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Phytochemical Investigation and Pharmacological Screening of *Mimusops elengi* Linn. bark for its in-vitro anti arthritic and wound healing activity

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ABSTRACT

Mimusops elengi is the most traditionally used medicinal plant that belongs to family Sapotaceae, commonly known as Spanish cherry is native to the Western Ghat region of the peninsular India. By looking the high traditional use of the plant , the present investigation was undertaken which deals with pharmacognostic (moisture content, ash value, extractive value) and phytochemical screening of *Mimusops elengi* bark .The main aim of this study was to evaluate the invitro anti- arthritic (Protein denaturation and Heat induced haemolysis) and wound healing (Chick chorioallantoic membrane model) activity for methanolic extract of bark. The maximum membrane stabilization of methanolic extract of *M. elengi* L bark was found to be 80.31% at a dose of 250mcg/ ml and that of inhibition of protein denaturation was found to be 82.37% at a dose of 250mcg/ml with regards to standard (Diclofenac sodium) in the anti-arthritic activity. The alcoholic extract was also capable of promoting angiogenesis in chick eggs which is an indication of its wound healing activity. From the results it can be concluded that *M. elengi* extract shows good in vitro anti-arthritic and wound healing activities.

Keywords: *Mimusops elengi*, Pharmacognostic study, Phytochemical screening, Phytochemistry, Anti-arthritic, Wound healing.

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INTRODUCTION

Herbal medicine also called botanical medicine or phytomedicine refers to using plants berries, seeds, roots, leaves, flowers or barks for medicinal purpose. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease. World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs for their primary health care needs[1]. These herbal drugs and Indian medicinal plants are also rich sources of beneficial compounds including antioxidants and components that can be used in functional foods. Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principles will yield rich dividends in the near future in improving health, especially among people who do not have access to the use of costlier western systems of medicine.[2]

Mimusops elengi belonging to the family Sapotaceae a small to large evergreen tree, grows up to 15 m high. Generally characterized by a short, dark and very rough trunk and wide spreading, the ends of which tend to rise and forms a thick globular head to the tree and the parts used are leaves, flowers, seeds, fruits and bark.[3] Bark of *Mimusops elengi* has been traditionally used by folks against arthritis. Since no scientific study has been carried out on a particular activity and on the basis of leads available from folk usage and literature collected, *Mimusops elengi* Linn. bark was selected to screen for its anti arthritic activity.

Rheumatoid arthritis (RA) is an auto immune disease that results in a chronic systemic inflammatory disorder that may affect many tissues and organs but principally attacks flexible (synovial) joints.[4] The anti arthritic study was done by an in vitro method i.e inhibition of protein denaturation and heat induced haemolysis method.

Wound healing is an intricate process in which the skin (or another organ tissue) repairs itself after injury.[5] The classic model of wound healing comprises three or four sequential phases :- Hemostasis, Inflammation, Proliferation and Remodelling. Upon injury to the skin, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage[6]. With the use of antibiotics, a new era in the management of wound infections commenced. Unfortunately, eradication of the infective plague affecting surgical wounds has not ended because of the insurgence of antibiotic-resistant bacterial strains and the nature of more adventurous surgical intervention in immuno compromised patients and in implant surgery. The

process of wound healing is promoted by several natural products which are composed of active principles like triterpenes, alkaloids, flavanoids and other biomolecules.

MATERIALS AND METHOD

Materials

Bovine serum albumin (5% aqueous solution), 1N HCI, Phosphate Saline, buffer (pH 6.3), Diclofenac sodium, Methanolic extract of *Mimusops elengi*, Whole human blood, Normal saline, Methyl cellulose disk loaded with *Mimusops elengi* extract. All the reagents were obtained and prepared in college laboratory.

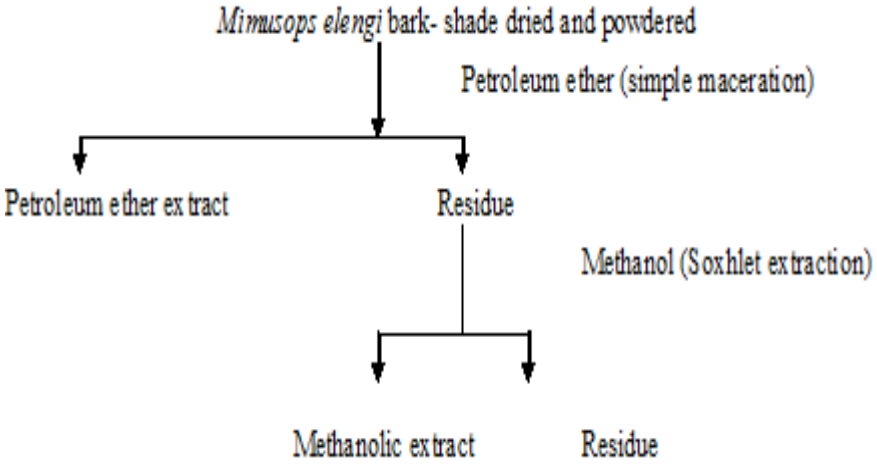
Methods

A. Collection and authentification of sample

The *M. elengi* bark used in the present study were collected from Piravom, Ernakulam district, Kerala. The first step in the standardization of a plant is its correct identification. The plant was authenticated by the botanist, Dr. M E Kuriakose, Department of Botany, K G College, Kottayam.

B. Extraction of *Mimusops elengi* bark

Barks were collected from the tree and dried at room temperature to remove moisture, and size reduced. Extraction was carried out by continuous hot extraction using alcohol as the solvent.



C. Phytochemical screening

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, carbohydrates, proteins/amino acids, glycosides, fixed oils & fats phenolics, tannins, phytosterols, flavonoids, saponins. [7, 8.]

D. Physicochemical investigation

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material.

E. Biological evaluation

ANTI-ARTHRITIC ACTIVITY

Inhibition of Protein Denaturation:- [9]

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of plant extract (250 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl . The samples were incubated at 37°C for 20 min and then heated at 57°C for 3min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition = 100 - (O.D. of test – O.D. of product control) /O.D. of Control x 100

Heat Induced Haemolysis:-[10]

Preparation of RBC suspension:-

The blood (10 ml) was collected from healthy human volunteer who has not taken NSAIDs for 2 weeks prior to the experiment and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed 3 times with equal volume of normal saline. The volume of blood was measured and re constituted as 10%v/v suspension with normal saline.

Heat induced haemolysis:-

The reaction mixture (2ml) consisted of 1ml test sample of concentration (250mcg/ml) and 1ml of 10% RBC suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was used as standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath 56ºC for 30 minutes. At the end of incubation the tubes were cooled at running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicate for all the test samples.

Percentage inhibition of haemolysis = (Absorbance of control-Absorbance of sample) / Absorbance of control. X 100

WOUND HEALING ACTIVITY:-[11, 12.]

Preparation of methyl cellulose disk loaded with extract: 10 mgs each of extract and methyl cellulose is dissolved in 15 ml of distilled water. Added a drop of glycerine. Stirred well and poured into a petri plate. It is then modified into a film by keeping in a hot air oven at 70ºC for 4 hrs.

Grouping of eggs:

In this experiment a total of 6 eggs were used. They were divided into 2 groups according to the following manner.

Group 1- Control treated with simple methylcellulose disk

Group 2- Test group treated with methyl cellulose disk loaded with alcoholic extract of *Mimusops elengi* bark.

Chick chorioallantoic (CCA) membrane model:

This model was used to assess the angiogenic activity of the extract ten day old fertilized chick eggs were selected and a small window of 1.0cm² made in the shell. A small hole was drilled at the air space and air was sucked using a rubber bulb, as a result of which the membrane fell. The window was opened and a sterile disk of methylcellulose loaded with *Mimusops elengi* bark extract was placed in at the junction of two big vessels. The window was resealed by tape and the eggs were incubated at 37ºC in a well humidified chamber for 72 hrs. The eggs were then opened. New vessel formation was observed and compared with that in eggs containing disk without plant extract.

RESULTS AND DISCUSSION:-

All the results generated from the present study are represented in the respective tables. The powdered bark of *Mimusops elengi* were subjected to physicochemical and preliminary phytochemicals analyses which were found to be very promising.

Phytochemical screening

Preliminary phytochemical screening mainly revealed the presence of carbohydrates, proteins /amino acids, terpenoid, sterols, glycosides, saponins, flavonoids, and phenolics/ tannins in alcoholic extract and petroleum ether extract contains only terpenoids and saponins.[13, 14, 15 ,16, 21.] The results pertaining to this investigation were presented in Table 1.

Table.1: Phytochemical Screening

Sl.no	Phytoconstituents	Petroleum ether extract	Alcoholic extract
1.	**Alkaloids**		
a.	Mayer's test	-	-
b.	Wagner's test	-	-
c.	Hager's test	-	-

d.	Dragendroff's test	-	-
2.	**Glycosides**		
a.	Legal test	-	+
b.	Baljet test	-	+
3.	**Phenolics**		
a.	Ferric chloride test	-	+
b.	Lead acetate test	-	+
4.	**Flavones and Flavonoids**		
a.	Aqueous sodium hydroxide test	-	++
b.	Shinoda test	-	++
5.	**Carbohydrates**		
a.	Molisch's test	-	+
b.	Benedict test	-	+
c.	Fehlings test	-	+
6.	**Terpenoids**		
a.	Isoprenoid test	++	+
7.	**Sterols**		
a.	Libermann Burchard test	-	+
b.	Salkowski's test	-	+
8.	**Proteins and aminoacids**		
a.	Millon's test	-	+
b.	Biuret test	-	+
c.	Ninhydrin test	-	+
9.	**Saponins**		
a.	Foam/Froth test	+	+
10.	**Tannins**	-	+
	Ferric chloride test		

Physicochemical parameters

Table.2: Physicochemical Parameters

Sl.no.	Parameters	% w/w
1.	**Ash value**	
	a) Total Ash	5.80
	b) Acid insoluble ash	1.44
	c) Water soluble ash	4.23
2.	**Loss on drying**	2.305
3.	**Extractive value**	
	a) Alcohol soluble	12.2
	b) Water soluble	10.33

The determination of various physicochemical parameters i.e. total ash, acid insoluble ash, water soluble ash, loss on drying, alcohol soluble extractive and water soluble extractive values were calculated as per Indian Pharmacopoeia.[17,18, 22.] The results are tabulated in the Table 2.

Biological Evaluation

ANTI ARTHRITIC ACTIVITY [19,20,23]

1. Inhibition of protein denaturation

The extracts of *M. elengi* were analyzed for its anti-arthritic activity and it is compared with the

activity of the standard Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that the extracts are capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease. Control shows 100% denaturation. The results are tabulated in Table 3. The comparison of the anti- arthritic activity is given in the figure1.

Table.3: Percentage Inhibition of Protein Denaturation

Sample	Concentration(µg/ml)	% Inhibition
Standard	250	96.88±0.37
Test	250	80.31±0.64

Values expressed as mean ± SD

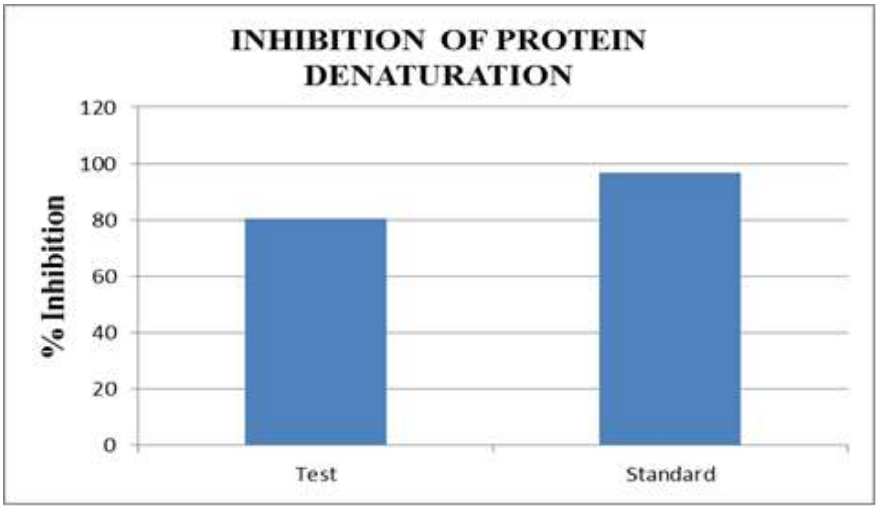


Figure 1: Inhibition of protein denaturation

2. Heat induced haemolysis

The extracts of *M. elengi* were analyzed for its anti-arthritic activity and it is compared with the activity of the standard Diclofenac sodium. From the results of present study, it can be stated that the extracts are capable of stabilizing erythrocyte membrane in rheumatic disease. Control shows 100% denaturation. The results are tabulated in Table. 4. The comparison of the anti- arthritic activity is given in the figure 2.

Table.4: Percentage Inhibition of Haemolysis

Sample	Concentration(µg/ml)	% Inhibition
Standard	250	90.24±0.52
Test	250	82.37±0.76

Values expressed as mean ± SD

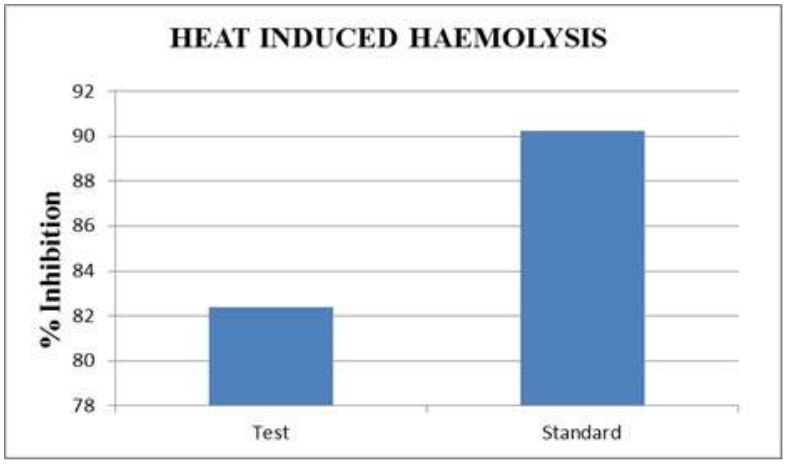


Figure 2: Heat induced haemolysis

WOUND HEALING ACTIVITY [13,14]

From the results obtained it is found that the alcoholic extract is capable of promoting angiogenesis which is an indication of its wound healing activity. The constituents in the bark extract like terpenoids and flavonoids may be responsible of promoting wound healing process, due to their astringent and antimicrobial properties.The wound healing activity are shown in Figure 3, 4 and 5

Figure. 3: The photograph of the egg taken after ten days of incubation

Figure. 4: Chorioallantoic membranes of chick egg, 13-days-old showing vessel formation without drug treatment

Figure. 5: Chorioallantoic membrane of chick eggs (13-days-old) showing increased number of vessels after treatment with *M. elengi* bark extract impregnated in methylcellulose disks.

CONCLUSION:-

The present study revealed significant anti arthritic activity of the alcoholic extract of *Mimusops elengi* at the dose of 250mcg/ml. The extract showed maximum inhibition percentage. This inhibition was found to be highly significant. This may be due to the presence of phytoconstituents like flavanoids and phenolics. Several studies indicate that a fore mentioned phytoconstituents possess significant anti arthritic activity. Angiogenesis plays an important role in wound healing and newly formed blood vessels comprise 60% of the repair tissue. Neovascularization helps hypoxic wounds to attain the normoxic conditions. *Mimusops elengi* promoted angiogenesis in invitro models as indicated by the formation of new blood vessels in CAM model. The increased vessel growth can facilitate both the extent and direction of fibroplasias. Improved angiogenesis, therefore, would be contributing significantly to wound healing activity of *Mimusops elengi*. A detailed research on wound healing and anti arthritic activities using different screening models, isolation of more active constituents possessing therapeutic activities etc can be assessed.

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