
Antimicrobial and Wound Healing Properties of Stem Bark of *Toddalia Asiatica* Linn.

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The antimicrobial activity of the different extracts of the stem bark of *Toddalia asiatica* was performed on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, respectively. Screening for wound healing activity was performed by excision wound model and incision wound model with both uninfected and infected wound with cocktail of bacteria comprising *Staphylococcus aureus* and *Escherichia coli*. The results of antimicrobial activity revealed that all the extracts exhibited varying degree of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* but none of the extracts were effective against *Candida albicans*. The studies on excision and incision wound healing models with and without infection showed that there is almost complete healing on the 12th post wounding day with all tested extracts. The degree of wound contraction with all extracts were in order of petroleum ether extract > chloroform extract > acetone extract > ethanol extract > aqueous extract. After infection with cocktail of tested bacteria, the order of tensile strength of the wounds of the animals treated with different extracts remained unchanged. The above findings justify potential wound healing properties of the stem bark as suggested in the folklore claims.

Toddalia asiatica Linn. (Fam. Rutaceae) is a very variable rambling, sarmentose and prickly shrub distributed almost throughout India, ascending to an altitude of 2500 m¹. The plant is highly valued in the traditional system of medicine in India for treating a variety of diseases including diarrhoea, dyspepsia, pain and inflammation, malaria and intermittent fever, wound and filthy ulcers, epilepsy, gonorrhoea and general debility^{1,2}. As per the folklore information, the tribes of Orissa apply the poultice of stem bark over severe open wounds and claim its tremendous effectiveness towards healing of wounds.

The occurrence of alkaloids, toddalinine, toddamine, pimpinellin, isopimpinellin, cyclohexylamine in the plant have been reported earlier³. Presence of coumarin derivatives such as toddaculin, coumurrayin, toddalenol, toddalenone, toddalosin, toddasin and toddanone has also

been reported^{4,5}. Earlier reports on pharmacological activity of the stem bark are scarce. In the present communication we report the antimicrobial and wound healing properties of *T. asiatica* stem bark.

MATERIALS AND METHODS

The plant *Toddalia asiatica* Linn. was identified at the Department of Botany, Utkal University. After authentication, the stem bark was collected in bulk from young mature plants from the hills of Khandagiri, Bhubaneswar during early winter, washed, shade dried and then milled in to coarse powder by a mechanical grinder. The resulting plant material (250 g) was then successively extracted with 600 ml each of petroleum ether (60-80°), chloroform, acetone, ethanol and water in a Soxhlet apparatus. The liquid extracts were concentrated separately under vacuum and the resulting dried extracts (yield: petroleum ether-0.83 %, chloroform-1.26 %, acetone-3.86 %, ethanol-4.72 % and aqueous-10.5 %, respectively with respect to dried material) were preserved in a desiccator until further use. Preliminary phy-

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tochemical tests of different extracts were performed by using specific reagents through standard procedures^{6,7}. The dried extracts were suspended in PEG 400 and used for the wound healing studies. For the antimicrobial study, the extracts were dissolved in DMSO. All the chemicals used were of analytical grade obtained from standard suppliers. Nitrofurazone served as reference standard for wound healing screening. Ampicillin trihydrate and fluconazole served as reference standards for the antimicrobial study. Adult Wistar rats of either sex, weighing between 180 to 250 g supplied by M/s Ghose enterprises, Calcutta, were used for the study. The animals were supplied with food and water *ad libitum*.

Antimicrobial activity:

The antimicrobial activity of the crude extracts was performed by Agar cup plate method⁸. The extracts were dissolved separately in DMSO at a concentration of 10 mg/ml. Ampicillin trihydrate (1 mg/ml) in DMSO was used as reference standard for the antibacterial study. Fluconazole (0.5 mg/ml) in DMSO served as reference control for the antifungal study. Solvent control (only DMSO) was also maintained throughout the experiment. The selected microorganisms included *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* respectively. The results are depicted in Table 1.

Wound healing evaluation:

Screening for wound healing activity was performed by excision wound model⁹ and incision wound model¹⁰ with

both uninfected and infected wound with cocktail of bacteria containing *Staphylococcus aureus* and *Escherichia coli*, respectively. The experimental protocols have been approved by the institutional animal ethics committee. The hair on the skin of back surface of animals was removed by using a suitable depilatory (Anne French hair removing cream). The selected animals were divided into seven groups of six in each. All the test samples were applied topically.

Excision wound model:

Circular wounds of 8 mm diameter were inflicted on the cleared skin by cutting under mild ether anesthesia. The areas of the wounds were measured (sq. mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This was taken as the initial wound area reading. Group 1 served as negative control to which no treatment was given. Group 2 served as positive control to which nitrofurazone (0.2 % w/v in PEG 400) was applied topically. Group 3 to 7 animals were treated with different extracts (500 mg/ml) in a similar manner. All the test samples were applied once daily. In the infected excision wound model, cocktail of the microorganisms was inoculated on the wound area on the first day itself. After 24 h of inoculation, the test samples were applied in a similar manner and measurement of wound area was done as above. The wound area of each animal was measured on days 0, 3, 6, 9 and 12 after inflicting the wound. Percent wound contraction was calculated from the days of measurements of wound area (Table 2).

TABLE 1: ANTIMICROBIAL ACTIVITY OF *T. ASIATICA*.

Test substance	Concentration (mg/ml)	Zone of inhibition (mm)		
		<i>Staph. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
Petroleum ether extract	10	30	28	-
Chloroform extract	10	22	17	-
Acetone extract	10	20	16	-
Ethanol extract	10	10	6	-
Aqueous extract	10	7	5	-
Ampicillin trihydrate	01	42	41	-
Fluconazole	0.5	-	-	38
Solvent (DMSO)	-	-	-	-

All the test extracts were evaluated for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* at the concentration of 10 mg/ml.

TABLE 2: EFFECT OF TOPICAL APPLICATION OF *T. ASIATICA* ON EXCISION WOUNDS.

Treatment	Concentration (mg/ml)	Percentage wound contraction on 12 th post wounding day							
		Uninfected wound				Infected wound			
		3 rd	6 th	9 th	12 th	3 rd	6 th	9 th	12 th
Pet ether extract	500	67.3	74.8	86.3	92.8	25.5	44.5	60.3	70.2
Chloroform extract	500	62.7	73.6	84.3	90.1	20.6	32.0	51.9	68.7
Acetone extract	500	60.2	70.5	81.1	89.9	24.3	34.6	60.2	62.4
Ethanol extract	500	50.6	67.6	78.0	88.3	23.4	32.5	54.7	60.6
Aqueous extract	500	49.4	66.3	78.5	86.3	10.3	31.4	45.2	53.4
Nitrofurazone	2	60.1	78.5	85.4	90.7	11.7	36.8	51.6	65.3
Solvent	-	24.5	43.4	53.9	56.9	-	-	-	-

n=6, Values expressed as percentage wound healing in each group. Control group was administered PEG 400 and test groups were administered different extracts (500 mg/ml).

Incision wound model:

Light incisions were made on the cleared surface by cutting the skin of the animals under mild ether anesthesia. The wounds were created at a length of about 1.5 cm. After the incision, the parted skin was kept together and stitched with black silk at both the ends of the created wound. The test samples were applied as above in a similar manner. The tensile strength was measured by using tensiometer on 12th post wounding day. In the infected excision wound model, cocktail of the microorganisms were inoculated on the wound on the first day. After 24 hours of inoculation, the test samples were applied in a similar manner and measurement of tensile strength was done as above (Table 3).

Histopathological studies:

The histopathological studies of the skin newly formed on the wounds was carried out on 12th post wounding day by fixing the skin in 10% buffered neutral formalin. Paraffin sections (5-10 μ) were prepared, stained with haematoxylin and eosin, and finally mounted in neutral DPX medium. Histopathological examinations were performed to study the process of epithelization on the excised wounds and to find out evidence of granuloma, dysplasia, oedema and

malignancy in the skin under examination¹¹.

Statistical analysis:

Results were analysed by student's *t*-test where applicable. The minimum level of significance was fixed at $p < 0.01$.

RESULTS AND DISCUSSION

The preliminary phytochemical studies of different extracts show the presence of steroids and sterols, triterpenoids, alkaloids, tannins and phenolic substances, flavonoids, carbohydrates and proteins. The results of antimicrobial activity revealed that all the extracts exhibited varying degree of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* but none of the extracts were effective against *Candida albicans*. However, the petroleum ether extract exhibited highest activity than all other extracts under similar conditions.

The studies on excision wound healing model without infection showed that there is almost complete healing on the 12th post wounding day with all tested extracts. The petroleum ether and chloroform extract exhibited comparably better wound contraction (92.8% and 90.1% respectively, nitrofurazone 90.7%). The degree of wound contraction on

TABLE 3: EFFECT OF TOPICAL APPLICATION OF *T. ASIATICA* ON INCISION WOUNDS.

Treatment	Concentration (mg/ml)	Tensile strength (g) on 12 th post wounding day	
		Uninfected	Infected
Petroleum ether extract	500	518±7.26*	493±6.28*
Chloroform extract	500	502±7.03*	488±4.78*
Acetone extract	500	483±6.67*	478±3.85*
Ethanol extract	500	478±7.04*	448±5.26*
Aqueous extract	500	436±5.23*	437±4.01*
Nitrofurazone	2	512±7.58*	472±4.36*
Solvent (PEG 400)	-	231±6.38	219±4.72

n= 6, Results expressed as Mean ± SEM, All p values are calculated with respect to vehicle control, *denotes statistical significance at p< 0.001.

the 12th post wounding day with all extracts were in order of petroleum ether extract >chloroform extract>acetone extract >ethanol extract>aqueous extract. In the infection model, the percentage of wound contraction on 12th post wounding day was found to be 70.2% and 68.7% in petroleum ether and chloroform extract respectively where as in other extracts, it showed lesser contraction.

In incision model, the tensile strength of the uninfected animals on 12th post wounding day with all tested extracts was found to be significant (p<0.01). The petroleum ether and chloroform extracts showed greater tensile strength (518 and 502 g, respectively) compared to the other extracts. The order of tensile strength was petroleum ether extract> chloroform extract>acetone extract>ethanol extract>aqueous extract. After infection with cocktail of tested bacteria, the order of tensile strength of the wounds of the animals treated with different extracts remained unchanged.

The histopathological studies of the newly formed skin on the wounds revealed healed ulcer and fibrosis with normal epithelization with no evidence of granuloma, dysplasia, oedema and malignancy in all tested extracts other than the aqueous extract where moderate inflammation and mild oedema was observed. In solvent control group, focal ulceration covered by granulation tissue with moderately dense inflammation, minimal fibrous tissue and presence of oedema was observed. In conclusion, the above findings suggest potential wound healing properties of the stem bark that is comparable with nitrofurazone.

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REFERENCES

- Anonymous, Eds. In; The Wealth of India: Raw materials, Vol. X, Sp-W, Publications and Information Directorate, CSIR, New Delhi, 1976, 258.
- Kirtikar, K.R. and Basu, B.D., Eds. In; Indian Medicinal Plants, 2nd Edn., Vol. I, Bishen Singh Mahendrapal Singh, Dehradun, 1994, 465.
- Tsai, I.L., Wun, M.F., Teng, C.M., Ishikawa, T. and Chen, I.S., *Phytochemistry*, 1998, 48, 1377.
- Chen, I.S., Tsai, I.L., Wu, S.J., Sheen, W.S., Ishikawa, J. and Ishii, H., *Phytochemistry*, 1993, 34, 1449.
- Ishii, H., Tan, S., Wang, J.P., Chen, I.S. and Ishikawa, T., *Yakugaku Zasshi*, 1991, 11, 376.
- Trease, G.E. and Evans, W.C., Eds. In; Pharmacognosy, 12th Edn., ELBS Publication, Delhi, 1985, 734.
- Tyler, V.E., Brady, L.R. and Robbers, J.E., Eds. In; Pharmacognosy, 9th Edn., Lea & Febiger Publication, Philadelphia, 1985, 21.
- British Pharmacopoeia, Vol II, Her Majesty's Stationary Office, London, 1988, 146.
- Dash, G.K., Suresh, P. and Ganapaty, S., *J. Nat. Remedies*, 2001, 1, 105.
- Udupa, S.L., Udupa, A.L. and Kulkarni, D.R., *Fitoterapia*, 1994, 65, 141.
- Bergmeyer, H.U., *Clin. Chem.*, 1972, 18, 1305.