World Journal of Agricultural Sciences 6 (6): 735-739, 2010
ISSN 1817-3047
© IDOSI Publications, 2010

Preliminary Phytochemical Investigation on Mango (*Mangifera indica* L.) Leaves

[1]*Abdelnaser Abdelghany Elzaawely and* [2]*Shinkichi Tawata*

[1]Department of Agricultural Botany, Faculty of Agriculture, Tanta University, Tanta, Egypt
[2]Department of Bioscience and Biotechnology, Faculty of Agriculture,
University of the Ryukyus, Okinawa, Japan

Abstract: Mango (*Mangifera indica* L.) is one of the most important tropical plants. Most studies on the exploitation of mango have been dealing with mango peels, juices and stem bark, however a little attention has been given to mango leaves. The main objective of this study was to develop a commercially phenolic-enriched product from mango leaves to be used as a nutritional supplement or as herbal tea. In this study, contents of total phenolics and total flavonoids were determined in ethyl acetate fraction prepared from mango leaves and were measured as 386.3 and 127.3 mg gallic acid and rutin equivalents per gram of extract, respectively. By using GC-MS, eight phenolic compounds including benzoic acid, pyrogallol, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, ethyl gallate and gallic acid were detected in the EtOAc fraction. It was concluded that mango leaves contain high amounts of total phenolics and flavonoids that assess the possibility to utilize mango leaves as a natural source for phenolic compounds in food and beverage industry.

Key words: *Mangifera indica* · Phenolics · Flavonoids · GC-MS

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical plants in the world [1]. It grows in the tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies [2]. Many phenolic compounds have been detected in mango peels [3-5], mango bark [6], mango puree concentrate [7], mango pulps and seed kernels [8-10]. Several pharmacological activities of mango extracts have been reported including anti-inflammatory [2], antioxidant [8-10], antiallergic and anthelmintic [11] and antiamoebic [12].

Herbal drinks are very popular, as they contain natural constituents especially phenolic compounds [13]. Vimang is an aqueous decoction of mango stem bark that has been developed in Cuba on an industrial scale used as a nutritional supplement, cosmetic and phytomedicine [14]. It mainly contains phenolic compounds and it showed a potent anti-inflammatory and antioxidant activities [15].

Previously, most studies conducted for the exploitation of mango importance have been dealing with mango fruits, peels, juice and stem bark. However, a little attention has been given to mango leaves. This study was designed to prepare a phenolic-enriched and healthy-natural beverage from mango leaves that could be used as a nutritional supplement or as herbal tea.

MATERIALS AND METHODS

Mango leaves were collected from Okinawa, Japan. Benzoic acid, pyrogallol, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, ethyl gallate and gallic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethanol, methanol, hexane, acetone and ethyl acetate solvents were of the highest purity needed for each application and purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of the Extracts: The fresh leaves (500 g) were boiled in water for 30 min. After cooling at room temperature, the water crude extract was filtered and subsequently extracted with hexane (10 × 150 ml) and ethyl acetate (2 × 150 ml). The obtained EtOAc fractions and water residue were collected, filtered and dried under vacuum at 40°C.

Corresponding Author: Abdelnaser Abdelghany Elzaawely, Department of Agricultural Botany, Faculty of Agriculture, Tanta University, Tanta, Egypt. Tel: +2 010 1397749, E-mail: elzaawely@yahoo.com.

Determination of Total Phenolic Content: The amount of total phenolics was determined according to the Folin-Ciocalteu procedure [16]. Briefly, 1.0 ml Folin-Ciocalteu's reagent (50%) and 0.8 ml 7.5% (w/v) Na_2CO_3 were added to 0.2 ml (500 ppm) of methanolic solution of EtOAc fraction and water residue of mango leaves. After shaking, the mixture was incubated at room temperature for 30 min. Absorption was measured at 765 nm using a Shimadzu UV-160A spectrometer, Kyoto (Japan). Total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram extract.

Determination of Total Flavonoids: The amount of total flavonoids was determined according to the method described by [17]. Briefly, 1.0 ml of methanolic solution of EtOAc fraction and water residue of mango leaves (1000 ppm) was mixed with 1 ml aluminum chloride (2% in methanol). After shaking, the mixture was incubated at room temperature for 15 min and then the absorption was measured at 430 nm using a Shimadzu UV-160A spectrometer, Kyoto (Japan). Total flavonoids content was expressed as rutin equivalents (RE) in milligrams per gram extract.

GC-MS Analysis: A 1µl aliquot of acetone solution of EtOAc fraction from Mango leaves was injected into the GC–MS (QP-2010, Shimadzu Co., Kyoto, Japan). The DB-5MS column was 30 m in length, 0.25 mm id and 0.25 µm in thickness (Agilent Technologies, J&W Scientific Products, Folsom, CA, USA). The carrier gas was helium. The GC oven temperature program was as follows: 50°C hold for 6 min, raised at 5°C/min to 280°C and hold for 5 min. The injector and detector temperatures were set at 250°C and 280°C, respectively. The mass range was scanned from 20 to 900 amu. The control of the GC-MS system was carried out by means of Shimadzu's GC-MS solution software, version 2.4.

RESULTS AND DISCUSSION

Contents of Total Phenolics and Total Flavonoids: The amounts of total phenolics and total flavonoids of the EtOAc fraction as well as water residue of mango leaves are presented in Figure 1. The EtOAc fraction contained a higher amount of total phenolics than water residue (386.3 and 127.3 mg GAE/g extract, respectively). In the same manner, a higher quantity of total flavonoids was found in the EtOAc fraction (127.3 mg RE/g extract) than in water residue (41.3 mg). Folin-Ciocalteu procedure is a very common method as it gives a rapid and useful evaluation for the phenolic content of plant samples [18]. Phenolic compounds are among the most important plant components as they possessed a variety of biological activities including antioxidant [19-20], therefore it is quite important to evaluate the total phenolic content in tested extracts.

GC-MS Analysis: GC-MS was used to elucidate the phenolic profile of ethyl acetate fraction of mango leaves and a total of 8 compounds including benzoic acid, pyrogallol, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, ethyl gallate and gallic acid were tentatively identified on the basis of spectral data and standard chemicals (Figs. 2 and 3 and Table 1). It was noted that different parts of mango contain a wide array of phenolic substances and the key biological compound in its parts is a polyphenol called mangiferin [1]. However, mangiferin could not be detected by GC-MS in our study, as it is considered to be a water-soluble compound and the reversed-phase high performance liquid chromatography is usually used for its determination. It was stated that water-soluble plant compounds have been determined by liquid chromatography, while GC-MS has been used to analyze the volatile substances [21].

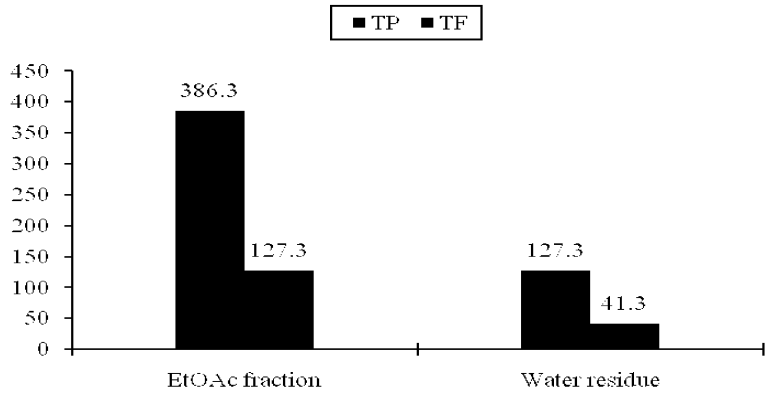

Fig. 1: Total phenolics and total flavonoids in EtOAc fraction and water residue of mango leaves

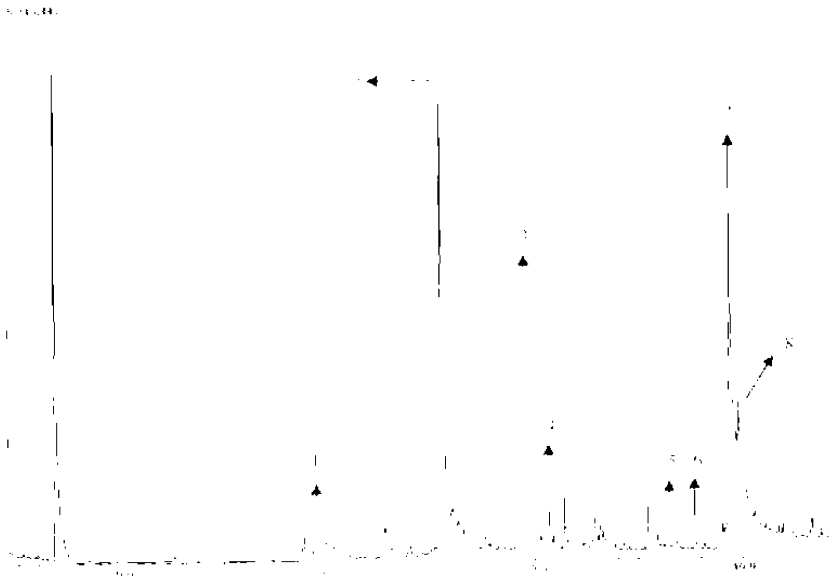

Fig. 2: GC chromatogram of phenolic compounds detected in EtOAc fraction of mango leaves, Peaks 1: Benzoic; 2: Pyrogallol; 3: *p*-hydroxybenzoic; 4: Vanillic; 5: Syringic; 6: Ferulic; 7: Ethyl gallate; 8: Gallic

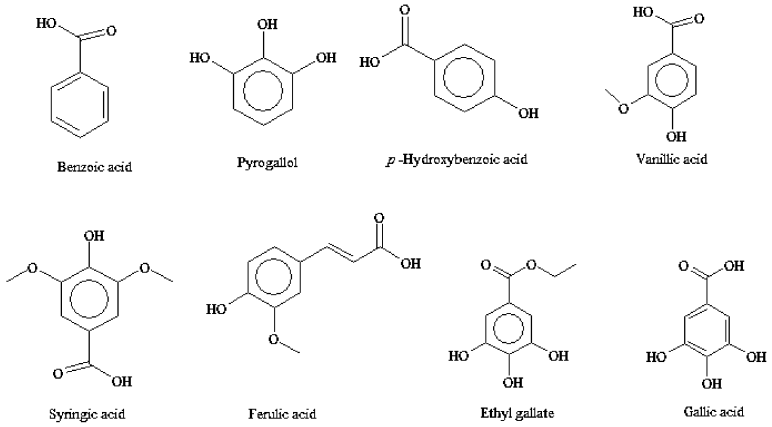

Fig. 3: Phenolic compounds detected in EtOAc fraction of mango leaves

Table 1: Retention time, molecular weight and major peaks of phenolic compounds detected by GC-MS in EtOAc fraction of mango leaves

Compound	Retention time (min)	Molecular weight	Major peaks
Benzoic acid	19.1	122	105, 122, 77
Pyrogallol	25.5	126	126, 52, 80
p-hydroxybenzoic acid	29.6	138	121, 138, 93
Vanillic acid	30.7	168	168, 153, 97
Syringic acid	36.5	198	198, 183, 127
Ferulic acid	37.8	194	194, 179, 133
Ethyl gallate	39.4	198	153, 198, 170
Gallic acid	40.7	170	170, 153, 125

Fruits, vegetables and plant beverages are natural sources for phenolic compounds that have health-promoting properties and are associated with a low risk of cancer and cardiovascular diseases [22]. Therefore, there is now much interest to use phenolic-rich products in food industries.

CONCLUSSION

Based on our findings, the ethyl acetate fraction of mango leaves contains high amount of total phenolics and total flavonoids. Furthermore, several phenolic compounds have been detected in this fraction by GC-MS. However, this study is a preliminary one and further research is still needed to study the antioxidant properties of this fraction and be analyzed by HPLC and this would expand the scope of its application to the food industry as a natural drink.

REFERENCES

1. Severi, J.A., Z.P. Lima, H. Kushima, A.R.M.S. Brito, L.C. Dos Santos, W. Vilegas and C.A. Hiruma-Lima, 2009. Polyphenols with antiulcerogenic action from aqueous decoction of mango leaves (*Mangifera indica* L.). Molecules, 14: 1098-1110.
2. Garrido, G., D. González, Y. Lemus, D. García, L. Lodeiro, G. Quintero, C. Delporte, A.J. Núñez-Sellés and R. Delgado, 2004. *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract (Vimang). Pharmacol. Res., 50: 143-149.
3. Schieber, A., N. Beàardini and R. Carle, 2003. Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv. "Tommy Atkins") peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem., 51: 5006-5011.
4. Beàardini, 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.
5. Beàardini, N., M. Knödler, A. Schieber and R. Carle, 2005. Utilization of mango peels as a source of pectin and polyphenolics. Innov. Food Sci. Emerg., 6: 442-452.
6. Nong, C., W. He, D. Fleming, L. Pan and H. Huang, 2005. Capillary electrophoresis analysis of mangiferin extracted from *Mangifera indica* L. bark and *Mangifera persiciformis* C.Y. Wu et T.L. Ming leaves. J. Chromatogr. B., 826: 226-231.
7. Schieber, A., W. Ullrich and R. Carle, 2000. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innov. Food Sci. Emerg., 1: 161-166.
8. Ribeiro, S.M.R., J.H. De Queiroz, M.E.L.R. De Queiroz, F.M. Campos and H.M.P. Sant'Ana, 2007. Antioxidant in Mango (*Mangifera indica* L.) Pulp. Plant Foods for Hum. Nutr., 62: 13-17.
9. Ribeiro, S.M.R., L.C.A. Barbosa, J.H. Queiroz, M. Knödler and A. Schieber, 2008. Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chem., 110: 620-626.
10. Maisuthisakul, P. and M.H. Gordon, 2009. Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. Food Chem., 117: 332-341.
11. García, D., M. Escalante, R. Delgado, F.M. Ubeira and J. Leiro, 2003. Anthelminthic and antiallergic activities of *Mangifera indica* L. stem bark components Vimang and mangiferin. Phytother. Res., 17: 1203-1208.
12. Tona, L., K. Kambu, N. Ngimbi, K. Cimanga and A.J. Vlietinck, 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol., 61: 57-65.
13. Kruawan, K. and K. Kangsadalampai, 2006. Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. The Thai J. Pharm. Sci., 30: 28-35.
14. Sellés, A.J.N., H.T.V. C astro, J. Agüero-Agüero, J. González-González, 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.
15. Pardo-Andreu, G.L., D.J. Dorta, R. Delgado, R.A. Cavalheiro, A.C. Santos, A.E. Vercesi and C. Curti, 2006. Vimang (Mangifera indica L. extract) induces permeability transition in isolated mitochondria, closely reproducing the effect of mangiferin, Vimang's main component. Chem. Biol. Interact., 159: 141-148.
16. Elzaawely, A.A., T.D. Xuan, H. Koyama and S. Tawata, 2007. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) B.L. Burtt. and R.M. Sm. Food Chem., 104: 1648-1653.
17. Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal, 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97: 654-660.
18. Luximon-Ramma, A., T. Bahorun and A. Crozier, 2003. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. J. Sci. Food Agric., 83: 496-502.

19. Elzaawely, A.A., T.D. Xuan and S. Tawata, 2005. Antioxidant and antibacterial activities of *Rumex japonicus* Houtt. Aerial Parts. Biol. Pharm. Bull., 28: 2225-2230.
20. Ao, C., A. Li, A.A. Elzaawely, T.D. Xuan and S. Tawata, 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. Food Control, 19: 940-948.
21. Kivilompolo, M., V. Obùrka and T. Hyötyläinen, 2007. Comparison of GC–MS and LC–MS methods for the analysis of antioxidant phenolic acids in herbs. Anal. Bioanal. Chem., 388: 881-887.
22. Atmani, D., N. Chaher, M. Berboucha, K. Ayouni, H. Lounis, H. Boudaoud, N. Debbache and D. Atmani, 2009. Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chem., 112: 303-309.