Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2009; 1(2): 85-87

Research Article ISSN 0975 1556

Study on the Wound Activity Potential on the Aqueous Extract of the Bark of *Myrica esculenta* Buch. & Ham.

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ABSTRACT
Myrica esculenta belongs to the family *Myricaceae* is a sub-temperate evergreen tree found throughout the mid-Himalayas, starting from about 1,300 meters and going up to about 2,100 meters. The tree yields a drupaceous fruit which is one of the tastiest wild fruits of the sub-Himalayan region. It is medium to large woody, evergreen, dioecious tree, 12 to 15 meters high; trunk girth, 92.5 cm; the male and the female trees have almost similar appearance.as ethnomedically bark was claimed to treat wounds and infection also, hence validation was done for its activity.
The results show that the aqueous extract of *Myrica esculenta* has potent wound healing capacity as evident from the wound contraction and increased tensile strength. The results also indicated that *Myrica esculenta* extract possesses potent antioxidant activity by inhibiting lipid peroxidation and increase in the superoxide dismutase (SOD) and Catalase activity, besides antioxidant activity to understand the mechanism of wound healing activity.

Keywords: Antioxidant; Wound healing; *Myrica esculenta,* SOD.

INTRODUCTION

Myrica esculenta is a sub-temperate evergreen tree found throughout the mid-Himalayas, starting from about 1,300 meters and going up to about 2,100 meters. [1] The tree yields a drupaceous fruit which is one of the tastiest wild fruits of the sub-Himalayan region. It is medium to large woody, evergreen, dioecious tree, 12 to 15 meters high; trunk girth, 92.5 cm; bark, light brown to black; the male and the female trees have almost similar appearance. [2] The bark is antirheumatic, antiseptic, aromatic, astringent, carminative, ophthalmic and stimulant. It has proved useful in the treatment of fevers, asthma and coughs. [3-4] The juice is applied to treat rheumatism. Mixed with ginger, it is used as a rubifacient in the treatment of cholera. The juice of the bark is taken internally in the treatment of catarrh and headaches, and is applied externally to cuts and wounds and also is used in the treatment of fevers, asthma and diarrhea. The decoction is boiled to form a gelatinous mass that is applied as a poultice on sprains. Combined with the bark of *Quercus lanata*, it is used as a decoction in the treatment of dysentery. The juice of the unripe fruit is used as an anthelmintic. [5]

MATERIALS AND METHODS

Collection and preparation of the plant material

The plant was selected as per its ethnomedical use in Indian system of Medicine. The local vernacular name of the plant is *Kafal*. Proper identification was done by Department of Botany, Government Post Graduate College, Gopeshwar, Uttrakhand, from its herbarium section no. (GPG/BOT/*ME*-415). After collection, of the bark it was sun dried for 7 days and powdered using pestle and mortar and stored at 35 - 37°C until required.

Extraction

One hundred grams of the powdered drug was soaked into 400 ml distilled water (Chloroform: water, 10:100) in a 1 liter of round bottom flask. This was allowed to stand for 72 h at 35 - 37°C with occasional shaking and then filtered by using Whatman No.1 filter paper. The filtrate was kept in Freeze drier for drying and to achieve the crude extract for further studies. Again one hundred gram of powdered drug was kept for maceration with ethanol 70 %, for 24 h with 6 h of occasional shaking. Then the filtrate was kept for distillation under controlled temperature (30-35°C) to remove out the solvent and to achieve the extract for further studies.

Preliminary phytochemical screening

The extract was screened for the presence of various secondary metabolites like steroids, alkaloids, carbohydrates, proteins, flavonoids, tannins and glycosides using the standard methods. The results are shown in Table 1.

Pharmacological activity

Animal Used

Albino rats (180-220 g) of either sex were purchased from the animal house of the Central Drug Research Institute, Lucknow. They were kept in the departmental animal house

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at 26±2 °C at relative humidity 44–55 % and light and dark cycles of 10 and 14 h, respectively, for 1 week before the experiment. Animals were provided with rodent diet and water ad libitum. All studies were conducted in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals". Ethical clearance for the animal study was obtained from the institutional animal ethics committee. In the experiment, the rats were divided into three groups, six in each.

Group 1 was the control group which received simple ointment base.

Group 2 was treated with reference standard (0.2 %, w/w nitrofurazone ointment).

Group 3 received *Myrica esculenta* ointment (100 mg/500mm^2) topically on wound created on the dorsal back of rats daily till the wounds completely healed. [5]

Excision wound model

An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear using a round seal of 2.5 cm diameter on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500mm^2 diameter. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions, which contribute for wound closure in the first 2 weeks, were studied by tracing the raw wound. Wound area was measured by retracing the wound on a millimeter scale graph paper. The degree of wound healing was calculated [6] and hydroxyproline was measured using the method of Neuman and Logan. [7]

Incision wound model

Rats 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. Full aseptic measures were not taken and no local or systemic antimicrobial was used throughout the experiment. [9] 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 thread on both wound edges were tightened for good closure of the wounds. The wound was left undressed. *Myrica esculenta* ointment, along with water soluble base ointment (control) and nitrofurazone ointment were applied topically twice a day for 9 days. When wounds were cured thoroughly the sutures were removed on the 9th day and tensile strength was measured with a tensiometer.

Tensile strength

The tensile strength of a wound represents the degree of wound healing. Usually wound healing agents promote a gain in tensile strength. The sutures were removed on the 9th day after wounding and the tensile strength was measured on the 10th day. The herbal ointment along with standard and control were applied throughout the period, twice daily for 9 days. The mean tensile strength on the two paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of *Myrica esculenta* ointment treated wounds was compared with control and nitrofurazone ointment as standard. The tensile strength increment indicates better wound healing stimulated by the applied herbal formulation. Further epithelization period and scar area were measured daily for 25 days after determination of tensile strength. [6]

Antioxidant activity

Lipid peroxidation was assayed by the measurement of malondialdehyde (MDA) levels in liver on the basis of reaction with thiobarbituric acid. [8] The activity of SOD was determined in liver by monitoring the inhibition of the autoxidation of pyrogallol. [10] CAT activity in liver was determined according to the standard method. [11] Proteins were determined using bovine serum albumin as a standard. Values were represented as mean ±S.E.M and data were analyzed by paired *t*-test using SPSS software package.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the plant the extracts showed presence of alkaloids, flavonoids, tannins, saponins, glycosides and proteins (Table 1). The wound healing activity results showed that upon application of *Myrica esculenta* ointment there was a decrease in the epithelization period, along with a visibly decreased scar area (Table 2). There was also a significant increase in the tensile strength and hydroxyproline content when compared to the control group and comparable to the standard drugs nitrofurazone (Table 2). The observations and results obtained in this study indicated that the crude extract of *Myrica esculenta* significantly stimulated wound contraction. Thus, the plant extract might be useful as a wound healing agent. Since there is a definite role of free radicals in the pathogenesis of wound. [12] The results indicate that *Myrica esculenta* possesses potent antioxidant activity by inhibiting lipid peroxidation and increase in the SOD and catalase activity (Table 3). This again validates the potent wound healing activity. Thus the wound healing activity may be because of the potent-radical-scavenging activity.

Table 1: Phytochemical screening of *M.esculenta* extract

S. No.	Test	Aqueous extract
1	**Carbohydrates**	+
2	**Glycosides**	—
3	**Alkaloids**	—
4	**Phytosterols**	—
5	**Flavonoids**	+
6	**Saponins**	+
7	**Proteins and amino acids**	—
8	**Phenolic compounds**	+
9	**Tannins**	+
10	**Gums and mucilage**	—
11	**Fixed oils and fats**	—

(+) Present, (—) Absent

Table 2: Wound healing activity of *M .esculenta* extract

Topical treatment	Epithelization period (days)	Tensile strength (g)	Scar area (mm)^2
Control	30.7±1.7	275.5±15.9	64.7±4.4
M. esculenta ointment (100 mg/500mm^2)	17.3±0.71 [a]	391.6±17.8[b]	33.4±2.3[b]
Nitrofurazone (2%) ointment	12.7±1.3[b]	417.2±11.7 [b]	29.7±2.5[c]

Values are mean ±S.E.M. for six rats; statistically significant differences in comparison with control group.
[a] $p < 0.01$, [b] $p < 0.001$, [c] $p < 0.02$.

Table 3: Antioxidant activity of the Myrica esculenta extract.

Sample concentration	TBARS(nmoles/mg protein)	GSH Level (µg/mg protein)	Catalase Activity(µg/g liver)
Control	1.59±0.07	6.06±0.75	113.33±13.66
M.E extract	0.88±0.11	4.43±0.97	248.11±14.97

Values are Mean ± S.E.M of six replicates

Conclusion

Myrica esculenta showed potent wound healing and antioxidant activities suggesting that and ethnopharmacological approach in selecting the plant for study may be useful. There is not much information available on the phytochemical and pharmacological studies on *Myrica esculenta*. The report of the efficacy of this plant as wound healing may be due to its action against dermatophyte which in turn can also be correlated to the effect on antioxidant enzymes.

ACKNOWLEDGEMENTS

I acknowledge my deep regards to Dr. B.C. Behera, Principal, KMIPS, Orissa, for providing the facility for conducting the experiments and co-operate up to the successful completion of the work.

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