
Departamento de Farmacognosia y Medicamentos Orgánicos, University of the Andes, Mérida, Venezuela

Email: nurbyrios@ula.ve

Propolis has has analgesic, anti-inflammatory, antibacterial, fungicidal, antiviral, anesthetic, antiulcer, immunostimulants, hypotensive, cytostatic, and antioxidant activities. It is used for the treatment of various diseases such as rhinitis, tonsillitis, bronchial asthma, ulcers, eczema, antiseptic, particularly eye infections, throat, urinary tract (Lesser, 1987; Graça and Antunes, 2011; Kusumoto et al., 2001).

The chemical composition of propolis essential oil has been little studied. Mainly Brazilian propolis has been explored. De Albuquerque et al. (2008), determined the chemical composition of the essential oil produced by *Apis mellifera* propolis from Minas Gerais. They found 17 compounds (91%) of which three were the major ones (E)-nerolidol (17.1%), β - caryophyllene (13.4%), and petrolatum – 3.7 (11) - diene (10.4%). Kusumoto et al. (2001), isolated and identified nine components from propolis essential oil, seven were known and two new 2,2-dimethyl-8-prenyl-6-vinilcromeno and 2,6- diprenil -4-vinylphenol. Also Oliveira et al. (2010) studied the essential oil collected by *Apis mellifera* in Rio de Janeiro, identifying 26 compounds (67.45%). The three major found were β - caryophyllene (12.69%), acetophenone (12.26%) and linalool (6.47%). Finally, Bancova et al. (1998), analized the chemical composition of the essential oil of Brazilian propolis in different seasons and the main components found were espatulenol (3.0 to 13.9%), (2Z, 6E)-farnesol (1.6 to 14.9%) benzyl benzoate (0.3 to 18.3%) and prenylated acetophenones (3.4 to 17.1%). These common compounds only differ in quantity in the different essential oils. From the chemical point of view, in Venezuela, propolis has been explored finding only some few chemical components presented in work of Tomás-Barberán et al. (1993), Trusheva et al. (2004) and Trusheva et al. (2004). Therefore, this work focuses on determining the chemical composition of the essential oil obtained from *Apis mellifera* propolis from Paraguana Peninsula, Falcon State, Venezuela.

Materials and Methods

The collection of propolis was performed using the scraping method, with a stainless steel spatula, without much edge to avoid dragging wood shavings. The propolis was obtained from the inner surface of the hive discarding what was in the background that usually is much polluted. Propolis was collected in 2010, in the Paraguana Peninsula, Falcón State, Venezuela.

Isolation of the essential oil

Oil extraction was performed from 800 g of propolis, which were extracted by the method of hydrodistillation using a Clevenger-type apparatus for 4 hours. The oil was dried over anhydrous sodium sulphate and stored at 4ºC.

Gas chromatography

GC analyses were performed using a Perkin-Elmer AutoSystem gas chromatograph equipped with flame ionization detector and data handling system. Two capillary columns of different polarities were used: a 5% phenylmethyl polysiloxane fused-silica column (AT-5, Alltech Associates Inc., Deerfield, IL) 60 m x 0.25 mm, film thickness 0.25 μm, and a polyethylene glycol fused-silica column (AT-WAX, Alltech Associates Inc., Deerfield, IL) of the same dimensions. For the AT-5 column an oven temperature of 60°C was used. It was then heated to 260°C at 4°C/min, the final temperature was maintained for 20 min. For the AT-WAX column an initial temperature of 50ºC was used. The oven was heated to 200ºC at 3ºC/min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 ml/min. The sample was injected using a split ratio of 1:20. Retention indices were calculated relative to C8-C24 n-alkanes, and compared with values reported in the literature. The normalization method from the peak areas was used to calculate the percentage composition of the essential oil.

Gas Chromatography-Mass Spectrometry

GC-MS analyses were carried out on a Model 5973 Hewlett-Packard GC-MS system fitted with a HP-5MS fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm, Hewlett-Packard). The oven temperature program was the same as that used for the HP-5 column for GC analysis; the transfer line temperature was programmed from 150ºC to 280ºC; source temperature, 230ºC; quadrupole temperature, 150ºC; carrier gas, helium, adjusted to a linear velocity of 34 cm/s; ionization energy, 70 eV; scan range, 40:500 amu; 3.9 scans/s. Sample (1.0 μL) was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. The identity of the oil components was established from their GC retention indices, by comparison of their MS spectra with those of standard compounds available in the laboratory, and by a library search (Nist, 05) (Adams, 1995; Skoog et al., 2001).

Results and Discussion

The Venezuelan propolis hydrodestilation produced 0.5ml of yellow essential oil (0.06%

yield). The analysis by gas chromatography - mass spectrometry allowed the identification of twenty compounds (93.6%) (Table 1) of which the three major ones were D-germacrene (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%) (Figure 1). The 60.86% are non-oxygenated sesquiterpenes and 39.13% are oxygenated sesquiterpenes. We found that the most abundant oxygenated sesquiterpene was α-cadinol. It is observed that similar to Brazil propolis; Venezuelan propolis contains β-caryophyllene in representative amount β-caryophyllene in representative amount (from 10% to 13%) (De Albuquerque et al., 2008; Oliveira et al., 2010; Kusumoto et al, 2001). However it is interesting to note, that as the essential oil composition of Brazilian propolis varies depending on the collection site, the Venezuelan propolis presents differences in composition according to the flora accessed from *Apis mellifera* (De Albuquerque et al., 2008).

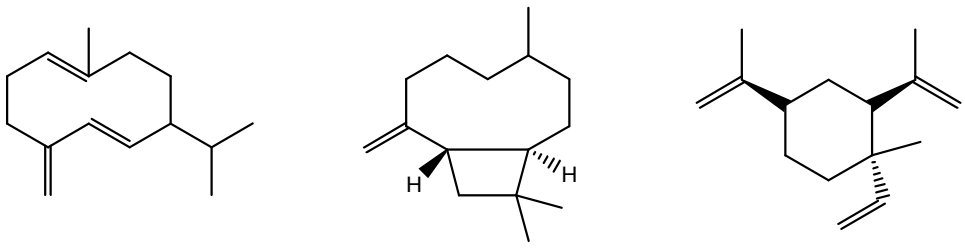

Figure 1. Major components of the essential oil of propolis

Table1. Chemical Composition of Propolis Essential Oil

N°	Compuesto	Área%	RT	Ik_{cal}	Ik_{lit}
1	α-copaene	0,8	18,451	1384	1379
2	β-bourbonene	0,8	18,738	1392	1388
3	β-elemene	8,1	18,966	1397	1389
4	β-caryophyllene	10,2	19,840	1429	1421
5	β-cubenene	1,1	20,123	1439	1440
6	α-guaiene	2,4	20,405	1450	1437
7	α-humulene	2,9	20,882	1466	1455
8	γ-muurolene	2,9	21,592	1490	1478
9	Germacrene D	26,5	21,760	1496	1479
10	β-silinene	2,2	21,891	1500	1489
11	Bicyclogermacrene	6,8	22,195	1510	1494
12	α-murolene	0,9	22,292	1514	1496
13	Germacrene A	4,4	22,461	1519	1503
14	γ-cadinene	1,2	22,702	1527	1513
15	δ-cadinene	5,0	22,980	1536	1522
16	Z-neronidol	1,5	24,137	1571	1564
17	Spathulenol	1,0	24,584	1584	1578
18	Isoaromadendrene epoxide	1,3	24,766	1590	1579
19	Viridiflorol	1,1	25,000	1595	1592
20	Guaiol	1,1	25,163	1602	1600
21	1-epi-cubenol	1,0	26,032	1639	1628
22	τ-muurolol	2,7	26,416	1655	1640
23	α-cadinol	7,8	26,775	1669	1652
	Total	93,6			

* The compounds were identified by comparing the mass spectrum of each component with Wiley library database GC / MS data and logarithmic retention index (LRI). Area% was determined by GC-FID.
RT: retention time; Ikcal: Kovats index calculated; Ik lit: Literature Kovats Index

Conclusions

This work represents the first report of the chemical composition of essential oil obtained from *Apis mellifera* propolis from Venezuela. The essential oil is mainly composed of sesquiterpenes. A total of twenty three compounds (93.6%) were identified, the three major ones in the oil, are the D-germacrene (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%). There are significant differences with the chemical composition of the essential oils obtained from propolis from Brazil.

Acknowledgements

This work was funded by the Consejo de Desarrollo Científico, Humanístico, Tecnológico y de la Artes, Universidad de los Andes, Mérida, Project: FA-480-10-08-B). We thank Dr. Cesar Rengifo who supplied *Apis mellifera* propoplis sample.

References

Adams, R. 1995. Identification of essential oil components by gas chromatography/ mass spectroscopy. Allured Publishing. Illinois. p.804.

Bancova, V., B. Krasteva, S. Popov, J. Sforcin and F. Cunha. 1998. Seasonal variations in essential oil from Brazilian Propolis. J. Essent. Oil Res. 10:603-695.

Chaillou, L., H. Herrera and J. Maidana. 2004. Estudios de propóleos de Santiago del Estero, Argentina. Ciênc Tecnología Alimentos; 24:11-15.

De Albuquerque, I., L. Alves, T. Lemos, C. Dorneles and M. De Morais. 2008. Constituents of the essentials oil of brazilian green propolis from Brazil. J. Essent. Oil Res. 20:1-2.

Graça, M. and M. Antunes. 2011. Is propolis safe as an alternative medicine? J. Pharm. BioAllied Sci. 3:479-495.

Kusumoto, T., T. Miyamoto, R. Higuchi, S. Dot, H. Sugimoto and H. Yamada. 2001. Isolation and structures of two new compounds from the essential oil of brazilian propolis. Chem. Pharm. Bull. 49:1207-1209.

Mendizabal, F. 2005. Abejas. 1ra Ed. (Argentina): Albatros SACI. p. 65.

Lesser, R. 1987. Manejo y Crianza Practica de la Abeja. Como usar los productos de las abejas en la salud del hombre. 1ra Ed. (Chile): Andrés Bello. p. 122.

Oliveira, A., H. França, R. Kuster, L. Teixeira and L. Rocha. 2010. Chemical composition and antibacterial activity of Brazilian propolis essential oil. J. Venom Anim. Toxins Incl. Trop. Dis. 16:121-130.

Tomás-Barberán, F., C. García-Viguera, P. Vit-Olivier, F. Ferreres and F. Tomás-Lorente. 1993. Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. Phytochemistry 34:191-196.

Trusheva, B., M. Popova, H. Naydenski, I. Tsvetkova, J. Rodriguez and V. Bankova. 2004. New polyisoprenylated benzophenones from Venezuelan propolis. Fitoterapia 75:683–689.

Skoog, D., J. Holler and T. Nieman. 2001. Principios de Análisis Instrumental. 5th Ed. (España): McGraw-Hill-Interamericana. P. 221.

Emir. J. Food Agric. 2014. 26 (7): 643-646
doi: 10.9755/ejfa.v26i7.18199
http://www.ejfa.info/

SHORT COMMUNICATION

Gas Chromatography-Mass Spectrometry study of the pulp of *Garcinia tinctoria* fruit

Migdalia Arazo[1*], Maivis Hernández[1], Adonis Bello[2], David Marrero[3] and Ahmed Al-Shagdari[4]

[1]*Department of Food Science, Pharmacy and Food Institute, University of Havana, Cuba*
[2]*Department of Pharmacy, Pharmacy and Food Institute, University of Havana, Cuba*
[3]*Center of Natural Products, National Center for Scientific Researchs, Cuba*
[4]*Yemen Economic Corporation (YEC0), Sanaa, Yemen*

Abstract

The aim of this work was to identify the compounds in the hexane extract of the mature fruits of *Garcinia tinctoria* by means of capillary gas chromatography-mass spectrometry (GC-MS). The study allowed identifying 51 chromatographic peaks. The mass spectra allowed the identification of 20 paraffins, 13 carboxylic acids and 15 phenolic and/or alcoholic compounds in the volatile fraction of hexane extract of pulp. The major compounds were: the stearic, palmitic and oleic acids.

Key words: Clusiaceae, *Garcinia tinctoria*, GC-MS

Introduction

The large tropical genus *Garcinia* (Clusiaceae) contains about 400 species of polygamous trees or shrubs, distributed in the tropical Asia, Africa and Polynesia (Chattopadhyay and Kumar, 2006). *Garcinia* species are characterized by the production of a yellow latex in the endocarp of the fruit, in the bark and perhaps also in the wood (Negi et al., 2008). Fruits of *Garcinia* can be widely used for many culinary purposes and as folk medicine to treat skin infections, wounds, and diarrhea (Mahabusarakam, et al., 1987; Joseph et al., 2005).

Garcinia is well known as a rich source of bioactive compounds such as xanthones (Komguem et al., 2005; Ali et al., 2005; Quan-Bin et al., 2008), benzophenones (Harrison et al., 2005; Pereira et al., 2010) and biflavonoids (Deachathai et al., 2005; Okunji et al., 2007). Secondary metabolites of *Garcinia* species have shown antioxidant, antibacterial and antitumoral activities (Xing-Cong et al., 2004; Verdi et al., 2004; Rui-Min et al., 2009; Jawed et al., 2010).

Received 26 September 2013; Revised 10 January 2014; Accepted 17 January 2014; Published Online 18 May 2014

*Corresponding Author

Migdalia Arazo
Department of Food Science, Pharmacy and Food Institute, University of Havana, Cuba

Emails: migdister@gmail.com; migdi@ifal.uh.cu

G. tinctoria (Wight) is an important under-utilized crop distributed in the lower hills of Eastern Himalayas, Western Ghats and Andaman Islands (Rema and Krishnamoorthy, 2000). *G. tinctoria* is well known as yellow mangosteen and its fruits are highly acidic and are used to flavour curries. The dried fruits and leaves have been used widely as a traditional folk medicine for bilious condition, diarrhea, and dysentery (Pedraza-Chaverri et al., 2008).

There is little information about the chemical composition of *G. tinctoria* (yellow mangosteen) fruits. In this work the compounds from the hexane extract of the mature fruits of *Garcinia tinctoria* were identified using GC-MS.

Materials and Methods

Plant material

Fresh mature fruits of *Garcinia tinctoria* were collected in the Jardín Botánico Nacional (Habana, Cuba) in February-April 2012. A voucher specimen has been deposited at HAJB Herbarium (Havana, Cuba) under number 700.

Sample preparation and extraction

Fruits were washed and peeled in order to separate the peels from pulps and seeds. Pulp was ground into a paste using a mortar and pestle and it was preserved in refrigeration (4°C) until the moment of utilization. In order to evaluate chemical composition of the apolar extract of *Garcinia tinctoria* fruits pulp, it was prepared maceration (1g of pulp) with enough hexane during 7 days at room

temperature. Sample was filtered using Whatmann No. 4 filter paper and concentrated under reduced pressure at 45°C using a rotary vacuum evaporator. The dried extract was weighed and stored until the analysis moment.

Determination of the components from the hexane extract

It was weighed 10 mg of the extract and was dissolved in 0.5 mL of internal standard solution (tridecanoic acid at 0.05 mg/mL in chloroform, Sigma, USA). The solution was derivatized with 100 µL of N metil N trimetilsililtrifluoroacetamide (MSTFA, Sigma, USA) to 70°C during 30 min in a dry thermostat Multiblock and flow control (LabLine Instruments Inc., EE UU).

Sample was separated on a HP-5 Ms 30 m x 0.25 mm installed on a gas chromatograph 6890N with a mass detector 5975 (Agilent, EE UU).

The temperature was programmed as follows: isothermic initial 60°C for 2 min, then temperature was increased up to 200°C at 20°C/min, of 200°C up to 320 at 8°C/min, and isothermic final at 320 for 30 min. The carrier gas was helium at a flow rate of 1.0 mL/min. Injector temperature was 320°C. The energy of ionization was of 70 eV. Mass spectra were obtained in mode scan since 40 to 800 m/z. The volume of injection was of 0.5 µL.

Identification was made by comparison of retention times (tr) and spectra from available commercial standards and similar compound spectra at the libraries Wiley MS, 6ª ed. and NIST 11.The quantification was made using internal standard.

Statistical analysis

Three independent analyses were done on five samples. Statistical analysis was performed by Mann - Whitney U Test.

Results and Discussion

Identification of the constituents from the apolar extract of pulp

The analysis of the apolar extract of *G. tinctoria* allowed identifying fifty one compounds. The compounds, their percentages and retention times are listed in Table 1. Three compounds were identified as the major constituents: stearic acid (21.72%), palmitic acid (20.31%) and oleic acid (19.32%) and these represented approximately 61% of all the compounds of the fraction.

Table 1. Constituents of *Garcinia tinctoria* pulp.

Components *	RT	%±SD
Lactic acid	7.382	1.702 ± 0.081
Hexanoic acid	7.688	0.273 ± 0.010
Citronellal	8.274	0.984 ± 0.008
2-Hydroxyphenol	8.731	0.979 ± 0.009
2-Hydroxyphenol (isomer)	8.776	0.237 ± 0.002
Benzoic acid	9.091	0.295 ± 0.004
Catechol	9.669	0.327 ± 0.007
etil 2-butiletoxy	9.766	1.997± 0.092
Nonanoic acid	9.905	0.162 ± 0.005
Capric acid	10.561	0.237 ± 0.005
3-Hydroxy-benzoic acid	11.291	0.216 ± 0.006
Hexadecane	11.452	0.119 ± 0.010
Lauric acid	11,806	0.842 ± 0.013
Heptadecane	12,115	0.228 ± 0.016
Octadecane	12,820	0.259 ± 0.012
3,4-dihydroxy-benzoic acid	13,077	3.757 ± 0.162
Myristic acid	13.197	1.755 ± 0.113
Nonadecane	13,577	0.272 ± 0.008
Pentadecanoic acid	13,972	0.553 ± 0.009
1-hexadecanol	14,071	0.662 ± 0.010
Eicosane	14,390	0.332 ± 0.007
Palmitoleic acid	14,601	0.925 ± 0.015
Palmitoleic acid (isomer)	14,726	1.994 ± 0.018
Palmitic acid	14,807	20.314 ± 0.042
Henicosane	15,250	0.392 ± 0.008
Margaric acid	15,662	0.443 ± 0.007
1-octadecanol	15,764	0.887 ± 0.015
Docosane	16,139	0.678 ± 0.010
Linoleic acid	16,295	0.629 ± 0.009
Oleic acid	16,341	19.320 ± 0.032
Oleic acid (isomer)	16,489	3.779 ± 0.015
Stearic acid	16,565	21.716 ± 0.060
Tricosane	17,044	0.457 ± 0.008
Tetracosane	17,955	0.627 ± 0.012
Eicosanoic acid	18,359	0.660 ± 0.018
Pentacosane	18,861	0.645 ± 0.021
Hexacosane	19,756	1.054± 0.054
Docosanoic acid	20,127	0.420 ± 0.008
Heptacosane	20,633	0.637 ± 0.023
NI	21,462	0.597 ± 0.019
Octacosane	21.475	0.613 ± 0.022
Squalene	21,802	1.032 ± 0.061
Nonacosane	22,326	0.620 ± 0.030
Triacontane	23,136	0.403 ± 0.010
NI	23,197	1.797 ± 0.058
Henatriacontane	23,929	0.459 ± 0.009
1-Octacosanol	24,276	0.769 ± 0.018
Sterol (cholesterol)	24,585	0.530 ± 0.012
Tetratriacontane	25,445	0.258 ± 0.009
1-Triacontanol	25.760	0.438 ± 0.011
Sterol (NI)	26,540	0.722 ± 0.060

*Components are listed in order of elution on HP-5 (30 m) column, NI: not identified [a] FID area percents were corrected to wt % according to total weight. Data are the means + SD of five experiments performed in triplicate.

Ajayi and Adesanwo (2009) reported that the principal fatty acids of pulp and seed of *Dacryodes edulis* were oleic and palmitic acids. Oleic acid is the most widely distributed fatty acid on nature and it's the principal responsible of health benefits of the Mediterranean diet. Some investigations have demonstrated that oleic acid can reduce the risk to suffer breast cancer and other diseases (Win, 2005).

Other compounds detected were oleic acid isomer (3.78%); 3,4-dihydroxibenzoic acid (3.76%); eter 2-butoxy etinil (1.99%); palmitoleic acid isomer (1.99%); miristic acid (1.75%), lactic acid (1.70%) and unidentified sterol (0.72%) by the data base.

The abundance of fatty acids could contribute to acidity that characterizes the fruits of *Garcinia* species, particularly *Garcinia tinctoria* (Cavalcante et al., 2006; Rittirut and Siripatana, 2006). In a chemical study of volatile constituents of *Garcinia dulcis* fruits using gas chromatography (Pino et al., 2003), it was reported a higher amounts of fatty acids that could be responsible for the acidic and pungent notes observed in fruit.

In general, it's was observed that most of compounds are derived of paraffins with different degrees of oxidation. The composition of the classes of compounds was as follows: organic acids (22%); phenols (15%); alcohols (11%) and others (13%). The presence of phenols corresponds with the antioxidant activity detected in *G. tinctoria* using techniques like DPPH y FRAP (Arazo et al., 2012). Numerous investigations have determined the ability of the extracts from fruits of *Garcinia* species to capture free radicals (Yu et al., 2007; Okonogi et al., 2007; Sulaiman and Udaya, 2009).

Very few studies on the composition of volatile compounds in *Garcinia* genus have been accomplished (Pino et al., 2003). However, there are no data in the literature concerning the possible pharmacological effects and the chemical constituents of this plant.

Acknowledgements

The authors are grateful to the National Botanical Garden for kindly providing the plant material.

This work was financially supported by University of Havana and the Center of Natural Products.

References

Ajayi, I. A. and O. Adesanwo. 2009. Comparative study of the mineral element and fatty acid composition of *Dacryodes edulis* pulp and seed. World J. Agric. Sci. 5(3):279-283.

Ali, D. M. H., K. C. Wong and P. K. Lim. 2005. Flavonoids from *Blumea balsamífera*. Fitoterapia 76(1):128-130.

Arazo, M., A. Bello, L. Rastrelli, M. Montelier, L. Delgado and C. Panfet. 2011. Antioxidant properties of pulp and peel of yellow mangosteen fruits. Emir. J. Food Agric. 23(6):517-524.

Cavalcante, Í. H. L., N. D. Jesus and A. B. G. Martins. 2006. Physical and chemical characterization of yellow mangosteen fruits. Rev. Brasil. Fruticult. 28(2):325-327.

Chattopadhyay, K. and K. Satyanshu. 2006. Identification and quantification of two biologically active polyisoprenylated benzophenones, xanthochymol and isoxanthochymol in *Garcinia* species using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B. 844(1):67-83.

Deachathai, S., W. C. Taylor, W. Mahabusarakam and S. Phongpaichit. 2005. Phenolic compounds from the fruit of *Garcinia dulcis*. Phytochemistry 66(1):2368-2375.

Jawed, A. S., G. Swarnkar, K. Sharan, B. Chakravarti, G. Sharma, T. P. Rawa, M. Kumar, F. M. Khan, D. Pierroz, R. Maurya and N. Chattopadhyay. 2010. 8,8″-Biapigeninyl stimulates osteoblast functions and inhibits osteoclast and adipocyte functions: Osteoprotective action of 8,8″-biapigeninyl in ovariectomized mice. Article Mol. Cell. Endocrinol. 323(2):256-267.

Joseph, G. S., G. K. Jayaprakasha, A. T. Selvi, B. S. Jena and K. K. Sakariah. 2005. Antiaflatoxigenic and antioxidant activities of *Garcinia* extracts. Internat. J. Food Microbiol. 101(1):153-160.

Komguem, J., A. L. Meli, R. N. Manfouo, D. Lontsi and F. N. Ngounou. 2005. Xanthones from *Garcinia smeathmannii* (Oliver) and their antimicrobial activity. Phytochemistry 66(1):1713-1717.

Mahabusarakam, W., P. Iriyachitra and W. C. Taylor. 1987. Chemical constituents of *Garcinia mangostana*. J. Nat. Prod. 50:474-478.

Negi, P. S., G. K. Jayaprakasha and B. S. Jena. 2008. Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne

pathogens and spoilage bacteria. Food Sci. Technol. 41(1):1857-1861.

Harrison, L. J. 2002. Xanthones from the heartwood of *Garcinia mangostana*. Phytochemistry 60(5):541-548.

Okonogi, S., C. Duangrat, S. Anuchpreeda, S. Tachakittirungrod and S. Chowwanapoonpohn. 2007. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chem. 103:839–846.

Okunji, C., S. Komarnytsky, G. Fear, A. Poulev, D. M. Ribnicky, P. I. Awachie, Y. Ito and I. Raskin. 2007. Preparative isolation and identification of tyrosinase inhibitors from the seeds of *Garcinia kola* by high-speed counter-current chromatography. J. Chromatogr. A. 1151(1):45–50.

Pedraza-Chaverri, J., N. Cárdenas-Rodríguez, M. Orozco-Ibarra and J. M. Pérez-Rojas. 2008. Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem. Toxicol. 46:3227-3239.

Pereira, I., M. Marque, A. Pavan, B. Codonho, C. Barbiéri, L. Beijo, A. Doriguetto, E. Martin and M. Dos Santos. 2010. Leishmanicidal activity of benzophenones and extracts from *Garcinia brasiliensis* Mart. fruits. Phytomedicine 17(1):339-345.

Pino, J., R. Marbot, A. Rosado and C. Vázquez. 2003. Volatile constituents of fruits of *Garcinia dulcis* Kurz. from Cuba. Flavor Fragr. J. 18:271-274.

Quan-Bin, H., Y. Nian-Yun and T. Hong-Lei. 2008. Xanthones with growth inhibition against HeLa cells from *Garcinia xipshuanbannaensis*. Phytochem. 69(1):2187-2192.

Rema, J. and B. Krishnamoorthy. 2000. *Garcinia species* of economic importance – Distribution and uses. Indian Spices 37(1):20–23.

Rittirut, W. and C. Siripatana. 2006. Drying characteristics of *Garcinia atroviridis.* Walailak J. Sci. Tech. 3(1):13-32.

Rui-Min, H., T. Yu-Xi, L. Yin, C. Chang-Hui, A. Xi-Cheng, Z. Jian-Ping and L. H. Skibsted. 2009. Comparison of flavonoids and isoflavonoids as antioxidants. J. Agric. Food Chem. 57(9):3780-3785.

Sulaiman, A. and K. Udaya. 2009. A study of antioxidant properties from *Garcinia mangostana* L. pericarp extract. Acta Sci. Pol. Technol. Aliment. 8(1):23-34.

Win, D. T. 2005. Oleic acid-The anti-breast cancer component in olive oil. AU J. Technol. 9:75-78.

Verdi, L. G., M. G. Pizzolatti and A. B. Montanher. 2004. Antibacterial and brine shrimp lethality tests of biflavonoids and derivative of *Rheedia gardneriana*. Fitoterapia 75(1):360-363.

Xing-Cong, L., S. I. Khan, M. Jacob, L. A. Walker and D. Ferreira. 2004. Absolute configuration and biological activity of biflavonoids from *Rheedia acuminate*. Abstracts of Papers, 228th ACS National Meeting, Philadelphia, PA, United States, August 22-26.

Yu, L., M. Zhao, B. Yang, Q. Zhao and Y. Jiang. 2007. Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities. Food Chem. 104:176–181.

Emir. J. Food Agric. 2014. 26 (7): 647-651
doi: 10.9755/ejfa.v26i7.18200
http://www.ejfa.info/

SHORT COMMUNICATION

Ethnobotanical records of not yet documented therapeutic effects of some popular Bulgarian medicinal plants

Ekaterina Kozuharova[1*], Niko Benbassat[1] and Ilko Getov[2]

[1]*Department of Pharmacognosy and Botany, Faculty of Pharmacy, Medical University, Sofia, Bulgaria*
[2]*Department of Social pharmacy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria*

Abstract

Despite the existence of many relevant publications, and of reliable records of the traditional empiric data about medicinal plants in Bulgaria, over the last few years we found quite a lot of hitherto undocumented data about the healing properties of plants. In order to test the contemporary status of the traditional knowledge about medicinal plants, we performed the study as a Rapid Ethnobotanical Appraisal in the way of structured interview based on fixed questions namely what plants are used against certain health disorders. We therefore interviewed 183 people during the summer of 2011: some whom we knew to be particularly interested in medicinal plants and traditional ways of healing, and also a random sample of people of different ages. These subjects were all over l8. The survey was called "Granny had a cure for this." (or "Granny knew how to cure this.") In this paper we present data for remedial properties of popular medicinal plants which have not been documented in the literature yet - *Pulsatilla pratensis* - fresh leaves used as compress against exostoses, *Sambucus ebulus* - immature fresh leaves used for compress in case of exostoses, *Primula veris* - leaves infusion used against vertigo and *Nepeta nuda* - application of decoction internally against cystitis and prostate gland inflammation as well as externally against wounds and on the stock udder against mastitis. Comparative analysis of relevant studies was also done.

Key words: Medicinal plants, *Pulsatilla pratensis, Sambucus ebulus, Primula veris, Nepeta nuda,* Remedial properties, Bulgaria

Introduction

Etnobotanical data are very important base for further pharmacological tests. For example the results of pharmacological tests are leading to the hypothesis that *Lonicera tatarica* and *Viburnum opulus* species could be used for the development of novel antimicrobial products or strategies for fighting medical biofilms (Bubulica et al., 2012). The background for these tests is again some data about traditional use of the plants. In the huge pool of traditional data sometimes appears information about therapeutic effect that is not quite popular. For instance pharmacological tests inspired by ethno botanical knowledge revealed that leaf extracts of *Melissa officinalis* can improve cognitive performance and mood and may therefore be a valuable adjunct in the treatment of Alzheimer's disease. This plant species that is widely used as a mild sedative and sleep aid has been traditionally attributed with memory-enhancing properties (Kennedy et al., 2003).

In the contemporary reality of globalization and urbanization in Bulgaria, traditional empiric ethnobotanical knowledge is disappearing. Despite that discouraging tendency, we have found several examples of traditional empiric data which had not been documented and so we were inspired to see what more had been overlooked.

As a whole, the traditional knowledge of medicinal plants and their uses, preserved and transmitted from generation to generation, is quite well documented in Bulgaria. The collecting of common names was pioneered by teachers, university professors, naturalists, folklorists and physicians during 19th and 20th century. These pioneers recorded their use for conventional remedial purposes and also their use in traditional spells and magical rituals (Stanev, 2010). This collection of ethnobotanical data led to the

Received 26 July 2013; Revised 13 January 2014; Accepted 22 January 2014; Published Online 18 May 2014

*Corresponding Author

Ekaterina Kozuharova
Department of Pharmacognosy and Botany, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Email: ina_kozuharova@yahoo.co.uk

publication of valuable scientific works (Petkov, 1982; Pamukov, 1992; Nikolov, 2006) etc. The traditional knowledge documentation bases the list of 741 taxa recognized in Bulgaria by the law as medicinal plants, although the therapeutic effect and application is not specified (Medicinal plant act, 2000). The formulas of the famous Bulgarian healer, Peter Dimkov, are well known for their efficacy, as is accepted by the vast majority of Bulgarians. His books have gone into numerous editions, the last one after his death (Dimkov, 2001). The last decades ethno botanical research was performed both by national and foreign scientists (Ivancheva and Stantcheva, 2000; Leporatti and Ivancheva, 2003; Nedelcheva, 2009; Nedelcheva and Dogan, 2009).

The development of new drugs has often taken place as the result of clues provided by local and traditional knowledge of the medicinal or toxic effects of vascular plants, algae, fungi etc. This traditional expertise is not always acknowledged, but many scientists have seen this to be one of the driving forces of ethnobotanical research (Giovannini and Heinrich, 2009; Heinrich, 2010).

In this paper we focus on the data for remedial properties which we found apparently new or as yet not documented in the literature for generally acknowledged medicinal plants. We aimed to document this empiric information on the traditional use of plants 1) and thus to preserve the knowledge that has still not been recorded in the literature and 2) also to provide opportunities for further study of chemical constituents and/or biological activities. Comparative analysis of relevant studies dealing with same health disorders or same plants is done.

Material and Methods

In order to test the contemporary status of the traditional knowledge about medicinal plants we performed the study as a Rapid Ethnobotanical Appraisal in the way of structured interview based on fixed questions namely what plants are used against certain health disorders (Gerique, 2006).

During the summer of 2011, 183 interviews were carried out. The interviews were performed in towns and villages from several districts of Bulgaria (Sofia, Ichtiman, Vidin, Plovdiv, Dobrinishte, Pleven, Kavarna, Tzarevo). As a result of urbanization strict topographic localization of the knowledge is impossible. Even the data obtained from town inhabitants originate from different villages localized nearby or rather distant ones. Target groups were both few people whom we knew to be particularly interested in medicinal plants and traditional ways of healing and also the majority was a random sample of people – male and female of different social status and different ages, above 18 and the oldest informant was at the age of 92. Basically we aimed to test the attitude of a random sample of people to the herbs and the traditional way of healing: 1) to find out what herbs were most popular among the population of Bulgaria; 2) what was most popular application; 3) to test how negative or positive attitude to the traditional use of medicinal plants was related to age and gender of people.

Data analysis

Ethnobotanical data were analyzed and summarized by using Microsoft excel and statistics to determine relative frequencies of citations so as to identify the most common and popularly used plants in the study area. The detailed results of the analysis are published elsewhere (Kozuharova et al, 2013). Here we summarize and emphasize some of our new findings concerning the therapeutic effect that are not documented yet in the scientific literature dealing with Bulgarian official or folk medicine application of plants. In this paper we focus on data that are preserved by transmitting verbally from generation to generation and we did not find records for this in the scientific publications.

Our research team consisted of lecturers in pharmaceutical botany and pharmacognosy and undergraduate students. We devised a questionnaire listing the main groups of medicinal problems: problems with vision, inflammation of the eyes, inflammation of the ears, skin inflammations, rashes, warts, joint pains, rheumatism, sciatica, exostoses, failures of the immune system, colds, bronchitis or other problems of the, respiratory tract, contraception, miscarriage, breast feeding, mastitis, colic, bedwetting, blood disease, hypertonia, heart disease, *g*astrointestinal tractdiarrhea, constipation, kidneys, problems of the urinary tract, cystitis, menstrual disorders, prostatitis, treatment of trauma/wounds, memory loss and insomnia. In order to trigger the informants and obtain as much as possible information without boring and repelling them**,** we tried to balance between not enough detailed and too heavy list. The key point concerned the origins of the information – the source of knowledge had to be that it had been transferred from generation to generation in the traditional way, not learned from books. In other words, our motto was “Granny’s cure for this was ….”.

Ideally, we to obtained a sample of the plant in question, or 'voucher material', but most often we were given a description which would go with the common name. From all listed in this paper plant species we obtained voucher materials of only *Pulsatilla vulgaris* and *Nepeta nuda* (blend). They are deposited in the Herbarium of the Faculty of Pharmacy. The voucher materials were identified after Jordanov (Jordanov, 1963-1995) (namely *Pulsatilla vulgaris*) or by comparison to reference material in the herbarium (registered herbaria of University of Sofia and Institute of Botany, SO, SOM namely *Nepeta nuda*). Data are grouped according to the therapeutic effect in the Results and Discussion part of this paper. We include a comparative analysis of the published literature concerning data for the content of secondary compounds and also an analysis of the popular use of these compounds as well as a summary of other plants recommended for such disorders in folk medicine.

Results and Discussion

Totally 77 plant species (including the ones in the formula combinations) were mentioned during the investigation. They belong to 38 families. The most important families are Lamiaceae, Rosaceae, Asteraceae and most popular plants as "Granny's cure" were *Hypericum perforatum*, *Cotinus coggigria*, *Plantago major*, *Sempervivum* sp. div., *Calendula officinalis*, *Melissa officinalis*, *Allium sativum*, *Aesculus hippocastanum*, *Matricaria chamomilla* and *Cornus mass.* The great number of herbs is used to treat disorders of CNS (Central Nervous System), bones, skin, gastro-intestinal and respiratory system – details are provided in other paper of ours (Kozuharova et al., 2013). In this paper we emphasize on data for remedial properties which we found apparently new or as yet not documented in the literature for generally acknowledged medicinal plants.

Most of the new data that we found were connected to healing of exostoses and problems related to Central Nerve System (CNS) in one or another form. Each new fact presented in this paper was reported once (0.6% of all reports). In other words these are not very popular folk medicine applications.

Exostoses healing

Pulsatilla vulgaris Mill. Ranunculaceae (Figure 1). Voucher material was provided and reported that fresh leaves are used as compress against exostoses. Warning was that in case of prolonged application "muscles could be melted" was given too. This is quite unusual use which we found to be new. The plant is known as sedative and anaphrodisiac (Petkov, 1982). It is prescribed as central-acting analgesic (Yarnell, 2002). In homeopathy Pulsatilla 6X is used against migraine disorders, vertigo, neuralgic pain, venous stasis (Gottwald and Weiser, 2000).

Figure 1. Voucher material of *Pulsatilla vulgaris* Mill. presented by the informant.

Sambucus ebulus L. Adoxaceae (Caprifoliaceae). Immature fresh leaves are used for compress in case of exostoses. It is popular herb recognized by Medicinal plant act (Medicinal plant act, 2000). It is known mainly for diuretic, antiseptic and expectorant therapeutic effects and additionally it is used as laxative, anti-hemorrhoidal, anti-tuberculosis, against neuralgia and rheumatism. Root flowers/inflorescences and fruits are mainly used. Additionally leaves in folk medicine are used as decoction against intestinal colic (Petkov, 1982).

Central nerve system healing

Primula veris L. (*P. officinalis* (L.) Hill.) Primulaceae. Folium infusion was found to be used against vertigo and this information we did not find documented in the literature. *P. veris* is rather popular herb recognized by Medicinal plant act (Medicinal plant act, 2000). It is known mainly as expectorant. Additionally empiric data are available

for neurosis and insomnia cure (Petkov, 1982; Nikolov, 2006). The only mentioning of this plant against vertigo is not related to herbal medicine but to homeopathy (Anonimous, 2011).

Other therapeutic effects

Nepeta nuda L. Lamiaceae – we were given aerial parts plant substance as a voucher material of this plant species (Figure 2). The informant reported application of decoction internally against cystitis prostate gland inflammation. Externally decoction was applied against wounds and on the stock udder against mastitis. So far in Bulgarian literature is listed *Nepeta cataria* L. It is recognized by Medicinal plant act (Medicinal plant act, 2000).This plant species is popular for its antimicrobial, spasmolytic and calming effect (Nikolov, 2006) and it is considered to be especially effective against sepsis (Petkov, 1982). Essential oil of *N. nuda* was found to show weak antioxidant activities and the results suggested that *N. nuda* essential oil could be a natural antibacterial agent (Alim et al., 2009). Another research revealed that Antioxidant activity of *Nepeta nuda* L. ssp. *nuda* is proven - the essential oil had significant effects on lipid peroxidation (Gkinis et al., 2010).

Figure 1. Voucher material of *Nepeta nuda* L. presented by the informant.

Conclusion

The ethnobotanical data should always be treated with caution when it concerns medicinal properties of plants and further pharmacological tests are required in order to accept officially the application. Sometimes plants used traditionally in folk medicine are officially rejected due to some toxicity or negative side effects. At the same time ethnobotanical data are valuable source of basic information. We consider that some of the unrecorded knowledge that we found as a result of our study deserves further research, especially the therapeutic effects of *Pulsatilla vulgaris* and *Primula veris*. Such effect on CNS reported for *Primula veris* is quite unusual for this plant well known for its expectorant properties.

Acknowledgements

The study was conducted with the financial support of project 5/2011, (contract 32/2011), Medical University Sofia, Council of Medical Sciences. We are grateful to the undergraduate students M. Tzvetkova, N. Tzenova, D. Damjanova and D. Malchev for their assistance in the performance of the interviews. Special thanks to Prof. S. Ivancheva and Prof. A. Nedelcheva for their interest to our work and useful comments.

References

Alim, A., I. Goze, A. Cetin, A. D. Atas, S. A. Cetinus and N. Vural. 2009. Chemical composition and in vitro antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp. albiflora (Boiss.) Gams. Afr. J. Microbiol. Res. 3:463-467.

Anonimous 2011. Homeopathic Materia Medica Primula veris (prim-v) http://www.vithoulkas.com/en/books-study/online-materia-medica/3746.html 15.11.2013

Bubulica, M. V., I. Anghel, A. M. Grumezescu, C. Saviuc, G. A. Anghel, M. C. Chifiriuc, I. Gheorghe, V. Lazar and A. Popescu. 2012. In vitro evaluation of bactericidal and antibiofilm activity of Lonicera tatarica and Viburnum opulus plant extracts on Staphylococcus strains, Farmacia, 60:80-91.

Dimkov, P. 2001. Natural healing methods and living with nature; Bulgarian traditional medicine. 1-3. Astrala (Bulgarian language)

Gerique, A. 2006.An introduction to ethnoecology and ethnobotany: Theory and Methods. Integrative assessment and planning methods for sustainable agroforestry in humid and semiarid regions. Advanced Scientific Training – Loja, Ecuador September

Giovannini, P. and M. Heinrich. 2009. Xki yoma' (our medicine) and xki tienda (patent

medicine)—Interface between traditional and modern medicine among the Mazatecs of Oaxaca, Mexico. J. Ethnopharmacol. 121:383-199.

Gkinis, G., B. Bozin, N. Mimica-Dukic and O. Tzakou. 2010. Antioxidant activity of Nepeta nuda L. ssp. nuda essential oil rich in nepetalactones from Greece. J. Med. Food 13:1176-1181.

Gottwald, R. and M. Weiser. 2000. Treatment of rheumatic diseases with a homeopathic preparation. Biomed. Therap. 18:281-285.

Heinrich, M., 2010. Galanthamine from Galanthus and Other Amaryllidaceae - Chemistry and Biology Based on Traditional Use. In: Geoffrey A. Cordell, editors, The Alkaloids. Chennai: Academic Press, Volume 68 Elsevier Inc., 157-165.

Ivancheva, S. and B. Stantcheva. 2000. Ethnobotanical inventory of medicinal plants in Bulgaria, J. Ethnopharmacol. 69:165-172.

Jordanov, D. (Ed.) 1963-1995. Fl. Reipubl. Popularis Bulgaricae, In Aedibus Acad. Sci. Bulgaricae, Serdicae 1-9. (Bulgarian language)

Kennedy, D. O., G. Wake, S. Savelev, N. T. J. Tildesley, E. K. Perry, K. A. Wesnes and A. B. Scholey. 2003. Modulation of mood and cognitive performance following acute administration of single doses of *Melissa officinalis* (Lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. Neuropsychopharmacol. 28:1871–1881.

Kozuharova, E., H. Lebanova, I. Getov, N. Benbassat and J. Napier. 2013. Descriptive study of contemporary status of the traditional knowledge on medicinal plants in Bulgaria Afr. J. Pharm. Pharmacol. 7(5):185-198

Leporatti, M. and S. 2003. Ivancheva. J. Ethnopharmacol. 87:123-142.

Medicinal Plants Act (Promulgated, SG No. 29/7.04.2000, Amended, SG No. 23/1.03.2002, 91/25.09.2002). http://lex.bg/laws/ldoc/2134916096

Nedelcheva, A. M. 2009. Plants related to the life and medicinal practice of St. Ivan Rilski, In: J-P. Morel and A. Mercuri (Eds.), pp. 175-178. Plants and Culture: seeds of the cultural heritage of Europe, Bari, Edipuglia.

Nedelcheva, A. and Y. Dogan. Folk botanical nomenclature and classification in Bulgarian traditional knowledge. In: Morel J-P., A. Mercuri (eds), Plants and Culture: seeds of the cultural heritage of Europe, 2009, Bari, Edipuglia, 169-173.

Nikolov, S. (ed.) 2006. Specialized Encyclopedia of medicinal plants in Bulgaria. “Bulgarian encyclopedia” – Bulgarian Academy of Sciences, Faculty of Pharmacy MU, Publishing House “Trud”, Sofia, (Bulgarian language)

Pamukov, D. 1992. Home pharmacy. Sofia, (Bulgarian language)

Petkov, V. 1982. Contemporary phytotherapy. Medicine and physculture, Sofia, (Bulgarian language)

Stanev, S. 2010. History of the Bulgarian Botany. Tafprint, Plovdiv, (Bulgarian language)

Yarnell, E. 2002. Phytotherapy for theTreatment of Pain. Modern Phytother. 7:1-12.

EMIRATES JOURNAL OF
FOOD AND AGRICULTURE

GUIDELINES FOR AUTHORS

The Emirates Journal of Food and Agriculture [EJFA] is a peer-reviewed international journal publishing basic and applied research articles in the field of Food and Agricultural Sciences by the College of Food and Agriculture, United Arab Emirates University, United Arab Emirates. The journal also publishes invited reviews relevant to current Agricultural, Food and Nutrition Science topics. The Journal's full-text articles are published both in print and online, accessible electronically via the College page http://cfa.uaeu.ac.ae/ as well as through the journal website at http://www.ejfa.info/ EJFA is publishing **12 issues** every year (monthly). Issues will appear online prior to printing with DOI numbers as *e-Preview* issues.

Manuscript submission

Before submission, please ensure that the manuscript is in accordance with the EJFA Policies given in the journal's website: http://ejfa.info/index.php/ejfa/pages/view/journal_policies. Manuscripts should be submitted to the Editorial office of EJFA through the advanced Online Submission, Peer Review and Tracking System available at http://www.ejfa.info/

Journal language

Only English language articles submitted for publication will be considered.

Peer Review

The submitted papers will pass through initial screening in the editorial office followed by internal review by Internal Board of Reviewing Editors. Then the paper will be checked through the Advanced Plagiarism Detection Software (CrossCheck® powered by iThenticate®). After this, the paper will be assigned to Senior Editor for conducting the External Peer Review by two reviewers. After revision, again the paper will undergo CrossCheck® screening.

Manuscript preparation

Manuscripts should be typed double-spaced and with lines numbered. A font size of 12 points (Times New Roman) text is preferred. Manuscripts should be written in sound, clear and concise language. Page numbers should be located on the bottom-center of every page of the manuscript including the title page, references, tables etc. The title should be centered at the top of page 1 of the manuscript. Capitalize only the first letter of the first word and any proper nouns or abbreviations that require capitalization. The title should not be more than 100 characters followed by the author(s) name(s), affiliation(s), and address(es) and additional contact information (e-mail). For authors' names use only initials for all but the family or last name. Indication of other professional titles should not be used.

Structure of manuscripts

A detailed guide to authors is given in the journal web page.

Abstracts

Abstracts should be concise, clear and include an objective description of the contents and the major significant findings of the article. Abstract should not exceed 250 words in one paragraph.

Key words

At the end of abstract list, in alphabetical order, up to five key words that best describe the research.

Introduction

Should reflect a brief background of the research and the purpose of its initiation. Extensive discussion of relevant literature should be included in the discussion of results, not in the introduction.

Materials and Methods

A clear description or specific original reference is required for all procedures including statistical procedures.

Results and Discussion

These can either be pooled together or presented under separate sections.

Conclusions

The conclusion should not be more than 1000 characters plus spaces in one paragraph.

Acknowledgement(s)

If necessary.

References
References should be arranged alphabetically by author's last name then chronologically per author. Publications by the same author(s) in the same year should be listed by year followed by the letters a. b. c. etc. (e.g. 2002a, 2002b, 2002c).

Examples of reference listing format

Journal articles
Jenkins, T. G., C. L. Ferrell and A. Roberts. 2000. Lactation and calf weight traits of mature crossbred cows fed varying daily levels of metabolizable energy. J. Anim. Sci. 78:7-14.

Proceedings
Johnson, E. H., D. Muirhead, R. Al-Busaidy and B. E. Musa. 1998. The ultrastructure of the camel eosinophil. In: proceedings of the third annual meeting for animal production under arid conditions 'the international conference on camel production and future perspectives'. Publisher UAE University, United Arab Emirates. P 88-95.

Abstracts from conferences and meetings
Hymadan, H. S. 1983. Impact of seedborne pathogens on international movements of seeds. Phytopathology. 73:784. (Abstr.).

Books and chapters within edited books
AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

O'toole, J. C. and T. T. Chang. 1979. Drought resistance in cereals: Rice-a case study, In: H. Mussel and R. C. Stafle (Eds.), pp. 373-406. Stress Physiology of Crop Plants. Wiley-Interscience. N.Y.

Handbooks, Technical bulletins and Dissertation
Goering, H. K., and P. J Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379. ARS, USDA, Washington, DC.

Nouri, L. K. and A. R. Hassan. 1973. Studies on soil fertility and fertilizers in Iraq. Tech. Bull. No. 43. Ministry of higher education and scientific research. Baghdad. Iraq.

Alhadrami, G. A. 1991. Effect of preservatives and maturity on the nutritional value of alfalfa hay for lactating dairy cows. Ph.D. dissertation, University of Arizona, Tucson.

Tables
Should be organized similar to the text, so that the table can be read without turning the page sideways (if possible). Large tables should be avoided. They should be typed on separate pages and numbered sequentially according to the order of their respective appearance in the text. Each table should have a brief and self-explanatory title. Column headings should include the International Standard abbreviations(s) of their respective units of measurement included between parentheses. Footnotes should be indicated by superscript letters starting with (a) in each table and kept as short as possible.

Illustrations
Drawings and photographs should be numbered sequentially according to their respective order of citation in the text. Explanations should be given in the typewritten legend, which should contain sufficient details to permit figure interpretation without reference to the text. Colored and black and white photographs are accepted. All illustrations should be limited to an A4 size paper. In case of using MS Excel program for diagrams, the original MS Excel file should be sent also.

Footnotes
Footnotes are permitted in the tables but not in the rest of the text.

Citations
Citations should be referenced in the text in one of two ways, depending on the sentence structure: 1) At the beginning of the sentence - Alhadrami et al. (2001); 2) At the end of the sentence - (Alhadrami et al., 2001). When a citation has one or two authors, cite the reference using the name(s) and the date. The name of the first author should be used followed by 'et al' when there are more than two authors in the citation. However, 'et al' should not be used in the list.

Review articles and short communication
Can also be published in the journal and they need not be divided into the above-mentioned sections. Normally, original papers and review articles should not exceed 20 typewritten double spaced pages while short communications should be limited to a maximum of five pages.

Proofs
One set of galley proofs will be sent to the corresponding author for typographical checking only. It should be returned within three days of receipt.

Reprints
Authors will receive a complimentary copy of printed journal upon request.