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# Evaluation of Analgesic and Anti-Inflammatory (*In vitro* and *In vivo*) Activity of Petroleum Ether, Ethyl Acetate and Methanol Extracts of *Malpighia emarginata* (DC)

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**Abstract:** In the present study, analgesic and anti-inflammatory (*In-vitro* and *in-vivo*) activity of petroleum ether, ethyl acetate and methanol extracts of *Malpighia emarginata* (*M. emarginata*, ME*)* leaves and fruits in experimental animals were evaluated. The analgesic activity by Tail flick model, x exhibited significant effect (PEME, EAME and MAME*;* 400mg/kg) in latency reaction time after 60 min . In *in-vivo* (carrageenan) and *in-vitro* (Human Red Blood Cell) anti-inflammatory activity models, PEME, EAME and MAME found to be significant (400 and 200 mg/kg) when compared with control group. The MAME leaves and fruits extract at a concentration of 200 mg/ml showed 70.22 and 65% protection respectively. The results obtained from the present study when compared with control revealed that the methanol extract of leaves and fruits shows potential analgesic and anti-inflammatory activity than that of petroleum ether and ethyl acetate extracts.

**Key words:** *Malpighia emarginata* · Analgesic · Anti-Inflammatory · Human Red Blood Cell diclofenac and Pentazocin

## INTRODUCTION

Currently the source of drugs was natural products due to the therapeutic properties and for a long time, mineral, plant and animal products also main source of drugs [1]. There is anindication of herbs being used in the treatment of diseases and for stimulating body systems in almost all ancient people. The primogenital book of library of manRig-Veda, supplies the information about some herbs.The field of medicine and its allied sciences has also made rapid strides with their rapid progress in various fields of human activities. The production of many chemicals and their introduction into therapeutics as drugs certainly revolutionized the management of diseases. Nowadays we have a big number of synthetic drugs which are effective in diverse diseased situations [1].

The privileges of therapeutic efficiency and lack of toxicity of many plants have been scientifically demonstrated in recent years. However, a large number of plants were doubtful value among the enormous repertory of indigenous drugs. It will be a worthy exercise if one attempts to select the best out of them. There large number of plants, which have to be studied thoroughly for useful activity or lack of it [1].

There is an extensive confidence that the natural products are less toxic when compared to pure chemicals. Although this belief has been challenged by a number of scientists, one cannot altogether rule out the belief. In many circumstances, it is found that the plant or extract of the plant has some therapeutic activity which is not seen in the pure components isolated from it. It is possible that some ingredients nullify the toxic effects of other components of a plant and the whole plant extract becomes less toxic and more useful. The drugs of plant origin can be simply prepared and hence are cheaper than the synthetic drugs [1]. The plants and herbs are used for humans in india because of the diverse cultural traditions associated to cure the health.

However, very few plants are used by locals for medicine is imperiled to scientific exploration. The need for the conservation of medicinal plants and modern

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knowledge, particularly in developing countries like India, taking into account the sociocultural and economic conditions is urgent [2].

Acerola (*Malpighia emarginata* DC.) belongs to the family Malpighiaceae and comprises 30 species of shrubs inborn to the West Indies. It has good adaptation to soil and climate grows in central and South America including Brazil [3]. Acerola is native to South America, southern Mexico, Puerto Rico, Brazil and Central America, but is now also being grown as far north as Texas and in subtropical areas of Asia, such as India [4]. The plant bears a small trilobate cherry-like red fruit. The pulp is very juicy and refreshing with a sweet, fruity flavor. It is principally known for its high vitamin C content, being one of the most important natural sources for this vitamin.

This useful effect is due to the action of antioxidant compounds, which are capable of nullifying free radicals and reduce oxidative damage in the body [5]. However, recent research showed that besides vitamin C, acerola fruits may be also a good source of phytochemicals such as-anthocyanin [6, 7] flavonoids and phenolic acids [8] and polyphenols [6, 9]. With respect to bioactivities, acerola showed antioxidant [10] antimicrobial [11] hepatoprotective [12] and anti-hyperglycemic [13] effects.

Oxidative stress has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurologic disorders, as well as in the process of aging [14].

Therefore, the aim of this work was to evaluate the analgesic and anti-inflammatory (*in-vitro* and *in-vivo*) activity of leaf and fruit extracts due to their antioxidant activity.

## MATERIALS AND METHODS

**Chemicals:** Carrageenan, purchased from Sigma Aldrich, Mumbai, India. Pentazocin, Diclofenac sodium was purchased from Microlabs, Bangalore, India. Petroleum ether, ethyl acetate and Methanol were Analytical grade, Merck India.

**Animals:** Wistar albino rats (150-200 gm) and mice (20-25gm) of both sexes were selected and procured from Mahaveer enterprises, Hyderabad, India. The selected animals were maintained under standard laboratory conditions temperature at 25±1°C, relative humidity 55±10% and with 12 h light/dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. Seven days' time to get acclimatized to the laboratory conditions. All experiments were performed according to the Institutional Animal Ethics Committee (IAEC) (Approval No: 1047/ac/07/CPCSEA).

**Plant Material:** The leaves and fruits of MEwere collected from the botanical garden of Acharya N G Ranga Agricultural University, Hyderabad, Telangana, India, in the month of September – October 2016. This plant species was identified and authenticated by Prof K. S. Raju Botanist Department of Botany K.U. Warangal, Telangana, India from different trees.

**Preparation of Extracts:** Coarsely powdered leaves and fruits of ME were subjected to extraction by using a Soxhlet extractor for 72 hrs at a temp 60-80 °C not exceeding the boiling point of the solvent to increasing polarity from petroleum ether (PE), ethyl acetate (EA) and methanol (MA). The extract was filtered using Whattman filter paper (No. 1) and then concentrated in vacuum and dried at 45 °C for the removal of solvents and the extracts were kept in sterile bottles under refrigerated conditions until use.

**Experimental Design**

**Analgesic Activity of Leaves and Fruits Extracts of *M. emarginata* by Tail Flick Method:** Swiss Albino mice were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animal that failed to withdraw its tail within 5sec was rejected from the study. The selected animals are divided into fourteen groups each group consists of six animals. The grouping details are:

The group-I was served as normal control received the 2% acacia, the group-II was served as standard received pentazocin 30 mg/kg, group-III to XIV test groups received multiple doses of *M. emarginata* leaf and fruit extracts (200 and 400 mg/kg, b. w) as follows treatment PEME (L) (III & IV), EAME (L) (V & VI), MAME (L) (VII & VIII), PEME (F) (IX & X), EAME (F) (XI & XII) and MAME (F) (XIII & XIV) respectively.

Each mouse was placed individually in actophotometer for 10 min and basal reaction time is noted. Scoring was done after 60 min administration of different doses of test substances and standard drug. Reduction in the motor activity indicates depressants and increases in the motor activity indicate the stimulant property of the drugs [15, 16].

***In vivo* Anti-inflammatory Activity of Leaves and Fruits Extracts of *M. emarginata* by carrageenan Method:** Wistar albino rats (150-200 gm) were taken and they were divided into fourteen groups of six animals each were fasted for 12 hours prior to induction of oedema although water was available *ad libitum*. Rats were deprived of water only during the experiment to ensure uniform hydration and minimize any variability in oedematous response. Inflammation of the hind paw was induced by injecting 0.1 ml of 1 % carrageenan in normal saline into the sub plantar region of the right hind paw.

The group-I was served as normal control received the 2% acacia, the group-II was served as standard received diclofenac at a dose of 10 mg/kg, group-III to XIV test groups received multiple doses of *M. emarginata* leaf and fruit extracts (200 and 400 mg/kg, b. w) as follows treatment PEME (L) (III & IV), EAME (L) (V & VI), MAME (L) (VII & VIII), PEME (F) (IX & X), EAME (F) (XI & XII) and MAME (F) (XIII & XIV) respectively. All the doses were given 1 hr before the carrageenan injection, oedema was expressed as the increase in paw volume. The paw volume was measured with a digital Plethysmometer (UgoBasile, 7140) before and 1, 2, 3 and 4 hrs after the carrageenan injection. Percentage of paw volume inhibition was calculated using the following formula [17].

Percent inhibition (%) = (PC – PT) /PC X 100

where PC and PT is the increase in paw volume in control and test respectively.

***In-vitro* Anti-Inflammatory Activity of *M. emarginata* Leaves and Fruits Extracts by HRBC Method:** The *in vitro* anti-inflammatory activity was determined by human red blood cell (HRBC) membrane stabilization method. Collect the blood sample from the healthy human volunteers who was not taken any NSAIDS for 2 weeks prior to the study and for the separation of packed cells, blood sample mixed with equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% critic acid and 0.42% NaCl) and centrifuged at 3, 000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made with isosaline. For the estimation of anti-inflammatory property HRBC suspension were used. Different concentrations (100 and 200 mg/ml) of extracts using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. These mixtures were incubated at 37 °C for 30 min and centrifuged at 3, 000 rpm. The supernatant liquid was transferred and the hemoglobin content was assessed spectrophotometrically at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100 %. Diclofenac (100 g/ml) was used as reference standard [18].

% protection = [1- (OD sample/OD control)] x 100

**Statistical Analysis:** Results were expressed as Mean ± SD, statistical significance was calculated by applying one way ANOVA. $P<0.05$ was considered as significant (Dunnett's-test) [19].

## RESULTS

**Analgesic Activity of Leaves and Fruits Extracts of *M. emarginata* by Tail Flick Method:** The orally administered leaves and fruits of PEME, EAME and MAME extracts with 400mg/kg delayed the duration of reaction time at 30 and 120 min significantly ($P<0.01$), while in 60 min ($P<0.001$) and 200 mg/kg also shows significantly ($P<0.05$) at 120 min when compared with control group. Standard Pentazocin (30 mg/kg) reached a maximum ($P<0.001$) increase in latency reaction time at 30, 60 and 120 min, when compared with control and extract groups (Table 1). The effectiveness of analgesic agents in the tail flick pain model is highly correlated with relief of human pain.

***In vivo* Anti-Inflammatory Activity of Leaves and Fruits Extracts of *M. emarginata* by Carrageenan Method:** With the oral administration of leaves and fruits of PEME, EAME and MAME extracts against carrageenan induced paw edema in rats. At 400 mg/kg and 200 mg/kg exhibited significant ($P<0.001$, $P<0.05$) anti-inflammatory activity respectively when compared with control group at 3hr and 4hr (Table 2). Standard Pentazocin (30mg/kg) exhibited significant effect ($P<0.001$) at 3hr and 4hr, while in 1hr also shows significant effect ($P<0.05$) when compared with control and extract groups.

***In-vitro* Anti-Inflammatory Activity of *M.emarginata* Leaves and Fruits Extracts by HRBC Method:** The PEME, EAME and MAME extracts of the leaves of *M. emarginata* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Among all the extracts MAME leaves and a fruit extracts showed

Table 1: Analgesic Activity of *M. emarginata* Leaves and Fruits Extracts

| | | Reaction time in sec (Mean ± SD) at time (min) | | | | |
|---|---|---|---|---|---|---|
| Treatment | Dose (mg/kg) | 0 min | 15 min | 30 min | 60 min | 120 min |
| Control | ---- | 3.14±0.40 | 3.40±0.35 | 3.55±0.38 | 3.52±0.30 | 3.41±0.82 |
| Pentazocin | 30 | 3.62±0.15 | 4.53±0.38 | 6.24±0.46*** | 7.68±0.54*** | 5.50±0.42*** |
| PEME(L) | 200 | 3.53±0.36 | 4.04±0.62 | 4.42±0.47 | 5.15±0.24** | 4.65±0.41* |
| | 400 | 3.62±0.22 | 4.32±0.80 | 5.29±0.53** | 6.81±0.63*** | 4.70±0.46** |
| EAME(L) | 200 | 3.92±0.51 | 4.08±0.39 | 4.35±0.23 | 5.28±0.42** | 4.62±0.23* |
| | 400 | 3.98±0.36 | 4.13±0.42 | 5.09±0.49** | 6.77±0.67*** | 4.71±0.57** |
| MAME(L) | 200 | 3.34±0.26 | 3.56±0.46 | 4.08±0.38 | 5.25±0.45** | 4.68±0.43* |
| | 400 | 3.54±0.71 | 3.74±0.69 | 5.10±0.39** | 7.09±0.86*** | 4.80±0.75** |
| PEME(F) | 200 | 3.96±0.50 | 4.15±0.42 | 4.40±0.35 | 5.19±0.42** | 4.68±0.37* |
| | 400 | 3.92±0.38 | 4.18±0.45 | 5.05±0.48** | 6.72±0.56*** | 4.82±0.41** |
| EAME(F) | 200 | 3.35±0.19 | 3.52±0.36 | 3.83±0.87 | 5.21±0.44** | 4.66±0.23* |
| | 400 | 3.43±0.14 | 3.62±0.29 | 5.19±0.47** | 6.74±0.52*** | 4.81±0.48** |
| MAME(F) | 200 | 3.83±0.55 | 4.05±0.68 | 4.59±0.56 | 5.22±0.67** | 4.67±0.34* |
| | 400 | 3.95±0.33 | 4.22±0.74 | 5.07±0.36** | 6.82±0.61*** | 4.73±0.51** |

Values are Mean ± SD, n=6 in each group.$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with control group (Dunnett test).

Table 2: *In-vivo* Anti-Inflammatory Activity of *M. emarginata* Leaves and Fruits Extracts

| | | Mean paw volume in ml (% inhibition) | | | |
|---|---|---|---|---|---|
| Treatment | Dose (mg/kg) | 1hr | 2hr | 3hr | 4hr |
| Control | --- | 0.32±0.05 | 0.40±0.06 | 0.64±0.03 | 0.43±0.03 |
| Diclofenac | 10 | 0.27±0.06(10.52) | 0.30±0.04*(25.00) | 0.37±0.05***(42.18) | 0.19±0.03***(55.81) |
| PEME(L) | 200 | 0.31±0.05(5.26) | 0.37±0.06(7.50) | 0.52±0.04*(18.75) | 0.34±0.06*(20.93) |
| | 400 | 0.30±0.03(7.89) | 0.36±0.05(10.00) | 0.49±0.03***(23.43) | 0.31±0.07***(27.90) |
| EAME(L) | 200 | 0.31±0.02(2.63) | 0.38±0.04(5.00) | 0.52±0.06*(18.75) | 0.32±0.06*(25.58) |
| | 400 | 0.30±0.05(7.36) | 0.37±0.07(7.50) | 0.47±0.08***(26.56) | 0.30±0.02***(30.23) |
| MAME(L) | 200 | 0.31±0.07(6.31) | 0.35±0.03(12.50) | 0.53±0.04*(17.18) | 0.33±0.05*(23.25) |
| | 400 | 0.30±0.06(7.89) | 0.34±0.02(15.00) | 0.50±0.03***(21.87) | 0.28±0.08***(34.88) |
| PEME(F) | 200 | 0.30±0.03(6.84) | 0.36±0.05(10.00) | 0.53±0.05*(17.18) | 0.33±0.04*(23.25) |
| | 400 | 0.29±0.05(8.42) | 0.35±0.02(12.50) | 0.48±0.02***(25.00) | 0.31±0.02***(27.90) |
| EAME(F) | 200 | 0.31±0.08(6.31) | 0.37±0.03(7.50) | 0.51±0.08*(20.31) | 0.33±0.02*(25.00) |
| | 400 | 0.29±0.06(7.89) | 0.36±0.05(10.00) | 0.45±0.05***(29.68) | 0.26±0.04***(39.53) |
| MAME(F) | 200 | 0.31±0.05(2.63) | 0.36±0.06(10.00) | 0.51±0.06*(20.31) | 0.32±0.05*(25.88) |
| | 400 | 0.30±0.09(5.26) | 0.35±0.03(12.50) | 0.44±0.09***(32.81) | 0.24±0.04***(44.18) |

Values are Mean ± SD, n=6 in each group.$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with control group (Dunnett test).

Table 3: *In-Vitro* Anti-inflammatory activity of *M.emarginata* Leaves and Fruits Extracts by HRBC method

| Extracts | Concentration (mg/ml) | % Inhibition of denaturation |
|---|---|---|
| Control | 2% acacia | ---- |
| Diclofenac | 100 | 79.20±1.60 |
| PEME(L) | 100 | 35.22±1.20 |
| | 200 | 63.10±1.06 |
| EAME(L) | 100 | 28.20±1.31 |
| | 200 | 45.28±1.72 |
| MAME(L) | 100 | 43.22±1.66 |
| | 200 | 70.22±1.42 |
| PEME(F) | 100 | 48.28±1.85 |
| | 200 | 61.80±1.26 |
| EAME(F) | 100 | 26.33±1.20 |
| | 200 | 31.28±1.32 |
| MAME(F) | 100 | 28.80±1.90 |
| | 200 | 65.20±1.32 |

significant anti-inflammatory activity in a concentration dependent manner. The MAME leaves and fruits extract at a concentration of 200 mg/ml showed 70.22% and 65% protection of HRBC in hypotonic solution. All the results were compared with standard diclofenac which showed 79.20% protection. The results were tabulated in Table 3.

## DISCUSSION

It is also established that tail flick well-established method for measuring the central analgesic effects of drugs through opioid receptor. In this model the data showed that the extract dose-dependently increased pain threshold, the increase in the pain threshold tail flick latency profiles of the extract were less than that of the standard drug, pentazocin. The $\mu$ receptor stimulation is generally associated with pain relief and has been shown to be potent in regulating thermal pain. Non-analgesic effects via μ receptors include respiratory depression and most importantly for therapeutic considerations, is its induction of physical dependence [20].

Activation of μ 2 opioid subtype leads to spinal analgesia and commonly causes constipation as adverse effect. Therefore, taking all these data together we believe that the analgesic activity of the extract is most likely to be mediated by central action (Spinally and supra spinally) and indicates a codeine-like mechanism by binding with opioid receptors [20].

Our present study demonstrated that pet ether, ethyl acetate and methanol extracts were effective against all these models at 400 mg/kg doses which were comparable with standard drug pentazocin. Narcotic analgesics are active against both peripheral and central pain, while NSAIDs inhibit peripheral pain. Our findings suggested that extracts may act like narcotic analgesic drugs [20].

Pretreatment of rats with *M. emarginata* extract inhibited the development of paw oedema induced by carrageenan across the three phases of the test. However, the inhibitory effects were more prominent in the second and third phases. Carrageenan-induced paw edema is a highly sensitive tool to evaluate the efficacy of acute inflammation [19]. The development of edema in the rat hind paw following the injection of carrageenan has been described as a biphasic, age weight dependent event in which various mediators operate in sequence to produce the inflammatory response [22].The histamine, serotonin and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation, prostaglandins (PGs) are involved in the increased vascular permeability and are detectable in the late phase of inflammation. The initial phase of edema induced by carrageenan is not inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin or aspirin, while the second, accelerating phase (which has been correlated with elevated production of prostaglandins and induction of inducible cyclooxygenase (COX-2) in the hind paw) can be blocked by the NSAIDs. [22, 23]. Local neutrophil infiltration and activation also contribute to this inflammatory response by producing, among other mediators, oxygen-derived free radicals such as superoxide anion (O2-) and hydroxyl radicals [22]. Induced reduction in the levels of interleukin-8 (IL-8), IL-1β, tumor necrosis factor-α (TNF-α), NO via iNOS inhibition and PGE2 via COX-2 inhibition has been associated with diminution in carrageenan-induced paw swelling[19]. It was reported that carrageenan causes the production and release of NO at the injured site [22]. The effects of *M.emarginata* in the carrageenan-induced rat paw edema test in this study suggest that it elicits anti-inflammatory activity possibly via inhibition of production of prostaglandins and diminution of inducible cyclooxygenase, NO and other mentioned cytokines.

The presence of saponins, tannins, flavonoids, phenols, reducing sugars, glycosides, terpenoids, steroids and phlobatannins. Saponins and related phytosterols have been associated with anti-inflammatory, antipyretic and immune-modulating properties, phenols including flavonoids have been shown to possess various pharmacological and biochemical actions including antipyretic and anti-inflammatory properties [24]. There is abundant literature regarding medicinal plants establishing relations between anti-inflammatory, analgesic and phenol/flavonoid content [25]. Specific flavonoids and related compounds including genistein, kaempferol, quercetin, resorcinol and resveratrol have been reported to exhibit cyclooxygenase (COX)-2 inhibition activity and dose-dependent decreases in inflammatory-mediating cytokine TNFα [22]. Tannins [24] and some glycosides [26] have been reported to possess anti-nociceptive and/or anti-inflammatory activities. The established analgesic, anti-inflammatory activities of *M. emarginata* extract in this study are likely due to the presence of these phytochemicals.

The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes [27]. Stabilization of lysosomal membrane is important in

limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [28]. Some of the NSAIDs are known to possess membrane stabilization due to osmotic loss of intracellular electrolyte and fluid components [29]. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components.

The methanol extract showed significant *in-vitro* anti-inflammatory activity and also evaluated for *in- vivo* anti-inflammatory activity by carrageenan induced paw oedema model in rats. The MAME (L) extract showed (30%) and MAME (F) showed (44%) significant anti-inflammatory activity at the dose of 400 mg/kg while thestandard diclofenac showed 55% inhibition of oedema. From the above results it can beconcluded that the methanol extract showed significant anti-inflammatory activity studied by *in-vitro* and *in- vivo* models. The study also provides astrong evidence for the use of the leaves and fruits of *M. emarginata* in folkloric treatment as anti-inflammatory agent. The activity may be due tothe presence of one or more phytochemicalconstituents present in the extract further study iswarranted, for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity.

## CONCLUSION

In the present study, the administration of petroleum ether, ethyl acetate and methanol fractions of *M. emerginata* (ME) has significant analgesic, anti-inflammatory activity (*in-vitro* and *in-vivo*). The present study indicates that ME can be used in the treatment of inflammatory mediated induced diseases.It is further suggested that it can also be used as a supportivedrug with other Ayurvedic drugs.

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