JOURNAL OF PHARMACOLOGY AND BIOMEDICINE
www.rbscience.co.in
E-ISSN: 2456-8244

Evaluation of anti-inflammatory action of Melaleuca bracteata F. Muell. leaf extract

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Abstract

The present work focused on preparing ethanolic extract of *Melaleuca bracteata* F. Muell. and evaluating its anti-inflammatory potential using *in vitro* and *in vivo* models. The ethanolic extract was dark brown in color and had an yield of 46.3% with respect to the weight of the leaf powder used for extraction. The total phenolic content in the ethanolic extract, expressed as gallic acid equivalents was found to be 74.27±0.59 GAE mg/g. The ethanolic extract of *Melaleuca Bracteata* exhibited the inhibition of albumin denaturation at all doses in a dose dependent manner. The 500 µg/mL concentration of the extract had shown the greatest inhibition capacity (58.9±2.869%) whereas the lowest inhibition capacity was exhibited by concentration 100 µg/mL (11.52±3.291%). MBE at dose level of 200 mg/Kg exhibited maximum reduction in edema after 3h with 27.74% reduction whereas the change decreased at the 4th hour to 16.57%. MBE dose of 400 mg/Kg exhibited 42.77% reduction after 3h. The results obtained led to the conclusion that *Melaleuca bracteata* leaves are a rich source of potential phytochemicals and exhibits anti-inflammatory action.

Keywords: Antiinflammatory, *Melaleuca bracteata*, albumin denaturation, carageenan, edema, extract

Received 12/07/2021; Revised 10/08/2021; Accepted 12/08/2021

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Introduction

Inflammation is a primary immune response presented by the host for the removal of harmful stimuli as well as for healing the damage occurred to tissues (Ghasemian et al, 2016). Several medicines including steroids, non steroidal anti-inflammatory drugs, and immunosuppressants have been used frequently for management of inflammatory crisis. These medications present wide array of adverse effects while the aim of therapy practically remains to achieve maximum efficacy at lowest possible dose and adverse actions (Uttara et al, 2009). Hence natural anti-inflammatory therapies have to be considered and herbs need to be investigated.

Melaleuca bracteata is a fast growing evergreen perennial that is widely grown for its attractive golden foliage. It was found from the literature that though the species is rich in flavonoids and phenolics (Siddque et al, 2020), it has not been much explored for its pharmacological potential. Most of the work on the species was directed towards it potential antimicrobial action (antibacterial and antifungal) (Sharifi-Rad et al, 2017; Goswami et al, 2017). Indeed some work has been done on its antisecretory (Adesanwo et al, 2009) and antirheumatoid (Cock et al, 2015) potentials. The essential oil obtained from the leaves are also said to be carminative and have the capability to absorb UV radiation. Despite all the reported activities, our focus was stuck on to the presence of phenolics and flavonoids in the leaves which may render them an array of pharmacological actions.

The present was therefore undertaken with an objective to extract the components of the leaf of *Melaleuca bracteata* F. Muell using ethanol as the extraction solvent and assessing its anti-inflammatory potential using *in vitro* and *in vivo* models.

Material and Methods

The leaves of *M. bracteata* were collected from the plant obtained from a local plant nursery of Bhopal, Madhya Pradesh in the month of February and authenticated wide voucher number RB/Herbarium/09.

Preparation of the plant material

The leaves were washed with distilled water, dried under shade and powdered using a blender at low speed. The powdered leaves were sieved to remove any unwanted debris and were stored in air tight container until taken for use.

Extraction of leaves (Sahira Banu and Cathrine, 2015)

100 g of powder was evenly packed in the extractor of the soxhlet apparatus and subjected to successive solvent extractions using solvent of increasing polarity (n-hexane, chloroform, and ethanol by hot continuous extraction process for about 18 h. The extracts were concentrated by distillation to reduce the volume to one-tenth. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The

dried extracts were stored in desiccators for further processing.

Preliminary phytochemical screening (Arora and Arora, 2019)

The extract was evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation during the testing was used as analytical responses to these tests.

Total Phenolic Content

5 g dried powder of leaves was mixed with 80 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 100 mL with methanol. For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples.

Evaluation of anti-inflammatory potential

Inhibition of albumin denaturation (In vitro method)

The technique of inhibition of albumin denaturation reported by Singh and Mishra, 2020 was used for the present study. The *Melaleuca bracteata* extract (MBE) rich in flavonoids was dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentration. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control. The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the extract solution. Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of the extract solution. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Carrageenan Induced Paw edema in Rats (In vivo method)

The animal (male wistar rats, weighing 180-200 g) were housed in the animal house in cages with free access to food and water and maintained on a 12 h

dark and light cycle. Paw edema was induced by subcutaneous injection of 0.1ml (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat (Amdekar et al., 2012). The extract was administered in dose of 200 mg/kg and 400 mg/Kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 4 groups (n = 5) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III – MBE (200 mg/Kg)

Group – IV – MBE (400 mg/Kg)

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter every hour up to four hours using a vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation produced by the synthesized compounds was calculated by using following formula:

$$\% \text{ inhibition} = C\text{-}T/\ C \times 100$$

C= Paw thickness (mm) in vehicle treated group (control)

T= Paw thickness (mm) in drug treated group

Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result were considered statistically significant when P- value less than 0.05 ($P<0.05$) *vs* control.

Results and Discussion

The ethanolic extract was dark brown in color an had an yield of 46.3% with respect to the weight of the leaf powder used for extraction. The findings of the phytochemical analysis suggest the presence of saponin glycosides, phenolics, terpenoids, protein, and flavonoids in the leaves. The presence of flavonoids and long chain acids has been previously reported in the studies on *M. bracteata* and other species of the genus (Goswami et al, 2017). The extract was tested for the presence of various categories of phytochemicals and it was found to contain glycosides, phenols, tannins, steroids and flavonoids.

Total Phenolic content

The ethanolic extract of *M. bracteata* was evaluated for quantifying the total phenolic content concentrations in extracts by using standard calibration curve of gallic acid at 765 nm. The total phenolic content in the ethanolic extract, expressed as gallic acid equivalents was found to be 74.27±0.59 GAE mg/g.

In vitro anti-inflammatory activity

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis (Saso et al, 2001). According to Opie (1962), tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity.

The ethanolic extract of *Melaleuca Bracteata* exhibited the inhibition of albumin denaturation at all doses in a dose dependent manner (Table 1, Figure 1). The 500 µg/mL concentration of the extract had shown the greatest inhibition capacity (58.9±2.869%) whereas the lowest inhibition capacity was exhibited by concentration 100 µg/mL (11.52±3.291%). The inhibition protein denaturation by 100 µg/mL solution of standard drug Ibuprofen was found to be 78.73 ± 3.561%.

Table 1 Albumin denaturation inhibition activity

Treatment	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Ibuprofen	78.73±3.561	-	-	-	-
MBE	11.52±3.291	20.16±2.657	32.6±2.194	51.5±3.167	58.9±2.869

Results are expressed as mean ± SD, n = 5

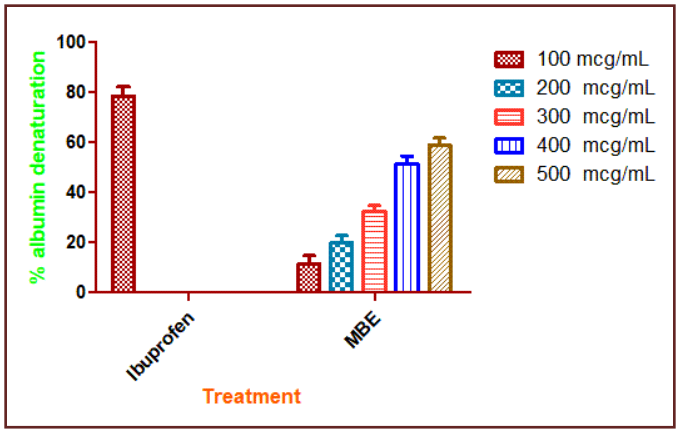

Figure 1 % albumin denaturation by MBE

In vivo anti-inflammatory activity

The percent change in rat paw volume with respect to the control group was calculated and the results are presented in table 2. The change in thickness of paw was measured with the aid of vernier caliper and the % change in thickness was determined. It can be seen from the results that after 4h of administration, Ibuprofen was able to reduce 75.90% of edema caused by carageenan injection. On the other hand, the two doses of MBE were able to reduce the volume very vividly. MBE at dose level of 200 mg/Kg exhibited maximum reduction in edema after 3h with 27.74% reduction whereas the change decreased at the 4th hour to 16.57%. MBE dose of 400 mg/Kg was indeed able to exhibit better reduction in edema as compared to the control with 42.77% reduction after 3h. The reduction decreased to 34.34% after the 4th hour. The effect of MBE was therefore found to be short acting.

Table 2 Effect of MBE on rat paw thickness

Group	Change in Paw thickness (mm) [% inhibition of edema]			
	1h	2h	3h	4h
Vehicle Control	1.35 ± 0.043	2.93 ± 0.072	3.46 ± 0.070	3.32 ± 0.064
Ibuprofen	0.43 ± 0.056 [68.14%] **	1.02 ± 0.075 [65.18%] **	0.98 ± 0.061 [71.67%] **	0.80 ± 0.064 [75.90%] **
MBE (200 mg/Kg)	1.31 ± 0.039 [2.96%]	2.67 ± 0.04 [8.87%]	2.50 ± 0.041 [27.74%]	2.77 ± 0.043 [16.57%]
MBE (400 mg/Kg)	0.96 ± 0.036 [28.89%]	1.93 ± 0.051 [34.13%]	1.98 ± 0.053 [42.77%]	2.18 ± 0.059 [34.34%]

Results are expressed as mean ± SD, n=5

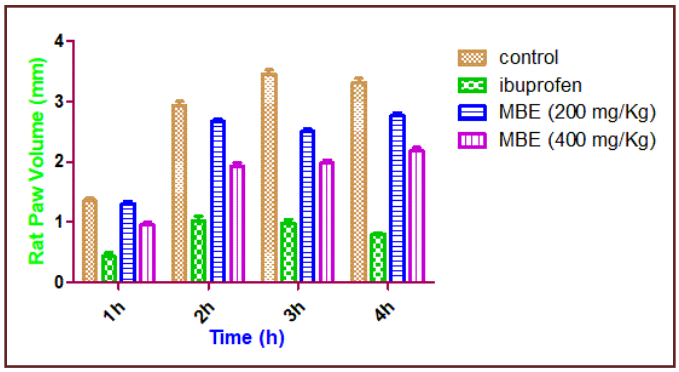

Figure 2 Statistical representation of effect of MBE on rat paw edema

As depicted from the results the extracts were not able to significantly decrease the edema in the rats though they exhibited anti-inflammatory activity. The percent change in paw thickness is presented in figure 3.

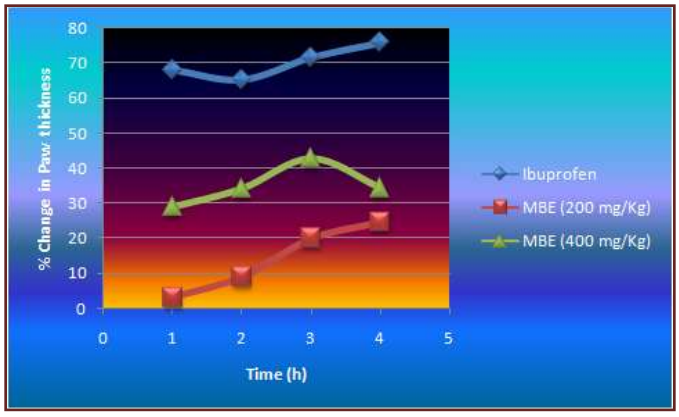

Figure 3 Percent change in paw thickness

Conclusion

The objective of the present study was to assess the anti-inflammatory potential of ethanolic extract of leaf of *Melaleuca bracteata* using the *in vitro* and *in vivo* models. The results obtained led to the conclusion that *Melaleuca bracteata* leaves are a rich source of potential phytochemicals and exhibits anti-inflammatory action.

Acknowledgement

The authors are thankful to the management of IPS College of Pharmacy, Gwalior for providing necessary facilities to carry the research work. Authors would also like to thank RB Science for authentication of the plant material.

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