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ANTIOXIDANT, ANTIMICROBIAL AND WOUND HEALING POTENTIAL OF *MACARANGA PELTATA* BARK EXTRACTS

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ABSTRACT

The aim of the present study was to evaluate the wound healing activity of bark of *Macaranga peltata* in excision and incision wound models in rats. Antimicrobial and antioxidant activities were studied to understand mechanism of wound healing process. Total phenolic content (TPC) was estimated to screen the prepared extracts by using Folin-Ciocalteu phenol reagent method. Methanol extract showing highest TPC was undertaken for detailed antioxidant, antimicrobial and wound healing activities. Methanol extract showed moderate antioxidant activity on scavenging DPPH, ABTS radicals and by pyrogallol red bleaching method with the determined IC_{50} values of 85.67±1.85, 49.10±2.07 and 336.50±5.74 ug/ml respectively. Methanol extract also showed antimicrobial activity against wound pathogens by cup plate method. The wound healing activity was assessed by the rate of wound contraction, period of epithelialization and skin-breaking strength. In excision wound model, the gel containing methanolic extract of *Macaranga peltata* bark treated group exhibited 96.05±4.14% reduction in wound area on 20^{th} day when compared to control group animals which was 89.61±2.66%. Additionally, epithelialization period of animals treated with gel containing methanolic extract was found to be lower (22.67±4.08) when compared to control group animals (25±1.67). In incision wound model, breaking strength of animals treated with the gel containing methanolic extract of *Macaranga peltata* bark was found to be significantly ($p < 0.001$) higher (508.17±8.64) as compared to the control group animals (406.50±9.16). The present study revealed that gel containing methanol extract of *Macaranga peltata* posses wound healing activity.

KEYWORDS: Incision wound, Excision wound, Antioxidant, Antimicrobial activity

INTRODUCTION

Healing of a wound is a complex multifactorial process that results in contraction and closure of the wound and restoration of a functional barrier.[1] Healing occurs in three stages: inflammation, cell proliferation and contraction of collagen lattice formation.[2] These stages are accomplished by the release of eicosanoids, prostaglandins, leukotrienes and reactive oxygen species (ROS). Among these, ROS play a vital role in healing and serve as cellular messengers that drive numerous aspects of molecular and cell biology. ROS are produced in high amounts at wound site as defense mechanism against invading bacteria. But excess production of ROS can lead to oxidative stress and destruction of newly formed tissues.[3] and delay natural wound healing process and leading to severe complications. Antioxidants play an important role in wound healing because of their free radical scavenging activity. They reduce burn-mediated mortality and tissue lipid peroxidation and thus protect the microvascular circulation.[4] Topical applications of compounds with free-radical-scavenging properties have shown to improve wound healing significantly and protect tissues from oxidative damage.[5]

Though the healing process takes place by itself, there are many potential factors that can interfere with healing. Wound infection resulting from impaired immunity and exposure to pathogens or poor hygiene is one of the most commonly encountered and clinically important impediments to effective wound healing. The injured skin remains vulnerable to invasive microbial infections of all kinds, with the subsequent development of wound sepsis that seriously delays the healing process.[6] *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeroginosa*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* are some important organisms causing wound infection[7]. An injury becomes infected, because the wound area is an ideal medium for the multiplication of the infecting organism. Topical antimicrobial therapy is one of the most important methods of wound care. A wide range of antibiotics is being used at present for treating wound infections, but they produce adverse effects in the human body, also these pathogens develop resistance to the antibiotics targeted against them[8]. Hence, there is a need to develop herbal formulation which can treat wound infection.

A large number of plants have been used by tribal and folklore, in many countries for the treatment of wounds and burns. *Macaranga peltata* (*Euphorbiaceae)* is commonly known as Chandwar. In tribal medicine, gum powder of *Macaranga peltata* bark is used to join fractured bones. In a preliminary study in our laboratory, bark extract of *Macaranga peltata* showed antioxidant activity. There are no literature reports with respect to scientific evaluation of wound healing activity of bark of *Macaranga peltata.* Hence it is thought to investigate in detail *in vivo* wound healing activity of bark of *Macaranga peltata.* The antioxidant and antimicrobial activities were also studied to understand the mechanism behind the wound healing activity.

MATERIALS AND METHODS

Chemicals

Gallic acid, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid (AA), Folin-Ciocalteu phenol reagent, pyrogallol red, 2,2'azino-bis (3- ethylbenthiazoline-6-sulphonic acid) (ABTS), potassium persulphate and all other substances used were obtained from Sigma-Aldrich Co. Ltd. Nutrient agar was obtained from Himedia (Mumbai, India). All chemicals used, including the solvents were of analytical grade.

Plant materials

The bark of *Macaranga peltata* were collected from Malvani area in Malad, Mumbai and authenticated by Agharkar Institute, Pune.

Extraction of plant materials

Autheticated plant material was further dried in shade, powdered and used for extraction. Extraction was carried out using various solvents such as petroleum ether (60-80^{0}C), chloroform, methanol, 50% hydroalcohol and water. The extracts were concentrated in a rotary evaporator under pressure, kept in desicator and used for further studies.

Determination of extractive value

10 gm of powdered material was extracted with 100 ml solvent using soxhlet extraction apparatus. The % yield of each extracts was determined.

In vitro antioxidant assay methods

Plant phenolic constitutes one of the major groups of compounds acting as primary antioxidants or free terminators.[9] Hence total phenolic content of the prepared extracts were determined to screen the active extract.

Determination of Total Phenolic Content (TPC)

The total phenolic content was measured using Folin-Ciocalteu reagent based on procedures described by Singleton et al. (1999), with some modifications[10]. Briefly, 1 ml of extract solution (1 mg/ml) or (0.1 mg/ml) was added to 0.5 ml of Folin Ciocalteau reagent and 5 ml of distilled water. The mixture was incubated at room temperature for 10 min. Then 1.5 ml of anhydrous sodium carbonate solution (10% w/v) was added and the final volume was made upto 10 ml. The final mixture was allowed stand at room temperature for 2 hr with intermittent shaking. Then the absorbance of the dark blue colour that developed was measured at 725 nm using UV-Vis spectrophotometer. The experiment was carried out in triplicates. Gallic acid was used for preparing the standard curve (10 µg/ml to 100 µg/ml). The total phenolic content in the plant extract was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.

The extract showing maximum TPC was further used for various *in vitro* antioxidant assays, antimicrobial activity and *in vivo* pharmacological activities.

DPPH radical scavenging activity

The free radical scavenging activity of active extract was measured by DPPH using the method of Blios[11]. An aliquot of 1 ml of the extract solution in various concentration range was added to 3 ml of 0.1 mM DPPH solution. The decrease in absorbance was determined at 517 nm after 30 min. The percentage scavenging activity was calculated from $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. A blank is the absorbance of the control reaction (containing all reagents except the test compound). The % scavenging activity and IC_{50} value of extracts were calculated for the various concentrations. Ascorbic acid was used as standard antioxidant for comparison.

Peroxynitrite Pyrogallol Red bleach Method

Pyrogallol Red solution (100 µM) was prepared in 100 mM phosphate buffer, pH 7.4. 1 ml of extract solution was added to 2 ml of 100 µM Pyrogallol Red solution.0.5 ml of 200 µM/Litre peroxynitrite solution was added to the mixture and immediately vortexed. After 15 min the absorbance was measured using UV-vis spectrophotometer at 540 nm. The % inhibition of pyrogallol red bleaching was determined using the formula $[(A_1-A_2)/A_1] \times 100$, where A_1 is the absorbance in presence of antioxidants and A_2 is the absorbance in absence of antioxidants. The IC_{50} values yielding 50% inhibition of Pyrogallol Red bleaching were estimated. Ascorbic acid was used as standard antioxidant for comparison.[12]

ABTS assay

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 hr. The $ABTS^{+\cdot}$ solution was diluted with a phosphate buffer (2 mM, PH 7.4) to achieve an absorbance of 0.8 ±0.014 at 734 nm. Extract solutions were mixed with $ABTS^{+\cdot}$ solution, and after 1 min the absorbance was read using UV-vis spectrophotometer at 734 nm. Phosphate buffer solution was used as a blank. The % radical-scavenging activity

of the samples was determined using the formula $[(A_{control}-A_{test})/ A_{control}] X 100$, where $A_{control}$ is the absorbance of the control (ABTS+• solution without test sample) and A_{test} is the absorbance of the test sample (ABTS+• solution with extract). The IC_{50} values scavenging 50% of $ABTS^{+\cdot}$ were estimated. Ascorbic acid was used as standard antioxidants for comparison.[13]

Antimicrobial activity

In vitro antibacterial and antifungal activities of methanolic extract of bark of *Macaranga peltata* were examined by the agar cup plate method against wound pathogens.[14]

Bacterial such as *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus aureus* (NCIM 5022), *Streptococcus pyogenes* (NCIM 2608), *Clostridium perfringens* (NCIM 2677), *Escherichia coli* (NCIM 2065), *Klebsiella pneumonia* (NCIM 5082), *Klebsiella aerogens* (NCIM 2239) and fungal such as *Candida albicans* (NCIM 3471), *Aspergillus niger* (NCIM 1196) were used as test organisms. The organisms were procured from NCL (National Chemical laboratory) Pune, India and tested.

The petri plates were prepared by pouring melted nutrient agar inoculated with 16 to 18 hr old culture test organisms in a sterile petridish. Cups were bored in agar by means of sterile cork borer and were filled with either extract to be tested or standard or control and incubated at 37^0C for 18-20 hours. Mixtute of Dimethyl sulfoxide and water were used as control. Chloramphenicol was served as standard when efficacy was tested against bacterial organisms while fluconazole was served as standard for *fungi*. Diameter of each inhibition zone was measured and compared with standard.

***In-vivo* pharmacological activities**

Methanol extract of *Macaranaga peltata* bark was formulated into 1.5 % Carbopol 971 P NF gel by using extract (1%), ethanol, propylene glycol, triethanolamine and distilled water. Prepared gel was evaluated for skin irritation and wound healing activities.

Animals

Albino Wistar rats of either sex weighing 180 - 200g were used for the study. The animals were procured from Bharat Serum and Vaccines Pvt. Ltd., Thane. All animals were housed in polypropylene cages under standard experimental conditions with 26 ± 2^0C ambient temperature and 12 h light-dark cycle. The animals were fed standard pellet diet and were provided water *ad libitum*. All experimental protocols were approved by the Institutional Animal Ethic Committee (CUSCP/IAEC/10/2013) of C. U. Shah College of Pharmacy, Santacruz (W).

Skin irritation studies

In order to evaluate safety of prepared topical gel, skin irritation study was conducted on albino rats as per OECD guide lines No. 404 (OECD, 2004).[15] The back of the albino rat was shaved to remove the fur carefully, 24 hours before application of the sample. Prepared topical carbopol gel containing methanolic extract of *Macaranga peltata* bark was applied on the skin patches of albino rats and the site of application in terms of erythema and edema was examined at 24, 48 and 72 hours for changes in any dermal reactions. The irritation index was calculated to assess the irritation potential of the prepared carbopol gel according to Draize Test.[16]

***In vivo* evaluation of wound healing**

Incision and excision wound models were used to evaluate the wound-healing activity of prepared topical carbopol gel containing methanolic extract of *Macaranga peltata* bark.

Grouping of animals

Animals were randomized into four groups of six animals each.

Treatment (Group I): Received with topical application of carbopol gel containing methanol extract of *Macaranga peltata* bark.

Positive control (Group II): Received topical application of standard drug ointment i.e. Betadine.

Negative (vehicle) control (Group III): Received with topical application of plain carbopol gel.

Negative control (group IV): Animals were left without any treatment For both excision and incision wound model, the animal groups were classified and treated in the same manner.

Excision wound model

Rats were anaesthetized by open mask method using ether before wound creation. The particular skin area was shaved using hair remover cream (Veet) one day prior to the experiment. A full thickness of the excision wound of circular area (approx. 500 mm^2) and 2 mm depth was made on the shaved back of the rats. The wound was left undressed to the open environment. Carbopol gel containing methanol extract of *Macaranga peltata* bark, plain carbopol gel and betadine were topically applied once a day, to different group of rats, starting from day 0 till complete epithelialization. The parameters studied were % wound closure and epithelialization time. Wound closure was measured as a percent contraction in wound area in each four days over a period of 30 days. Wound closure was studied by tracing the raw wound using transparent paper and a permanent marker on every 4^{th} day for 16 days. Wound area was measured by retracing the wound on a millimeter scale graph paper. The period of epithelialization was calculated as the number of days

required for falling off of the dead tissue remnants (eschar) without any residual raw wound.[17]

Incision wound model

Rats were anaesthetized by open mask method using ether before wound creation. The particular skin area was shaved using hair remover cream (Veet) 1 day prior to the experiment Incision of 6 cm was made through the skin and cutaneous muscles using sterile scissors and forceps. The incision was then closed with interrupted sutures with stitches 1cm apart using sterile absorbable sutures. Carbopol gel containing methanol extract of *Macaranga peltata* bark, plain carbopol gel and Betadine were applied to different groups of rats, once daily from day 0 to day 9 post-wounding. The sutures were then removed on the 8th post – wounding day and the tensile strength of 10-day old wound was measured by tensiometer.[18]

Statistical analysis

Results were expressed as means ± SEM (Standard Error of The Mean). Comparisions between groups were performed using One way ANOVA with post test on GraphPad Instat 3 statistical software. The values of p less than 0.001 were considered as statistically significant.

RESULTS

Extraction

The extraction process yielded 0.18 % w/w of petroleum ether extract, 3.18 % w/w of chloroform extract, 13.02 % w/w of the methanol extract, 5.10 w/w % of the of water extract and 17.08 % w/w of 50% hydroalcoholic extract. (Table 1).

***In vitro* antioxidant assay methods**

Determination of Total Phenolic Content (TPC)

The estimation of total phenolic contents among the different extracts revealed a high phenol content in the methanol extract i.e. 126.57±0.2706 mg/g of gallic acid equivalent (GAE) followed by hydroalcoholic extract (120.91±2.141mg/g GAE), water extract (92.45±2.838 mg/g GAE), chloroform extract (64.82±1.064 mg/g GAE) and petroleum ether extract (20.09±1.846 mg/g GAE) by reference to standard curve (y=0.011x+0.011and r2=0.998) (Table 2).

DPPH radical Scavenging activity

Table 3 shows the amount of extract/standard needed for 50% inhibition (IC50). Methanol extract of *Macaranga peltata* bark showed DPPH scavenging activity at higher IC_{50} value of 85.67 ug/ml as compared to standard ascorbic acid.

Assessment of pyrogallol red bleaching by peroxynitrite

The plant extract and standard exhibited inhibition of bleaching by Pyrogallol Red method indicating peroxy nitrite scavenging activity. Standard ascorbic acid was able to inhibit bleaching of Pyrogallol Red at IC_{50} value of 38.08 µg/ml. However methanol extract of *Macaranga peltata* bark showed less inhibitory activity with higher IC50 value of 331.36 µg/ml as compared to standard ascorbic acid (Table 3).

ABTS scavenging assay

Standard ascorbic acid was able to scavenge ABTS radical at IC_{50} values of 7.23 µg/ml. Methanol extract of *Macaranga peltata* bark exhibited moderate free radical scavenging activity by ABTS method with IC_{50} values of 49.10 µg/ml(Table 3).

Antimicrobial ativity

The results of inhibitory effect of methanol extract from the bark *Macaranga peltata* against nine pathogens were shown in Table 4 and 5. The results showed that different bacterial and fungal species exhibited different sensitivities towards the extract. Smaller zones of inhibition were observed with 50 mg/well of methanol extract of *Macaranga peltata*. However, methanol extract at concentration of 100 mg/well showed better antimicrobial activity against the wound pathogens. The highest antibacterial activity was exhibited against *Klebsiella pneumonia* (12.67±0.58mm), moderate activity against *Staphylococcus aureus* and *Clostridium perfringens* (9.33±2.31 and 10.67±1.53mm), *Pseudomonas aeruginosa* (8±1.00 mm) least activity was exhibited by *Streptococcus pyogenes* (5.33±0.58), *Escherichia coli* (5.67 ± 0.58) and *Klebsiella aerogens* (6.33±0.58mm). Methanol extract showed no activity against *Candida albicans* and *Aspergillus niger* at concentration of 50mg/well. However methanol extract showed antifungal activity against *Candida albicans* at concentration of 100mg/well. Hence methanol extract of *Macaranaga peltata* bark was further evaluated for *in vivo* wound healing activity.

***In vivo* pharmacological activities**

Skin irritation study

Carbopol gels containing methanol extract of *Macaranga peltata* showed no erythema or oedema on intact rat skin. The primary skin irritation index of the gels was calculated as 0.00. The results indicated that all carbopol gels did not cause any skin reaction after examining at 24, 48 and 72 hrs. Since the primary skin irritation index of the creams was calculated as 0.00, it can be classified as non-irritant and were found to be safe for topical application.

Excision Wound Study

The results of wound healing activity by excision wound model are presented in Table 6 and 7. The values presented in the table represent percentage wound contraction at 4, 8, 12, 16, 20 and 24 days for control, vehicle control, standard (povidone iodine treated group) and the test group viz. methanolic extract (1% w/w). It is noticed that wound contraction of rats treated with ointment containing 1 % (w/w) methanol extract was found to be greater on day 20th as compared to control group and vehicle control group. Additionally, wounds

of animals treated with methanol extract showed faster epithelialization (22.67±4.08 days) when compared to control (25±1.67 days) group. On 16^{h} day, wound contraction of standard group rats was found to be significant *(P < 0.001)* in comparison to control group rats. The period of epithelization of standard group was also found to be significantly *(P<0.001)* low as compared to control group.

Incision wound model

The results of the Incision wound healing studies are presented as mean weight in gram±SEM required to break open the resutured wound (Table 8). The animals treated with methanolic extract and standard showed statistically significant increase (p <0.001) in the breaking strength (508.17±8.64 gm) as compared to the control group animals (406.50±9.16 gm). This indicates that the wounds of treated rats were stronger.

Table 1: Determination of % yield of extracts of bark of *Macaranga peltata*

Sr. No.	Extracts	Yield (% w/w)
1	Pettoleum ether (60-80^{0}C)	0.175
2	Chloroform	3.18
3	Methanol	13.02
4	Water	5.10
5	Hydroalcoholic	17.08

Table 2: Determination of Total Phenolic Content (TPC) of extracts of *Macaranaga Peltata*

Sr. No.	Extracts	mg GAE/gm of extract ± SD
1	Petroleum ether (60-80^{0}C)	20.09±1.846
2	Chloroform	64.82±1.064
3	Methanol	**126.57±0.2706**
4	Water	92.45±2.838
5	Hydroalcoholic	120.91±2.141

Values are presented as mean ± SD (*n* = 3).

Table 3: Free radical scavenging activity of methanol extract of *Macaranga peltata* bark by DPPH, ABTS scavenging and pyrogallol red bleaching methods.

Sr. No.	Samples	IC_{50} values of samples (µg/ml)		
		DPPH scavenging	ABTS scavenging	Pyrogallol red bleaching
1	Methanolic extract of *Macaranga peltata*	85.67 ± 1.85	49.10 ± 2.07	336.50 ± 5.74
2	Ascorbic acid	10.99 ± 0.22	7.23 ± 0.61	38.08 ± 0.44

Values are presented as mean ± SD (*n* = 3).

Table 4: Antimicrobial activity of methanol extract of *Macaranga peltata* bark against bacteria.

S.N.	Microorganisms	*Zone of inhibition (mm)*		
		Macaranga Peltata (50mg/well)	*Macaranga Peltata* (1000mg/well)	Chloramphenicol (50ug/well)
1	*Pseudomonas aeruginosa*	4.33 ± 1.16	8.00±1.00	10 ± 0.00
2	*Staphylococcus aureus*	6.00 ± 0.00	9.33±2.31	14.33 ± 0.58
3	*Streptococcus pyogenes*	2.00 ± 0.00	5.33±0.58	12.67 ± 0.58
4	*Escherichia coli*	2.00 ± 0.00	5.67±0.58	17 ± 0.00
5	*Klebsiella pneumonia*	5.33 ± 0.58	12.67±0.58	11 ± 1.00
6	*Klebsiella aerogens*	3.67 ± 0.58	6.33±0.58	12 ± 1.73
7	*Clostridium perfringens*	6.00 ±0.00	10.67±1.53	18 ± 0.00

Values are presented as mean ± SD (*n* = 3).

Table 5: Antimicrobial activity of methanol extract of *Macaranga peltata* bark against fungi.
Values are presented as mean ± SD (*n* = 3)

S.N.	Microorganisms	*Zone of inhibition (mm)*		
		Macaranga Peltata **(50mg/well)**	***Macaranga Peltata*** **(100mg/well)**	**Fluconazol (50ug/well)**
1	*Candida albicans*	--	9.67±0.58	13 ± 0.00
2	*Aspergillus niger*	--	--	12.33 ± 0.59

-- No zone of inhibition

Table 6: Effect of topical application of carbopol gel containing methanol extract of *Macaranga peltata* bark on wound contraction of excision wound.

Post Wounding Days	% wound Contraction			
	Group I	**Group II**	**Group III**	**Group IV**
	Methanol extract of *Macaranga peltata* bark	Standard (Betadine)	Vehicle control (Plain gel)	Control (Without treatment)
4	46.34 ± 6.82	56.04 ± 20.10	28.59 ± 7.29	30.47 ±16.65
8	64.57 ± 10.45	83.47± 17.162	62.36 ± 8.36	59.52 ± 9.13
12	73.62±9.262	95.92± 3.80	78.98± 6.01	76.21± 4.805
16	85.52 ±9.90	98.7± 2.06***	88.52 ± 3.00	84.05 ± 3.40
20	96.05±4.14	100	93.06±1.38	89.61±2.66
24	100		100	96.61± 2.85

*Values are presented as mean ± SD (n = 6). ***p<0.001 vs control*
The treated groups are compared by One way ANOVA with post test.

Table 7: Effect of topical application of carbopol gel containing methanol extract of *Macaranga peltata* bark on epithelialization period of excision wound

S.N.	Groups	Period of epithelialization (days)
1	I: Methanol extract of *Macaranga peltata* bark	22.67±4.08
2	II: Standard (Betadine)	**16.67±2.94*** **
3	III: Vehicle control (Plain gel)	24.33±2.16
4	IV: Control (Without treatment)	25±1.67

Values are presented as Mean ± SD (n = 6). ****p<0.001* vs control
The treated groups are compared by One way ANOVA with post test.

Table 8: Effect of topical application of carbopol gel containing methanol extract of *Macaranga peltata* bark on breaking strength of incision wound

S.N.	Groups	Tensile strength (gm)
1	I: Methanol extract of *Macaranga peltata* bark	508.17 ± 8.64***
2	II: Standard (Betadine)	614.33± 8.80***
3	III: Vehicle control (Plain carbopol gel)	409.17 ±9.56
4	IV: Control	406.50±9.16

*Values are presented as mean ± SD (n = 6). ***p<0.001 vs control*
The treated groups are compared by One way ANOVA with post test.

DISCUSSION

Wound healing is natural healing process. Though it does take place by itself and does not require much help, various risk factors have brought attention to promote this process. Especially oxidative stress resulting from overproduction of reactive oxygen species (ROS) can be extremely toxic and damage various tissues resulting in delayed wound healing. Wounds are also known to be complicated with infection. Wound area is an ideal medium for the multiplication of infecting organism. When wound occurs and it is exposed to external environment, it is more prone to attack by microbes which invade through skin and delay natural wound healing process. Topical antimicrobial therapy is one of the most important methods of wound care. Hence, if a compound having antioxidant potentials and antimicrobial activity additionally, it can be a good therapeutic agent for accelerating the wound-healing process.

Hence in present study, potentials of *Macaranga peltata* in wound care have been studied along with its

antioxidant and antimicrobial activities. The bark extract exhibited antioxidant, antimicrobial and wound healing activities suggesting that the bark of *Macaranga peltata* can play a useful role in wound care.

Numerous plant constituents have been proven to possess free radical scavenging or antioxidant activity[19]. The antioxidant activity may be due to potent-radical-scavenging activity of the phenolics present in the extract. Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines etc.) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation[20]. The activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

The estimation of phenolic content of extracts of *Macaranga peltata* bark was carried out using Folin–Ciocalteu reagent that produced blue colour by reducing yellow hetero polyphosphomolybdate–tungstate anions[21]. The formation of intense blue coloured complex clearly suggests the presence of large number of hydrogen donating groups in the phenolic compounds. The methanol extract had a higher concentration of total phenolic compounds than other extracts. Hence methanol extract was further evaluated for various free radical scavenging and antimicrobial activity.

Antioxidant activity of methanol extract of bark of *Macaranga peltata* was further assessed by DPPH, ABTS scavenging and pyrogallol red bleaching methods. Extracts showed moderate antioxidant activity on scavenging DPPH, ABTS radicals and by pyrogallol red bleaching method with the determined IC_{50} values of 85.67±1.85, 49.10±2.07 and 336.50±5.74 ug/ml respectively. Therefore, such phenomena suggest that the methanol extract may act as free radicals scavenger and may react with radicals to convert them to stable products and terminate radical chain reaction[5] have reported that topical application of compounds with free radical scavenging properties in patients have shown to improve wound healing significantly and protect tissues from oxidative damage. A higher amount of total phenolics in methanol extract of *Macaranga peltata* also supports these results as phenolic compounds are known for free radical scavenging property.

In the preliminary screening of antimicrobial activity, methanol extract at concentration of 50 mg/well and 100 mg/well showed antimicrobial activity against the wound pathogens. Hence, because of antimicrobial activity of methanol extract, the external application of the gel containing methanol extract of *Macaranga peltata* bark on wound could prevent the microbes to invade through the wound, resulting protection of wound against the infection of the various organisms. Microbial infection of wounds delays healing and causes a more pronounced acute inflammatory reaction[22,23] which can lead to further tissue injury and damage. Thus, the antimicrobial activity of the extract on wound pathogens partly contribute to the wound healing effect by eliminating infection thus allowing the natural tissue repair processes to start.

Methanol extract having antioxidant and antimicrobial activities was further evaluated for wound healing activity in fresh wound model in rats. The wound healing process consists of different phases such as homeostasis, inflammation, granulation, collagenation, collagen maturation, and scar maturation which are concurrent but independent of each other [24]. Therefore, it may not be possible to draw firm conclusion about the influence of a given agent on healing by studying only one phase of healing. Hence, in this study three different models (incision, excision and dead space wound models) were used to assess the effect of bark of *Macaranaga peltata* on various phases.

The tensile strength of a wound is determined by the rate of collagen synthesis and more so, by the maturation process where there is a covalent binding of collagen fibrils through inter and intra molecular cross linking.[25] In the incision wound study, there was a significant increase in the tensile strength of incised wound due to treatment with the carbopol gel containing methanolic extract of *Macaranga peltata* and standard drug. The increase in tensile strength of treated wounds may be due to an increase in collagen concentration and stabilization of the fibers facilitating wound healing. This increase in collagen synthesis may be due to the antioxidant effect of the extract which enhances wound healing. The increased tensile strength reveals that the disrupted surfaces are firmly knit by collagen.

Wound contraction, the process of shrinkage of area of the wound depends on the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue.[26] In the excision wound model, the methanol extract of the *Macaranga peltata bark* showed increase in percentage closure of the wounds by enhanced epithelialization. This enhanced epithelization may be due to the antioxidant effect of the extract, which augments collagen synthesis.

The wound healing activity of *Macaranga peltata* bark extract showed that upon application of gel there was increase in wound contraction and increased tensile strength. It can be also concluded that the synergistic effect of both antimicrobial and antioxidant activity accelerated the wound healing process in albino rats. Thus, methanol extract of *Macaranga peltata* might be useful as a wound healing agent.

CONCLUSION

In conclusion, this study confirms the promising wound healing activity of *Macaranga peltata* bark. Results obtained in the present study have shown that methanol extract is active against the growth of the wound pathogens. It also possesses antioxidant activity. Hence,

the external application of methanol extract of *Macaranga peltata* bark on the wound prevented the microbes to invade through the wound, resulting protection of wound against the infections of the various microorganisms. At the same time, external application of the extracts entrapped the free radicals liberated from the wound surrounding cells, which are having inherent machinery to protect the cells from the microbes. Hence, the synergistic effect of both antimicrobial and antioxidant activity accelerated the wound-healing process.

Competing interests

The author(s) declare that they have no competing interests.

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