Formulation and evaluation of immune boosting herbal tea

Sushmita L Bhandare and Smita P Borkar

Abstract
The evaluation of anti-inflammatory activity was carried out on the crude aqueous extract of the flowers of Moringa oleifera. The aim of this study was to evaluate the anti-inflammatory activity of Moringa oleifera flowers against Diclofenac Sodium, also to evaluate the Anti-inflammatory activity of prepared Herbal Tea formulation against Diclofenac Sodium as an standard. The anti-inflammatory activity of aqueous flower extract was determined in vitro, by inhibition of thermally induced protein denaturation.

The Moringa oleifera flower extract showed significant inhibition of denaturation of egg albumin in dose dependent manner. This result provide valuable information that Moringa oleifera hold great promise as highly effective as an anti-inflammatory agent.

Keywords: Moringa oleifera, anti-inflammatory activity, pharmacological activities, diclofenac sodium

Introduction
The sensory appeal of tea, like a all food products, is an important consideration in new product development. Tea in general and herb tea in particular are gaining increasing consumer attention due to growing awareness of health benefits derived from their consumption. Even though several under utilized plants exits with potential for processing into herb tea, research in product development of herb tea is limited [1]. The herbal teas are made from herbs, fruits, seeds, roots steeped in hot water. Instant tea may contain very little amounts of actual tea and plenty of sugars. A pharmaceutical branch of Ayurveda has contributed several innovative dosage forms. Conversion of dosage form into more suitable for modern era with additional benefits of palatability and presentation is always essential [1].

Need of present investigation
- Research work on same plant was done by various ways, in current investigation we will the study about flowers of the plant.
- Formulation of investigation plant is unique and easy to use and shall take regular as a health drink.

Fig 1: Drumstick tree

Aim
“Formulation and evaluation of immune boosting herbal tea”.

Objectives
- To prepare immune boosting herbal tea.
- To evaluate the formulation with respect to various physical parameter.
• To evaluate the phytochemical screening of given crude drug.

• To evaluate the formulation with respect to anti-inflammatory activity.

Table 1: Plan of work

<table>
<thead>
<tr>
<th>S. No</th>
<th>Title</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Literature review.</td>
<td>It discovered coagulant extract from Moringa oleifera seed by salt solution.</td>
</tr>
<tr>
<td>2</td>
<td>Selection of drug.</td>
<td>It estimate the study of hypoglycemic agents on rats.</td>
</tr>
<tr>
<td>3</td>
<td>Crude drug profile.</td>
<td>It discovered that antioxidant provide protection against degenerative diseases including cancer, Alzheimer disease.</td>
</tr>
<tr>
<td>4</td>
<td>Materials and method.</td>
<td>It includes aqueous extract of leaves of Moringa oleifera was investigated and rationalized for its wound healing activity.</td>
</tr>
<tr>
<td>5</td>
<td>Experimental work.</td>
<td>It estimate the study of hypotensive, spasmodic activity exhibited by Moringa oleifera constituent.</td>
</tr>
<tr>
<td>6</td>
<td>Formulation of herbal tea.</td>
<td>Nutritional values of Moringa oleifera compared with other food.</td>
</tr>
<tr>
<td>7</td>
<td>Evaluation of herbal tea.</td>
<td>Reminder that heroic lengths and modern science are not always necessary to combat antimicrobial pathogens in remote regions where modern medicine are not available.</td>
</tr>
<tr>
<td>8</td>
<td>Result and conclusion.</td>
<td>M. oleifera mainly contains alkaloids, flavonoids, anthocyanins, proanthocyanidins and cinnamates.</td>
</tr>
</tbody>
</table>

Literature review

Table 2: List of reference papers

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Title of paper</th>
<th>Author</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isolation and characterization of coagulant extracted form of Moringa oleifera seed and salt solution.</td>
<td>Tetsuji Okuda; Aloysius U. Baes.</td>
<td>It discovered coagulant extract from Moringa oleifera seed by salt solution.</td>
</tr>
<tr>
<td>2</td>
<td>Effect of Moringa oleifera leaves aqueous extract therapy on hypoglycemic rats</td>
<td>Dolly Jaiswal, Prashant kumar Rani, Amit kumar</td>
<td>It estimate the study of hypoglycemic agents on rats.</td>
</tr>
<tr>
<td>3</td>
<td>Effect of antioxidant activity of Moringa oleifera leaves</td>
<td>Shahid Iqbal, M.I. Bhaeter</td>
<td>It discovered that antioxidant provide protection against degenerative diseases including cancer, Alzheimer disease.</td>
</tr>
<tr>
<td>4</td>
<td>Evaluation of Aqueous leaves extract of Moringa oleifera for wound healing in albino rats</td>
<td>B.S. Rath, S.L. Bodhankar and A.M. Baheti</td>
<td>It includes aqueous extract of leaves of Moringa oleifera was investigated and rationalized for its wound healing activity.</td>
</tr>
<tr>
<td>5</td>
<td>Pharmacological studies on Hypotensive and spasmodic activities of Moringa oleifera</td>
<td>Anwar H. Gilani, Khalid Afub</td>
<td>It estimate the study of hypotensive, spasmodic activity exhibited by Moringa oleifera constituent.</td>
</tr>
<tr>
<td>6</td>
<td>The review work deals with nutritional, therapeutic, traditional uses or benefits of Moringa.</td>
<td>Khawaja Tahir Mahmood, Tahir Mugal and Ikram UL Haq.</td>
<td>Nutritional values of Moringa oleifera compared with other food.</td>
</tr>
<tr>
<td>7</td>
<td>Potential uses of Moringa oleifera and examination of antibiotic efficacy conferred by M. oleifera seed and leaf extract used by crude extraction techniques.</td>
<td>Rockwood J.L., Anderson B.G, Casamatta D.A.</td>
<td>Reminder that heroic lengths and modern science are not always necessary to combat antimicrobial pathogens in remote regions where modern medicine are not available.</td>
</tr>
</tbody>
</table>

Selection of drug

Moringa oleifera is one of the miracle tree, is widely cultivated throughout India, belong to Family Moringaceae. It is widely used as a nutritive herb and possess valuable pharmacological activities. present article describes habitat, pharmacognostic features hytochemistry, nutritive values and pharmacological activities like anticancer, antimicrobial, anti-inflammatory, antihyperlipidemic, hypotensive, anti diabetic, hepatoprotective, antiasthmatic, anthelminic, anti-fertility, etc of moringa. It is one of the rich sources of vitamin C, milk protein, etc. Present review gives the information of all essential nutrients that are needed to improve immunity [8].

Pharmacological activities

1. Anti-cancer activity

Various extracts of leaves and ethanolic extract of seeds of Moringa oleifera shows anti tumor activity in-vitro tests, Thiocarbamate and isothiocyanate related compounds were isolated, which act as inhibitor of tumor promoter teleocidin B-4-induced Epstein Barr virus (EBV) activation in Raji cells [8].

2. Anti-fertility activity

Aqueous extract of Moringa oleifera was found be effective as an anti-fertility in presence as well as absence of estradiol dipropionate and progesterone and shown incresed histoarchitecture of uterine [8].

3. Anti-oxidant activity

Moringa oleifera exhibit strong anti-oxidant and radical scavenging activity [8].

4. Cardiovascular activity

Ethanol extract of Moringa oleifera shows antihypertensive or hypotensive activity. It was found that thiocarbamate and isothiocyanate glycosides are responsible for this promising hypotensive activity [8].

5. Anti-epileptic activity

Methanolic extract of Moringa oleifera were investigated its anti-convulsant activity using pentylentetrazole (PTZ) and maximum electric shock (MES) on male albino mice [8].

6. Anti-asthmatic activity

Moringa oleifera were found spasmodytic in Acetylcholin, histamine, BacI2 and 5HT induced bronchospasm [8].

7. Anti-ulcer activity

Antiucler activity in various animal models on adult Holtzman albino rats of either sex [8].
8. Anti-inflammatory activities
Methanolic extract of leaves and flowers as well as ethanolic extract of seeds of *Moringa oleifera* has shown anti-inflammatory activity in carrageenan induced paw edema model. Aurantiamide acetate and 1, 3 dibenzyl urea, isolated from roots shown this anti-inflammatory activity so they responsible for anti inflammatory activity of *Moringa oleifera*. anti-inflammatory agents are used to cure inflammation caused by prostaglandin (PGE2). Drugs with analgesic, antipyretic, and anti-inflammatory effects -they reduce pain, fever, inflammation [8].

**Classification**
1. Salicylic acid derivatives- sodium salicylate, Aspirin
2. p-Aminophenol derivatives - paracetamol, phenacetin
3. Pyrazolidinedione derivatives- Plenylbutazone
4. Anthranilic acid derivatives - Mefenamic acid, Meclofenamate
5. Aryl alkanoic acid derivatives-
   a) Indoleacetic acid: Indomethacin
   b) Indeneacetic acid: Sulindac
   c) Pyroleacetic acid: Tolmentin,
   d) Phenylacetic acid: Ibuprofen, Diclofenac

6. Oxicams- Piroxicam

7. Miscellaneous
Nimesulide

**MOA**
Inhibitors of the enzyme cyclo-oxygenase, inhibiting both the cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) isoenzymes. Cyclo-oxygenase catalyses the formation of prostaglandins, prostacyclin and thromboxane from arachidonic acid (Which is derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act as messenger molecules in the process of inflammation.

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**Crude drug profile**
**Introduction to Moringa oleifera**
Name: *Moringa oleifera*

**Synonyms** [8]
Sanskrit: Subhanjan
Marathi: Shevga
Hindi: Saguna, sainjna
English: Drumstick tree
Tamil: Morigkai
Family: Moringaceae

Fig 2: Mode of action of Anti-inflammatory drugs
**Taxonomic classification** [8]

Kingdom: Plantae
Sub kingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliopsida
Class: Magnoliopsida
Sub class: Dilleniidae
Order: Capparales
Genus: Moringa
Species: oleifera

**Termeric**

**Synonyms** [4]: Curcuma longa, Curcuma herb

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**Biological Source:** It is dried root and rhizomes of *Curcuma longa.*

**Family:** Zingiberaceae

**Taxonomical Classification** [4]

Kingdom: Plantae.
Division: Magnoliophyta
Class: Liliopsida
Subclass: Commelinids
Order: Zingiberales
Genus: Curcuma Species Curcuma longa

The wild turmeric is called *C. aromatica* and the domestic species is called *C. longa.*

**Chemical composition**

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has α-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%) Curcumin was first isolated in 1815 and its chemical structure was determined by Roughley and Whiting in 1973 melting. It has a point at 176–177 °C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform.

**Pharmacological actions of curcumin**

1. **Effect on cardiovascular system**

Curcumin decreases severity of pathological changes thus protects from damage caused by myocardial infarction. Curcumin improves Ca2+ transport and its slippage from the cardiac muscle sarcoplasmic reticulum, thereby raising the possibility of pharmacological interventions to correct the defective Ca2+ homeostasis in the cardiac muscle. Curcumin has significant hypocholesteremic effect in hypercholesteremic rats [4].

2. **Effect on nervous system**

Curcumin and manganese complex of curcumin offer protective action against vascular dementia by exerting antioxidant activity [4].

3. **Anti-inflammatory activity**

Curcumin is effective against carrageenin-induced oedema in rats and mice. The natural analogues of curcumin, viz. FHM and BHM, are also potent anti-inflammatory agents. The volatile oil and also the petroleum ether, alcohol and water extracts of Curcuma longa show anti-inflammatory effects [4].

4. **Anticoagulant activity**

Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta [4].

5. **Antidiabetic effect**

Curcumin prevents galactose-induced cataract formation at very low doses. Both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes in rat. Curcumin also decreases advanced glycation end products induced complications in diabetes mellitus [4].

6. **Antifungal effect**

Ether and chloroform extracts and oil of *C. longa* have antifungal effects. Crude ethanol extract also possesses antifungal activity. Turmeric oil is also active against Aspergillus flavus, A. parasiticus, Fusarium moniliforme and Penicillium digitatum [4].

7. **Antivenom effect**

Ar-turmerone, isolated from Curcuma longa, neutralizes both haemorrhagic activity of Bothrops venom and 70% lethal effect of Crotalus venom in mice. It acts as an enzymatic inhibitor of venom enzymes with proteolytic activities [4].

**Cardamom**

**Synonym** [9]: Choti – Ilalchi (Hindi)

**Biological source:** Ripe fruit of *Elettaria cardamomum* var

**Family:** Zingiberacea

**Taxonomic classification** [5]

Kingdom: Plantae
Order: Zingiberales
Genera: Elettaria Amomum
Fig 5: Cardamom seeds

Chemical composition
Seeds of Elettaria cardamomum are rich in volatile oil that mainly includes phenolic and flavonoid components. Starch, protein, waxes and Sterols are other components of the oil.

Pharmacological activities
1. Antibacterial activity
Ethanolic extract of E. cardamomum possess antibacterial effect at the dose of 512 μg/mL. Toxicity of the extract was observed at 0.3 mg/g, which showed inflammation in brain, oxidative stress and cells necrosis in heart. The use of E. cardamomum as spice should not exceed the 0.003 mg/g since at this amount no negative effects were observed [5].

2. Gastroprotective activity
Gastroprotective activity of E. cardamomum was best found in the petroleum ether soluble extract which inhibited lesions in the stored product insects attacking wheat, e.g. Tribolium castaneum and Sitophilus zeamais, via contact and fumigant action. This activity at this amount no negative effects were observed [5].

3. Antioxidant activity
Cardamom oil is effective as an antioxidant and can increase levels of glutathione, a natural antioxidant in body. The effect is increased by increasing the content of the oil from 100 to 5000 ppm [5].

4. Insecticidal activity
The volatile oil from cardamom acts as a potential grain protectant by killing various life stages of the stored product insects attacking wheat, e.g. Tribolium castaneum and Sitophilus zeamais, via contact and fumigant action [5].

Materials and Methods
Materials
- Moringa oleifera flowers.
- Turmeric.
- Cardamom.

Methods
To determine anti-inflammatory activity of Moringa oleifera flowers.

Chemicals

Table 3: List of chemicals used

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of ingredients</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diclofenac Sodium tablet IP 50mg</td>
<td>Acme Generics LLP Davni, Tehsil Nalegarh.</td>
</tr>
<tr>
<td>2.</td>
<td>Phosphate Buffer Saline pH 6.4</td>
<td>A.G.C.O.P. Satara</td>
</tr>
</tbody>
</table>

Equipments

Table 4: List of equipments used

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Instrument</th>
<th>Make &amp; Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Incubator</td>
<td>Quality, QBOD-05</td>
</tr>
<tr>
<td>2.</td>
<td>Hot air oven</td>
<td>Bio-Techniques India, BTI-26</td>
</tr>
<tr>
<td>3.</td>
<td>Vacuum pump</td>
<td>Value, VE115N</td>
</tr>
<tr>
<td>4.</td>
<td>Refrigerator</td>
<td>Blue Star, CHF150C</td>
</tr>
<tr>
<td>5.</td>
<td>Electronic balance</td>
<td>Shimadzu, BL-22OH</td>
</tr>
<tr>
<td>6.</td>
<td>UV Spectrophotometer</td>
<td>Dynamic, Halo DB-20</td>
</tr>
<tr>
<td>7.</td>
<td>Muffle Furnace</td>
<td>Hally Instruments, HI-25</td>
</tr>
</tbody>
</table>

Experimental work
Preparation of herbal tea [9]

General method of preparation emphasized for shatavari granules is follow preparation of Moringa oleifera herbal tea. Coarse powder of Moringa oleifera flowers

Mixed with sugar syrup
The mixture was heated on mild fire (Mandagni) i.e 90 °C - 100 °C till it attained more than two thread consistency of sugar syrup
- 1 hrs 30 min of heating - adhesion of syrup to spoon.
- 1 hrs 50 min of heating-syrup was found to be in a two thread consistency.
- 2 hrs 5 min of heating - not instant dissolution in water.

Add Turmeric as a Anti-inflammatory agent and also as a colouring agent
Add cardamom as a flavouring agent
The contents were removed from heat source
Thus obtained mass was dried in hot air oven and subjected to multi mill sieve to obtain granules

Herbal tea

Formulation of herbal tea

Table 5: Formula for herbal tea

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1(gm)</td>
<td>F2(gm)</td>
</tr>
<tr>
<td>Moringa oleifera Flowers</td>
<td>2</td>
</tr>
<tr>
<td>Sugar</td>
<td>10</td>
</tr>
<tr>
<td>Turmeric</td>
<td>0.030</td>
</tr>
<tr>
<td>Cardamom</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Extraction of plant material
In the present study, the dried powdered flower were extracted by aqueous extraction method at room temperature with occasional shaking. The extract was filtered and reextracted by same process until plant material were exhausted. The collected filtrates were pooled and evaporated to dryness under reduce the pressure to yield dry extract and was stored at 4°C until used.

Result and Discussion
Evaluation of in-vitro anti-inflammatory activity
Anti-inflammatory activity of M. oleifera flower extract was evaluated by protein denaturation method. Diclofenac Sodium, a powerful non-steroidal anti-inflammatory drug was used as standard drug.
Reaction mixture consists of 2 ml of different concentrations of *M. oleifera* flower extract (ug/ml) or standard Diclofenac Sodium (ug/ml) and 2.8 ml of Phosphate Buffered Saline pH (6.4) was mixed with 0.2 ml of egg albumin (From fresh hen’s egg) and Incubated at (27 °C) for 15min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate and average was taken. The percentage inhibition of protein denaturation was calculated by using following formula [6].

\[
\% \text{ inhibition} = \frac{A_t - A_c \times 100}{A_c}
\]

Were,

- \( A_t \) = Absorbance of Test
- \( A_c \) = Absorbance of Standard/control

### Evaluation parameters

#### Determination of ash value

Ash value are helpful in determining the quality and purity of crude drug, especially in powder form. The objective of ash vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.

1. **Total Ash value**

Weight accurately about 2 gm of powdered drug in a tarred silica crucible. Incinerated at a temperature not exceeding 450 °C for 4hrs, until free from carbon, cooled and weighted.

\[
\% \text{ Total Ash value} = \frac{\text{Wt. Of total ash}}{\text{Wt. Of crude drug taken}} \times 100
\]

= 7.9 %w/w

2. **Water soluble ash value**

The ash boiled with 25 ml of water, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in tarred crucible at temperature not exceeding 450 °C for 4 hrs cooled in desiccatior and weighted. Calculated of percentage of acid insoluble ash with the reference to the air dried drug.

\[
\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of total ash} - \text{Wt of water insoluble ash}}{\text{Wt of crude drug taken}} \times 100
\]

= 3.45 %w/w

3. **Acid insoluble ash value**

Boiled the ash for 5 min with 25 ml of 2 M HCL. Filtered and collected the insoluble matter on ash less filter paper, washed with hot water and ignited in tarred crucible the temperature not exceeding 450 °C for 4 hrs. Cooled in dissociator and weighted. Calculated percentage of acid insoluble ash with the reference to the air dried drug.

4. **Bulk density (gm/ml)**

- Bulk volume in ml
- Mass of Granule in gram

\[
\text{Bulk density} = \frac{\text{Mass}}{\text{Tapped Volume}} = 0.78 \text{ g/ml}
\]

5. **Tapped density (gm/ml)**

- Tapped volume in ml
- Mass of granule in gram

\[
\text{Tapped density} = \frac{\text{Mass}}{\text{Tapped Volume}} = 0.78 \text{ g/ml}
\]

6. **Angle of repose**

- Height of pile in cm
- Average radius of circle in cm

\[
\text{Angle of repose (} \theta \text{)} = \tan^{-1}(h/r) = 18.26
\]

### Preliminary phytochemical analysis of *M. oleifera* flower extract [3]

#### Table 6: Preliminary phytochemical screening of *M. oleifera* flower extract

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Name of test</th>
<th>Flower extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>5%FeCL&lt;sub&gt;3&lt;/sub&gt;,Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowski test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Hagers test, Meyers test, Wagners test</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molishs test</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski test</td>
<td>+</td>
</tr>
<tr>
<td>Cardic Glycoside</td>
<td>Keller killiani test</td>
<td>+</td>
</tr>
</tbody>
</table>

### In vitro Anti-inflammatory effect of *M. oleifera* flower extract

#### Table 7: % inhibition of protein denaturation by *M. oleifera* flower extract

<table>
<thead>
<tr>
<th>Conc.(ug/ml)</th>
<th>Absorbance of test (Extract)</th>
<th>Absorbance of reference (Diclofenac sodium)</th>
<th>% Inhibition of protein denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.0837</td>
<td>0.0489</td>
<td>71.16</td>
</tr>
<tr>
<td>400</td>
<td>0.1553</td>
<td>0.0621</td>
<td>150.08</td>
</tr>
</tbody>
</table>
**In vitro Anti-inflammatory effect of prepared herbal tea formulation**

Table 8: % inhibition of protein denaturation by prepared herbal tea formulation

<table>
<thead>
<tr>
<th>Conc.(ug/ml)</th>
<th>Absorbance of test (formulation)</th>
<th>Absorbance of reference (Diclofenac sodium)</th>
<th>% inhibition of protein denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.0927</td>
<td>0.0489</td>
<td>89.57</td>
</tr>
<tr>
<td>400</td>
<td>0.1270</td>
<td>0.0621</td>
<td>104.50</td>
</tr>
</tbody>
</table>

**Conclusion**

The Medicinal Plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to presence of secondary plant metabolites such as alkaloids, flavonoids, glyacosides, tannin etc. The result of our study suggest that the aqueous extract of *Moringa oleifera* flowers shows marked *in-vitro* anti-inflammatory activity in dose dependent manner.

**References**


