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Original Article

# Evaluation of Pharmacological Activities of Leaves and Bark of *Myristica fatua* var. magnifica Extracts

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## Abstract

*Myristica fatua* var. magnifica (Beddome) Sinclair belongs to the family Myristicaceae. It is a tree used as an important plant to cure various diseases. In the present study, petroleum ether, ethyl acetate and methanolicextracts of*Myristica fatua* were evaluated for its toxicity, potential analgesic, depressant and anti-inflammatory (*invitro* &*in vivo*) activities. The extracts of different concentrations wereused to evaluate the toxicity in albino rats and revealed that they are nontoxic at the studied concentration. The appropriate dose range for preclinical study was found to be 200 & 400 mg/kg and used for above said activities through oral administration and exhibited dose dependent response.In anti-inflammatory (*in vitro*) studies, the percentage of stabilization was found to be 79.66, 80.63 and 85.22 at concentration of 200 mg/ml of MF *(Myristicafatua*) leaves, bark and standard respectively. Hence present investigation has established some pharmacological evidences to support the folklore claim of MFas analgesic, depressant and anti-inflammatory.

*Key words*: *Myristicafatua,*analgesic, depressant, anti-inflammatory

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## 1. Introduction

The traditional medicine that encompasses the exploitation of plants plays a significant protective role in humans and animals particularly in developing and under-developing countries. Plants are an essential part of daily life as humans use herbal medicine for therapy. It is assessed that 80% of world's population depends on plant based formulations for healthcare needs. Traditional medicine is frequently practiced in India. Plants represent an integral part of several systems of medicine such as Ayurveda, Siddha, and Unani.

Plants are known to be the sources lead compounds for the development of modern drugs such as aspirin, digoxin, quinine, vincristine, vinblastine, reserpine and morphine. Nowadays, huge interest and survey of medicinal values of medicinal plants is increased because of developing resistance and side effects associated with the use of modern drugs [1-9].

*Myristica fatua* var. magnifica (Beddome) Sinclair, a wild relative of *Myristica fragrans*, endemic to the Western Ghats belongs to family Myristicaceae. It is a large arborescent tree having significant ecological importance and is restricted to freshwater swamps [10].

The studies suggest that using extracts of aril, seed, leaves, bark and fruits of MF, as a source of natural food preservative for pharmaceutical industries and showed good antioxidant, antimicrobial activity against different pathogens and anticancer activity [11-13].There are no reports on the central nervous system (depressant and muscle relaxant) and inflammation mediated (anti-inflammatory and analgesic) properties of various parts of MF. So, barks and leaves of MFwere selected for the above studies because the plant contains abundant of flavonoids, saponins, tannins, phenolic, resins and alkaloids [12].

## 2. Materials and Methods

### *2.1. Animals*

Wistar rats (150-200 gm) and albino mice (20-25gms) 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).

### *2.2. Drugs*

Carrageenan, purchased from Sigma Aldrich, Mumbai, India. Pentazocin, Diclofenac sodium from Microlabs, Bangalore, India. Petroleum ether, ethyl acetate and methanol from Merck India. All the reagents and solvents used for the study are analytical grade.

### *2.3. Plant Material*

The leaves and bark of MFwere collected directly from the tree in the month of December 2016 from the Thiruvananthapuram, Kerala, India. This plant species was identified and authenticated by Prof K. S. Raju Botanist Department of Botany, Kakatiya University, Warangal, Telangana, India.

### *2.4. Preparation of Extracts*

Coarsely powdered leaves and bark ofMF were subjected to extraction using Soxhlet extractor for 72 hrs at a temperature not exceeding the boiling point of the solvent by increasing polarity from petroleum ether (PE), ethyl acetate (EA) and methanol (MA). The extract was filtered using Whatmann filter paper (No.1), then concentrated under vacuum.

Finally dried at 45°C and the extracts were kept in sterile bottles and refrigerated until use.

*2.5. Experimental Design*

*2.5.1. Acute Toxicity and Gross Behavioral Changes*

The lethal dose ($LD_{50}$) for petroleum ether (PEMF (L) &PEMF(B)), ethyl acetate (EAMF (L) &EAMF(B)) and methanolic (MAMF (L) &MAMF(B)) extracts of MF leavesand bark was determined by the method of Miller and Tainter [14] modified by Irwin [15] using female albino mice.Toxicity studies were carried out to check whether extract ofMF produced any mortality, gross behavioraland adversereactions when given at maximum concentration (2000 mg/kg).

The selected animals were divided in to different groups (n=6) and administered with the dose of 100, 200, 400, 600, 1000 and 2000 mg/kg, while control group received only the vehicle (2-5% acacia, *p.o.*). After administration animals were observed for their behavior and mortality up to 24 hrs.

*2.5.2. Analgesic Activity of Leaves and Bark Extracts of MFby Tail Flick Method*

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 (n=6):Group-I received 2 % acacia as control, group-II received pentazocin (30 mg/kg), group-III to XIV are test groups received MF leaves and bark extracts (200 and 400 mg/kg) as PEMF (L) (III & IV), EAMF (L) (V & VI), MAMF (L) (VII & VIII), PEMF (B) (IX & X), EAMF (B) (XI & XII) and MAMF (B) (XIII & XIV) respectively. Individually each mouse was placed on radiant heat source and basal reaction time was noted at 0 min, after15,30,60 and 120 minthe reaction time for endpoint was noted. Increase in reaction time indicates good analgesic activity [16, 17].

*2.5.3. Depressant Activity of Leaves and Bark Extracts of MFby Actophotometer*

The depressant activity of extracts was done by actophotometer. Each mouse was placed individually in actophotometer for 5 min and basal reaction time is noted.Now theselected animals are divided into fourteen groups (n=6):Group-I received 2% acacia as control, group-II received diazepam (4 mg/kg), group-III to XIV received MFleaves and bark extracts (200 and 400 mg/kg) as PEMF (L) (III & IV), EAMF (L) (V & VI), MAMF (L) (VII & VIII), PEMF (B) (IX & X), EAMF (B) (XI & XII) and MAMF (B) (XIII & XIV) respectively [18].

*2.5.4. Muscle Relaxant Activity of Leaves and Bark Extracts of MFby Rota Rod*

The muscle relaxant effect was assessed by using rota rod apparatus. The selected animals were trained to remain for 3 min on rotating rod (25 rpm) for a week.After training the animals are divided into fourteen groups (n=6):Group-I received 2% acacia,group-II received diazepam (4 mg/kg), group-III to XIV test groups received MFleaves and bark extracts (200 and 400 mg/kg) as PEMF (L) (III & IV), EAMF (L) (V & VI), MAMF (L) (VII & VIII), PEMF (B) (IX & X), EAMF (B) (XI & XII) and MAMF

(B) (XIII & XIV) and their ability to remain on the rotating rod was assessed before and after 30 min. The fall off time from the rod was observedand noted at 0, 0.5, 1, 2 and 3hrsfor each animal [19].

*2.5.5. In vivo Anti-inflammatory Activity of Leaves and Bark Extracts of MFby carrageenan Method*

Wistar rats (150-200 gm) were taken and they were divided into fourteen groups (n=6) 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 right hind paw was induced by injecting carrageenan (1 %; 0.1 ml) in normal saline into the sub plantar region.Group-I received 2% acacia, group-II received diclofenac (10 mg/kg), group-III to XIV test groups received MFleaves and barkextracts (200 and 400 mg/kg) as PEMF (L) (III & IV), EAMF (L) (V & VI), MAMF (L) (VII & VIII), PEMF (B) (IX & X), EAMF (B) (XI & XII) and MAMF (B) (XIII & XIV) respectively. All the doses were given 1 hr before the carrageenan injection. The oedema was expressed as increase in paw volume and measured with a digital Plethysmometer (UgoBasile, 7140) at 0, 1, 2, 3 and 4 hrs. Percentage of paw volume inhibition was calculated using the following formula [20].

Percent inhibition (%) = (PC – PT) /PC X 100
Where PC and PT is the increase in paw volume in control and test respectively.

*2.5.6. In-Vitro Anti-inflammatory activity of MFLeaves and Bark Extracts by HRBC method*

The *in vitro* anti-inflammatory activity was determined by human red blood cell (HRBC) membrane stabilization method. Blood sampleswere collected from healthy human volunteers who was not received NSAIDs for 2 weeks prior to the study. Packed cells were separated using equal volumes of blood sample and Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% critic acid and 0.42% NaCl), centrifuged at 3,000 rpm for about 10 min. The separated packed cells were washed and 10 % suspension was prepared with isosaline. Different extract (100 and 200 mg/ml) solutions were prepared in distilled water and to each sample, phosphate buffer (1 ml), hyposaline (2 ml) and HRBC suspension (0.5 ml) were added, incubated at 37 °C for 30 min and centrifuged at 3,000 rpm. The supernatant liquid was used to assess hemoglobin content by spectrophotometer at 560 nm. The % hemolysis was calculated assuming that the hemolysis produced in the control is 100 % [21].

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

*2.5.7. Statistical Analysis*

Results were expressed as Mean ± SD, statistical significance were calculated by applying one-way ANOVA (Dunnett test). $P<0.05$ was considered as significant.

## 3. Results and Discussion

### *3.1. Acute Toxicity and Gross Behavioral changes*

To find the appropriate dose range, acute toxicities were studied. Rats were observed for visible signs of toxicity and mortality after administration of MFextracts.The study revealed that there was no mortality up to 2000 mg/kg, but some gross behavioral changes were noticed (sleep, sedation and loss of muscle tone) and were recovered within 24hrs. So, 200&400 mg/kg doses were selected for conducting further pharmacological studies because every drug need deeper evaluation of their efficacy and safety due to its growing demand over allopathic medicines.

### *3.2. Analgesic Activity of Leaves and Bark Extracts of MFby Tail Flick Method*

The leaves and bark of PEMF, EAMF&MAMFextracts were evaluated for central analgesic activity using tail flick method (Table 1) [22].The results revealed that all the extracts exhibited dose dependent effect.Among the extracts, MAMFis more significant for analgesic activity at 60 min with both the doses 200 and 400 mg/kg ($P$<0.05,$P$<0.001). On the other hand, Pentazocin is also significant ($P$<0.001) when compared with control.

Based on the results, it implies that the MFextracts exert analgesic activity by interfering with central nervous system for transmission of painful messages in mice.

**Table 1**. Analgesic Activity of *M. fauta* Leaves and Bark Extracts by Tail flick method.

| Treatment | Dose (mg/kg) | Reaction time in sec (Mean ± SD) at time (min) | | | | |
|---|---|---|---|---|---|---|
| | | 0min | 15 min | 30 min | 60 min | 120 min |
| **Control** | 2% acacia | 3.76±2.34 | 3.95±0.84 | 4.82±0.84 | 4.91±0.65 | 4.65±0.55 |
| **Diazepam** | 4 | 4.49±0.79 | 4.67±0.74* | 7.21±0.35** | 8.0±0.65*** | 4.92±0.35 |
| **PEMF(L)** | 200 | 4.01±0.69 | 4.02±0.22 | 5.30±0.44 | 6.86±0.52* | 4.20±0.79 |
| | 400 | 4.07±0.85 | 4.23±0.54 | 6.66±0.32* | 7.32±0.45** | 4.36±0.57 |
| **EAMF(L)** | 200 | 4.04±0.65 | 4.04±0.29 | 5.24±0.25 | 6.08±0.54* | 4.32±0.56 |
| | 400 | 4.30±0.78 | 4.28±0.37 | 6.69±0.42* | 7.31±0.33** | 4.49±0.62 |
| **MAMF(L)** | 200 | 4.06±0.75 | 4.21±0.59 | 5.82±0.78 | 6.87±0.78* | 4.39±0.29 |
| | 400 | 4.26±0.82 | 4.53±0.52 | 7.07±0.22* | 7.94±0.35*** | 4.64±0.38 |
| **PEMF(B)** | 200 | 3.99±0.62 | 4.10±0.25 | 5.28±0.23 | 6.78±0.55* | 4.72±0.73 |
| | 400 | 4.02±0.55 | 4.28±0.44 | 6.62±0.34* | 7.34±0.48** | 4.82±0.46 |
| **EAMF(B)** | 200 | 3.84±0.42 | 4.14±0.26 | 5.34±0.35 | 6.88±0.53* | 4.30±0.48 |
| | 400 | 4.23±0.66 | 4.39±0.39 | 6.78±0.48* | 7.68±0.32** | 4.89±0.52 |
| **MAMF(B)** | 200 | 3.99±0.56 | 4.25±0.59 | 5.89±0.65 | 6.98±0.74* | 4.32±0.45 |
| | 400 | 4.38±0.82 | 4.54±0.52 | 6.90±0.28* | 7.97±0.36*** | 4.90±0.62 |

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).

Several mediators like kinins, acetylcholine, substance P, calcitonin-gene-related peptide and different prostaglandins (PGs) take part in visceral pain model and transmission of the nociceptionfrom the viscera [22,23].The tail flick is thermally induced nociception model where radiant heat is used as a source for flicking reaction (end point) which is due to spinal reflex [24].

### *3.3. Depressant Activity of Leaves and Bark Extracts of MFby Actophotometer*

Theleaves and bark extracts of PEMF, EAMF and MAMFshown significant ($P$<0.05) reduction in locomotor activity at 1 and 3hrs with 400 mg/kg whereas treatment with 200, 400 mg/kg and diazepamsignificantly reduced ($P$<0.01, $P$<0.001) when compared with control at 2hrs. The results were summarized in Table 2. Most of the centrally active analgesic agents influence the locomotor activities in human beings and rodents mainly by reducing the motor activity because of their CNS depressant property [25].Locomotor activity is considered as an index of wakefulness or alertness of mental activity and a decrease may lead to calming and sedation as a result of reduced CNS excitability[26].The results of the

**Table 2**. Depressant Activity of *M. fauta* Leaves and Bark Extracts by Actophotometer.

| Treatment | Dose (mg/kg) | Locomotor activity (Scores) in 5 Minutes | | | | |
|---|---|---|---|---|---|---|
| | | Before Treatment | After Treatment | | | |
| | | 0hrs | 1hrs | 2hrs | 3hrs | 4hrs |
| **Control** | 2% acacia | 150.63±0.45 | 152.13±0.37 | 144.38±0.44 | 127.26±0.64 | 142.67±0.84 |
| **Diazepam** | 4 | 149.32±0.32 | 148.68±0.39* | 112.64±0.34*** | 120.22±0.36** | 141.21±0.26 |
| **PEMF(L)** | 200 | 150.39±0.38 | 150.11±0.52 | 141.66±0.54** | 126.85±0.64 | 142.65±0.25 |
| | 400 | 149.62±0.54 | 149.01±0.62* | 133.29±0.53*** | 125.44±0.62* | 142.84±0.44 |
| **EAMF(L)** | 200 | 150.30±0.33 | 151.25±0.82 | 141.51±0.29** | 127.56±0.43 | 143.60±0.56 |
| | 400 | 149.10±0.42 | 149.66±0.26* | 130.33±0.32*** | 124.42±0.34* | 141.35±0.34 |
| **MAMF(L)** | 200 | 150.34±0.83 | 151.89±0.56 | 141.62±0.43** | 127.28±0.32 | 143.83±0.82 |
| | 400 | 150.68±0.36 | 149.94±0.38* | 125.45±0.52*** | 123.47±0.48* | 141.32±0.38 |
| **PEMF(B)** | 200 | 150.45±0.69 | 150.95±0.56 | 142.24±0.66** | 126.39±0.62 | 142.74±0.41 |
| | 400 | 149.38±0.27 | 149.13±0.84* | 133.42±0.38** | 124.58±0.42* | 142.39±0.75 |
| **EAMF(B)** | 200 | 150.25±0.74 | 151.38±0.73 | 141.38±0.42** | 127.35±0.27 | 143.20±0.54 |
| | 400 | 149.29±0.34 | 149.30±0.47* | 129.82±0.64*** | 122.52±0.47* | 141.58±0.39 |
| **MAMF(B)** | 200 | 150.23±0.56 | 151.82±0.50 | 141.32±0.66** | 126.50±0.49 | 144.30±0.45 |
| | 400 | 149.60±0.84 | 149.60±0.64* | 128.62±0.64*** | 121.44±0.36** | 141.22±0.78 |

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).

present study disclosed significant influence in locomotor activity hence indicating its CNS depressant property in mice.

*3.4. Muscle Relaxant Activity of Leaves and Bark Extracts of MFby Rota Rod*

Mice treated with MAMF (200 and 400 mg/kg) were more significant towards muscle relaxant effect ($P$<0.01,$P$<0.001) than PEMF and EAMF ($P$<0.05,$P$<0.01) when compared with control at 1 hr. Diazepam showed significant muscle relaxant effect at all-time intervals when compared with control groups (Table 3).The muscle relaxant effect was due to the loss of muscle gripimplying skeletal muscle relaxation [20]. Demonstration of marked muscle relaxant effect by the rota-rod study indicated that MF induced neurological deficit accompanied with taming or calming effect supports CNS-depressant effect. It is possible that extracts of MF may acts by potentiating GABAergic inhibition in the CNS through membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts because Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system may be due to the presence flavonoids, saponins and tannins[27].

**Table 3.** Muscle relaxant Activity of *M. fauta* Leaves and Bark Extracts by Rota rod.

| Treatment | Dose (mg/kg) | Fall of time in seconds (Mean ± SD) | | | | |
|---|---|---|---|---|---|---|
| | | 0hrs | 0.5hrs | 1hrs | 2hrs | 3hrs |
| **Control** | 2% acacia | 150.63±0.45 | 152.13±0.37 | 144.38±0.44 | 127.26±0.64 | 142.67±0.84 |
| **Diazepam** | 4 | 149.32±0.32 | 148.68±0.39* | 112.64±0.34*** | 120.22±0.36** | 141.21±0.26 |
| **PEMF(L)** | 200 | 150.39±0.38 | 150.11±0.52 | 141.66±0.54** | 126.85±0.64 | 142.65±0.25 |
| | 400 | 149.62±0.54 | 149.01±0.62* | 133.29±0.53*** | 125.44±0.62* | 142.84±0.44 |
| **EAMF(L)** | 200 | 150.30±0.33 | 151.25±0.82 | 141.51±0.29** | 127.56±0.43 | 143.60±0.56 |
| | 400 | 149.10±0.42 | 149.66±0.26* | 130.33±0.32*** | 124.42±0.34* | 141.35±0.34 |
| **MAMF(L)** | 200 | 150.34±0.83 | 151.89±0.56 | 141.62±0.43** | 127.28±0.32 | 143.83±0.82 |
| | 400 | 150.68±0.36 | 149.94±0.38* | 125.45±0.52*** | 123.47±0.48* | 141.32±0.38 |
| **PEMF(B)** | 200 | 150.45±0.69 | 150.95±0.56 | 142.24±0.66** | 126.39±0.62 | 142.74±0.41 |
| | 400 | 149.38±0.27 | 149.13±0.84* | 133.42±0.38** | 124.58±0.42* | 142.39±0.75 |
| **EAMF(B)** | 200 | 150.25±0.74 | 151.38±0.73 | 141.38±0.42** | 127.35±0.27 | 143.20±0.54 |
| | 400 | 149.29±0.34 | 149.30±0.47* | 129.82±0.64*** | 122.52±0.47* | 141.58±0.39 |
| **MAMF(B)** | 200 | 150.23±0.56 | 151.82±0.50 | 141.32±0.66** | 126.50±0.49 | 144.30±0.45 |
| | 400 | 149.60±0.84 | 149.60±0.64* | 128.62±0.64*** | 121.44±0.36** | 141.22±0.78 |

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).

*3.5. In vivo Anti-inflammatory Activity of Leaves and Bark Extracts of MFby carrageenan Method*

The leaves and bark extracts ofMFwere evaluated for *in vivo* anti-inflammatory activity by paw edema method (Table 4). The results revealed that leaves and bark MAMF extracts (400 mg/kg) reduced the induced edema by 50 and 54.16 %at 3 hr, whereas standard drug reduced to 56.25 % when compared with control group (Table 4). All the extracts exhibited dose dependent anti-inflammatory activity.

**Table 4**. Anti-Inflammatory Activity of M. fauta Leaves and Bark Extracts by Carrageenan Method.

| Treatment | Dose (mg/kg) | Mean paw volume in ml (% inhibition) | | | |
|---|---|---|---|---|---|
| | | 1hr | 2hr | 3hr | 4hr |
| **Control** | 2%acacia | 0.34±0.06 | 0.65±0.02 | 0.48±0.05 | 0.45±0.06 |
| **Diclofenac** | 10 | 0.29±0.05(14.70) | 0.32±0.04***(50.76) | 0.21±0.05***(56.25) | 0.35±0.05(22.22) |
| **PEMF(L)** | 200 | 0.33±0.04(2.94) | 0.50±0.06*(23.07) | 0.32±0.07**(33.33) | 0.39±0.06(13.33) |
| | 400 | 0.32±0.02(5.89) | 0.47±0.07**(27.69) | 0.29±0.04***(39.58) | 0.38±0.08(15.55) |
| **EAMF(L)** | 200 | 0.33±0.04(2.94) | 0.50±0.08*(23.07) | 0.30±0.06**(37.50) | 0.40±0.05(11.11) |
| | 400 | 0.32±0.06(5.89) | 0.45±0.06***(30.76) | 0.26±0.09***(45.83) | 0.39±0.04(13.33) |
| **MAMF(L)** | 200 | 0.33±0.09(2.94) | 0.51±0.05*(21.53) | 0.31±0.07**(35.41) | 0.37±0.06(17.77) |
| | 400 | 0.32±0.03(5.89) | 0.43±0.04**(33.84) | 0.24±0.05***(50.00) | 0.36±0.09(20.00) |
| **PEMF(B)** | 200 | 0.32±0.02(5.89) | 0.51±0.06*(21.53) | 0.31±0.06**(35.41) | 0.38±0.03(15.55) |
| | 400 | 0.31±0.07(8.82) | 0.46±0.09**(29.23) | 0.28±0.04***(41.66) | 0.37±0.08(17.77) |
| **EAMF(B)** | 200 | 0.33±0.06(2.94) | 0.49±0.05*(24.61) | 0.31±0.06**(35.41) | 0.39±0.06(13.33) |
| | 400 | 0.31±0.05(8.82) | 0.43±0.03**(33.84) | 0.25±0.08***(47.91) | 0.38±0.09(15.55) |
| **MAMF(B)** | 200 | 0.33±0.07(2.94) | 0.49±0.08*(24.61) | 0.30±0.06**(37.50) | 0.38±0.04(15.55) |
| | 400 | 0.31±0.09(8.82) | 0.42±0.06**(35.38) | 0.22±0.05***(54.16) | 0.37±0.06(17.77) |

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).

Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-edematous effects of natural products [28] and believed to be triphasic, mediated by prostaglandins [29].Apart from prostaglandins inflammation was also due to monocytes that differentiate locally into macrophages leading to the release of inflammatory mediators such as cytokines, TNFα, chemokines and inducible enzymes (COX-2 and iNOS).Diclofenac sodium is acyclooxygenase inhibitor and said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant activity against carrageenan induced paw edema [28]. So, inhibition of carrageenan induced paw edema by the MF extract could also be due to its inhibitory activity on the lipoxygenase and cycloxygenase enzyme.

*3.6. In-Vitro Anti-inflammatory Activity of MF Leaves and Bark Extracts by HRBC Method*

In the *in-vitro* anti-inflammatory activity screening it was observed that MF extracts of leaves and barkshowed significant activity in membrane stabilization of HRBC and found to be 79.6, 80.63 and 85.22% at 200 mg/ml of MF leaves, bark and standard respectively (Table 5).The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. As the

**Table 5**. In-Vitro Anti-inflammatory activity of M. fauta Leaves and Bark Extractsby HRBC method.

| Extracts | Concentration (mg/ml) | % Inhibition of denaturation |
|---|---|---|
| **Control** | 2% acacia | ---- |
| **Diclofenac** | 100 | 85.22±2.21 |
| **PEMF(L)** | 100 | 45.58±2.32 |
| | 200 | 70.88±2.13 |
| **EAMF(L)** | 100 | 51.28±2.49 |
| | 200 | 76.69±2.80 |
| **MAMF(L)** | 100 | 56.28±2.62 |
| | 200 | 80.63±2.46 |
| **PEMF(F)** | 100 | 43.52±2.34 |
| | 200 | 69.78±2.10 |
| **EAME(F)** | 100 | 50.24±2.45 |
| | 200 | 74.67±2.86 |
| **MAME(F)** | 100 | 55.23±2.65 |
| | 200 | 79.60±2.44 |

erythrocyte membrane is analogous to lysosomal membrane, [30] stabilization of erythrocyte membrane may also stabilize lysosomal membranes. Stabilization of lysosomal membrane prevents the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause tissue inflammation and damage upon extra cellular release.

## 4. Conclusion

From the present study, it can be concluded that MAMF leaves and bark possessed promising centrally mediated anti-nociceptive, locomotor, skeletal muscle relaxant and also possess anti-inflammatory effects. Further studies require purification of the chemical constituents and investigation of biochemical pathway for better therapeutic index with low toxicity.

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