ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 4, 2013

ISSN - 0974-2441

Research Article

PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACT OF *TRICHODESMA INDICUM* (LINN)R.Br.

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Received: 13 June 2013, Revised and Accepted: 5 July 2013

ABSTRACT

The methanolic extract of the whole plant of *Trichodesma indicum* Linn R.Br. was screened for its *in-vitro* antioxidant, anti bacterial and wound healing activity. The antioxidant activity was screened by Nitric oxide scavenging and reducing power assay method. The methanolic extract showed good antioxidant potential. The antibacterial and wound healing potency of the extract was screened by preparing an ointment formulation using simple ointment base. It has good antibacterial activity against the tested organisms when compared with control base. It has complete rate of contraction (closure 99.63%) when compared with standard Neosporin treated and control base treated group when studies for wound healing potency.

Keywords: *Trichodesma indicum* Linn R.Br, Antioxidant, Wound healing, Antibacterial activity.

INTRODUCTION

Trichodesma indicum (Linn) R.Br., is a common weed available in India. It has numerous medicinal values such as anti diarrhoeal[1], cough suppressant[2] and anti inflammatory[3]. In folklore medicine, the paste of the leaves of *Trichodesma indicum* along with rhizomes of *Acorus calamus* and *Allium sativum* were used for the wound healing potentials[4]

Acorus calamus[5] and Allium sativum[6 & 7] had wound healing potency. From our knowledge there is no evidence for wound healing potency of *Trichodesma indicum.* Hence our present study was planned for its wound healing potency along with its antioxidant and antibacterial activity.

EXPERIMENTAL DESIGN

Preparation of extract

The whole plants were collected form the campus of Madurai Medical College, Madurai. And authenticated by Dr. Stephen Lecturer in Botany, The American College, Madurai. A herbarium was deposited in the Department of Pharmacognosy as PCG/001/2010 in Madurai Medical College, Madurai.

The plant materials were cleaned, shade dried and powdered. The powdered plant materials were first extracted with petroleum ether for 72h, filtered and the marc dried and further extracted with methanol for 72h then filtered and the filtrate was evaporated under vacuum by using Rota vacuum pump. The dried methanolic extract was used for the further studies and also screened for its preliminary phytochemical constituents

In-vitro antioxidant property

Method A : Nitric oxide scavenging activity assay[8-9]

The reaction mixture contained 1mL of 10mM sodium nitroprusside, 2.5mL of phosphate buffered saline (pH 7.4) and 1mL of methanolic extract at various concentrations ranging from 44.44–266.67 µg/mL and incubated at 25°C for 30min. After 30min, 1.5mL of the reaction mixture was mixed with 1.5mL of Greiss reagent. Then the absorbance was measured at 546nm. Ascorbic acid was used as a standard. The percentage inhibition was calculated using the formula: % inhibition = [(Control-Test) Control] * 100. The IC_{50} was calculated using linear regression equation analysis. The results are presented in Fig. 1.

Method B: Reducing power assay [10-11]

The methanolic extract (0.1–0.5mL) was mixed with 0.75mL of phosphate buffer and 0.75mL of potassium ferric cyanide and incubated at 56°C for 20min. After 20min, 0.75mL of trichloro acetic acid was added and the final volume was made up to 3mL with buffer solution. It was then centrifuged at 3000rpm for 10min. 1.5mL of the supernatant solution was mixed with 1.5mL of distilled water and 0.1mL of ferric chloride solution. Then the absorbance was measured at 700nm using spectrophotometer. Ascorbic acid was used as a standard. Increasing absorbance indicates the stronger reducing power. The results are shown in Fig. 2.

Preparation of herbal ointment

The methanolic extract of the plant was mixed with simple ointment base (5% each of wool fat, cetostearyl alcohol, hard paraffin and 85% of white soft paraffin) at two concentrations such as 5% and 10% level. The prepared ointment was screened for its antibacterial activity and wound healing potency.

Dermal Toxicity Study [12]

The prepared ointments were tested for toxicity by topical application in Wistar albino rats. Anesthetic ether was used as an anesthetic agent. The dorsal surface of the rats were depilated and the prepared ointments at 10% concentration were applied once daily on the depilated dorsal surface for 14 days. The skin was observed for erythema, edema and necrosis once a day for 14 days.

Wound healing activity

The protocol of the study was approved by Madurai Medical College Institutional animal Ethical Committee. Reference number is 10196/E2/4/2010. Swiss albino rats were divided randomly into four groups of 6 rats per group. The rats weighing between 140-180g was used for wound healing study. The animals were acclimatized to the animal room conditions and were maintained on standard pellet diet and tap water throughout the period of study.

Experimentally induced excision wound [13]

The dorsal surface of the skin of the rat was shaved. A predetermined area of 2.0 2.5cm diameter of the skin in its full thickness was excised out under ether anaesthesia. Group 1 – 4 were treated with simple ointment base, 5% *Trichodesma indicum* ointment, 10% *T. indicum* ointment and Standard Neosporin ointment[15] respectively. All the ointments were applied topically

once daily for 16 days[16]. The wounds were left undressed to open environment and the animals were housed separately in polypropylene cages.

Wound area measurement[17]

The progressive changes in the wound area were monitored by tracing the wound on a tracing paper at every 4 days interval. The percentage reduction of the wound area was calculated with reference to control group periodically.

Histological examination [18]

On 16thday the regenerated tissues were removed for the histological examination. The sections of the tissues were stained with haematoxylin and eosin and evaluated for the extent of reepithelization, maturation and organization of the epidermal squamous cells, thickness of the granular cell layer and the degree of tissue formation. The photographs of the sections were presented in fig: 3

Antibacterial activity [19]

Well diffusion method was used for its antibacterial screening; 100mg of the prepared ointment at their two concentrations were placed in each well. Simple ointment base and Neosporin ointment were used for control and standard respectively. The results are presented in the Fig. 4.

RESULTS

The dried methanolic extract showed the presence of polyphenols, flavonoids, tannins, carbohydrate, proteins and sterols.

Antioxidant activity

Method A: The methanolic extract had maximum scavenging capacity and it was found to be 76.21 ± 0.34% and standard ascorbic acid had 80.03 ± 1.97 % and the IC_{50} was found to be 130.04 µg/mL and 84.64 µg/mL for methanolic extract and standard respectively.

Method B: The absorbance of the extract was 0.346 ± 0.010 while the ascorbic acid had 0.443 ± 0.001. The antioxidant potency can also be expressed in terms of ascorbic acid equivalent and it was found to be 0.747g/g of extract.

Dermal toxicity studies

The prepared ointment at their 10% level did not show any symptomps of erythema, itching and allergic reactio and hence it could be used for topical application.

Wound healing activity

Wound area

A better healing with complete wound closure was observed in rats treated with *Trichodesma indicum* ointment treated group (16 days). The neosporin treated and control groups needed 19 days and 21 days respectively for complete wound closure. The percentage rate of contraction of wound area was presented in table 1.

Histological examination

The histological examination was carried out for the treated and untreated control group tissue samples. The sections of the skin tissues of animals treated with *T. indicum* ointment showed the presence of strips of lining squamous epithelium which was thinned out in some places and granulation tissues were also seen. The animals treated with standard Neosporin ointment showed the presence of lining squamous epithelium with areas of ulceration, inflammatory cell infiltration and granulation tissues. The sections of skin tissues of untreated animals showed the presence of lining squamous epithelium with areas of ulceration. No granulation tissues were seen.

Anti bacterial activity

From the results it showed that *T. indicum* at their 10% level it has more zone of inhibition than the standard Neosporin against the *E. coli* and even though effective against all the tested organisms the zone of inhibition was comparatively less than the standard.

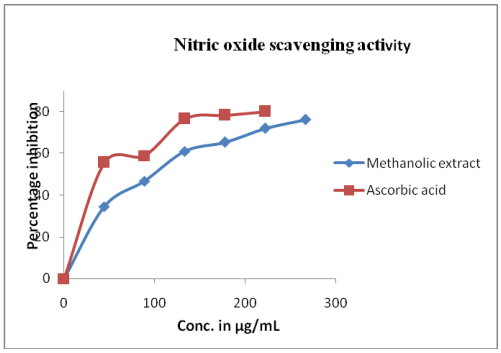


Fig. 1: Nitric Oxide Radical scavenging by methonolic extract of t. indicum

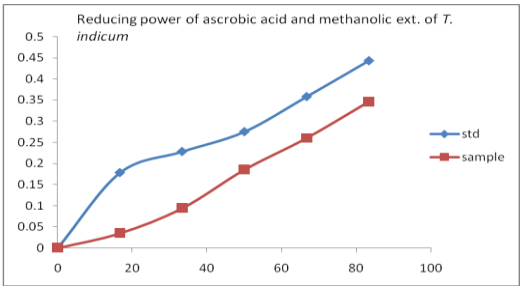
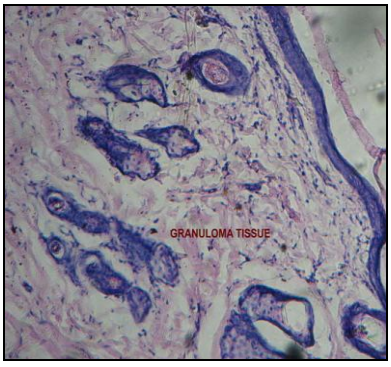
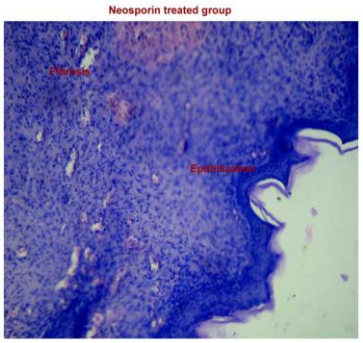


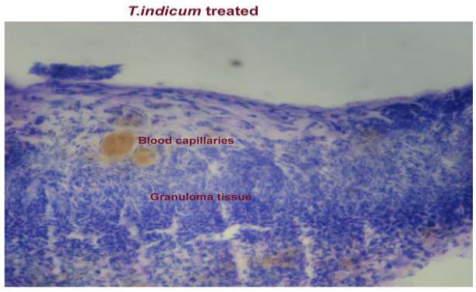
Fig. 2: Reducing Power Assay Of Methanolic extract of t. Indicum & ascorbic acid on potassium ferricyanide



Control treated group



Neosporin Treated Group



10% T.INDICUM TREATED GROUP

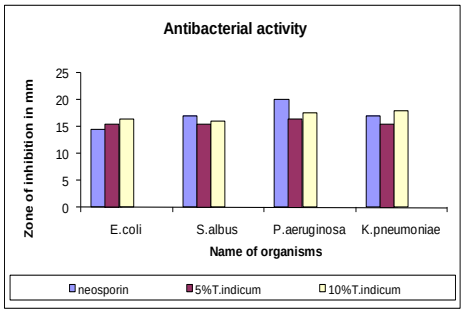


Fig.4: Anti bacterial activity

DISCUSSION

The excess production of nitric oxide is associated with several diseases like septic shock, acute and chronic and other auto immune diseases. The extract had good antioxidant activity against nitric oxide radical generation[8-9]. The reducing power assay was used to determine the electron donating capacity of the plant materials[10-11]. The extract had good electron donating capacity and may depend upon the presence of reductones in the extract.

From the results it showed *T. indicum* treated group showed faster healing than the other treated groups based on the percentage reduction of wound area and histological examination.

The antibacterial activity of the prepared herbal ointment showed the presence of inhibition against all the tested organisms.

CONCLUSION

The methanolic extract of *T. indicum* had good antioxidant activity against nitric oxide scavenging and reducing power assay. It also had good antibacterial activity against the tested organisms. It reached the granuloma tissue formation stage in wound healing activity. From the above, it may be concluded that the wound healing potency of the methanolic extract of *T. indicum* may be attributed to its antioxidant and antibacterial potency.

ACKNOWLEDGEMENTS

I express my sincere thanks to my Family Members and the entire Staff's of Madurai Medical College, Madurai. For their full support to me throughout the study.

Table 1: Rate Of Contraction Of Wound Area In Treated And Untreated Animals

S. No.	Treatment	Percentage rate of contraction of wound area			
		4th day	8th day	12th day	16th day
1	Control	29.14 ± 2.49	39.54 ± 1.52	64.55 ± 6.21	79.08 ± 4.38
2	Standard – Neosporin	42.30 ± 2.00[a]	72.30 ± 5.10[b]	90.77 ± 2.67[b]	96.37 ± 1.82[b]
3	*T. indicum* ointment 5%	46.64 ± 6.52[a]	72.88 ± 6.75[b]	89.48 ± 1.42[b]	93.94 ± 1.14[b]
4	*T. indicum* ointment 10%	53.50 ± 2.41[b]	75.58 ± 4.91[c]	91.37 ± 2.56[b]	99.74 ± 0.39[b]

NOTE: AP< 0.05; BP< 0.01; CP< 0.001 VS. CONTROL BY STUDENT'S T TEST

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