Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

# Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*

Mohan CG[1*], Deepak M[2], Viswanatha GL[3], Savinay G[4], Hanumantharaju V[1], Rajendra CE[5], Praveen D Halemani[4]

[1]*Department of Pharmacognosy, Government College of Pharmacy, Bangalore-560 027*
[2]*Department of Phytochemistry, Natural Remedies Pvt. Ltd. Bangalore- 560 100*
[3]*Department of Pharmacology, PES College of Pharmacy, Bangalore-560050*
[4]*Deparment of Pharmaceutics, Government College of Pharmacy, Bangalore-560 027*
[5]*Drug Testing Laboratory, Drugs Control Department, Palace road, Bangalore-560001*

ARTICLE INFO

*Article history:*
Received 15 November 2012
Received in revised form 27 December 2012
Accepted 28 January 2013
Available online 28 April 2013

*Keywords:*
*Mangifera indica*
Lipoxygenase
DPPH assay
Antioxidants
Anti-inflammatory

ABSTRACT

**Objective:** To evaluate the anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica* in *in vitro* conditions. **Methods:** *In vitro* DPPH radical scavenging activity and lipoxygenase (LOX) inhibition assays were used to evaluate the anti-oxidant and anti-inflammatory activities respectively. Methanolic extract (MEMI), successive water extract (SWMI) and ethyl acetate fraction (EMEMI), *n*-butanol fraction (BMEMI) and water soluble fraction (WMEMI) of methanolic extract were evaluated along with respective reference standards. **Results:** In *in vitro* DPPH radical scavenging activity, the MEMI, EMEMI and BMEMI have offered significant antioxidant activity with $IC_{50}$ values of 13.37, 3.55 and 14.19 $\mu$g/mL respectively. Gallic acid, a reference standard showed significant antioxidant activity with $IC_{50}$ value of 1.88 and found to be more potent compared to all the extracts and fractions. In *in vitro* LOX inhibition assay, the MEMI, EMEMI and BMEMI have showed significant inhibition of LOX enzyme activity with $IC_{50}$ values of 96.71, 63.21 and 107.44 $\mu$g/mL respectively. While, reference drug Indomethacin also offered significant inhibition against LOX enzyme activity with $IC_{50}$ of 57.75. Furthermore, MEMI was found to more potent than SWMI and among the fractions EMEMI was found to possess more potent antioxidant and anti-inflammatory activity. **Conclusions:** These findings suggest that the MEMI and EMEMI possess potent anti-oxidant and anti-inflammatory activities in *in vitro* conditions.

## 1. Introduction

Many natural substances of plant origin are reported to be biologically active, endowed with antimicrobial, allelopathic and antioxidant properties[1].

*Mangifera indica* L, belonging to the family Anacardiaceae and it is commonly found in tropical and subtropical regions and is one of the most popular edible fruits in the world[2]. The plant is widely used in the traditional system of medicine. The extracts from the stem bark and fruits are known to contain vitamins, polyphenols, terpenoids, steroids, fatty acids and trace elements and reported to possess various biological activities such as anti-tumour, anti-neoplastic, anti-oxidant, anti-inflammatory[3-9], analgesic[10] and immunomodulatory[11] properties. In folk medicine, the leaves of the plant had been widely used to treat various inflammatory conditions, but there is a paucity of scientific evidence for anti-oxidant and anti-inflammatory activity of the leaves. With this background, present study was aimed to evaluate the anti-oxidant and anti-inflammatory activity of various extracts and fractions of *Mangifera indica* leaves.

## 2. Materials and methods

### 2.1. Plant material

Leaves of *Mangifera indica* were collected from Natural Remedies Private Limited, Bangalore and the leaf

*Corresponding author: Mr.Mohan C.G, Department of Pharmacognosy, Government College of Pharmacy, Subbaiah Circle, Bangalore-560 027, Karnataka, India.
E-mail: mohangcp@gmail.com, glv_000@yahoo.com

material was authenticated at National Institute of Science Communication and Information Resources (NISCSIR), New Delhi. Fresh leaves were washed under running tap water, air dried in shade and then homogenized to make fine powder. The voucher specimen of the leaves were preserved in the herbarium of Pharmacognosy Department of the Institute.

### 2.2. Preparation of crude extracts

Ten grams of the air dried leaf powder was refluxed with methanol (MEMI) at 70 ℃ for 2 hours. It was filtered through muslin cloth and the filtrate was concentrated under vacuum at a temperature not more than 70 ℃ and dried in vacuum tray drier.

### 2.3. Fractionation of methanolic extract

The MEMI was heated on a water bath for 1 hour at 70 ℃ with 1 L of demineralized water (DM water) and filtered through fine muslin cloth. The filtrate was partioned with 500 mL of ethyl acetate (EMEMI) for 3 times, the combined ethyl acetate fraction was concentrated and dried in a vacuum tray dryer at a temperature not more than 70 ℃. the filtrate left after portioning with ethyl acetate was re-partitioned with 500 mL of *n*-butanol (BMEMI) for 3 times. The *n*-butanol fractions were combined, concentrated and dried in a vacuum tray dryer at a temperature not more than 70 ℃.

The aqueous layer which was left after partitioning with ethyl acetate and *n*-butanol was used as water soluble fraction (WMEMI). It was concentrated and dried under vacuum at a temperature not more than 80 ℃. The methanolic extract which was left after the sepration of water extract (WMEMI) was designed as water insoluble fraction. The marc left after extraction with methanol was extracted successively thrice with water at 70 ℃ and subjected to spray dried (SWMI).

### 2.4. In vitro lipoxygenase assay for anti-inflammatory activity

This assay measures the hydroperoxides generated from the incubation of a lipoxygenase (5-, 12- or 15 LO) with linoleic acid as substrate. The conversion of linoleic acid to 13-hydroperoxy linoleic acid by soybean lipoxygenase was followed spectrophotometrically by the appearance of conjugated dienes at 234 nm. Indomethacin was used as a positive control[12,13].

Preparation of borate buffer (2M, pH 9) was prepared as following: 6.18 g of boric acid was dissolved in 500 mL of deionized water and pH was adjusted to 9.0 with 1N NaOH.

25 mg of linoleic acid was suspended in 25 mg of tween 20, the suspension was dissolved and volume was made up to 25 mL with borate buffer (Stock I). 8.3 mL of the stock I was diluted to 50 mL with borate buffer (Stock II).

2.5 mg of lipoxygenase enzyme was dissolved in 5 mL of the cold borate buffer and stored at 20 ℃ (Stock I), 285 μL of stock I was diluted to 1 mL with cold borate buffer.

12 mg of the each extract and fraction was dissolved in 280 μL of methanol, the volume was made up to 10 mL with borate buffer.

60 μL of lipoxygenase enzyme was added to 940 μL of the borate buffer/vehicle/positive control/test solution separately and incubated for 5 min at room temperature.

After incubation at room temperature for 5 min, 2 mL of the substrate (linoleic acid) was added and the reaction was monitored for 240 seconds (4 mins) at 234 nm in UV Spectrophotometer. Percentage inhibition of the lipoxygenase activity was estimated using the equation[12,13].

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 2.5. DPPH radical scavenging activity

The free radical scavenging activity of the test samples s were measured by 1, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay[14,15].

1.5 mg of each extract and fraction was dissolved in DM water to made volume up to 5 mL with DM water and further dilutions were made to get concentration from 1 to 100 μL.

2.6 mg of DPPH was dissolved in methanol and made up to 10 mL with the same solvent and 2 mg of gallic acid was dissolved in 10 mL methanol (Stock I) and 100 μL of stock I was diluted to 1 mL with methanol.

50 μL of DPPH in methanol was added to 200 μL of the vehicle control/positive control/test solution and incubated for 20 min at room temperature. The absorbance of DPPH radical was measured at 520 nm using spectrophotometer. The DPPH radical scavenging activity was calculated according to the following formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## 3. Results

Present study was undertaken to evaluate the anti-oxidant and anti-inflammatory activity of extracts and fractions of leaves of *Mangifera indica* in *in vitro* conditions.

In anti-oxidant study, the MEMI and SWMI were evaluated for DPPH radical scavenging activity; the MEMI has offered better radical scavenging activity compare to SWMI with $IC_{50}$ value of 13.37 μg/mL. Based on the results, the MEMI was further fractioned in to EMEMI, BMEMI and WMEMI and evaluated similarly. Results have revealed that, EMEMI and BMEMI have significantly and dose dependently scavenged the free radicals with $IC_{50}$ values of 3.55 and 14.19 μg/mL; however the EMEMI was found to be more potent than BMEMI. Reference dug gallic acid has dose dependently scavenged the free radicals with $IC_{50}$ value of 1.88 μg/mL and it was found to be most potent (Table 1).

**Table 1**

*In vitro* DPPH radical scavenging activity of methanolic leaf extract and fractions of *Mangifera indica*.

| Test drug | Concentration (μg/mL) | % Inhibition | $IC_{50}$ (μg/mL) |
|---|---|---|---|
| Gallic acid | 0.5 | 16.45 | 1.88 |
| | 1 | 41.98 | |
| | 2.5 | 62.87 | |
| | 5 | 91.17 | |
| MEMI | 1 | 4.79 | |
| | 10 | 13.37 | |
| | 25 | 34.56 | |
| | 50 | 95.06 | |
| SWMI | 1 | 2.56 | 31.42 |
| | 10 | 29.56 | |
| | 25 | 43.63 | |
| | 50 | 72.02 | |
| EMEMI | 1 | 14.48 | 3.55 |
| | 5 | 72.64 | |
| | 10 | 84.01 | |
| WMEMI | 25 | 11.66 | 96.26 |
| | 50 | 22.92 | |
| | 100 | 52.57 | |
| BMEMI | 1 | 2.47 | 14.19 |
| | 10 | 29.47 | |
| | 25 | 89.21 | |

Values are expressed as mean of three trials, average of the optical density (OD) recorded in three trials were used for calculating percentage inhibition and $IC_{50}$ Values.

**Table 2**

*In vitro* LOX inhibition activity of methanolic leaf extract and fractions of *Mangifera indica*.

| Test drug | Concentration (μg/mL) | % Inhibition | $IC_{50}$ (μg/mL) |
|---|---|---|---|
| Indomethacin | 40 | 21.28 | 57.75 |
| | 60 | 54.37 | |
| | 80 | 85.12 | |
| MEMI | 50 | 31.44 | 96.71 |
| | 100 | 40.37 | |
| | 150 | 86.05 | |
| | 200 | 98.42 | |
| SWMI | 50 | 5.98 | 139.26 |
| | 100 | 20.29 | |
| | 150 | 52.00 | |
| | 200 | 89.62 | |
| EMEMI | 50 | 29.68 | 63.21 |
| | 75 | 58.00 | |
| | 100 | 82.56 | |
| | 150 | 100.00 | |
| WMEMI | 50 | 1.02 | 1 272.15 |
| | 100 | 3.65 | |
| | 200 | 7.05 | |
| BMEMI | 50 | 15.65 | 107.44 |
| | 100 | 37.56 | |
| | 150 | 81.68 | |
| | 200 | 94.47 | |

Values are expressed as mean of three trials, average of the optical density (OD) recorded in three trials were used for calculating percentage inhibition and $IC_{50}$ Values.

Similarly, the extracts and fractions of the leaves of *Mangifera indica* were evaluated for anti-inflammatory activity by *in vitro* lipoxygenase assay; among the extracts MEMI was found to more potent with $IC_{50}$ value of 96.71 μg/mL, while the EMEMI was found to be more potent among all the fractions with $IC_{50}$ value of 63.21 μg/mL; Indomethacin a well known non-steroidal anti-inflammatory drug (NSAID) offered significant and dose dependent inhibition of LOX activity with $IC_{50}$ value of 57.75 μg/mL (Table 2).

## 4. Discussion

Free radicals are the atoms or molecules containing an odd number of electrons, which results in an odd electron in the external orbit. These are highly reactive species capable of wide spread, indiscriminate oxidation and peroxidation of proteins, lipids and DNA, which can lead to significant cellular damage and even tissue and/or organ failure[15]. Many studies have explored the role of oxidative stress in causing various life threatening diseases and/or disorders and also therapeutic role of antioxidant in preventing the number of serious diseases[14]. The involvement of free radicals in the pathological process such as aging, behavioral and psychiatric disorders, cancer, atherosclerosis and rheumatoid arthritis is well recognized[15].

Anti-oxidants works by intercepting with reactive oxygen species to quench the radicals and to produce less aggressive chemicals species likely to cause tissue damage. Much attention has been focused on the use of anti-oxidants because of their protective effect against damage from reactive oxygen species, on this basis the beneficial effect of anti-oxidants are being increased[16-18].

In present study, the leaf extracts and fractions of *Mangifera indica* were evaluated for anti-oxidant and anti-inflammatory activities in *in vitro* by DPPH radical scavenging activity and lipoxygenase inhibition assay respectively.

DPPH is a relatively stable nitrogen centered free radical, that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, as a result the electrons become paired off by forming corresponding hydrazines; the solution therefore looses its color stochiometrically depending on the number of electrons it takes up.

Anti-oxidants present in the plant extract can be detected on a TLC plate by spraying with DPPH radical; anti-oxidants reduce these radicals and produces yellow spots on a purple background, thus making it possible to identify the anti-oxidants present in the extract.

In present study, leaf extracts and fractions of *Mangifera indica* were evaluated for anti-oxidant activity; Gallic acid a well known anti-oxidant used as a reference standard. The findings of the study revealed that, the MEMI and EMEMI

and BMEMI could scavenge the DPPH radicals significantly and dose dependently. Lipoxygenases (LOX) are the members of a class of non-heme iron containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids containing a cis-1, 4-pentadiene system to give unsaturated fatty acid hydroperoxides. In mammals, LOX's catalyzes the first step in the arachidonic acid cascade[19,20]. The 5-LOX and 15- LOX's together lead to the formation of biologically active lipoxins, where as 5-LOX's lead to formation of 5,6-epoxy-leukotrienes which are involved in the variety of inflammatory responses, including neutrophil chemotaxis, vascular permeability and smooth muscle contraction. There was a good correlation exists between the inhibitory activity of mammalian 5-LOX's and soya bean LOX's[20,21].

In present study, the leaf extracts and fractions of *Mangifera indica* were evaluated for anti-inflammatory activity in *in vitro* using LOX's derived from soya bean and Indomethacin was used as a standard drug.

Our results demonstrated that, the MEMI and fractions of MEMI have showed highest lipoxygenase inhibition activity and the inhibition was found to be dose dependent, while Indomethacin a well known NSAID has offered significant and dose dependent inhibition of LOX's activity and found to be more potent than all the extracts and fractions.

These results suggest that, the methanolic extracts and fractions of the methanolic extract of *Mangifera indica* leaves (EMEMI and BMEMI) possess better anti-oxidant and anti-inflammatory activities in *in vitro* conditions.

These findings suggest that, the MEMI was found to possess more potent anti-oxidant anti-inflammatory activity among the extracts. Furthermore, among the fractions of MEMI, EMEMI fraction was found to most potent compared to BMEMI and WMEMI. However. further studies are under progress to isolate the anti-oxidant and anti-inflammatory actives from the fractions of methanolic extract.

## Conflict of interest statement

We declare that we have no conflict of interest.

## References


[1] Charu G, Amar PG, Ramesh CU. Antibacterial activity of Amchur (Dried pulp of Unripe *Mangifera indica*) extracts on some food borne bacteria. *J Pharm Res* 2008; **1**: 54-57.

[2] Ascota JE, Jauregui UH, Amaro DG, Sordo LM. Spray drying of aqueous extract of *Mangifera indica* L (Vimang): Scale up for the process. *World Appl Sci J* 2009; **6**(3): 408-412.

[3] Yutaka S, Keiko K, Masahiro K. Protective activity of mango (*Mangifera indica* L) fruit against a zinc-induced neuronal cell death is independent of its antioxidant activity. *Trace Nutr Res* 2005; **22**: 73-79.

[4] Nunez S, Velez HT, Agilero J, Gonzalez J, Gonzalez J, Naddeo F, et al. Isolation and quantitive analysis of phenolic antioxidants, free sugars and polyols from amngo (*Mangifera indic*a L) stem bark aqueous decoction used in Cuba as a nutritional supplement. *J Agric Food Chem* 2002; **50**: 762-766.

[5] Muanza DN, Euler KL, Williams L, Newman DJ. Screening antitumour and anti-HIV activities of nine medicinal plants form Zaire. *Int J Pharmacogn* 1995; **33**: 98-106.

[6] Guha S, Ghosal S, Chattopadhyay U. Antitumor, immunomodulatory and anti-HIV effect of manigferin, a naturally occurring glucosylxanthone. *Chemotherapy* 1996; **42**; 442-451.

[7] Leiro JM, Alvarez E, Arranz A, Gonzalez-Siso I, Orallo F. *In vitro* effects of mangiferin on superoxide concentrations and expression of the inducible nitric oxide synthase, tumor necrosis factor- β and transforming growth factor- β genes. *Biochem Pharmacol* 2003; **65**: 1361-1371.

[8] Born M, Carrupt PA, Zini R, Bree F, Tillement JP, Hostettmann K, et al. Electrochemical behavior and antioxidant activity of some natural polyphenols. *Helv Chim Acta* 1996; **79**: 1147-1158.

[9] Garrido G, Gonzalez D, Delporte C, Backhouse N, Quintero G, Nunez-Salles AJ, et al. Analgesic and anti-inflammatory effect of *Mangifera indica* L, extract (Vinmang). *Phytother Res* 2001; **15**: 18-21.

[10] Garcia D. Modulator effects of rat macrophage function by *Mangifera indica* L extracdt (Vinmang) and Mangiferin. *Int Immunopharmacol* 2002; **2**(6): 797-806.

[11] Blois MS. Antioxidant determination by the use of a stable free radical. *Nature* 1958; **181**: 1199-1200.

[12] Bouska JJ, Bell RL, Goodfellow CL, Stewart AO, Brooks CD, Carter GW. Improving the *in vivo* duration of 5-lipoxygenase inhibitors: application of an *in vitro* glucuronosyltransferase assay. *Drug Metab Dispos* 1997; **25**(9): 1032-1038.

[13] Kumaraswamy MV, Satish S. Free radical scavenging activity and lipoxygenase inhibition of *Woodfordia fructicosa* Kurz and *Betula utilis* Wall. *African J Biotechnol* 2008; (12): 2013-2016.

[14] Hall ED, Andrus PK, Althaus JS. Von Voigtlander PF. Hydroxyradical production and lipid peroxidation paralleles selective post-Bchemic vulnerability in gerbil brain. *J Neurosci Res* 1993; **34**: 107-112.

[15] Okuda T, Yoshida T, Hostano T, Wagner H, Farnsworth NR. *Economic and medicinal plant research*. London: Farnsworth Academic Press; 1991, p. 129-65.

[16] Kaur SJ, Grover IS. Kumar S. Antimutagenic potential of ellagic acid isolated from *Terminalia arjuna*. *Ind J Exp Biol* 1997; **35**: 478-482.

[17] Rakholiya K, Chanda S. Pharmacognostic, Physicochemical and Phytochemical Investigation of *Mangifera indica* L. var. Kesar leaf. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S680-S684.

[18] Rath S, Dubey D, Sahu MC, Debata NK, Padhy RN. Surveillance of multidrug resistance of 6 uropathogens in a teaching hospital and in vitro control by 25 ethnomedicinal plants used by an aborigine of India. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S818-S829.

[18] Uma Sankar A, Bharti O. *In vitro* 5-Lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. *J Med Plants Res* 2008; **2**(9): 207-212.

[19] Araruna1 K, Carlos B. Anti-inflammatory activities of triterpene lactones from *Lactuca sativa*. *Phytopharmacology* 2010; **1**(1): 1-6.

[20] Kiran I, Sarfraz AN, Abdul M, Naheed R, Naveen M, Pir M, et al. Isolation and lipoxygenase-inhibition studies of phenolic constituents from Ehretia obtusifolia. *Chem Biodivers* 2005; **2**(1): 104-111.