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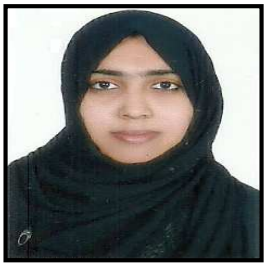

PHARMACOLOGICAL EVALUATION OF THE WOUND HEALING ACTIVITY OF *CINNAMOMUM VERUM* IN EXPERIMENTAL RATS

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ABSTRACT

The aim of the study was to perform pharmacological evaluation of *Cinnamomum verum* leaf extract for wound healing activity. The *Cinnamomum verum* leaves were successively extracted with various solvents according to their increasing order of polarity. The phytochemicals present in the extracts were identified by qualitative phytochemical screening, which revealed the presence of fats in petroleum ether extract; tannins, terpenes in chloroform extract; terpenes, flavanoids in ethyl acetate extract; saponin, flavanoids in water extract; and terpenes, phenolics, tannins and flavanoids in the alcoholic extract of *Cinnamomum verum* leaves respectively. The pharmacological evaluation for wound healing was carried out in three models in Wistar Albino rats and it was revealed that the isolated fraction of *Cinnamomum verum Linn* leaf possess good wound healing effect. This method involves histological examination of tissue for the study of details about the cellular morphology and other alteration at cellular levels during healing and regeneration. Hence, isolating the drug from the natural source may have possibilities of lesser side effects, which may be further helpful for the society.

KEYWORDS

Cinnamomum verum, Excision, Incision and Dead space model.

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INTRODUCTION

The skin has been described as the largest organ in the body. Its functions are numerous and any injury to its layers increases the vulnerability of the organisms to additional biological and physical hazards resulting in wound. Skin damage can be caused by radiation, chemicals, extremes of temperatures, invasion by microorganisms, dehydration, mechanical damage, arterial insufficiency and use of alkaline soaps. Maintaining

skin integrity is a complex process and is often taken for granted until damage occurs[1].

Wound Healing is a complex and dynamic process of restoring cellular structures and tissue layers that may take place in two ways viz. "Regeneration", the replacement of lost tissue by similar tissue and by contrast "Repair" is the replacement of lost tissue by a new structure known as "Granulation tissue", which matures to form the scar tissue.

Induction of the stress or injury by means of agents such as complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants results in the generation of the wounds and that are inescapable part of human life[2]. Because of poor hygienic condition, wound infection becomes the most ordinary disease in developing countries[3].

More than 80% of the world population still depends upon traditional medicines for their ailments[4], especially for wound management[5], as they provide a moist environment to encourage the establishment of the suitable environment. Several drugs of plant, mineral, and animal origin are described in the Ayurveda for their wound healing properties. Some of the plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models. This process is usually performed in a stepwise fashion starting with *in-vitro* testing, preclinical, and then clinical evaluations.

Wound healing activity has been done in various medicinal plants, but no such activity has been done in *Cinnamomum verum* leaves. The isolated and selected extracts of *Cinnamomum verum leaves* were made in the form of suspension and were evaluated for their wound healing activity in rats.

MATERIAL AND METHODS

Animals

About 33 Healthy, young, Wistar albino rats of either sex weighing between 120-150 gm were obtained from the animal house of Alshifa College of Pharmacy. They were individually housed at a temperature of 25^{o}C under standard conditions with 12hour day and night cycle. They were provided with standard pellet diet *ad libitum*. Animals were periodically weighed before and after experiments. All the animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study[91]. The study were conducted in conformity with and approved by the institutional animal ethical committee No-IAEC.015/13.

The rats were grouped in to five of 6 animals in each group.

Plant Material

Cinnamomum verum leaf extract was prepared in the lab using the plant materials collected from local areas of Malappuram. Authentication of the plant was done by Mr. A.K Pradeep, from Calicut University Herbarium which was certified that the given specimen no-88430 belonged to *Cinnamomum verum* Presl [Lauraceae]. Freshly collected leaves after cleaning were left for shade-drying on the floor above the newspaper for 10-15 days. After that, the leaves were dried in hot air oven at 40^{0}C for an hour just before starting the extraction process to remove the equilibrium moisture content.

EXTRACTION

The extraction of air dried and coarsely powdered leaves of *Cinnamomum verum Presl* was successively carried out in continuous hot soxhlet extractor with petroleum ether, chloroform, ethyl acetate, ethanol, and water in the order of increasing polarity. The percentage yield obtained for leaves extracts are tabulated in Table No.1. The extracts were then concentrated to dryness under reduced pressure and they were preserved in a refrigerator.

Phytochemical Screening

The extracts of *Cinnamomum verum* leaves were subjected to preliminary phytochemical evaluation[6,7] using standard procedures. The results are shown in Table No.2.

TOXICITY STUDIES

Acute toxicity studies were done by class method as per OECD guide lines. Here the animals can be limited to three and they were given the higher dose of 2000mg/kg body weight and observed for the mortality on the third day[8].

Wound Healing Activity

Here, 0.2% Nitrofurazone ointment was used as the standard drug. Topical route of administration was carried out. The isolate and selected extracts of *Cinnamomum verum leaves* were separately mixed with 1% carboxy methyl cellulose and made in the form of suspension and were observed for their wound healing activity in rats using the following models.

Excision Wound Model

Wistar rats were anesthetized prior to and during creation of the wounds, with anaesthetic ether. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat[9,10]. The dorsal fur of the animals was shaved and the anticipated area of the wound was created and outlined on the back using a circular stainless steel stencil. A full thickness of the excision wound of circular area of 500 mm2 and 2 mm depth was created using pointed scissors. The entire wound was left open; all surgical procedures were performed under aseptic conditions. The control group animals (Group I) treated with the vehicle, Standard (Group II) was applied with 0.2% w/w Nitrofurazone in simple ointment I. P[11,12]. Other groups of animals to be treated with the extracts. The wound closure rate was assessed by tracing the wound on days 1, 4, 6, 8, 11, 14, and 16 after wounding days using transparent paper and a permanent marker. The wound areas recorded to be measured using graph paper .The results are tabulated in Table No.3.

Incision Wound Model

The rats were anaesthetized prior to and during creation of the wounds, with anaesthetic ether. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back. After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed. Extracts were topically applied to the wound once a day. The sutures were removed on 8th post wound day and continued the application of the extract. The wound breaking strength was measured on the 10th day evening after the last application[13,14]. The results are tabulated in Table No.4.

Dead Space Wound Model

Dead space wounds was created by implanting two preweighed sterilized polypropylene tube (2.5 length × 0.25 cm diameter) beneath the dorsal paravertebral skin of the anaesthetized rats. The control group animals were provided with plain drinking water, and the other group rats were separately administered with the extract of the leaves. On the 10th post-wounding day, the granulation tissue formed on the implanted tubes was carefully detached from surfaces of the tubes. The wet weight of the granulation tissue collected was noted. The tissue samples was dried at 60°C for 12 hour and weighed to determine the dry granulation tissue weight. The results are tabulated in Table No.5.

Statistical Analysis

The results were expressed in Mean ± Standard Error Mean of six animals. Significance was calculated by one way ANOVA followed by dunnet test with the help of SPSS statistical software[15].

RESULTS

Extraction

The extraction of powdered leaves of *Cinnamomum verum* was carried with petroleum ether, chloroform, ethyl acetate, ethanol, and water in the order of increasing polarity. The percentage yield obtained for leaf extracts are tabulated in Table No.1.

Ethanolic fraction was obtained in good yield.

Phytochemical Screening

The Phytochemical screening of *Cinnamomum verum* Leaf revealed that the petroleum ether extract contains tannins. Chloroform and Ethyl acetate extract contain flavanoids. Water extract contain saponins, terpenes, flavanoids. Whereas, the ethanolic extract contains carbohydrates, saponins, flavanoids, terpenes, and phenols in higher amount.

Acute Toxicity Test

Observations included changes in skin and fur, eyes and mucous membranes, and respiratory and behaviour pattern. A special attention was directed to observations of tremors, convulsions, salivation,

diarrhea, lethargy, sleep and coma. All the characteristics of the animal appeared to be normal and no sign of toxicity was observed.

Excision Wound Model

The epithelialization period in the control animals was 16.32±0.54. Treatment with the isolate suspension resulted in significant decrease in epithelialization period (11.98±0.66), when compared with control group animals.

The isolate extract-treated animals showed faster epithelialization of wound than the animals treated with ethanolic and aqueous leaf extract. The period of epithelialization was 11.98±0.66 for the isolate extract treated group of animals as against 13.55 ± 45 for the standards drug-treated group.

Incision Model

In incision wound model , it was found that the ethanolic and isolate extract treated animals showed significant increase in breaking strength (198.32± 0.33 and 217.02±0.86 respectively) than water extract treated animals (176.84±0.85), when compared to the control (196.65±0.54). Here, the mean breaking strength was also significant in animals treated with standard drug Nitrofurazone (208.46±0.66). Thus, the isolate extract showed better activity than the Ethanolic and Water extract.

Dead Space Model

In dead space wound model, it was reported that the isolate extract-treated animals showed significant increase in dry weight of granulation tissue when comparing with the ethanolic and water extract.

Table No.1: Percentage yield of different extraction processes

S.No	Extracts	Percent Yield Value
1	Petroleum ether	2.23
2	Chloroform	5.41
3	Ethyl acetate	6.10
4	Ethanol	9.43
5	Water	8.01

Table No.2: Phytochemical screening of extracts of *Cinnamomum verum* leaves

S.No	Phytochemicals	PEE	CHE	EAE	ETL	WTR
1	Alkaloids					
2	Glycosides					
3	Carbohydrates				+	
4	Saponins				++	++
5	Flavones and Flavonoids		+	+	++	+
6	Terpenes				++	++
7	Tannins	+			+	
8	Phenols				++	+
9	Proteins and aminoacids					

PEE-Petroleum ether extract, CHE- Chloroform extract, EAE- Ethyl acetate extract, ETL-Ethanolic extract, WTE -Water extract.

Table No.3: Effect of various extracts of *Cinnamomum verum* leaves on period of Epithelization

S.No	Treatment	Wound contraction sq mm	Period of epitheliazation
1	Control	28.22±0.986	16.32±0.54
2	Standard	24.56±0.66*	13.55±.45*
3	Test 1	28.87±0.33	14.32±.36*
4	Test 2	32.4±0.66	16.98±0.94
5	Test3	19.55±0.91*	11.98±0.66*

All values are expressed in mean ±SEM n=6 *p<0.05 with control

Table No.4: Effects of extracts of *Cinnamomum verum* leaves on wound breaking strength

S.No	Treatment	Wound Breaking Strength in g
1	Control	196.65±0.54
2	Standard	208.46±0.66*
3	Test 1	198.32±0.33*
4	Test2	176.84±0.85
5	Test 3	217.02±0.86*

The values are expressed in Mean ± SEM n=6 *P<0.05 was considered as statistically significant with control.

Table No.5: Wound healing effects of various extracts of *Cinnamomum verum* leaves in dead space model

S.No	Treatment	Dry Granuloma Weight g/100g
1	Control	54.53±0.79*
2	Standard	34.55±0.81
3	Test 1	55.2±0.74*
4	Test 2	39.6±0.45*
5	Test 3	59.45±0.28*

The results were expressed in mean ±SEM n=6 *P<0.05 was considered significant statistically.

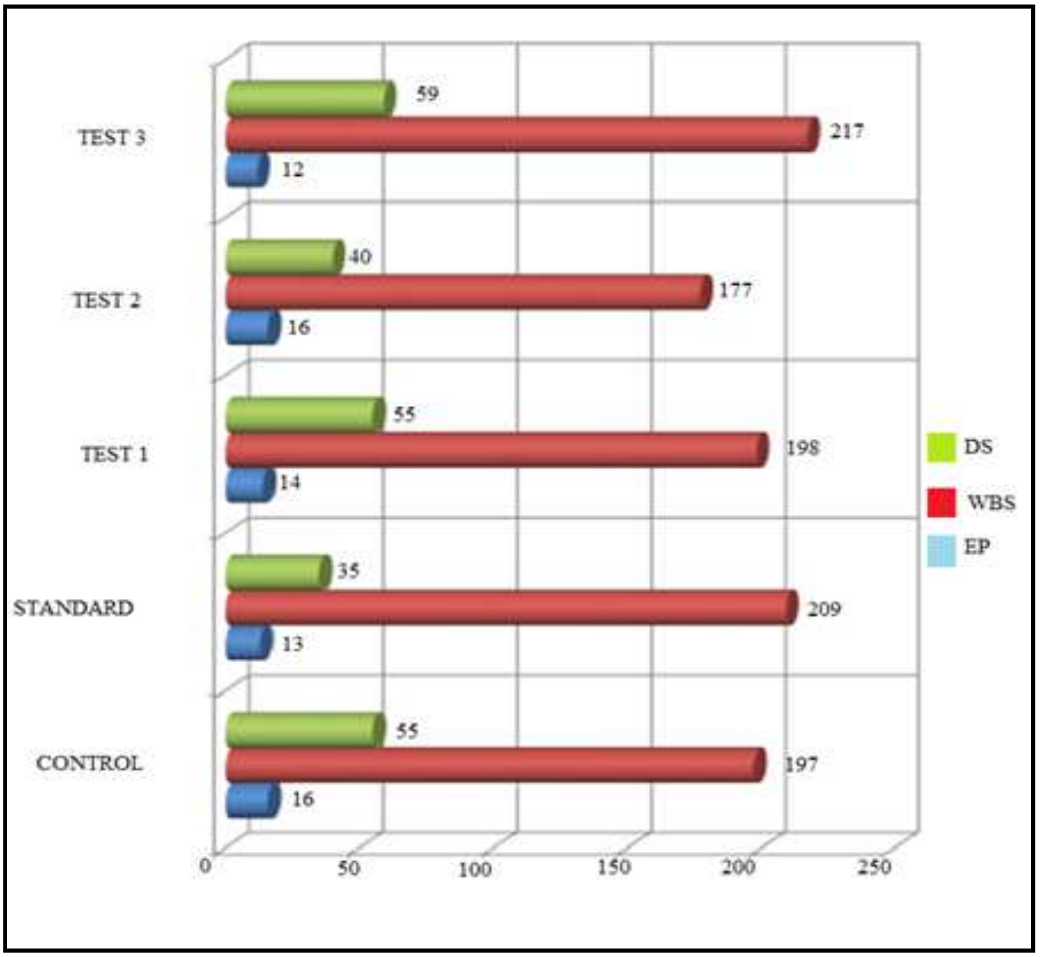

Figure No.1: Graphical representation of 3 models in wound healing of rats

CONCLUSION

In the present study, topical treatment of ethanolic isolate of *Cinnamomum verum* extract significantly increased the wound healing rate. By virtue of its antioxidant property, and presence of the flavanoid content, *Cinnamomum verum* leaves may be responsible for wound contraction and elevated rate of epithelization in wound healing. It may act either by increasing the myofibroblasts contractile property or causes enhancement of myofibroblasts number that was incorporated into mesenchymal cells of wound area. It can be hypothesized that *Cinnamomum verum* causes enhancement of epithelialization via its proliferation enhancement or by increasing the viability of epithelial cells. The present investigation provides pharmacological credence to the ethno botanical claims of *Cinnamomum verum* mentioned in the traditional Indian system of medicine.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Gilcrist B, Reid C. The bacteriology of chronic venous ulcers treated with occlusive hydrocolloid dressings, *British Journal of Dermatology,* 121(3), 1989, 337-344.
2. Enoch S, John Leaper D. Basic science of wound healing, *Surgery,* 23(2), 2005, 37-42.
3. Senthil Kumar M, Sripriya R, Vijaya Raghavan H, Sehgal P. Wound healing potential of *Cassia fistula* on infected albino rat model, *J Surg Res,* 131(2), 2006, 283-289.
4. Kumara P D, Jayawardane G L and Aluwihare A P. Complete colonic duplication in an infant Ceylon, *Med. Med. J,* 46(2), 2001, 69-70.
5. Purna S K and Babu M. Collagen based dressing- a review, *Burns,* 26(1), 2000, 54-62.
6. Roopashree T S, Dang R, Rani S R H, Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassia tora, Momordica charantia and Calendula officinalis*, *International Journal of Applied Research in Natural Products,* 1(3), 2008, 20-28.
7. Obasi N L, Egbuonu A C C, Ukoha P O, Ejikeme P M. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods, *African Journal of Pure and Applied Chemistry,* 4(9), 2010, 206-212.
8. Asare G A, Addo P, Bugyei K, Gyan B, Adjei S, Otu-Nyarko L S, Wiredu E K, Nyarko A. Acute toxicity studies of aqueous leaf extract of Phyllanthus niruri, *Interdiscip Toxicol,* 4(4), 2011, 206-210.
9. Morton J J, Malone M H. Evaluation of vulnerary activity by open wound procedure in rats, *Archives Internationales de Pharmacodynamie et de Therapie,* 196(1), 1972, 117-126.
10. Shivananda Nayak B, Steve Sandiford and Anderson Maxwell. Evaluation of the Wound-healing Activity of Ethanolic Extract of *Morinda citrifolia* L. Leaf, *Evidence Based Complementary and Alternative Medicine,* 6(3), 2009, 351-356.
11. Dash G K and Murthy P N. Studies on Wound Healing Activity of *Heliotropiumindicum* Linn. Leaves on Rats, *ISRN Pharmacology,* Article ID 847980, 2011, 8.
12. Mukherjee P K, Mukherjee K, Rajesh Kumar M, Pal M, Saha B P. Evaluation of wound healing activity of some herbal formulations, *Phytother Res,* 17(3), 2003, 265-268.
13. Reddy J S, Rao P R, Reddy M S. Wound Healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats, *Journal of Ethnopharmacol,* 79(2), 2002, 249-251.

14. Ehrlich H P, Hunt T K. The effects of cortisone and anabolic steroids on the tensile strength of healing wounds, *Annals of Surgery,* 170(2), 1969, 203-206.
15. Somashekar Shetty, Saraswati L. Udupa, Alaya L. Udupa, Venkata R. Vallala. Wound healing activities of *Jatropha curas Linn* in albino rats, *Saudi Med J,* 27(10), 2006, 1473-1476.
16. Patil. Pharmacological evaluation of ameliorative effect of aqueous extract of *Cucumis sativus L.* fruit formulation on wound healing in rats, *Chron Young Sci,* 2(4), 2011, 207-213.
17. Singh S D J, Krishna V, Mankani K L, Manjunatha B K, Vidya S M, Manohara Y N. Wound healing activity of the leaf extracts and deoxyelaphantopin isolated from Elaephantopus scaber Linn, *Indian Journal of Pharmacology,* 37(4), 2005, 238-242.
18. Singhal A K, Gupta H and Bhati V S. Wound healing activity of *Argyreia nervosa* leaves extract, *International Journal of Applied and Basic Medical Research,* 1(1), 2011, 36-39.
19. Audu S A, Mohammed I, Kaita H A. Phytochemical screening of the leaves of *Lophiralanceolata* (Ochanaceae), *Life Science Journal,* 4(4), 2007, 75-79.
20. Khandelwal K R, Practical Pharmacognosy: Techniques and experiments, *Nirali Prakashan, India,* 1, 1998, 78-80.
21. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur. Phytochemical screening and Extraction, A Review, *International Pharmaceutica Scientia,* 1(1), 2011, 98-106.
22. Remington J P. Remington: The science and practice of pharmacy, *Lippincott Williams and Wilkins,* 21st Edition, 2006, 773-774.
23. Eloff J N. Which extractant should be used for the screening and isolation of antimicrobial components from plants, *Journal of Ethnopharmacology,* 60(1), 1998, 1-8.
24. Das K, Tiwari R K S, Shrivastava D K. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends, *Journal of Medicinal Plants Research,* 4(2), 2010, 104-111.

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