Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 44 No. 3 (2023)

Assessment of wound healing action of ethanolic extract of *Melaleuca bracteata* leaf

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Abstract: The objective of the present investigation was to evaluate the wound healing action of *Melaleuca bracteata* leaf extract in rats. The powdered leaves were defatted with petroleum ether and extracted with ethanol using soxhlet apparatus. The extraction yield was found to be 21.4%. The total phenolic content of ethanolic extract of *M. bracteata* was 74.27±0.59 GAE mg/g. The topical application of 5 % w/w of the *Melaleuca bracteata* extract containing ointments on the wound resulted in an enhanced and statistically significant ($p < 0.05$) wound healing activity *in vivo* using the excision model. The plant extract exhibited 80.104 ± 5.86 % contraction of wound on the 20th day whereas only 47.739 ± 5.46 % contraction of wound was found in the control animals. The occurrence of tannins in the ethanolic extract could be attributed for the effective wound healing action by the ethanolic extract.

Keywords: *Melaleuca bracteata*, tannins, wound, excision,

1. Introduction

Wounds have affected humans since pre-historic times and the treatment and healing of wounds is an art as old as humanity.[1] Research on wound healing drugs is a rapidly developing area in modern biomedical sciences. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of disease and ailments. India is one of the twelve mega biodiversity centers having over forty five thousand plant varieties.

Melaleuca bracteata cultivar 'Revolution Gold' is a plant belonging to the Myrtaceae family native to Australia. It is a fast growing evergreen perennial that is widely grown for its attractive golden foliage. The genus of *Melaleuca* is known to contain around 230 species worldwide predominantly in Australia, Indonesia, tropical America and South Asia.[2-4]

The literature was reviewed for obtaining the available information on pharmacological potential of *Melaleuca* species. It was found from the literature that though the species is rich in flavonoids and phenolics, 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). Indeed some work has been done on its antisecretory and antirheumatoid potential. 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 a array of pharmacological actions.[5-10]

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 wound healing potential in rat/mice model.

2. Material and Methods

Collection and preparation of the plant material

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. 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[11]

100 g of powder was evenly packed in the extractor of the soxhlet apparatus and subjected to defatting with petroleum ether followed by extraction with ethanol using hot continuous extraction for 9 h. The extract were filtered while hot through Whatman filter paper to remove any impurity. 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[12]

The ethanolic 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[13]

The extraction of phenolic compounds was based on a modified method by Hsu et al. Briefly 1 g dried extract 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. The solution was stored at 4°C in amber bottles and served as the stock solution (50 mg/mL) for subsequent analyses.

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. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Pharmacological Evaluation

Animals

Healthy male Wistar male rats weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental periodadn maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Acute Toxicity Study[14]

A total of three animals were used which received a single oral dose (2000mg/kg) of ethanolic extract of *Melaleuca bracteata*. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks.

Preparation of test samples and standard drugs

The test samples were prepared by formulating the dried ethanol extract in simple ointment base (cetostearyl alcohol, wool fat, white paraffin, and hard paraffin) as a 5 % w/w ointment. Commercially available Povidone Iodine Ointment (5 %) was used as standard drug for comparison of action.

Preparation of simple ointment base[15]

Hard paraffin (5 g) and cetostearyl alcohol (5 g) were taken in a porcelain dish maintained on water-bath at 70°C. Wool fat (5 g) and white soft paraffin (85 g) are added to this mixture and stirred until all the ingredients were in molten state and mixed. The mixture was stirred until cold and packed in suitable container.

The animals were divided in to 4 groups of 5 rat each and the experiment was designed as per table 1.

Table 1: Experimental design for excision model

Group	**Nomenclature**	**Treament**
Group I	Control	Untreated
Group II	Vehicle Control	Simple ointment base
Group III	Standard	Povidone iodine ointment (5% w/w)
Group IV	Test	*Melaleuca bracteata* extract ointment (5% w/w)

All the test samples; vehicle and standard drug samples were applied topically on the wound of each of the animals daily, under sterile conditions.

Induction of wound[16-18]

On the day of inducing wound, each animal was anesthetized by the open mask method using short exposure to diethyl ether. The hair (fur) on the back of each rat was removed by shaving using an electric shaver. The area of the wound to be created was marked on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 1.5 cm in width (circular area 2.25 cm^2) created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. All the surgical procedures were carried out under sterile condition. After 24 h of wound creation, the ointments were applied gently to cover the wounded area once daily until complete healing. Wound area and wound contraction, were monitored on each day.

Measurement of wound contraction[16-18]

The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the wound size. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded.

$$\text{Percent wound contraction } = \frac{Healed\ area}{Total\ area}\ X\ 100$$

3. Results and Discussion

The extraction yield in ethanol was found to be 21.4% w/w and the extract revealed the presence of saponin glycosides, phenolics, terpenoids, and flavonoids.

Total Phenolic content

The ethanolic extract of *M. bracteata* was evaluated for quantifying the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be y = 0.0059x + 0.0057 (Figure 1). The total phenolic content in extract is expressed as gallic acid equivalents. The total phenolic content of ethanolic extract of *M. bracteata* was 74.27±0.59 GAE mg/g.

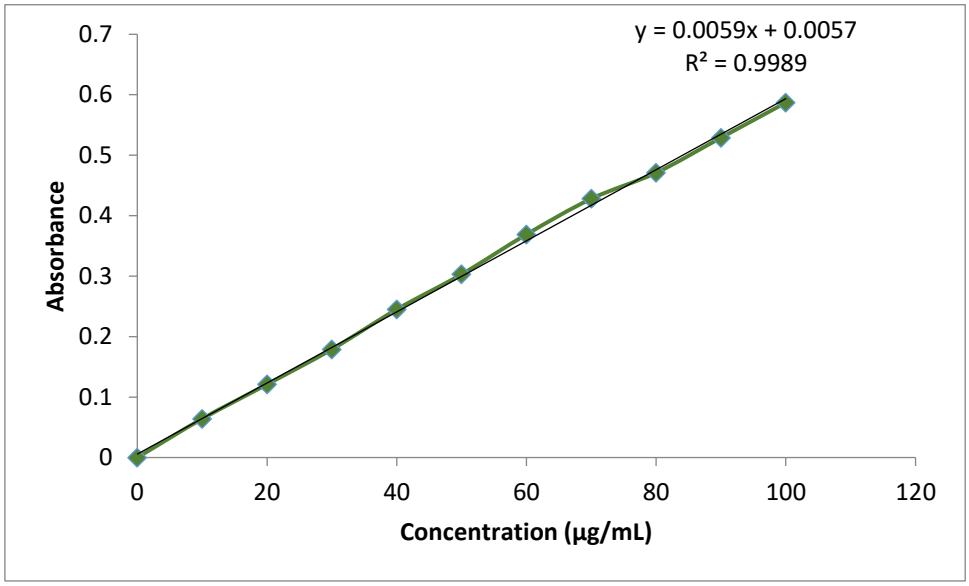

Fig 1: Calibration curve of gallic acid

Acute Toxicity Study

The acute toxicity test was performed by using the dried ethanolic and aqeuous extracts at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD_{50} was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of wound healing action.

Wound Healing action

The ethanolic extracts of *Melaleuca bracteata* leaves was tested to determine the *in vivo* wound healing effect by the excision model (n=5). The topical application of 5 % w/w of the *Melaleuca bracteata* extract containing ointments on the wound resulted in an enhanced and statistically significant ($p < 0.05$) wound healing activity *in vivo*. The wound area measurements and the percent wound contraction results of the progressive healing of the excision wounds for the control; vehicle control; standard reference drug and plant extract are presented in table 1. From the results it can be clearly seen that the ethanolic extract of the plant had an excellent wound healing potential with almost complete closure of the wound of the animals by 20 days. The plant extract exhibited 80.104 ± 5.86 % contraction of wound on the 20th day whereas only 47.739 ± 5.46 % contraction of wound was found in the control animals.

Table 2: Area of wound and % contraction of wound by ethanolic extract

Group	Control	Vehicle Control	Standard	*MB ethanolic extract*
Day	Area mm^2 (% contraction)			
Day 0	318.4 ± 21.1	326.4 ± 27.1	296.5 ± 24	309.1 ± 17.9
	-	-	-	-
Day 5	281.3 ± 24.26	237.4 ±16.49	161.3 ± 12.21	192.8 ± 19.35
	(11.652 ± 4.68)	(27.267 ± 2.55)	(45.599 ± 3.89)	(37.625 ± 6.18)
Day10	243.6 ± 23.21	181.9 ± 19.21	80.8 ± 18.42	125.5 ± 18.29
	(23.492 ± 7.18)	(44.271 ± 4.27)	(72.749 ± 7.32)	(59.398 ± 8.23)
Day 14	201.1 ± 15.7	135.5 ± 11.33	59.6 ± 9.59	99.7 ± 18.5
	(36.84 ± 4.42)	(58.487 ± 3.65)	(79.899 ± 3.28)	(67.745 ± 8.16)
Day 20	166.4 ± 16.3	121.9 ± 13.29	41.1 ± 11.07	61.5 ± 15.28
	(47.739 ± 5.46)	(62.653 ± 4.39)	(86.138 ± 4.20	(80.104 ± 5.86)

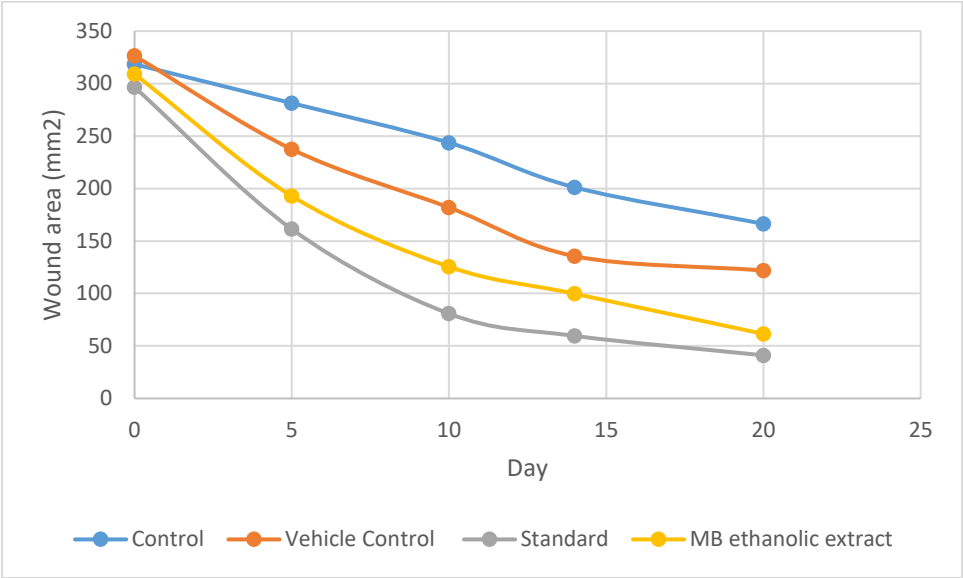

Fig 2: Wound healing efficacy of ethanolic extract of *Melaleuca bracteata* by *in vivo* excision model

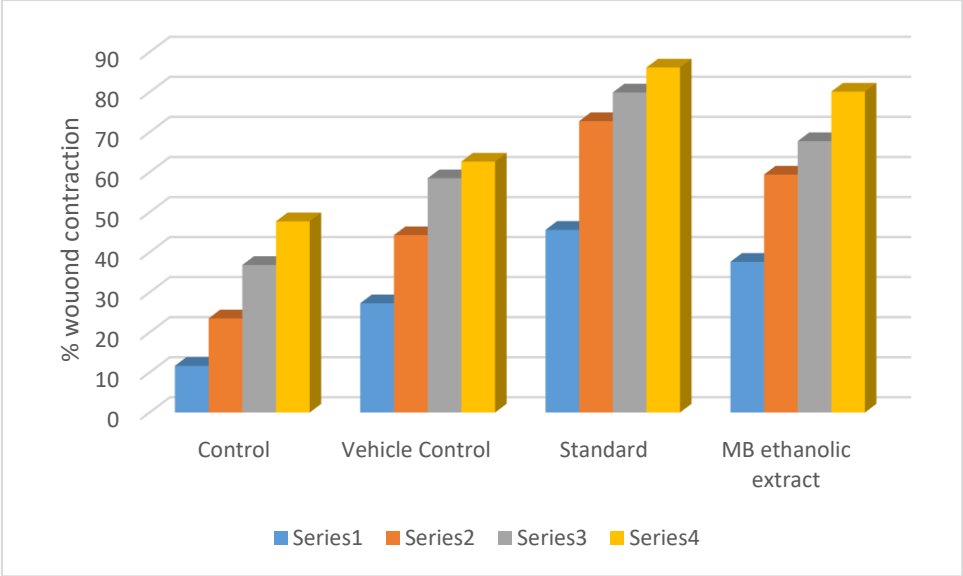

Fig 3: % contraction of wound exhibited by ethanolic extract of *Melaleuca bracteata* by *in vivo* excision model

As shown in the results above (Table 1, Figure 2 & 3) the ethanolic extract exhibited excellent wound healing capability when used as a 5% w/w ointment for topical application on the wound. The standard drug (povidone) was able to contract 86.138 ± 4.20 % of the wound in comparison to the 1st day and exhibited significant action ($p<0.01$).

The occurrence of tannins in the ethanolic extract could be attributed for the effective wound healing action by the ethanolic extract. Previous studies on wound healing action of the plant extracts have also linked the presence of tannins in the extract to its wound healing property.

4. Conclusion

The present investigation had thrown light on the remarkable potential of ornamental plant *Melaleuca bracteata* in terms of its pharmacological benefits it offers. The ethanol extract of the leaves of *Melaleuca bracteata* was found to be effective in the functional recovery of the wound. The result may be attributed to the phytoconstituents such as flavonoids, tannins and phenolics present in the extract which may be due to their individual or cumulative effect that enhanced wound healing.

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