ORIGINAL ARTICLE

Antimicrobial and analgesic activities of *Wendlandia thyrsoidea* leaf extracts

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The leaves of *Wendlandia thyrsoidea* were extracted with different solvents and screened for their antimicrobial and analgesic activities. The antimicrobial activity was evaluated using the minimum inhibitory concentration method and the analgesic activity was carried out by the acetic acid-induced writhing method. The ethyl acetate extract exhibited potent antimicrobial activity, whereas, the methanol extract showed a significant analgesic activity.

Key words: Analgesic activity, minimum inhibitory concentration, *Wendlandia thyrsoidea*

INTRODUCTION

Medicinal plants are important elements of traditional medicine in virtually all cultures. The idea that certain plants had healing potential was known long before human beings discovered the existence of pathogens. The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners. In recent years there has been a rising interest in the discovery of new antimicrobial compounds, due to an alarming increase in the rate of infections with multidrug resistant microorganisms.[1] Biologically active compounds present in medicinal plants have been of great interest to scientists working in the field. Efforts have been made to discover new antimicrobial compounds from various kinds of sources, such as, microorganisms, animals and plants. Systematic screening of these sources may result in the discovery of novel effective compounds.[2] There are several reports in literature regarding the antimicrobial activity of crude extracts prepared from plants.[3-6]

Wendlandia thyrsoidea belongs to the family Rubiaceae. It is a small tree or large shrub distributed in south India, Srilanka and frequently along Nemmaru-Kerekatte in Karnataka State.[7] In Kannada it is called as Neeru pale. In the present study pet-ether, ethyl acetate and methanol extracts of the leaves of *Wendlandia thyrsoidea* have been screened for antimicrobial and analgesic activities.

MATERIALS AND METHODS

Collection and Extraction

Wendlandia thyrsoidea leaves were collected from Hosanagara taluk, in Shimoga district, from the Western Ghats region of Karnataka, India, in the month of January. The plant was identified by the taxonomist, Department of Applied Botany, Kuvempu University, by comparing it with the authenticated specimen deposited at the Kuvempu University Herbaria (Voucher specimen KU/SD/HN115).

The leaves of the plant were shade dried and powdered. The powdered leaves were extracted with solvents of increasing polarity such as pet-ether (40-60°C), ethyl acetate and methanol, by the hot soxhlet successive extraction method. The solvent was removed under reduced pressure and controlled temperature by using a rotary flash evaporator. Standard methods[8-9] were used for preliminary phytochemical screening of the various extracts to know the nature of the phytochemicals present in it.

Microorganisms used

The following standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India, and were used for the study.
Bacteria: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli.*
Fungi: *Aspergillus niger, Candida albicans.*

The medium Mueller Hinton agar was obtained from Hi-media Laboratories Limited, Mumbai-400086, India.

Antimicrobial Activity

Determination of minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) of the extracts

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was performed using the broth dilution method,[10] with concentrations of the extracts ranging from 25 µg/ml to 500 µg/ml in Dimethyl sulfoxide (DMSO) against all test microorganisms [Table 1].

Analgesic Activity

This method is based on acetic acid-induced writhings in mice.[11] Male Swiss albino mice were procured from the Virus Diagnostic Laboratory, Shimoga. Five groups of six mice each (22-30 g) were selected and 0.6% acetic acid (dose 10 ml/kg) was injected intraperitoneally. The number of writhes was counted for 20 minutes, after 5 minutes of injection of acetic acid to each mouse. This reading was taken as the control. Next day the same groups of mice were used for evaluating the analgesic activity. Each group was administered orally with the suspension of test extract in 0.1% Tween-80 solution at a dose of 100 mg/kg body weight of the animal one hour before injection of acetic acid. After 5 minutes, the mice were observed for the number of writhes for the duration of 20 minutes. The mean value for each group was calculated and compared with the control. Acetyl salicylic acid was used as the standard for comparison of the analgesic activity. The percentage of protection was calculated using the formula,

Percent protection = (1 − Vc/Vt) × 100 [Table 2].

Where: Vt = Mean number of writhing in test animals
Vc = Mean number of writhing in control

RESULTS AND DISCUSSION

The antibacterial activity of the three different extracts, that is, pet-ether, ethyl acetate and methanol is shown in Table 1. The ethyl acetate extract showed significant activity against all the bacteria, whereas, the methanol extract showed good antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* as compared to the standard drug Ciproflaxacin.

The antifungal activity of the three different extracts is shown in Table 1. The ethyl acetate extract showed potent antifungal activity against tested fungi, whereas, the methanol extract showed moderate activity against *A. niger C. albicans* compared to the standard drug Clotrimazole.

The analgesic activity of pet-ether, ethyl acetate and methanol extracts are shown in Table 2. The methanol extract showed potent analgesic activity, whereas, the ethyl acetate extract exhibited good activity as compared to the standard drug Aspirin.

Phytochemical studies revealed the presence of steroids, alkaloids, flavonoids, glycosides and proteins in leaf extracts. Presence of constituent-like flavonoids in the extracts, as reported earlier, was probably responsible for the observed antimicrobial activity.[12] Preliminary phytochemical screening of ethyl acetate and methanol extracts revealed the presence of flavonoid compounds. Flavonoids are known to target prostaglandins, which are involved in the later phase of acute inflammation and pain perception.[13]

Further work on the profile and nature of the chemical constituents of *Wendlandia thyrsoidea* leaves will provide more information on the bioactive principles responsible for their pharmacological properties.

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Table 1: Antimicrobial activity of values of extracts of *Wendlandia thyrsoidea* leaves

Test samples	Minimum inhibitory concentrations (µg/ml)					
	SA	***BS***	***PA***	***EC***	***AN***	***CN***
Control	0.78 ± 0.07	0.36 ± 0.09	0.52 ± 0.11	0.57 ± 0.09	0.42 ± 0.14	0.43 ± 0.11
Ciproflaxacin	5.36 ± 0.05	5.36 ± 0.13	5.45 ± 0.12	5.48 ± 0.10	-	-
Clotrimazole	-	-	-	-	125.41 ± 0.13	125.09 ± 0.01
Petroleum ether extract	100.29 ± 0.03	150.49 ± 0.18	200.53 ± 0.10	100.46 ± 0.08	200.51 ± 0.19	200.41 ± 0.14
Ethyl acetate extract	50.63 ± 0.16	50.24 ± 0.07	25.37 ± 0.01	25.37 ± 0.02	50.34 ± 0.03	50.38 ± 0.01
Methanol extract	100.43 ± 0.10	100.57 ± 0.07	50.34 ± 0.05	50.35 ± 0.03	100.38 ± 0.01	100.65 ± 0.15

SA: Staphylococcus aureus; BA: Bacillus subtilis; PS: Pseudomonas aeruginosa; EC: Escherichia coli; AN: Aspergillus niger; CA: Candida albicans

Table 2: Analgesic activity of *Wendlandia thyrsoidea* leaves

Test		Mean no. of writhings		% Protection
		Without the administration of drugs	With the administration of drugs	
Standard	100	21.83 ± 2.31	08.83 ± 0.94	62.93
Petroleum ether extract	100	21.00 ± 36	9.5 ± 0.22	54.80
Ethyl acetate extract	100	23.50 ± 258	10.50 ± 0.22	55.40
Methanol extract	100	23.33 ± 1.41	9.33 ± 0.54	60.10

Values are mean ±; Index for analgesic activity study; *Method:* Acetic acid-induced writhing; Animal: Albino mice; No. of animals per group; 6; Route of administration: IP (Intraperitoneally); Standard drug used: Acetyl salicylic acid; SEM: Standard Error Mean.

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