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PHARMACOGNOSTIC EVALUATION, *IN VITRO* ANTI OXIDANT AND *IN VIVO* ANTI INFLAMMATORY STUDIES OF *MUNTINGIA CALABURA LINN*

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ABSTRACT

In the present work *Muntingia calabura linn* plant of muntinginaceae family was evaluated for its macroscopical and microscopical characterization studies. The fruit, seed and pollen grains were used in the current investigation to explore their anti oxidant and anti inflammatory studies. The macroscopic evaluation of fruit showed smooth, thin, tender skin and light brown, soft, juicy pulp, capsule shaped, 5-celled baccate, sub globose with small elliptic greyish yellow seeds, persistent stigma and flat embryo with copious endosperm in seeds confirmed their identity. In microscopical studies the fruits showed calcium oxalates crystals, starch grains, pollen grains and longitudinal section viewed with thick, short lobed stigmatic lobes, stigma consists of parenchymatous tissue with sclereids. The pericarp of the berry is 550µm thick with thin epidermal layer filled with dense, dark tannin and small clusters of narrow xylem elements and phloem cells. The seeds are circular and ovate with thick mucilage sheath and are 400µm wide and 850µm in length. The seed-coat consists of elliptical parenchyma cells secrete mucilage and the sclerotic layer was 20µm thick. The pollen grains with reticulate endings are seen in small clusters with 130µm long and 50µm wide abundant calcium oxalate crystals. The IC_{50} value obtained for the tested assay showed that lower the value higher was the antioxidant activity. The significant anti-inflammatory effect was dose dependent with 24.46% reduction observed for 200 mg/kg and 44.16% seen for 400 mg/kg dose of methanolic extract and 20.43% reduction observed for 200 mg/kg and 400mg/kg dose of aqueous extract.

Keywords: *Muntingia calabura linn, A*ntioxidant activity, Anti-Inflammatory activity

INTRODUCTION:

Over the last decade there has been a growing interesting drug of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment. Among the many problematic areas, the standardization of drugs is the most complex and difficult. In order to maintain efficacy Ayurvedic drugs need standardization at all stages-starting from identification of the source of plants to the finished product, including storage and shelf-life. *Muntingia calabura Linn,* is a

fast growing tropical American evergreen tree which has been widely cultivated and naturalized. The leaf infusion is drunk as a tea like beverage to reduce blood- sugar level. The leaf is reported to possess anti-nociceptive, anti- inflammatory and anti-pyretic activities. It is also a potential anti-bacterial agent. The leaves are reported to possess astringent, diuretic, tonic properties and also used for ulcers, healing wounds as well as digestive aid. The decoction of flowers is used in the treatment of abdominal cramps; it is also used as an emollient and an astringent [1-7]. The flower is reported in the treatment of spasms, cold and headaches. Flowers are said to possess anti-septic properties. An infusion of the flowers is valued as an anti-spasmodic. It is taken to relieve first symptoms of cold. Base on the above information the present study is designed to establish the pharmacognostic, morphological and microscopical characters of leaves and fruits of the plant assist in standardization and identification. Further the *in vitro* anti oxidant and *in vivo* anti inflammatory studies of *Muntingia calabura linn* was also investigated.

MATERIALS:

Muntingia calabura Linn is a small sized tree, widely seen on road side and in waste lands was procured from local area and authentification was done by qualified botanist. The leaves were separated, dried, coarsely powdered, passed through sieve no. 40, and stored in a closed container for further use. All the chemicals used were of analytical grade and were obtained from Merck Limited India and Hi-Media Laboratories, Mumbai, India.

METHODS:

Organoleptic evaluation:

Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation[8].

Preliminary Phytochemical screening:

Preliminary screening was done using standard methodology[8].

Determination of pharmacognostic parameters:

Microscopic examination of the powdered samples was carried out using Abbe type camera Lucida (at a magnification of 180×) while the quantitative parameters were determined using standard methods in Pharmacopoeia[9].

Macroscopic and microscopic analysis:

The macroscopic and microscopy of the plant were studied according to the method of Brain and Turner. For the microscopical studies, transverse sections were prepared and stained. The powder microscopy was performed according to the methods of Kokate and Khandelwal [10, 11].

Acute toxicity study:

The toxicity study was carried out using female and male albino mice (20-35 g).The acute toxicity studies were conducted as per the OECD guidelines 420(OECD 2000) where the limit test dose of 2000 mg/kg was used. The animals were divided into one control group and one treated group, each group consisting of six animals. Observations were made at 2,4,8 hrs for seven days for bodyweight, treatment related changes like respiration rate and heart rate and behavioral signs like apathy, reduced locomotor behaviour[11].

Antioxidant activity by DPPH radical scavenging assay:

The effect of fruit extracts on DPPH (1, 1-diphenyl picracyl hydrazine) radical was determined. Different concentrations of the extracts (500, 400, 300, 200, 100 µg/ml) were prepared and subjected to antioxidant tests. To 1 ml of each of the extracts, 5 ml of 0.1mM methanol solution of DPPH was added, vortexes, followed by incubation at 27 °C for 20 min.

The control was prepared without any extract and absorbance of the sample was measured at 517 nm using UV/VIS Spectrophotometer (ELICO) using methanol to set 0. The ability to scavenge DPPH radical was calculated [12].

Anti-inflammatory Activity:

The anti-inflammatory activity of the fruit sample was investigated in Carrageenan-induced inflammatory model. Acute inflammation was induced in rats. [43] The control group was administered with the saline solution only, while the third group was treated with Indomethacin (10 mg/kg p.o.). The fourth, fifth sixth and seventh groups were administered with the fruit alcoholic and aqueous extract (200 and 400 mg/kg/day p.o.) respectively. One hour after the administration of fruit extract, the standard Indomethacin acute inflammation was produced. Acute inflammatory edema was induced by sub plantar injection of 0.1 ml 1% Carrageenan in the right hind paw of each rat in all the groups except the control group [13, 14].

Results and Discussion:

The fruits *Muntingia calabura* Linn, belonging to family Elaeocarpaceae was selected for the dissertation on the basis of ethanobotanical information which reveals its use against widely spreader chronic disease Anti-inflammatory. The literature review revealed that few research works has been pursued in *Muntingia calabura* Linn, especially on fruit claiming maximum therapeutic uses hence it has high therapeutic value. The fruits of *Muntingia calabura* Linn, have been investigated in a systemic way covering Pharmacognostical, Phytochemical and Pharmacological aspects in an attempt to rationalize its use as drug of therapeutic importance.

Macroscopical studies: The fruits of *Muntingia calbura* Linn, showed berry globes, red when ripe, the abundant fruits are round, 1-1.25cm wide, with red or sometimes yellow, smooth, thin, tender skin and light brown, soft, juicy pulp, with very sweet musky, somewhat fig-like flavor, filled with exceedingly minute, yellowish seeds, too fine to be noticed in eating. Fruit is a drupe or capsule, 5-celled baccate, sub-globose with much small (1/2mm) elliptic greyish yellow seeds, stigma persistent. Seeds are with copious endosperm; embryo flat.

Microscopical studies:

In microscopical studies the fruits showed calcium oxalates crystals, starch grains, pollen grains etc. The fruit when viewed in longitudinal section (LS-VIEW), the fruit appears circular in outline with thick, short lobed stigmatic lobes at the top of the fruit and numbers scattered free seeds. The stigma consists of parenchymatous ground tissue in which thick masses of sclereids. The pericarp of the berry is 550µm thick. It consists of a thin epidermal layer which represents the epicarp of the pericarp. The epidermal cells are filled with dense, dark tannin. The vascular strands are less prominent; they consist of small clusters of narrow xylem elements and phloem cells. The berry has a thick central placental tissue is seen from which five thick septa vadiate towards. The placental tissue consists of two wide fan-shaped vascular stands; densely crowded wide, thick walled xylem elements are seen in the vascular stands.The seeds are circular in transactional view (fig.no.6) and ovate in longitudinal sectional view**.**

It has thick mucilage sheath all around. The seeds are 400µm wide and 850µm in length. The mucilage sheath is more or less uniform in thickness**.** The seed-coat consists of a thin outer layer of elliptical parenchyma cells which seem to secrete the mucilage. Inner to this mucilaginous epidermis is a thick unistratose sclerotic layer lignified walls and wide lumen and stain blue with O-toluidine blue dye**.** The sclerotic layer is 20µm thick.

Powder microscopy:

The pollen grains sticking on the surface of the berries are seen in small clusters in the powder. The pollen grains are measuring. Pollen grains show reticulate endings. Calcium oxalate crystals of various shapes are abundant in the powder. They are club shaped, elliptical or rectangular in outline. The elongated crystals are 130µm long and 50µm wide**.**

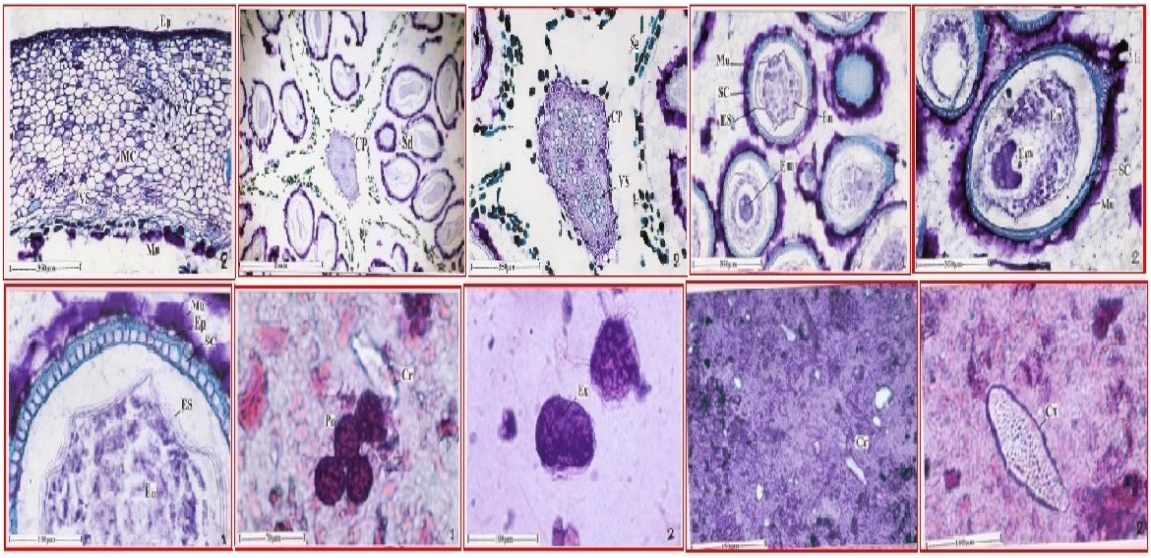

Figure 1: Powder microscopic characteristics of *Muntingia calabura* Linn plant

Physico chemical parameters:

Total ash value was found to be 8.5%w/w, water insoluble ash 2.8% w/w, acid insoluble value 3.6 %w/w, sulphated ash value 2.3 %w/w, alcohol soluble extractive 2.5%w/w, water soluble extractive value 2.8, loss on drying 9.2 %w/w were evaluated and the above studies enable the identification of plant material for further investigation and forms an important aspects of drug studies. The result of Ash value showed the presence of inorganic matters, which is significant .The result of loss on drying gives information about the presence of moisture in the plant. Florescence studies indicate the spectral nature of plant constituents present in the plant.

Phytochemical parameters: Phytochemical studies reveal the presence of alkaloids, carbohydrates, tannins and phenolic compounds, flavonoids, proteins and amino acids, fixed oils and fats, gums and mucilage's etc.

Pharmacological studies:

Acute toxicity studies: No toxicity or death was observed for these given dose level, in the selected and treated animals. No toxicity of the Ethanolic extract of *M. calabura* is found at 1000mg/kg. The dose selected was 20mg/kg and 400 mg/kg.

Table 7: The percentage inhibition on DPPH radical by *M. calabura* fruit extract

Conc. in µg/ml	% inhibition
100	55
200	65
300	78
400	85
500	94
IC_{50} value 90 µg/ml	

Anti oxidant activity:

The antioxidant activity of the fruits of *M. calabura* were evaluated by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. The IC_{50} value was obtained for the tested assay, which showed that the lower the IC_{50} value, the higher was the antioxidant activity.

***In vivo* anti-inflammatory activity:**

The methanolic and aqueous extract of *M. calabura* fruit at the dose levels of 200 and 400 mg/kg caused a dose dependent inhibition of localized swelling caused by Carrageenan at 4 hrs. The significant anti-inflammatory effect was dose dependent with 24.46% reduction observed for 200 mg/kg and 44.16% seen for 400 mg/kg dose of methanolic extract and 20.43% reduction observed for 200 mg/kg and 400mg/kg dose of aqueous extract. Further, the protection induced by 400 mg/kg methanolic extract was also found to be as potent as Indomethacin (84.28%) in reducing paw edema.

Table 8: The effect of fruit extract on percentage inhibition of paw volume

Groups	Initial paw thickness (mm)	Paw thickness after 4 hr (mm)	Difference in paw thickness (mm)	Inhibition percentage
Control	4.58 ± 0.28	4.58 ± 0.28	0	-
Induced	5.15 ± 0.33	4.33 ± 0.27	0.82	-
Standard	4.64 ± 0.37	4.48 ± 0.29	0.16	84.28
200 mg/kg	5.01 ± 0.31	4.31 ± 0.32	0.72	24.46
400 mg/kg	4.90 ± 0.25	4.36 ± 0.15	0.54	44.16
200 mg/kg	5.45 ± 0.34	4.56 ± 0.23	0.79	20.43
400 mg/kg	4.90 ± 0.25	4.48 ± 0.17	0.56	46.18
Values are mean ± SD of six samples in each group				

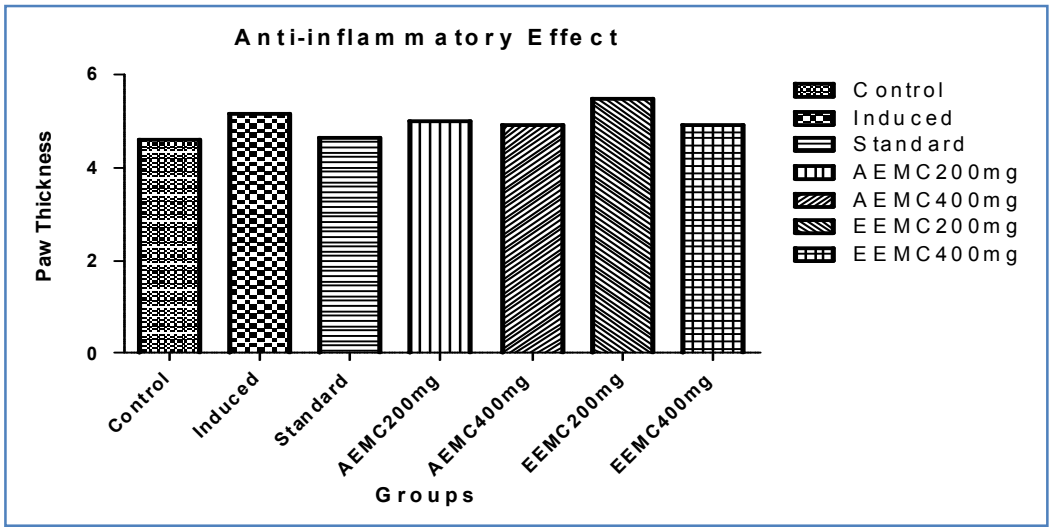

Figure 3: *In vivo* anti-inflammatory activity of *Muntingia calabura Linn*

CONSLUSION:

The fruits of *Muntingia calabura* Linn have been studied to compare and give detailed reports on its Pharmacognostical, Phytochemical and Pharmacological behaviour. The Pharmacognostical studies include macroscopical studies, microscopical studies, ash value, extractive value and loss on drying and Fluorescence analysis. The phytochemical investigation showed the presence of alkaloids, glycosides, carbohydrates, steroids, flavonoids and proteins and amino acids, terpenoids were identified. The pharmacological studies showed significant anti-inflammatory activity at a dose of 400mg/kg with ethanolic substance. Therefore these studies are needed to isolate and characterise the active principle of *Muntingia calabura* Linn, which can offer anti inflammatory activity to establish its mechanism of action.

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