INVESTIGATION OF ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF ETHANOL EXTRACT FROM *MUNTINGIA CALABURA* STEM BARK

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ABSTRACT
Muntingia calabura L. (Family: Elaeocarpaceae) is commonly known as Jamaican cherry, Panama berry or Singapore cherry. It is a very fast-growing tree and wildly distribute throughout the world. Present study was carried out for evaluation of ethanol extract of *M. calabura* (EEMC) stem bark at dose of 100, 250 and 500 mg/kg, p.o., for analgesic, anti-inflammatory and antipyretic activity. EEMC was screened for analgesic activity by writhing, tail immersion and hot plate method in mice. The anti-inflammatory activity by acute carrageenan induced paw oedema in rats. The antipyretic activity was evaluated using Brewer's yeast induced pyrexia in rabbits. Acute toxicity in mice was found to be higher than 2000 mg/kg., p.o. Ethanol extract of *M. calabura* stem barks showed a significat peripheral and centrally acting analgesic or antinociceptive effect in dose dependent manner in various tested models. Anti-inflammatory studies at 100, 250 and 500 mg/kg., p.o., of extract showed significant activity. The extract also possesses significant decrease in yeast-induced fever at dose of 500 mg/kg, p.o. This result seems to support the view that the extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be regulator of body temperature. Preliminary phytochemical tests revealed presence of saponins, carbohydrates, flavonoids, sterols, terpinoids, tannins and phenolic compounds in the ethanol extract of *M. calabura* stem bark. The present study demonstrated that ethanol extract obtained from stem bark of *Muntingia calabura* L., exhibited significant analgesic, anti-inflammatory and antipyretic properties in the different tested experimental animal models.
Keywords: *Muntingia calabura*, ethanol, analgesic, anti-inflammatory, antipyretic.

INTRODUCTION
The use of herbal heritage has become a part of general health care by the tribes since time immemorial. Herabal remidies are widely known to be used in the treatment of many infectious diseases and continue to provide a major source of natural therapeutic remedies. *Muntingia calabura* L. (Family: Elaeocarpaceae) is commonly known as Jamaican cherry, Panama berry or Singapore cherry. It is a very fast-growing tree and wildly distribute throughout the world [1]. The fruits sometimes eaten fresh and often cooked and made into jam, while the leaf infusion is drunk as a tea like beverage [2]. Traditionally the roots, stem barks, leaves, flowers and seeds of this plants are used as antiseptics, abortifacient, anemia, antiscorbutic, anthelmintic,

rubifacient, vesicant, carminative, tranquillizer and antispasmodic purpose [3, 4]. According to Peruvian folklore, leaves can either be boiled or steeped in water to provide relief from gastric ulcers or reduce swelling of prostate gland [1]. The leaves are reported to have cardiovascular depressive action, dose-dependent hypotensive effect [5], antioxidant properties[2], antidiabetics effect [6], invitro antimicrobial [7], antinociceptive [3], antiinflammatory and antipyretic activities [8, 9]. There are several scientific papers reporting on antitumour properties of the leaves and roots of *M. calaburar* [4, 10]. Phytochemical studies of various parts of *M. calaburar* are rich in flavonoids with flavones, flavanones, flavans, biflavans, chalcones, sesquiterpene and phenolic compounds [4, 5, 11]. Two new flavones, 8-hydroxy-7, 3, 4, 5-tetramethoxyflavone and 8, 4-dihydroxy-7, 3, 5-trimethoxyflavone, thirteen known compounds have been isolated from the stem of
M. calabura. Some flavonoids have beneficial effects on cardiovascular diseases and possess cytotoxic activities [11, 12]. In the present study, we investigated analgesic, anti-inflammatory and antipyretic properties of ethanol extract of *Muntingia calabura* (EEMC) stem bark in experimental animal model.

MATERIALS AND METHODS

Plant material

The plant materials were collected from the young and matured plants of *Muntingia calabura* (barks) and authenticated by the taxonomist Prof. Dr. S. K. Dash, Head of the Department of P.G. department of Biosciences, College of Pharmaceutical Sciences, Mohuda, Berhampur, Ganjam dist., Odisha-760002, India. The collected materials were washed, shade dried and pulverized by using a mechanical grinder to obtain coarse powder.

Preparation of the extract

The powdered plant materials were defatted with petroleum ether (60^0-80^0C) in a soxhlet extractor. The marc was then air-dried and extracted with ethanol (90%) and then the ethanolic extract was concentrated in rotary evaporator (Evator, Media Instrument Mfg. Co., Mumbai, India) at reduced pressure to obtain a deep brown residue (33.33%). Preliminary phytochemical studies were performed on the extract using standard procedures [13].

Animals

Animals used in this study were male Swiss albino mice (20-25 g), Wistar rats of both sex (150-210 g) and New Zealand white rabbits of both sex (1.5-2.0 kg). The animals were housed for at least one week in the laboratory animal room prior to testing in standard polypropylene cages at room temperature of 34 ± 2^0C and at 60-65% relative humidity. Food and water were given ad libitum unless otherwise specified. All experimental protocols were approved by the Institutional Animal Ethics committee of GITAM Institute of Pharmacy, Visakhapatnam, Andhra Pradesh, India (Regd. No.1287/ac/09/CPCSEA and protocol No: IAEC/GIP-1287/M Pharm/IP/SM-BU/04/2011-12). The experiments were designed in different groups containing six animals in each.

Acute toxicity study

The acute toxicity studies were conducted as per OECD guidelines 420, where the limit test dose of 2000 mg/kg, p.o., used. Observations were made and recorded continously for the first 4 h for any behaviorial changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any [14].

Evaluation of analgesic activity by writhing method

The test was performed according to Bose et al.,[15]. Writhing was induced in mice by single intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 min period. Group I serve as control received only vehicle (3 ml/kg, p.o.), the second group received aspirin (200 mg/kg, p.o.), which was used as reference standard for activity comparison; group III, IV and V received ethanol extract of *M. calabura* barks (100, 250 and 500 mg/kg, p.o.).

The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition was calculated.

Evaluation of analgesic activity by tail immersion method

The tail immersion test was carried out as described by Yeshwan et al., [16]. In this method, Swiss albino mice weighing between 20-25 g, deprived of food and water for 18 hours prior to the experiment, were divided in five groups of six mice in each. Group I served as control, which received only vehicle (3 ml/kg, p.o.). Other groups of animals received one of the following in a similar manner: pentazocine (15 mg/kg, p.o.) or ethanol extracts (100, 250 and 500 mg/kg, p.o.). The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0^0 C. The time taken to withdraw the tail was noted as reaction time. A cut off time 10 sec was maintained at 55.0^0 C to prevent tissue damage. The reaction time measured at 0, 30, 60, 90 and 120 min after treatment.

Evaluation of analgesic activity by hot-plate test

Mice (20-25g) of both sexes were fasted overnight before the study. Hot-pate was used to measure response latencies according to the methods of Reanmongkol, et al [17]. In this study, the hot-plate was maintained at 55 ± 1^0 C and the animals were individually placed on the heated surface. The time in seconds between placement and shaking, paw licking and jumping off the plate was recorded as response latency. Four groups of six animals each the first group received vehicle (3 ml/kg, p.o.); the second group received morphine sulphate (10 mg/kg, p.o.); other groups received doses of ethanol extract of *M. calabura* barks (100, 250 or 500 mg/kg, p.o.) respectively. Measurements were taken at zero, 30, 60, 90 and 120 minutes after the treatment of animals.

Evaluation of Carrageenan-induce anti-inflammatory activity

The test was performed as per the method of Mondal et al [18].The animals were divided into five groups. The control group received only vehicle (2 ml/kg) through oral route. Other groups received diclofenac (12.5 mg/kg, p.o.) or the ethanol extract of *M. calabura* barks at doses of 100, 250 or 500 mg/kg, p.o., in a similar manner. Carrageenan (0.1 ml of 1% solution in normal saline) was administered to the rats into the planter surface of the right hind limb to induce paw edema. Paw volume was measured with a plethysmograph after 90 and 120 minutes of carrageenan injection and paw swellings were compared with control. Percentage inhibition of oedema was calculated

Evaluation of antipyretic activity

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rabbits [15]. Fever was induced by injecting 3 ml/kg (s.c.) of 10% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck. After 18 hr, animals showing at least an increase of 1^0C of rectal temperature were selected for the experiment. Three groups of six animals each the first group received vehicle (3 ml/kg, p.o.), the second group received paracetamol 100 mg/kg, p.o., and 3rd group received of ethanol extract of *M. calabura* barks (500 mg/kg, p.o.) respectively. The rectal temperature was measured at 1, 3 and 5 h after treatment.

Statistical Analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A P-value < 0.05 were considered to be significant. All the values were expressed as mean ± SEM.

RESULTS

Preliminary phytochemical tests revealed the presence of saponins, carbohydrates, flavonoids, sterols, terpinoids, tannins and phenolic compounds in ethanolic extract of *Muntingia calabura* (EEMC) stem bark.

In acute toxicity study, EEMC when administered orally to mice in graded doses 100 to 2000 mg/kg, p.o., EEMC did not produce any significant changes in general behavior, cutaneous effects, breathing, sensory nervous system responses and GI effects during the study. However, there was no mortality in tested doses at the end of 14 days of observation.

Oral administrations of EEMC significantly ($P < 0.01$) reduce the writhings induced by acetic acid in mice; the activity was compared with that of aspirin (Table 1). Analgesic studies against thermal noxious stimuli the extract shows dose dependent analgesic effect (Table 2 and 3). In tail immersion method, EEMC at 500 mg/kg, p.o., showed significant activity (6.83 ± 1.22) ($P < 0.05$) after 30 minutes, whereas at a dose of 100 and 250 mg/kg, p.o., showed significant analgesic activity from 90 and 60 minutes respectively, pentazocine (15 mg/kg, p.o.) used as standard drug, which showed significant activity throughout the course of study. The preliminary study using the hot plate test demonstrated that EEMC possessed antinociceptive activity (Table 3). The test extract exhibited significant activity in a dose dependent manner untill the end of the experiment (120 min) when compare with control group animals.

The inhibitory activity on carrageenan-induced acute inflammation model is represented in Table 4. EEMC gave significant reduction of rat paw edema at all assessment times in dose dependent manner. The extract showed maximum inhibition of 29.57%, 36.57% and 50.74% at the dose of 100, 250 and 500 mg/kg, p.o., body weight, whereas standard drug diclofenac (12.5 mg/kg, p.o.) showed 57.74% of inhibition after 120 mins of drug treatment in carrageenan-induced paw edema.

The effect of yeast-induced pyrexia in rabbits is depicted in Table 5. It was found that ethanol extract of *M. calabura* stem barks at the dose of 500 mg/kg, p.o., also showed significant lowering of body temperature throughout the course of study. Subcutaneous (s.c) injection of yeast suspension markedly elevated the rectal temperature. EEMC treatment with tested dose reduced the rectal temperature of rabbits. Both the EEMC and Paracetamol (100 mg/kg, p.o.) standard drug significantly reduced the yeast elevated rectal temperature compared to control group.

DISCUSSION

Ethanol extract of *M. calabura* stem bark (EEMC) protected against both thermal and chemical induced stimuli, which were evidence from tail immersion, hot-pate and acetic acid induced writhing test.The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. Acetic acid causes analgesia by liberating endogenous substances that excite the pain nerve ending. This response is believed to be mediated by the prostaglandin pathways [19]. EEMC also produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways [20]. In the radiant heat tail immersion and hot-pate test the plant extract prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center [21] and the advantages to select hot plate as tools to evaluate central analgesic activity. This test produces at constant temperature and two kind of behavioural response can be evaluated like licking and jumping. Both of these are consider being supraspinally integrated response [16]. Centrally acting drugs, like morphine or pentazocine have been reported to produce an antinociceptive effect in both types of assays, while peripherally acting drugs, like aspirin produced an antinociceptive or analgesic effect only in the abdominal constriction test, thus from the results it is apparent that ethanol extract of *M. calabura* stem barks showed a significat peripheral and centrally acting analgesic or antinociceptive effect in dose dependent manner.

Carrageenan-induced rat paw edema is a biphasic process or prototype exudative phase of acute inflammation [19, 22]. The early phase is attribute release of histamine or serotonin and the next phase is associated with the production of lysosome, bradykinin, protease and prostaglandin [23]. Therefore, the inhibition of carrageenan-induced inflammation by EEMC could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis.

Ethanol extract of *M. calabura* stem barks at dose of 500 mg/kg, p.o. showed significant decrease in yeast-induced fever. This result seems to support the view that the extract has some influence prostaglandin biosynthesis because prostaglandin is believed to be regulator of body temperature [15].

Presence of phytoconstituents like saponins, alkaloids, tannins, flavonoids and phenolic compounds has been previously found to be responsible for analgesic, anti-inflammatory and antipyretic activities in plants [3, 15, 24]. The presence of the above said phytoconstituents in ethanol extract of *M. calabura* stem barks may be probably responsible for the observed activities.

CONCLUSIONS

From the above discussion, the present study demonstrated that ethanol extract obtained from stem bark of *Muntingia calabura* L., exhibited significant analgesic, anti-inflammatory and antipyretic properties in the different tested experimental animal models. The acute toxicity studies revealed no mortality was recorded. Further detailed investigation is underway to determine the exact phytochemical entities that are pesponsible for these activities.

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Table 1: Effect of ethanol extract of *M. calabura* (EEMC) stem barks by acetic acid induced writhing in mice.

Treatment	Dose	Avg. no. of writhing	Percentage Inhibition
Control	3 ml/kg, p.o.	65.33±6.1	-
Aspirin	200 mg/kg, p.o.	15.16±1.7**	76.79
EEMC	100 mg/kg,p.o.	31.16±1.11*	52.30
	250 mg/kg,p.o.	20.00±1.06**	69.38
	500 mg/kg,p.o.	16.00±1.89**	75.50

Values are expressed as mean ± S.E. (n = 6). *P<0.05, **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test)

Table 2: Evaluation of analgesic activity of ethanol extract of M. calabura stem bark by tail immersion method

Treatment groups	Dose	Average tail withdrawing time (sec)				
		0 min	30 min	60 min	90 min	120 min
Control	3 ml/kg, p.o.	3.08±0.41	1.43±0.15	1.55±0.15	1.88±0.22	1.58±0.16
Pentazocine	15 mg/kg, p.o.	2.52±0.42	4.38±0.53**	5.98±0.61**	7.05±0.5**	8.6±0.44**
EEMC	100 mg/kg, p.o.	2.15±0.39	3.95±0.71	6.80±0.79	7.76±0.87**	6.76±0.88**
	250 mg/kg, p.o.	2.06±0.21	4.11±0.80	7.16±0.64*	8.06±0.67**	7.65±1.20**
	500 mg/kg, p.o.	2.06±0.34	6.83±1.22*	7.33±0.85**	8.76±0.89**	9.66±0.12**

Values are expressed as mean ± S.E. (n = 6).

*P<0.05,**P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test)

Table 3: Effect of ethanol extract of *M. calabura* stem bark (EEMC) on pain induced by hot plate method

Treatment groups	Dose	Latency period				
		0 min	30 min	60 min	90 min	120 min
Control	3 ml/kg, p.o.	1±0.05	0.95±0.08	0.88±0.07	1.13±0.31	0.93±0.03
Morphine sulphate	10 mg/kg, p.o.	0.9±0.17	4.15±0.35**	6.04±0.33**	7.1±0.26**	8.05±0.07**
EEMC	100 mg/kg, p.o.	1.18±0.19	1.35±0.07	1.45±0.18*	2.78±0.31*	3.00±0.55*
	250 mg/kg, p.o.	1.41±0.22	1.56±0.23*	1.73±0.14**	3.00±0.37**	3.38±0.32*
	500 mg/kg, p.o.	1.6±0.23	2.01±0.26**	2.31±0.32**	.93*3.61±0*	4.15±1.08**

Values are expressed as mean ± S.E. (n = 6). *P<0.05,

**P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

Table 4: Effect of ethanolic extract of M. calabura stem bark (EEMC) on carrageenan induced rat paw oedema

Treatment groups	Dose	Time after 1% carrageenan injection			
		90 min		120 min	
		EV	% EI	EV	% EI
Control	2 ml/kg, p.o.	0.7±0.01	------	0.71±0.02	-----
Diclofenac	12.5 mg/kg, p.o.	0.35±0.02**	50.00	0.3±0.33**	57.74
EEMC	100 mg/kg, p.o.	0.49±0.01*	30.00	0.5±0.1**	29.57
	250 mg/kg, p.o.	0.47±0.02**	32.85	0.45±0.01**	36.61
	500 mg/kg, p.o.	0.39±0.05**	44.28	0.35±0.04**	50.74

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA.
* P<0.05, **P<0.01 when compared to control; Dunnet's t-test.
EV = oedema volume (ml) at time; %EI = percent oedema inhibition of test substance at time.

Table 5: Effect of ethanolic extract of M. calabura on yeast induced pyrexia in rabbit

Treatment groups	Dose	Rectal temperature (0C)		Rectal temperature after treatment (0C)		
		Initial	18 h after Brewer's yeast administration	1 h	3 h	5h
Control	5 ml/kg, p.o.	36.73±0.15	37.49±0.08	37.71±0.11	37.40±0.08	37.36±0.12
Paracetamol	100 mg/kg, p.o.	36.75±0.12	37.58±0.24	36.47±0.14**	36.00±0.11**	35.92±0.11**
EEMC	500mg/kg, p.o.	36.67±0.09	37.63±0.11	37.08±0.16**	37.00±0.13*	36.81±0.11**

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA.
* P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

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