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Research Paper

Evaluation of wound healing activity of the plant *Carmona retusa* (Vahl) Masam., in mice

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ABSTRACT

The evaluation of wound healing activity was conducted to study the effect of various extracts of *Carmona retusa* (Vahl) Masam. The ointment was prepared from the alcohol extract of root, stem and leaves with petroleum jelly as a base in 5% and 10% concentrations. Swiss albino mice were used for the evaluation of wound healing activity. The ointment prepared from various parts exhibited considerable response when compared with standard drug Nitrofurazone (0.2%) ointment in terms of wound contracting ability and wound closure time. Remarkable wound healing activity was observed with the root extract ointment at 5% and 10% concentration. This result showed that the ointment of plant extracts accelerate the wound healing process and specifically increased the epithelization in treatment groups when compared with control groups. Thus, this wound healing evaluation study demonstrates that *C. retusa* is effective in stimulating the closure of wounds.

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1. Introduction

Wounds are inescapable events of life, which arise due to physical or chemical injury or microbial infections. The healing of wounds often deviates from normal course, under-healing, over-healing or failure of wounds. Research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds¹. Medicinal plants are coming into prominence because of the overuse of conventional medicines such as antibiotics which has resulted in development of resistance with many infectious organisms. Thus, herbal preparations can be more effective than conventional medicines and their non-toxic nature reveals that they can be administered over long periods².

Wound healing is the process of repair that follows injury to the skin and other soft tissues. It is fundamentally a connective tissue response. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodelled to form scar³.

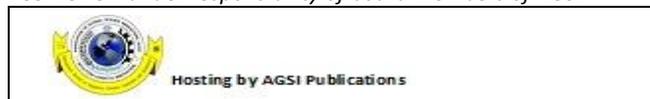
Carmona retusa (Vahl) Masam.,^{4,5,6} Fam.-Boraginaceae (Heliotrope family) previously known as *Ehretia microphylla* Lam. *Carmona* is a monotypic genus⁷. The only species of *Carmona retusa* is found from India eastward to southern China, Taiwan and Japan, and further south throughout Malesia to New Guinea and the Solomon islands. Synonyms: *Ehretia microphylla* Lam., *Ehretia buxifolia* Roxb., *Carmona microphylla* (Lamk) G. Don., *Cordia retusa* Vahl.⁴, *Carmona retusa* is a beautiful shrub (small tree) with many erect branches covered with glossy dark green coarse leaves.

Carmona retusa (Vahl.) Masam (*Ehretia microphylla* Lam.) is reported to be medicinally useful in Indigenous System of Medicine⁸. A novel

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natural product microphyllone has been isolated from *Ehretia microphylla* together with baurenol and ursolic acid⁹. The effects of *E. microphylla* promote the pituitary-ovary axis activities and cause an elevation in the serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol hormones, as well as increase the mean numbers of follicles and eventually ovarian weight¹⁰.

The leaves are used as a stomachic, and in the ailments of cough, fever and constitutional syphilis. The roots are used in southern India for Cachexia and syphilis and as an antidote for certain vegetable poisons^{11,12}.

In-vitro anti-inflammatory activity of alcohol extract of stem of *Carmona retusa* was investigated by human red blood cell membrane stabilization method and shows it as a potential source of anti-inflammatory agents¹³. *C. retusa* has a high potential in fighting the growth and multiplication of cancer cells^{14,15}.

However there is no scientific data on the use of this plant on wound healing activity. Hence the present study was carried out to evaluate the effect of alcoholic extract of various parts of *C. retusa* on experimentally induced excision wounds in mice. The activity of the alcoholic extract was more effective than the other extracts. The previous study on *in-vitro* anti-inflammatory activity was also carried out using the alcohol extract.

2. Materials and Methods

2.1 Collection of plant material

The whole plant *C. retusa* was collected from Chengalpattu District, Tamil Nadu, India. The plant was identified by Prof. Dr. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai-45.

2.2 Preparation of the plant extract

The various parts of the plant such as root, stem and leaves were shade dried and made into coarse powder. 500g of coarse powder of root, stem and leaves were extracted with alcohol and filtered using whatmann No. 1 filter paper. The filtrates were concentrated on water bath and finally in vacuum. The thick dark brown / dark green paste of alcoholic extract of *C. retusa* was stored in air tight container at 4°C till further use. These extracts were used for the evaluation of wound healing activity.

2.3 Experimental Animals

Healthy swiss albino mice of either sex of four months of approximately weighing above 25 to 40gms were used as experimental animals. No prior drug treatment was employed. The animals were housed in polypropylene cages with laced steel roofs in standard environmental conditions of temperature (22±3°C) with relative humidity (63±2%) under 12h light/dark cycles. Standard laboratory animal feed and water were fed *ad libitum*. The animals were allowed to acclimatize to laboratory condition for 10 days before starting the experiment. The experimental protocols and the procedures were approved by IEAC of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was carried out in Siddha Central Research Institute (SCRI), Arumbakkam, Chennai after getting the approval from Institutional Animal Ethical Committee (Reg. No. 141- Pharma/ SCRI-2013).

2.4 Acute toxicity study:

Acute oral toxicity test was carried out as per OECD-425 guidelines by acute class method of OECD¹⁶. Six animals of both sexes were orally treated with different concentrations of extracts like 5, 50, 300 and 2000mg/kg body weight respectively. Control group received similar volume of water. After dosing, all the animals were observed individually at least once during the first 30minutes and periodically during the first 24 hours to detect changes in behavioural, neurological and autonomic response viz. awareness, irritability, spontaneous activity, convulsions, righting reflex, corneal reflex, urinary, salivation and pilo-excitation according to method of Turner¹⁷. The experimental animals were observed for further 14 days for any toxic symptoms and mortality. There was no mortality observed in these doses. On the basis of acute toxicity study, the lowest dose levels of 100 and 200mg/kg body weight of *C. retusa* were chosen for wound healing activities.

2.5 Wound healing activity

The excision wound healing activity was studied by the method described by Luisa A DiPietro¹⁸ and Farahpour and Habibi¹⁹. The skin area on the dorsal thoracic region of the mice was removed by using a suitable depilatory (Anne French hair removing cream) one day prior to the experiment. Alcohol (70%) was used as antiseptic for the shaved region before making the wound. The surgical procedures were carried out under sterile conditions. The experimental animals were anesthetized with anesthetic ether. After successful anesthesia mice were fixed in a dorsal posture on a surgery table. Circular, full thickness surgical wounds with diameters of 5mm, 1 cm away from the backbone were made using 5mm biopsy punch. Using this excision wound method, the epidermal, dermal, hypodermal and panniculus carnosus layers were removed completely. After making surgical wounds, all mice were randomly marked using a non-toxic colour. The animals were divided into the following eight groups of six animals each (both male and female) and were treated as given below:-

- Group I - Normal control group received petroleum jelly
- Group II - Standard group received nitrofurazone (0.2% w/w) ointment
- Group III - Drug treated group received 5% w/w of AERCR
- Group IV - Drug treated group received 10% w/w of AERCR
- Group V - Drug treated group received 5% w/w of AESCR
- Group VI - Drug treated group received 10% w/w of AESCR
- Group VII - Drug treated group received 5% w/w of AELCR
- Group VIII - Drug treated group received 10% w/w of AELCR

The normal (control) and test (standard) group were applied topically with petroleum jelly and 0.2% nitrofurazone ointment respectively in the form of thin layer. The remaining groups were treated with 5% and 10% ointment prepared from the alcoholic extract using petroleum jelly as a base.

The drugs were topically applied daily until the formation of complete epithelial layer, starting from the first day of wound excision.

All the animals were monitored daily and observed for any wound fluid, evidence of infection and any other abnormalities. The diameters of the wound were measured immediately by using vernier caliper. The wound area of each animal was measured from the first day of wounding to the alternate days (1st, 3rd, 5th, 7th, 9th and 11th) until the healing was complete (Table-1). The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelization time that indicates the formation of new epithelial tissue to cover the wound.

The percentage of wound contraction was determined using the following formula:

$$\text{Percentage of wound contraction} = \frac{\text{Initial day wound size} - \text{Specific day wound size}}{\text{Initial day wound size}} \times 100$$

The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelization.

2.6 Statistical analysis

Statistical analysis was performed by using one way ANOVA followed by Duncan Multiple Range Test (DMRT) to compare the mean values of each treatment among groups. Significant differences between the means of parameters were determined by using DMRT. All statistical analysis was performed using SPSS Statistical version 18.0 software packages.

3. Results and Discussion

Plant products are potential wound healing agents and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations. The wound healing activity of various extracts of the plants like *Lantana camara* Linn.²⁰, *Achyranthes aspera* Linn.²¹, *Adhatoda vasica* Nees.²², *Ageratum conyzoides* Linn.²³ etc., have also been reported in rats and mice.

The topical application of drugs is an efficient therapy method of destroying microbial populations because the availability of the drug at the infected wound site leads to enhanced wound healing activity. The virulence capacity of microorganisms, amount of inoculums and host immune response are important factors that can cause massive damage during infection.

Ample numbers of secondary metabolites / active compounds isolated from plants have been demonstrated in animal models (*in-vivo*) as active principles responsible for facilitating healing of wounds²¹. Wound healing may be attributed to the phyto-constituents (phytosterols, glycosides, alkaloids, saponin, phenolic, tannins, flavonoid and alkaloids) present in the plant and rapid closer of wound could be a function of either the individual or additive effect of phyto-constituents in the various parts of the plant of *C. retusa*.

The topical application of various extract ointments at different concentrations (5% and 10%) elicited a significant reduction in the wound area (Table 1 and 2). There is no significant differences among all the groups upto 3rd day, but from 5th day there were significant differences among all groups, On 7th day, the wound contraction of standard and test were found to be significant when compared to control group. On 11th day, wound was completely healed for standard group while for test group healing was not complete. However on 13th day, the test group exhibited 99.30% healing with 10% root extract ointment and control group showed 61.57% healing. The time required for complete epithelization of the excision wound is an important parameter to assess the wound healing process. When compared with the control group, the wound healing activity exhibited by test group with extract ointments were found to be highly significant. More significant wound healing activity was observed with increased concentration of extract i.e., with 10% ointment prepared with extract.

Table 1 - Effect of alcohol extract of *Carmona retusa* on excision wound model - ANOVA for significant difference among groups.

S.No.	Group	Treatment	Wound size (mm)					
			3 rd Day	5 th Day	7 th Day	9 th day	11 th Day	13 th Day
1	Group I	Control	3.920 ^d (0.017)	3.570 ^d (0.031)	3.108 ^d (0.010)	2.740 ^e (0.070)	1.922 ^e (0.016)	1.288 ^f (0.012)
2	Group II	Standard 0.2% w/w	2.537 ^a (0.207)	1.895 ^a (0.022)	1.173 ^a (0.060)	0.503 ^a (0.012)	0.045 ^a (0.007)	(.)
3	Group III	AERCR 5% w/w	3.138 ^b (0.013)	2.317 ^{bc} (0.103)	1.700 ^{bc} (0.019)	1.218 ^b (0.075)	0.713 ^b (0.013)	0.236 ^b (0.033)
4	Group IV	AERCR 10% w/w	3.095 ^b (0.012)	2.237 ^b (0.185)	1.562 ^b (0.033)	0.532 ^b (0.040)	0.122 ^b (0.009)	0.033 ^a (0.006)
5	Group V	AESCR 5% w/w	3.308 ^{bc} (0.095)	2.625 ^c (0.123)	2.040 ^{cd} (0.108)	1.440 ^c (0.129)	0.643 ^c (0.067)	0.490 ^c (0.040)
6	Group VI	AESCR 10% w/w	3.232 ^b (0.019)	2.678 ^c (0.192)	1.835 ^{cd} (0.153)	1.215 ^b (0.014)	0.310 ^c (0.018)	0.275 ^b (0.019)
7	Group VII	AELCR 5% w/w	3.510 ^c (0.019)	2.660 ^c (0.077)	2.132 ^c (0.020)	1.688 ^d (0.012)	1.063 ^d (0.009)	0.882 ^d (0.026)
8	Group VIII	AELCR 10% w/w	3.323 ^{bc} (0.021)	2.597 ^c (0.071)	1.898 ^{cd} (0.019)	1.302 ^{bc} (0.015)	0.862 ^d (0.009)	0.730 ^d (0.017)
		F Value	22.855	17.319	61.869	135.419	522.437	313.567
		P Value	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**

Note:

1. n=6 animals in each group.
2. The value within bracket refers to SEM.
3. ** denotes significant at 1% level.
4. Different alphabet among Groups denotes significant at 5% level using Duncan Multiple Range Test (DMRT).

Table 2 - Effect of alcohol extract of *Carmona retusa* ointments on excision wound (% Wound closure)

S.No.	Groups		Wound Healing Area in Percentage					
			3 rd Day	5 th Day	7 th Day	9 th Day	11 th Day	13 th Day
1	Control	(Petroleum jelly)	21.60	28.60	37.83	45.20	61.57	74.23
2	Nitrofurazone	0.2% w/w	49.30	62.10	76.53	89.93	99.10	-
		5% w/w	37.23	53.67	66.00	75.63	85.74	95.29
3	Root	10% w/w	38.10	55.27	68.77	89.37	97.55	99.30
		5% w/w	33.83	47.50	59.20	70.87	87.13	90.20
4	Stem	10% w/w	35.37	54.93	63.30	77.50	93.80	94.50
		5% w/w	29.80	46.80	57.37	66.23	78.73	82.37
5	Leaf	10% w/w	33.87	48.07	62.03	73.97	82.77	85.40
		5% w/w	29.80	46.80	57.37	66.23	78.73	82.37

The wound healing activity observed with the alcoholic extract of the plant was significant and comparable with the result observed with standard drug. The wound healing activity of alcoholic extract of root of *C. retusa* is better in comparison with stem and leaves extract.

Since p values less than 0.01 there is significant difference among groups at 1% level with regard to wound healing activity at 3, 5, 7, 9, 11 and 13 day. Based on DMRT the control is significantly differs from standard group and test groups at 5% level also. The standard was significantly differ from all the test groups (Table 1)

The present study revealed that the alcoholic extract ointment of various parts of *C. retusa* possesses better wound healing potency, which was evidenced by the increased rate of wound contraction, reduction in the period of epithelization, increase in collagen deposition and increase in tensile strength in granulation tissues.

4. Conclusion

As infection is a major cause of morbidity and mortality in wound patients, these herbal extracts may be useful in preventing infection that leads to high risk of sepsis. Results demonstrate that the alcoholic extract ointment of various parts of the plant *C. retusa* would be capable of promoting wound healing activity. Further study on the fractionation of active components and the mutual effect of these plant extract machinery on infecting microbial species may provide a better understanding of the infection management in the process of wound healing.

Studies with purified chemical constituents and evaluation in clinical settings may provide more information on the complete mechanism of wound healing activity.

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