IJP (2017), Vol. 4, Issue 5 **(Research Article)**

Received on 10 February 2017; received in revised form, 20 April 2017; accepted, 25 April 2017; published 01 May 2017

ASSESSMENT OF MANGIFERIN IN THIRTY VARIETIES OF *MANGIFERA INDICA* L. (ANACARDIACEAE)

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Keywords:

Mangiferin,
Mangifera indica, HPLC

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ABSTRACT: Mangiferin content in aqueous extracts of 30 varieties of *Mangifera indica* leaves collected from Gujarat state of Western India has been estimated during this study, using a simple reverse phase HPLC-DAD analysis method. The proposed method has been validated according to ICH guidelines in terms of linearity, accuracy, intraday and interday precision, limit of detection and limit of quantitation. The calibration graph was found to be linear over a concentration range of 20-120 µg/mL, with a regression coefficient of 0.9943. Determination of accuracy by standard addition method at three different concentration levels returned a mean recovery value of 97.64%. Average intraday and interday precision values were found to be less than 2%.

INTRODUCTION: *Mangifera indica* L. belongs to Anacardiaceae and is commonly designated as the "King of fruits." It is an economically important fruit found throughout the world. Mango contains various classes of polyphenols, carotenoids, and ascorbic acid, which demonstrate different health-promoting properties, mainly from their antioxidant activities [1]. The tree is large, spreading, evergreen, with a dense rounded or globular crown. The bark is thick, sometimes with longitudinal bursts containing a little yellowing, transparent gum resin like juice. The wood is reddish grey, often streaked, moderately hard, coarse-grained and soft in young trees and is readily eaten by insects. The tree has the power of healing the wounds by covering it with the rapid growth of the cork.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.4(5).169-73

The article can be accessed online on
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.4(5).169-73

Leaves are simple, alternate, irregularly placed along the branchlets, sometimes remote and at other times (especially at the tips of the flowering branches) crowded, rather long-petioled, oblong-ovate to oblong-lanceolate, base acute to cuneate, narrowed, apex acute to acuminate, entire often with wavy margins, coriaceous, glabrous on both surfaces, leaf blade 10-32 cm, long, 2-9.5 cm, wide with resinous smell when bruised, pinnate nerved, distinctly reticulate veined, costa ronust, lateral secondary nerves numerous (12-13 pairs), conspicuous, yellowish green, prominent beneath and inarching near the margin, alternating with shorter intermediate nerves, young leaves violet (purplish yellow): petiole is terete, slightly thickened or swollen at the base, round, smooth, glabrous: mature leaves dark green.

Leaves are useful in numerous ways masticating to tone up the gums. Ash of burnt leaves is used as a household remedy for burns. They are also used as vegetables in Java and the Philippines. Mango leaves contain mangiferin which is an expectorant for cough caused by bronchitis, and also helps in preventing asthma.

Mango leaves are the main material sources for mango cough drops. It is effective in reducing fever, functions in tonifying the heart, diuresis, anti-melancholy, anti-inflammatory. In addition, mango has also been used as traditional medicine *i.e.* a bark infusion used in Samoa for mouth infections in children, mango leaves used as a remedy for treatment of relapse sickness in Tonga [2], in India [3], unripe fruit taken as a remedy for exhaustion and heat stroke, half-ripe fruit used for a treatment of gastrointestinal disorders, bilious disorders, blood disorders and scurvy, fresh leaves for treatment of diabetes, dried seed ground into flour for treatment of diarrhea, and bark extract for treatment of diarrhea and throat disorders [4]. The total number of distinct mango varieties cultivated in India has been variously estimated at 500 to 1000, out of which around 210 varieties are considered important. The most cultivated varieties of Gujarat are Kesar, Alphonso, Rajapuri, and Pairi.

EXPERIMENTAL ANALYSIS:

Materials: Samples of *M. indica* leaf varieties were collected from Junagadh Agriculture University, Junagadh. The leaf samples were washed, shade dried for a day and then dried completely in an oven at 38 °C. The plant materials were coarsely powdered using a rotary grinder, sieved through BSS mesh 85, and stored in airtight plastic containers to be used for HPLC analysis. Mangiferin standard was procured from SIGMA, Bangalore, India (CAS no. 4773-96-0). HPLC grade acetonitrile and water were procured from Qualigens, Mumbai, India.

Extraction: Fine leaf powder (1 g) of each sample was weighed into an Erlenmeyer flask. Ethyl alcohol (25 ml) was added and the flask subjected to shaking for overnight extraction in an orbital shaker. The extract was filtered using Whatman No. 41 paper. The solvent was removed under reduced pressure, and the residue was reconstituted in HPLC grade water, making the total volume of 10 ml in a volumetric flask. Exactly 0.5 ml of this diluted extract was pipetted out into a 5 ml volumetric flask, and the volume was made up using HPLC grade water and used directly for HPLC analysis. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Pall Corporation, Mumbai, India) before injection.

Preparation of Working Standards: Six concentration levels of mangiferin standard 20, 40, 60, 80, 100 and 120 µg/mL were prepared in HPLC grade water. These working standards were used for linearity study and preparation of calibration plot for quantization of mangiferin in the samples.

HPLC Analysis: HPLC analysis was carried out using a Shimadzu LC20AT series liquid Chromatograph equipped with a diode-array detector. The analytical column was a Phenomenex Luna C18 (250 × 4.6 lm; 5 µm) with a C18 guard column (Phenomenex, Torrence, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 1.0 ml per min for a total run time of 30 min, and the gradient programme was as follows: 90% for 5 min, 90% B to 80% B in 16 min, 80% B to 0% B in 9 min and 0% B to 90% B in 5 min. A post-run of 10 min was set for reconditioning. The injection volume was 20 µl, and peaks were monitored at 254 nm. Peaks were identified by congruent retention times and UV spectra when compared with those of the standard. All samples were prepared and analyzed in duplicate.

RESULTS: Mangiferin is a phenolic compound and occurs in response to stress. It is abundant in mango leaves. The present study showed mangiferin ranged from 1 to 47 mg/g in thirty varieties of mango from Junagadh (table). Maximum mangiferin was recorded in Ladoo **Fig. 2D** followed by Jahangir **Fig. 3D** and the minimum amount was seen in Kaju **Fig. 3F** followed by Aambadi **Fig. 3B**.

In the present study, mangiferin was isolated from leaves of thirty mango varieties cultivated in Gujarat (table). Mangiferin content ranged from 1.04 to 47.02 mg/g of leaf powder, where varieties Batli (1.04 mg/g) Aambadi (4.79 mg/g), Khodi (7.15 mg/g), had lesser values as compared to Ladvo (47.02 mg/g), Jahangir (38.71 mg/g) and Jhamrukhiyo (35.27 mg/g). Other varieties ranged in between, which is given in the table. Mangiferin (2-beta-D-glucopyranosyl-1, 3, 6, 7 – tetrahydroxy xanthen – 9 - one) has significant pharmacological properties including antidiabetic [5, 6], antioxidant [7, 8, 9], antitumor [10, 11, 12], vascular modulatory [13], immunomodulatory, and antiviral activities [14, 15].

TABLE 1: QUANTIFICATION OF MANGIFERIN EXTRACTED FROM 30 VARIETIES OF *M. INDICA*

Sample number	Variety	Mean Area	Mangiferin in mg/g of leaf powder
1	Cowasji	5418.767	17.49
2	Batli	3255.728	10.41
3	Jhumakhiya 1	3001.696	9.50
4	Sindoria	5872.641	18.9
5	Pairi	4995.308	16.10
6	Goto	2671.695	8.40
7	Jamadar	3966.123	12.73
8	Mulgoa	6761.328	21.88
9	Rucchado	5038.654	16.24
10	Ladvo	14439.990	47.02
11	Khodi	2259.688	7.15
12	Kesar	5191.940	16.75
13	Jhumakhiya2	4064.377	13.05
14	Sopari	8820.225	28.62
15	Langdo	3192.371	10.20
16	Aambadi	1541.022	4.79
17	Asadiyo	4118.159	13.23
18	Neelam	6664.538	21.57
19	Badshahpasand	9267.828	30.09
20	Desi	3852.857	12.36
21	Dudhpedno	5244.417	16.92
22	Alphonso	4804.176	15.48
23	Rajapuri	3244.844	10.37
24	Fazli	3292.698	10.53
25	Jahangir	11900.618	38.71
26	Totapuri	6846.395	22.16
27	Jhamrukhiyo	10849.272	35.27
28	Kaju	1133.178	3.46
29	Amirpasand	6661.742	21.56
30	Gajariyo	4407.681	14.18

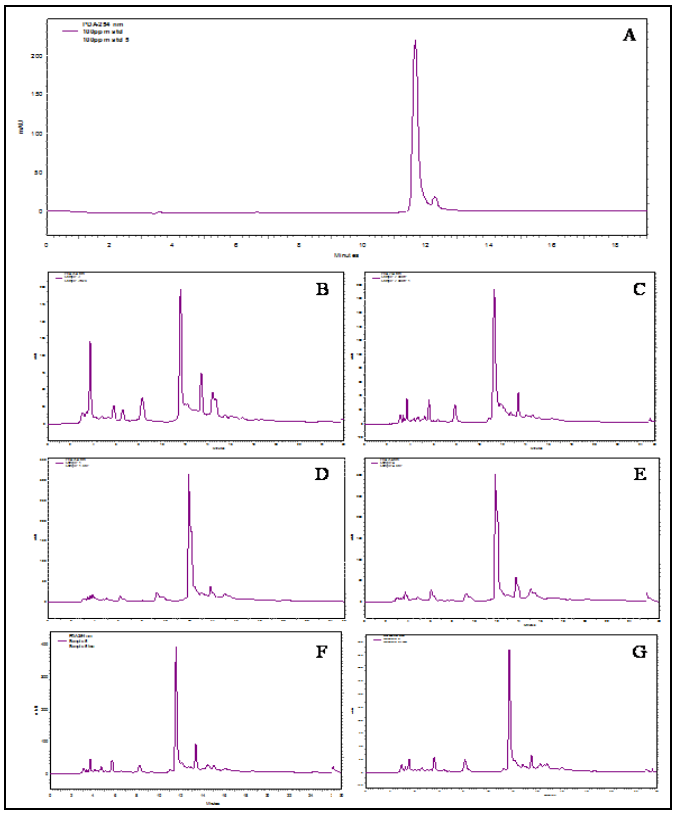

FIG. 1: HPLC OF MANGIFERIN A) STANDARD (100 PPM), B) SAMPLE 1, C) SAMPLE 2, D) SAMPLE 3, E) SAMPLE 4, F) SAMPLE 5, G) SAMPLE 6

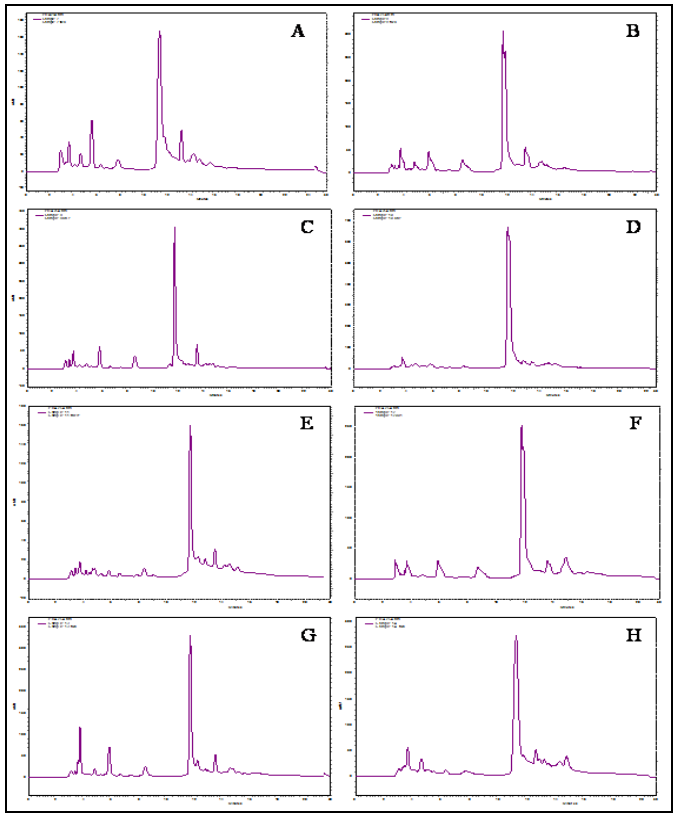

FIG. 2: HPLC OF MANGIFERIN. A) SAMPLE 7, B) SAMPLE 8, C) SAMPLE 9, D) SAMPLE 10, E) SAMPLE 11, F) SAMPLE 12, SAMPLE 13, SAMPLE 14

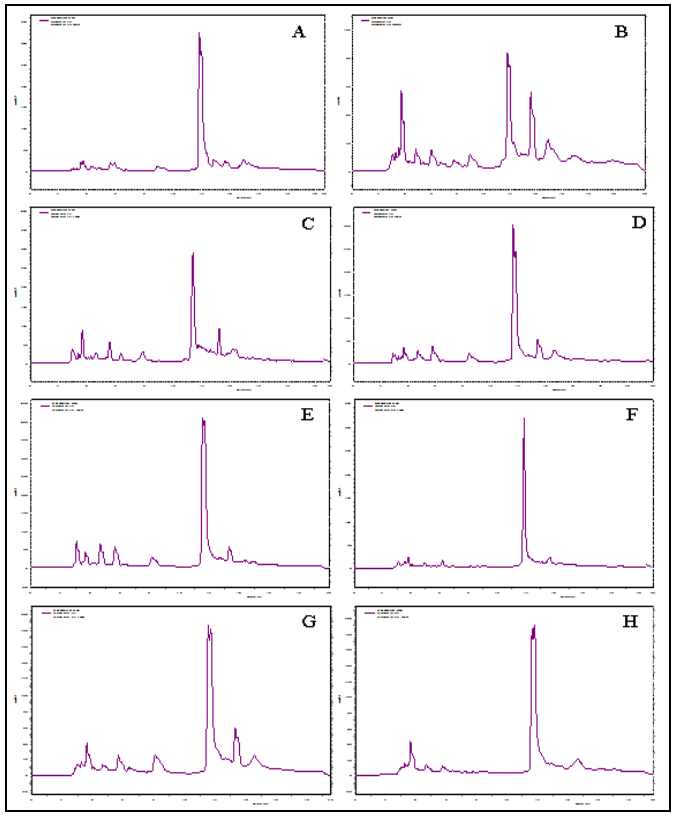

FIG. 3: HPLC OF MANGIFERIN, A) SAMPLE 15, B) SAMPLE 16, C) SAMPLE 17, D) SAMPLE 18, E) SAMPLE 19, F) SAMPLE 20, SAMPLE 21, SAMPLE 22

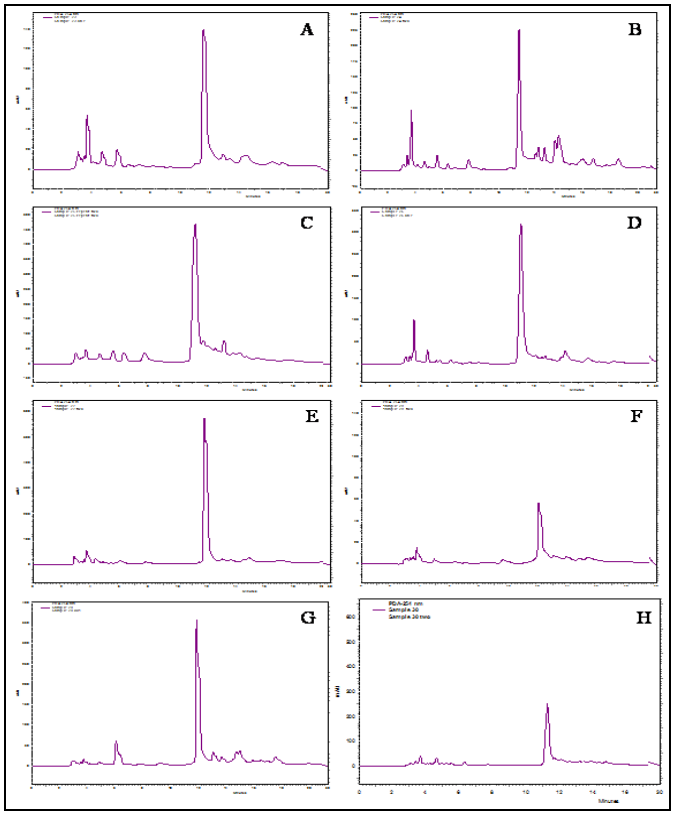

FIG. 4: HPLC OF MANGIFERIN, A) SAMPLE 23, B) SAMPLE 24, C) SAMPLE 25, D) SAMPLE 26, E) SAMPLE 27, F) SAMPLE 28, SAMPLE 29, SAMPLE 30

Other studies revealed antistress properties [16], anti-lipid peroxidation [17] and blood glucose control [18]. In Cuba, the stem bark aqueous decoction is used as a nutritional supplement [19,] and the standard aqueous extract is available in pharmaceutical formulations under the brand name Vimang and Salaretin [20]. Mangiferin is highly distributed in higher plants. It can be found in many other plants like *Coffea*, *Salacia chinensis* and *Anemarrhena asphodeloides* [21].

DISCUSSION: Mangiferin in particular, detected at high concentrations in young leaves (Coite = 172 g/kg), in bark (Momika = 107 g/kg), and in old leaves (Itamaraka = 94 g/kg), shows an exceptionally strong antioxidant capacity [22]. Studies concluded mango leaves as a natural source for phenolic compounds in the food and beverage industry [23].

Augustyn *et al.,* (2011) compared mangiferin content present in mango leaf of three cultivars with that of honeybush (*Cyclopia genistoides*), an indigenous South African herbal tea used for its antioxidant property and other health benefits [24]. Their results indicate that mango leaves may have more health benefits than honeybush tea. Jutiviboonsuk and Sardsaengjun, (2010) isolated mangiferin in three mango varieties cultivated in Thailand, out of which Nam Doc Mai showed a high amount of mangiferin [25]. In *Coffea* plants, localization of mangiferin is found in association with the photosynthetic tissue and also found accumulated at early stages of fruit formation. Franklin *et al.,* (2009) reported the presence of mangiferin for its antioxidant and antimicrobial properties along with plant defense against biotic stress. Mangiferin is a glycoside (2-β-D-glucopyranosyl-1, 3, 6, 7- tetrahydroxyxanthen-9-one, is the major component of mango leaves, fruit, bark and so on [26]. Studies have shown that mangiferin has anti-oxidant, anti-bacterial, anti-viral, immune regulation and anti-tumor activity.

CONCLUSION: The present study demonstrated that the leaves of thirty mango varieties were interesting sources of the pharmacologically active C-glycosyl xanthone, mangiferin. From the percentage amount of isolated mangiferin, variety Ladvo was a good source of mangiferin.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:
Sharma BG, Mammen D and Albert S: Assessment of mangiferin in thirty varieties of *Mangifera indica* L. Int J Pharmacognosy 2017; 4(5): 169-53. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.4(5).169-73.