

**Short Communication**

**SEASONAL VARIATION IN THE CONTENT OF MANGIFERIN IN LEAVES OF *MANGIFERA INDICA* L.**

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**ABSTRACT**

**Objective:** *Mangifera indica* L., a member of the genus of evergreen trees, is cultivated extensively in India. The various parts of the tree find its use in the Ayurvedic system of medicine against diseases like diarrhoea, dysentery, dyspepsia, sprue and carbuncle. The major constituent of the stem bark and leaves of *M. indica* is mangiferin and the various activities associated with the stem bark or the leaves are reported to be due to this component. Mangiferin is currently of interest due to its activities like antidiabetic, hypolipidemic, gastroprotective, antibacterial, chemopreventive and many more. The objective of the current study was to evaluate the variation in the mangiferin content with time, in leaves of *M. indica*, throughout the year.

**Methods:** Samples of *M. indica* leaves were collected every month for a period of one year and the content of mangiferin was assessed at these time points. Mangiferin was quantified using the HPLC method.

**Results:** Seasonal variation was seen in the mangiferin content of the leaves. Highest content was seen during the month of April.

**Conclusions:** Content of mangiferin changes with the different seasons and reaches its highest value during fruit ripening.

**Keywords:** HPLC, *Mangifera indica* L., Mangiferin, Seasonal variation.

Xanthenes, majorly representing yellow coloured compounds occur in their glycosidic forms, some being C-glycosides and other O-glycosides. Xanthenes are formed by cyclization of benzophenones resulting from the addition of two-carbon units (of malonyl-CoA) into a precursor in C<sub>6</sub>-C<sub>1</sub>, i. e. a benzoic acid arising from the shortening of a cinnamic acid. The biosynthesis of C-glycosyl xanthenes is thought to be analogous to that of flavonoids. Thus xanthenes represent the C<sub>6</sub>-C<sub>1</sub>-C<sub>6</sub> group of compounds. Mangiferin is a candidate of the xanthone class of compounds. Mangiferin is a C-glycoside, nomenclated as 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one.

An isomer of mangiferin, isomangiferin (4-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one) has also been reported and is found prominently in the *Anemarrhena asphodeloides* Bunge (Liliaceae). The major source of mangiferin is *Mangifera indica* L., an evergreen tree belonging to the Anacardiaceae family, widely growing throughout the Indian subcontinent. It is commonly called the mango tree. Mangiferin is also found in the leaves of *Bombax ceiba*, roots of *Salacia reticulata*, and the whole plant of *Swertia chirata*. Mangiferin is reported to possess activities such as antidiabetic, hypolipidemic, gastroprotective, antibacterial, chemopreventive, antiallergic, antiviral, anti-inflammatory and is also a potent antioxidant [1-5]. The various parts of the *Mangifera indica* tree find its use in the Ayurvedic system of medicine against diseases like diarrhoea, dysentery, dyspepsia, sprue and carbuncle [6]. Due to the array of properties mangiferin is reported to possess, it is a compound of importance to the pharmaceutical world.

Mangiferin has been isolated from the bark, leaves as well as the fruits of the mango tree [7]. The percentage of mangiferin in the bark has been reported to be as high as 10.2 per cent of the dry weight of the bark [8]. Since mangiferin is present in considerable amounts in the leaves of the tree it is also another source for mangiferin isolation. Gil *et al.* studied the changes in antioxidant compounds in mango pulp during ripening and observed the formation of polyphenolic compounds at the final stage of ripening. Thus the current study aims at studying the seasonal variation in the mangiferin content of the leaves of the mango tree. Samples of the leaves of mango trees were collected every month throughout one year and the mangiferin content estimated.

Leaves of *Mangifera indica* Linn. tree were collected from Matunga, Mumbai. Voucher specimen (ICT/MNPRL/2014/MI-01) was deposited at Medicinal Natural Products Research Laboratory, ICT, Mumbai. Samples were collected from five different trees every month over a period of one year from the same location. Chemicals used for the study were of analytical grade. HPLC grade solvents were sourced from Merck (India). The standard for mangiferin was isolated in-house [8]. Distilled water was filtered through 0.45 μm filter.

HPLC analysis was performed with a Jasco (Hachioji, Tokyo, Japan) system, using 250 mm × 4.6 mm i. d., RP-18 (5-μm particle size) column, an intelligent pump (PU-1580, PU-2080), a high-pressure mixer (MX-2080-31) and a manual sample injection valve (Rheodyne 7725i). Chromatographic data were processed with Borwin software.

Standard solution was prepared by dissolving mangiferin standard in methanol at a concentration of 1mg/ml. This was then diluted to obtain five concentrations for construction of the calibration plot in the range of 2 to 10 μg/ml. Leaves were dried at 50°C and then ground to a coarse powder using a domestic grinder. The dried leaf powder (three grams) was subjected to successive Soxhlet extraction using hexane, chloroform and finally methanol. The methanolic extract obtained was dried and the dry extract weight noted as in Table 1.

The content of mangiferin in the collected samples was estimated immediately after its collection (less than 15 days from collection) to avoid any changes due to degradation if any. Same standard was used throughout the entire experiment. At every time point (every month) whenever the estimation was done, a standard curve was established for ensuring uniformity in the results obtained throughout the year and to avoid any changes due to instrumental drift. The variation in the standard curves set in different months was not significant (less than 5%). The yields of methanolic extracts from 3 gm of the twelve different samples of *Mangifera indica* leaf collected throughout the year are reported in table 1.

Isocratic elution program of 15 min on the HPLC system with the mobile phase acetonitrile: water containing 0.1% v/v of o-phosphoric acid (15: 85), with a flow rate of 1.00 ml/min and an

injection volume loop of 20  $\mu$ l, showed a peak for standard mangiferin at  $R_t$  of 7.8 min. The presence of mangiferin in the extracts was ascertained by comparing the  $R_t$  of the peak with that of the standard mangiferin.

**Table 1: Yield of methanolic extract from 3g of *M. indica* leaf**

S. No.	Month of collection of sample	% yield of methanolic extract from 3 g sample (mean $\pm$ SD)
1	January	25 $\pm$ 0.5
2	February	32.3 $\pm$ 0.3
3	March	34 $\pm$ 0.4
4	April	31.13 $\pm$ 0.6
5	May	26.77 $\pm$ 0.5
6	June	14.33 $\pm$ 0.6
7	July	18.53 $\pm$ 0.4
8	August	22.53 $\pm$ 0.7
9	September	17.67 $\pm$ 0.4
10	October	22.83 $\pm$ 0.7
11	November	23.93 $\pm$ 0.6
12	December	32.03 $\pm$ 0.8

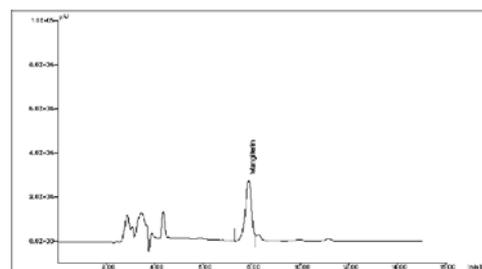
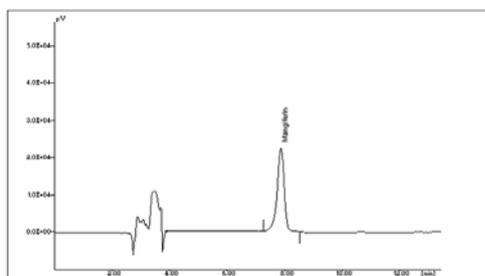
The detection wavelength for mangiferin was set up at 257 nm after recording the UV spectra for the same and choosing the wavelength showing maximum absorption [8]. The linear relationship between detector response and different concentrations of mangiferin (five levels) was confirmed in the range of 2-10  $\mu$ g/ml with correlation coefficient of 0.9968 and equation of  $y = 49591x + 33995$ . The mangiferin content of the different samples was estimated using the HPLC technique. Every month sample from five different trees was analysed. The content of mangiferin in terms of %w/w (mean  $\pm$  SD) of mangiferin with respect to dry weight of the sample is reported in the table 2.

**Table 2: Mangiferin content (%w/w  $\pm$  SD) of *M. indica* leaf sample**

S. No.	Month of collection of sample	Mangiferin content in %w/w (mean $\pm$ SD)
1	January	1.93 $\pm$ 0.5
2	February	2.70 $\pm$ 0.4
3	March	3.01 $\pm$ 0.6
4	April	4.29 $\pm$ 0.4
5	May	2.78 $\pm$ 0.8
6	June	0.8 $\pm$ 0.7
7	July	1 $\pm$ 0.4
8	August	1.72 $\pm$ 0.6
9	September	1.55 $\pm$ 0.5
10	October	1.7 $\pm$ 0.6
11	November	0.87 $\pm$ 0.3
12	December	1.91 $\pm$ 0.5

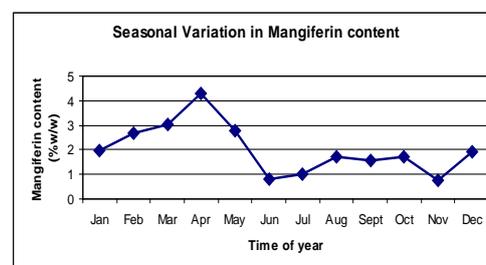
HPLC chromatogram for standard mangiferin and that for the sample are as shown in fig. 1.

Mangiferin, a C<sub>6</sub>-C<sub>1</sub>-C<sub>6</sub> compound of the xanthone class, has been reported to possess various properties right from it being antidiabetic, chemopreventive, hypolipidemic to antioxidant and anti-inflammatory. Hence it finds use in the various streams of medicine. A major source of mangiferin is the *Mangifera indica* (mango) tree and the compound has been isolated from its various parts.



**Fig. 1: HPLC chromatogram for standard mangiferin (A) and sample (B),**

The content variation of mangiferin (% w/w) in the various samples was plotted against time as shown in fig. 2



**Fig. 2: Seasonal variation in the mangiferin content of *Mangifera indica* leaves**

This study deals with the seasonal variation seen in the content of mangiferin in the leaves of this tree. Mangiferin is soluble in methanol and hence the methanolic extract from the successive extraction procedure is used for the estimation of mangiferin. Estimation was done using the HPLC method and hence to remove interference from other compounds successive method of extraction was employed. The chromatographic analysis has shown a variation in the content of mangiferin with time. The content of mangiferin has reached its highest value in the month of April (4.29%w/w) and its lowest in the month of June (0.8%w/w). The mango tree bears fruits approximately in the month of February and ripens during April-May. Thus the mangiferin content, highest in the month of April, and the fruit ripening time coincide as suggested by the data. Hence a connection could be possible in these two events. Hence we may say that the physiological changes taking place in the plant body during the ripening of the fruits is also responsible for the greater production of mangiferin in the leaves during this period. Thus the study suggests that there is seasonal variation in the content of mangiferin with its peak in the month of April and there could be the possible link in the fruit ripening and the increased mangiferin content due to the physiological changes involved.

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