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Sushmita L Bhandare
Department of Pharmacognosy, Arvind Gavali College of Pharmacy, Jaitapur, Satara, Shivaji University Kolhapur, Maharashtra, India

Smita P Borkar
Department of Pharmaceutics, Arvind Gavali College of Pharmacy, Jaitapur, Satara, Shivaji University Kolhapur, Maharashtra, India

Correspondence
Sushmita L Bhandare
Department of Pharmacognosy, Arvind Gavali College of Pharmacy, Jaitapur, Satara, Shivaji University Kolhapur, Maharashtra, India

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 standared. 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

S. No	Title
1	Literature review.
2	Selection of drug.
3	Crude drug profile.
4	Materials and method.
5	Experimental work.
6	Formulation of herbal tea.
7	Evaluation of herbal tea.
8	Result and conclusion.
9	Reference.

Literature review

Table 2: List of reference papers

S. No.	Title of paper	Author	Information
1.	Isolation and characterization of coagulant extracted form of moringa olefera seed and salt solution.	Tetsuji Okuda; Aloysius U. Baes	It discovered coagulant extract from Moringa olifera seed by salt solution.
2.	Effect of *Moringa oleifera* leaves aqueous extract therapy on hypoglycemic rats	Dolly Jaiswal, Prashant kumar Rani, Amit kumar	It estimate the study of hypoglycemic agents on rats.
3.	Effect of antioxidant activity of *Moringa oleifera* leaves	Shahid Iqbal, M.I. Bhanger	It discovered that antioxidant provide protection against degenerative diseases including cancer, Alzheimer disease.
4.	Evaluation of Aqueous leaves extract of *Moringa oleifera* for wound healing in albino rats	B.S. Rathi, S.L. Bodhankar and A.M. Baheti	It includes aqueous extract of leaves of *Moringa oleifera* was investigated and rationalized for its wound healing activity.
5.	Pharmacological studies on Hypotensive and spasmolytic activities of *Moringa oleifera*	Anwar H. gilani, Khalid Aftab	It estimate the study of hypotensive, spasmolytic activity exhibited by *Moringa oleifera* constituent.
6.	The review work deals with nutritional, therapeutic, traditional uses or benefits of moringa.	Khawaja Tahir Mahmood, Tahir Mugal and Ikram UL Haq.	Nutritional values of *Moringa oleifera* compared with other food.
7.	Potential uses of *Moringa oleifera* and examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extract used by crude extraction techniques.	Rockwood J.L, Anderson B.G, Casamatta D.A.	Reminder that heroic lengths and modern science are not always necessary to combat antimicrobial pathogens in remote regions where modern medicine are not available.
8.	Phyto-pharmacology of *Moringa oleifera* Lam, An overview.	Bhoomika R Goyal, Babita B Agrawal, Ramesh K Goyal and Anita A Mehta.	*M. oleifera* mainly contains alkaloids, flavonoids, anthocyanins, proanthocyanidins and cinnamates.

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, pharmacogonstic features hytochemistry, nutritive values and pharmacological activities like anticancer, antimicrobial, anti-inflamatory, antihyperlipidemic, hypotensive, antidiabetic, hepatoprotective, antiasthemetic, 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-fertiliy activity

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

3. Anti-oxidant acitivity

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

4. Cardiovascular activity

Ethanolic extract of *Moringa oleifera* shows antihypertensiv 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 pentylenetetrazole (PTZ) and maximum electric shock (MES) on male albino mice [8].

6. Anti-asthmatic activity

Moringa oleifera were found spasmolytic in Acetylcholin, histamine, Bacl2 and 5HT induced bronchospasm [8].

7. Anti-ulcer activity

Antiulcer 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 intiinflammatory 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-Aminoplenol 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) Pyrroleacetic 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 phospolipid bilayer by phospolipase A2). Prostaglandins act as messenger molecules in the process of inflammation.

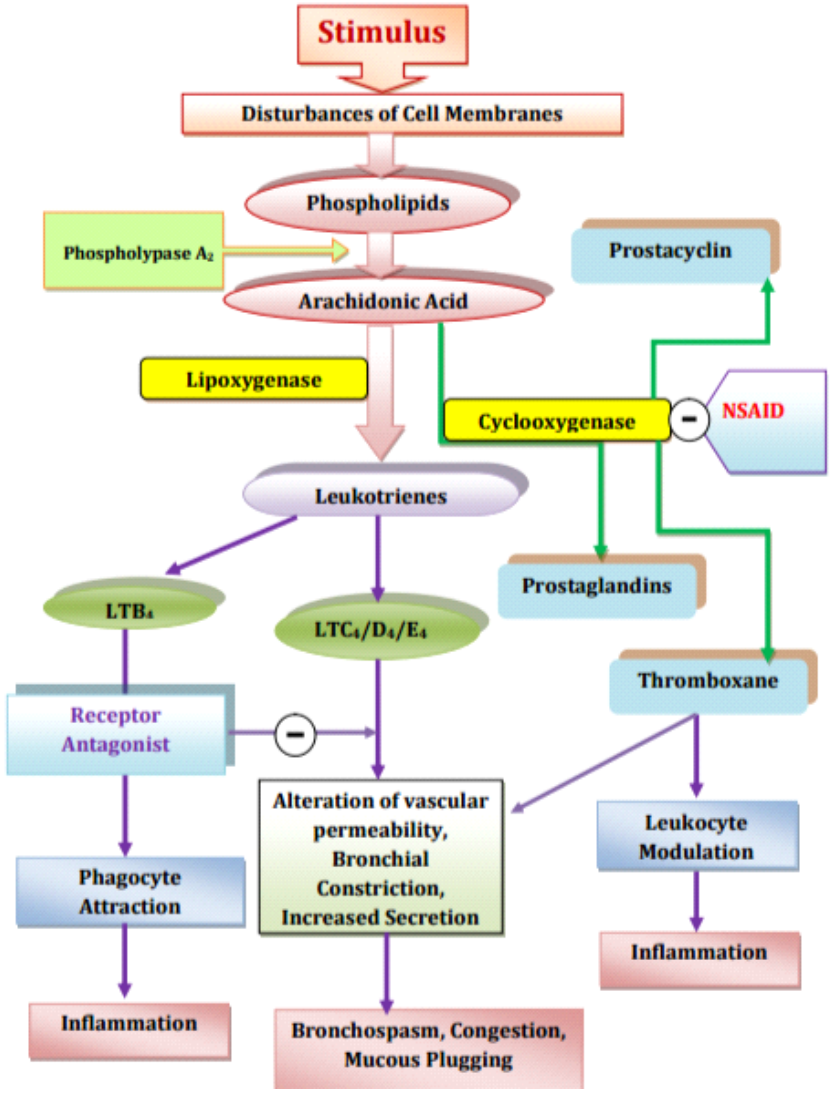

Fig 2: Mode of action of Anti-inflammatory drugs

Crud 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 3: *M. oleifera* flower

Taxonomic classification [8]
Kingdom: Plantae
Sub kingdom: Tracheobionata
Super division: Spermatophyta
Division: Magnoliopsida
Class: Magnoliopsida
Sub class: Dilleniidae
Order: Capparales
Genus: Moringa
Species: oleifera

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

Fig 4: Termeric

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 a-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 9 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 [4].

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 [5]**:** Choti – Ilalchi (Hindi)
Biological source: Ripe fruit of *Elettaria cardamomum* var
Family: Zingiberacea

Taxonomic classification [5]
Kingdom: Plantae
Order: Zingiberales
Genera: Elettarai 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 o.

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 by nearly 100% at 12.5 mg/kg in the aspirin-induced gastric ulcer. Methanolic extract also possess gastro protective effect [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

S. No	Name of ingredients	Source
1.	Diclofenac Sodium tablet IP 50mg	Acme Generics LLP Davni, Tehsil Nalegarh.
2.	Phosphate Buffer Saline pH 6.4	A.G.C.O.P. Satara

Equipments

Table 4: List of equipments used

S. No.	Instrument	Make & Model
1.	Incubator	Quality, QBOD-05
2.	Hot air oven	Bio-Techniqcs India, BTI-26
3.	Vaccum pump	Value, VE115N
4.	Refrigerator	Blue Star, CHF150C
5.	Electronic balance	Shimadzu, BL-22OH
6.	Uv Spectrophotometer	Dynamic, Halo DB-20
7.	Muffle Furnance	Hally Instruments, HI-25

Experimental work

Preparation of herbal tea [7]

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 ^{0}C-100^{0}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

Ingredients	Formulation	
	F1(gm)	F2(gm)
Moringa oleifera Flowers	2	8.33
Sugar	10	41.66
Turmeric	0.030	0.125
Cardamom	0.030	0.125

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 re-extracted 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^{0}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 standared 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 ^{0}C) for 15min. Denaturation was induced by keeping the reaction mixture at 70 ^{0}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].

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

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 ^{0}C for 4hrs, until free from carbon, cooled and wighted.

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

$$= 7.9 \text{ \%w/w}$$

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 a tarred crucible at temperature not exceeding 450 ^{0}C for 4 hrs cooled in desiccators, weighted and sub stracted wight off insoluble matter from the total weight of ash.

% water soluble ash value =

$$\frac{\text{Wt. of total ash-Wt of water insoluble ash}}{\text{Wt. Of crude drug taken}} \times 100$$

$$= 2.85 \text{ \%w/w}$$

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 ^{0}C for 4 hrs. Cooled in dissicator and weighted. Calculated of percentage of acid insoluble ash with the reference to the air dried drug.

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

$$= 3.45 \text{ \%w/w}$$

4. Bulk density (gm/ml)[2]

- Bulk volume in ml
- Mass of Granule in gram

$$\text{Bulk density} = \frac{\text{Mass}}{\text{Bulk Volume}}$$

Bulk Volume =0.60 g/ml

5. Tapped density (gm/ml)[2]

- Tapped volume in ml
- Mass of granule in gram

$$\text{Tapped density} = \frac{\text{Mass}}{\text{Tapped Volume}}$$

= 0.78 g/ml

6. Angle of repose[2]

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

Angle of repose (θ) = $\tan^{-1}(h/r)$
=18.26

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

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

Phytochemical tests	Name of test	Flower extract
Tannins	5%$FeCL_3$,Lead acetate test	+
Steroids	Salkowski test	+
Flavonoids	Shinoda test	+
Alkaloids	Hagers test Meyers test Wagners test	+
Carbohydrates	Molishs test	+
Terpenoids	Salkowski test	+
Cardic Glycoside	Keller killiani test	+

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

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

Conc.(ug/ml)	Absorbance of test (Extract)	Absorbance of reference (Diclofenac sodium)	% Inhibition of protein denaturation
100	0.0837	0.0489	71.16
400	0.1553	0.0621	150.08

In vitro Anti-inflammatory effect of prepared herbal tea formulation

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

Conc.(ug/ml)	Absorbnce of test (formulation)	Absorbance of reference (Diclofenac sodium)	% inhibition of protein denaturation
100	0.0927	0.0489	89.57
400	0.1270	0.0621	104.50

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.

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