Afr. J. Infect. Diseases
www.africanethnomedicines.net

Research Paper

ISSN: 2006-0165©2008

ANTIBACTERIAL AND WOUND HEALING ACTIVITIES OF *MELASTOMA MALABATHRICUM* LINN.

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Abstract

Melastoma malabathricum Linn. (Melastomataceae), locally known as senduduk putih, is a well-known plant in Malaysian traditional medicine. On the basis of its traditional use and literature reference, this plant was selected for evaluation of its wound healing and antibacterial activities. Methanol extract of *M. malabathricum* was examined for its wound healing activity in the form of an ointment in two types of wound model in rats: (i) the excision wound model and (ii) the incision wound model. The methanol extract ointment produced a significant response in both of the wound types tested. The results were also comparable with the standard drug, nitrofurazone, in terms of wound contracting ability, wound closure time, tensile strength and regeneration of tissues at the wound site. Regarding antibacterial activity, *M. malabathricum* extract inhibited the different clinical wound isolates of *S. aureus and P. aeruginosa* with MIC ranging from 3.0 mg/ml for 3 of the 4 clinical strains of *S. aureus* to 8.0 mg/ml for all the 3 clinical strains of *P. aeruginosa* tested.

Keywords*: Melastoma malabathricum*; Antibacterial; Methanol extract; Wound healing.

Introduction

Melastoma malabathricum Linn. (Melastomataceae) is an erect shrub or small tree 1.5 to 5 m tall, found more or less everywhere throughout Malaysia (Sulaiman et al., 2007). It is commonly called “Straits Rhododendron” and locally called “Senduduk” (Anonymous., 2007a). It is traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, infection during confinement, toothache, flatulence, sore legs and thrush (Anonymous., 2007b). The leaves are squeezed and the juice that is obtained is placed on wounds to obtain healing (Susanti and Rasadah, 2007).

Topical antimicrobial therapy is one of the most important methods of wound care(Meenakshi et al.,2006 ; Ranjith et al.,2006). The goal of topical antimicrobial therapy in wound care is to control microbial colonization and subsequent proliferation thus promoting the healing of the wounds (Veerapur et al., 2004). Some medicinal plants are commonly used in folk medicine for wound care (Rathi et al., 2004 ; Zakaria et al., 2007). The plant, *M. malabathricum,* is used for wound healing in traditional practices,so the present study was undertaken to evaluate scientifically the wound-healing and antibacterial activities .

Materials and methods

Plant materials

The leaves of *M.malabathricum* were collected from Taman Kemacahaya, Selangor, Malaysia in November 2007. The plant was identified by Dr.Adzhar, Department of Botany, Science university of Malaysia, Penang, Malaysia. A voucher specimen (No. MSCNH/M-1(8), 2007) was deposited in the Department of Pharmacy. Masterskill college of Nursing and Health,Selangor,Malaysia .. The leaves were shade- dried, powdered and sieved through a 40 mesh sieve.

Extraction and Formulation of Extracts

The powdered plant materials were extracted with methanol using a Soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The semi-solid mass (methanol free) thus obtained was used for antibacterial and wound-healing activities. The formulation (5% w/w) was prepared by incorporating 5 g of extract into 95 g of simple ointment base B.P. (Anonymous., 1953).

Drugs

0.5 g of extract ointment, simple ointment B.P. and Nitrofurazone ointment was applied once daily to treat different groups of animals, respectively, Nitrofurazone ointment (0.2%, w/w) (GSK pharmaceuticals, Bangalore, India) was used as a standard drug for comparing the wound-healing potential of the extract.

Animals used

Eight week old albino wistar rats (150-180 g) of either sex were selected for the experiment. Six rats were taken for each group. The rats were used after acclimatization to the laboratory environment for a 7 day period. They were provided with food and water *ad libiturn*. The experiments were authorized by the Institutional Animal Ethical Committee of the Masterskill University College of Health Sciences, Malaysia (IAEC NO.MUCH/F.19 (f)/40b).

Test microorganisms

The test organisms used for this study include 4 clinical isolates (A, B, C and D) of *S. aureus* and 3 clinical isolates (A, B, and C) of *Ps. aeruginosa* obtained from wounds and sores of patients undergoing treatment at the Master skill Clinic, Malaysia.

Excision wound model

Three groups with six animals in each group were anaesthetized by the open mask method with anaesthetic ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm 2 full thickness of skin from a predetermined area; the wound was left undressed to the open environment (Udupa et al., 1994a), and 0.5 g of extract ointment, simple ointment B.P. and Nitrofurazone ointment was applied once daily to treat the different groups of animals till the wound was completely healed (Chatterjee and Chakravorty., 1993). Wound contraction and wound closure were monitored at predetermined intervals. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day. From the healed wound, a specimen sample of tissue was isolated from each group of rats for histopathological examination. These tissues were stained with eosine I blue solution and viewed under microscope (Anderson., 1980).

Incision wound model

Three groups with six animals in each group were anaesthetized and two paravertebral-long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the

midline on each side of the depilated back of the rat (Udupa et al., 1994b). All the groups were treated in the same manner as mentioned in the case of the excision wound model. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5-cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed. The extract ointment along with simple ointment (control) and standard drug (0.2%, w/w, nitrofurazone ointment) were administered once daily for 9 days. When the wounds were thoroughly cured, the sutures were removed on the ninth day and tensile strength was measured with a tensiometer (.Anbu Jeba Sunilson et al., 2004).

Determination of tensile strength

The sutures were removed on the ninth day after wound and the tensile strength was measured on the tenth day. The sample drugs along with standard and control were administered throughout the period, once daily for 9 days. On the tenth day the rats were again anaesthetized and each rat was placed on a stack of paper towels on the middle of the board. The number of the towels could be adjusted in such a way that the wound was on the same level as the tips of the arms. The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally on to the polyethylene bottle and the position of the board was adjusted so that the bottle receive a rapid and constant rate of water from a large reservoir until the wound began to open. The amount of water in the polyethylene bag was weighed and considered as an indirect measure of the tensile strength of the wound. The mean determination of tensile strength on the two paravertebral incisions on both sides of the animals were taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of the extract-treated wounds were compared with controls. The tensile strength increment indicates better wound healing stimulation by the applied drug.

Antimicrobial sensitivity and minimum inhibitory concentration (MIC) determination

A solution of the methanol extract of *M. malabathricum* (10 mg/ml) was prepared in DMSO. This solution was introduced into equidistant wells of 6 mm bored on the surface of nutrient agar seeded with the laboratory isolates of test organisms The appropriate inoculum size is 10^5 CFU/ml. Blank DMSO were also placed in separate wells and served as controls. The plates were incubated at 37℃ for 24 h after a prediffusion period at room temperature. Inhibition zone diameter of 5 mm and above was taken as significant susceptibility of each test microorganism to the extract. The MICs of the *M. malabathricum* extract against the 4 clinical isolates (A,B, C, and D) of *S. aureus* and 3 clinical isolates (A, B, and C) of *P. aeruginosa* obtained from sores of different patients were determined using a modification of the agar dilution technique (NCCLS., 1990). Serial concentrations of the extract (0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 16 mg/ml) were incorporated into molten nutrient agar plates. Thereafter, a 24 h actively growing culture (10^5 CFU/ml) of the isolates were then streaked on the plates. MIC for each organism was taken as the lowest concentration of the extract in the nutrient agar that inhibited the visible growth of the organism after 24 h of incubation at 37°C.

Results and Discussion

The measurements of the progress of the wound healing by the nitrofurazone, ointment extract, and the control groups (i.e. simple ointment treated groups) in the excision wound method are shown in Table 1. It is observed that the wound contracting ability of the *M.malabathricum* extract in the form of ointment was significantly greater than that of the control. The time to wound closure of the nitrofurazone treated and the extract-treated groups was same (18 ± 2 days). In the incision wound studies, there was a significant increase in tensile strength of the 10 day-old wound due to treatment with the extract ointment and the standard drug nitrofurazone treated groups of animals when compared with the control. The measurements of the tensile strengths of wounds treated with the extract and the standard drug are shown in Table 2. The tensile strength of the nitrofurazone ointment (standard drug) and the extract

ointment treated groups was almost the same. The wound area treated with *M.malabathricum* extract ointment and Nitrofurazone ointment revealed that the original tissue regeneration was much greater in the case of the skin wounds treated with *M. malabathricum* extract ointment. The wound treated with 0.2% (w/w) Nitrofurazone showed more relative fibrosis than in the case of skin wounds treated with the extract ointment. However, though fibrosis was relatively less, the original tissue was regenerated much more in the case of the extract-treated animal wounds. The skin adrenal structures such as the Pilosebaceous glands, sweat glands, etc., were better presented in wounds treated with extract (ointment) compared to nitrofurazone-treated animal wounds.

Table 1: Effects of *M. malabathricum extract* and Nitrofurazone on wound contraction in albino rats (*N = 6)*

Post- wounding days	***Wound area (mm^2)±S.E.M. and (percentage of wound contraction)***		
	Simple ointment (control)	**Nitrofurazone ointment (0.2 %, w/w)**	**Extract ointment (5 %, w/w)**
0	538 ± 13.9 (0.0)	510 ± 23.6 (0.0)	527 ± 38.3 (0.0)
2	511 ± 16.31 (4.82)	452 ± 36.7 (11.34)	496 ± 19.12 (5.84)
4	463 ± 16.4 (13.04)	322 ± 15.5[a] (36.89)	403 ± 26.4 (22.61)
6	404 ± 11.6 (24.32)	266 ± 18.9[a] (46.31)	295 ± 21.6[a] (43.41)
8	372 ± 17.4 (32.18)	188 ± 27.8[a] (64.21)	171 ± 20.5[a] (65.19)
10	311± 13.1 (41.41)	103 ± 21.5[b] (78.64)	108 ± 18.46[b] (75.23)
12	280 ± 11.4 (47.12)	79 ± 16.8[b] (85.61)	78 ± 13.5[b] (84.03)
14	210 ± 13.5 (60.34)	39 ± 20.9[b] (91.85)	28 ± 11.3[b] (93.56)
16	170 ± 15.6 (67.91)	6 ± 4.5[b] (99.13)	9 ± 4.1[b] (98.34)
18	165 ± 13.4 (69.23)	0.0[b] (100.0)	0.0[b] (100.0)

P values vs. respective control by Student's *t*-test: [a]*P*< 0.01, [b]*P*< 0.001.

The *M. malabathricum* extract inhibited the different clinical wound isolates of *S. aureus and P. aeruginosa* with MICs ranging from 3.0 mg/ml for A, B and D clinical strains of *S. aureus* to 8.0 mg/ml (for all the 3 clinical strains of *P. aeruginosa* (Table 3). The extract inhibited the clinical wound isolates of *S. aureus* and *P. aeruginosa* obtained from sores of patients. This antibacterial property of

Table 2: Effects of *M. malabathricum* extract and Nitrofurazone on the Tensile strengths of wounds inflicted on albino rats by the incision wound model

Serial No	**No of animals**	**Treatment**	**Tensile strength (g) (mean ± S.E.)**
1	6	Simple ointment (ointment control)	418 ± 13.8
2	6	Extract ointment (5 %, w/w)	551 ± 16.9[a]
3	6	Nitrofurazone ointment (0.2%w/w)	576 ± 12 .5[a]

Results were compared with control and *P* values were calculated by student's *t*-test.. [a]*P* < 0.001

Table 3: Minimum inhibitory concentration (MIC) of the methanol extract of *M. malabathricum* against some clinical isolates. (*N*=3)

Organism	Clinical isolates (strains)	MIC ± S.E.M (mg/ml)
Staphylococcus aureus	A	3.0 ± 0.0
	B	3.0 ± 0.0
	C	7.0 ± 0.0
	D	3.0 ± 0.0
Pseudomonas aeruginosa	A	8.0 ± 0.0
	B	8.0 ± 0.0
	C	8.0 ± 0.0

M. malabathricum extract is very beneficial in wound care. Wounds are known to be easy portals for infections and provide suitable medium for the proliferation of microbial organisms. Wound infection has been identified as one of the most important factors that delays wound repair processes and outcome (Bowler et al., 2001). Several microorganisms, including *Ps. aeruginosa, S. aureus, S. faecalis, E.coli, Clostridium perfringens, Clostridium tetani, Coliform bacilli* and enterococcus have been found to infect wounds (Laurent et al., 1999). The leaves of *M. malabathricum* have been found to contain glycoside (Susanti et al., 2007). A glycosidal mixture extract of *Centella asiatica* has been reported to be responsible for enhanced repair only in incised wounds (Rosen et al., 1967) and in stimulating collagen in human skin fibroblast cells (Vogel and De Souza., 1980). The wound healing property of *M. malabathricum* may probably be due to the presence of the glycosides. However, the isolation of the active component is under way in our laboratory. These findings indicate the wound-healing potential of *M. malabathricum* extract and thus substantiate its use in folklore medicine.

Acknowledgements

We are grateful to Dr. Proom Promwichit , Academic Adviser & Mr. Edmund Santhara, CEO, Masterskill College of Nursing and Health, Malaysia, for encouragement and support.

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