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Comparative Study on Antimicrobial Activity of *Wedelia chinesis* and *Wedelia calendulaceae*

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Abstract— *The present study is aimed at preliminary phytochemical screening of the leaf extracts of Wedelia chinesis and Wedelia calendulaceae and evaluation of the same for potential antimicrobial activity. The ethanolic extracts of these plants were found to possess alkaloids, glycosides and flavanoids. The antimicrobial activity was evaluated against Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger. These plant extracts have great potential antimicrobial compounds that can be used in treatment of infectious diseases caused by a range of resistant microorganisms*.

Keywords— *Wedelia chinesis; Wedelia calendulaceae; antimicrobial activity; phytochemical screening*.

I. INTRODUCTION

Wedelia chinensis is a procumbent, perennial herb found in wet places in Uttar Pradesh, Assam, Arunachal Pradesh and all along the coastal areas[1]. Bengal, Burma, Konkan, plains districts of Madras Presidency, Ceylon- Malay Archipelago, China and Japan [2]. Parts used: leaves, stem, whole plant. The 'Bhringraj' has been included in the category 'Dasapuspam' in Ayurveda. Four plants viz *Wedelia calendulaceae* (*W. calendulaceae), Eclipta alba (E. alba), Heliotropium strigosum (H. strigosum) and Viscum album (V. album)* are known by the name Bhringaraj [3]. *Wedelia calendulacea* (L.) Less., an illegitimate name that is a synonym of *Sphagneticola calendulacea [4]*. *Wedelia chinensis* (osbeck) Merill, Asteraceae is a drug of natural origin (herbal medicine) and is most popular herbal medicine used in various systems of medicine like Ayurvedic, Siddha and Unani systems of medicine. [5,6]

The present study is aimed at preliminary phytochemical screening of the leaf extracts of *Wedelia chinesis and* Wedelia *calendulaceae* and evaluation of the same for potential antimicrobial activity.

II. MATERIALS AND METHODS

Fresh samples of *Wedelia chinesis* and *Wedelia calendulaceae* were collected from Thiruvananthapuram, southern part of Kerala. These plant samples were washed with tap water and dried under the shade and mashed with the help of mortar and pestle.

Extraction

250 g of air dried *powdered leaves of Wedelia chinesis and Wedelia calendulaceae* were defatted with 500ml of petroleum ether and extracted with chloroform, ethyl acetate and 90% alcohol in a continuous soxhlet extractor.

Phytochemical Screening

The extracts were screened for the presence of different phytoconstituents like saponins (Frothing test), tannins (Ferric chloride test and Lead acetate test) alkaloids, (Dragendroff's test, Meyer's test, Hager's test, and Wagner's test) glycosides (Brontrager's test for anthraquinone glycosides and Killer-Killani's test for cardiac glycosides), steroids (Salkowski test and Libarman-Burchard's test) and flavonoids (Shinoda's test, Ferric chloride test, and Pew's test). [7]

Microorganisms

The test organisms (*Staphylococcus aureus* MTCC 737, *Micrococcus luteus* MTCC 106, *Bacillus subtilis*MTCC 441, *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688, *Candida albicans*MTCC 3017 and *Aspergillus niger* MTCC 1344) were sub cultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques [8]. Stock cultures were maintained on nutrient agar slants at 4°C and sub cultured in nutrient broth at 37°C prior to each antimicrobial test.

Inoculums Preparation

Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria were added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be in 108 ml-1 and then plated out as inoculums [9].

Evaluation of Bactericidal Effects

The bactericidal effect of the extracts was evaluated with the modified Kirby-Bauer disc diffusion method [10]. Using a sterilized swab, aliquots from each tube were spread on dishes with Muller-Hinton agar (Hi-Media), disc with plant extract was plated and incubated at 37 °C for 24 hours. As negative control, discs were soaked with each solvent and used. Each

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sample was used in triplicate for the determination of antibacterial activity. The diameter of zone of inhibition was measured in mm.

A standard antibiotic Tetracycline (10µg/disc) as positive control and a negative control with only the solvent used for extraction were also maintained for reference.[11] From the plant extract 100mg of crude extract was dissolved in the respective solvent and was loaded onto the filter paper discs to get 100mg/disc concentration.

III. Results and Discussion

Chemical test

TABLE I. Chemical test.

Test	Petroleum ether		Chloroform		Ethyl acetate		Ethanol	
	W.Ch	W.cal	W.ch	W.cal	W.ch	W.cal	W.ch	W.cal
Alkaoids	-	-	-	-	+	+	+	+
Carbohydrates	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-
Sterols	+	+	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-
Proteins	-	-	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-	+	+
Flavonoids	-	-	-	-	+	+	+	+
Starch	-	-	-	-	-	-	-	-

W.ch- *Wedelia chinesis* W.cal- *Wedelia calendulaceae*

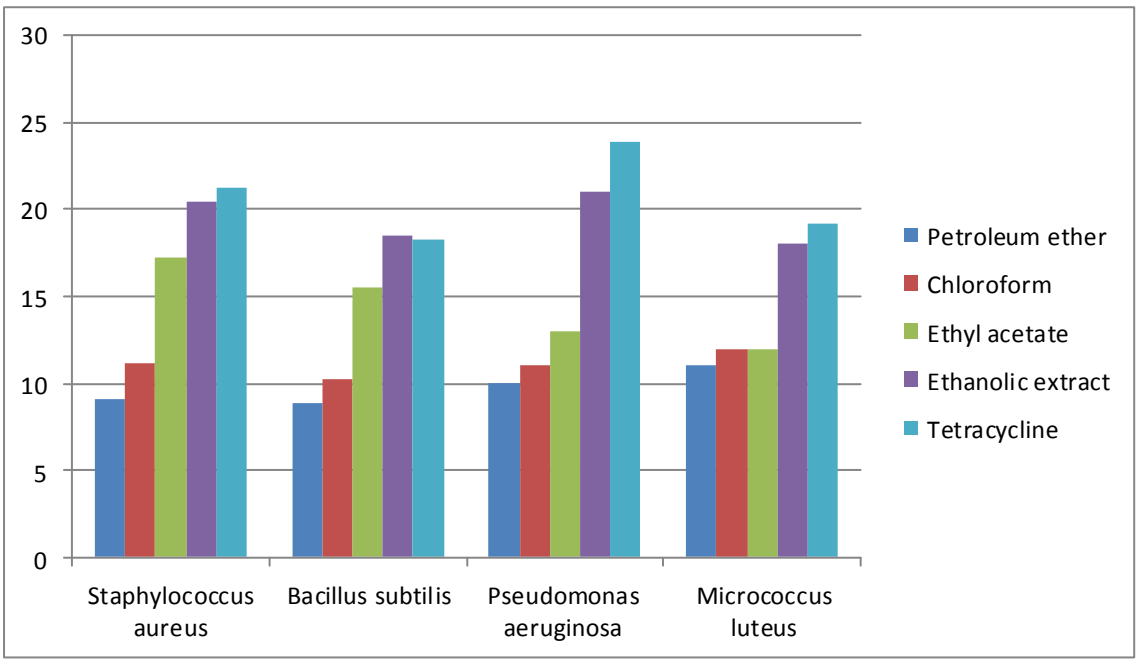
Antimicrobial activity

TABLE II. Antimicrobial activity.

Microorganism	Tetracycline	Petroleum ether extract		Chloroform extract		Ethyl acetate extract		Ethanolic extract	
		W.ch	W.cal	W.ch	W.cal	W.ch	W.cal	W.ch	W.cal
Staphylococcus aureus	21.22±0.8	9.1±2	8.9±1	11.2±	10.5±	17.2±0.35	17.1±0.28	20.5±0.2	19.8±0.6
Bacillus subtilis	18.22±0.5	8.9±0.13	8.7±0.6	10.2±1.2	11.1±2	15.5±1.4	15.2±1.5	18.5±0.8	17.9±0.9
Pseudomonas aeruginosa	23.86±0.4	10±0.9	11±0.75	11±0.50	12±0.21	13±1.1	13±1	21±0.2	20.9±0.2
Micrococcus luteus	19.2±0.9	11±0.72	11±1.1	12±0.9	13±0.35	12±1.1	12±1	18±0.2	17.9±0.2
Escherichia coli	24.92±0.8	8±0.8	9±0.9	9±0.75	10±1.1	14±0.1	15±0.2	21±0.9	22±0.85
Candida albicans	18.25±0.7	8±0.75	9±0.5	12±0.6	11±0.85	12±0.25	11±0.5	18±0.95	17±0.45
Aspergillus niger	21.33±0.5	9±0.78	9±0.66	11±0.74	10±0.32	14±0.43	15±0.43	20±0.72	21±0.87

Values are expressed as Mean ± Standard Error of 3 replicates

Antimicrobial activity of Wedelia chinesis



Graph 1. Antimicrobial activity of *Wedelia chinesis.*

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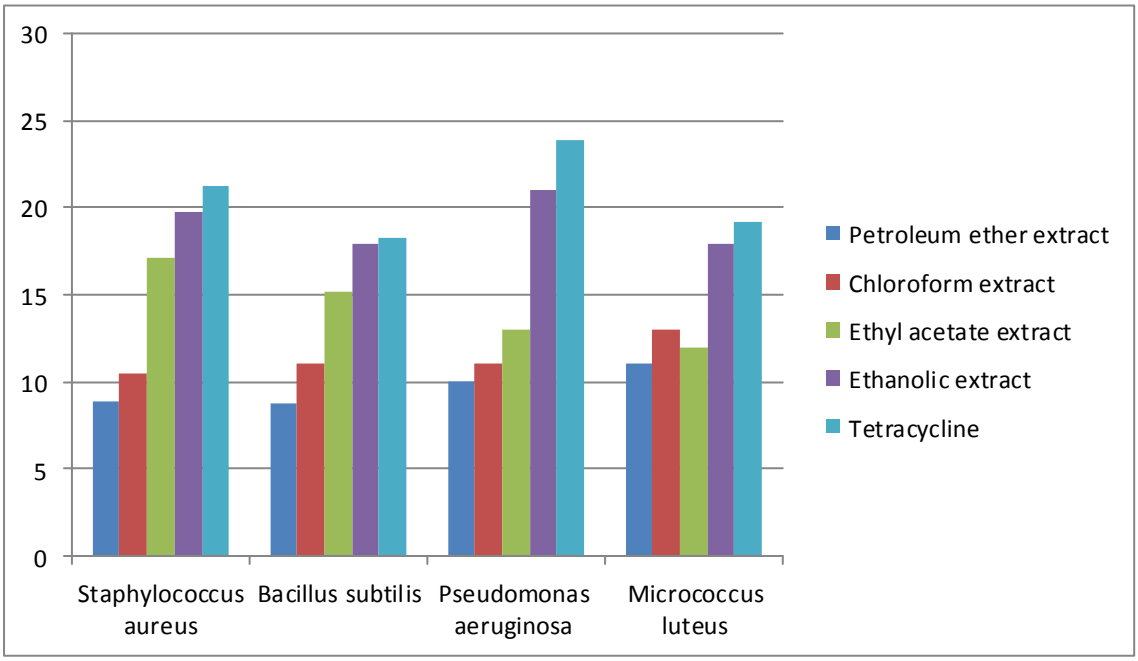
Antimicrobial activity of Wedelia calendulaceae



Graph 2. Antimicrobial activity of *Wedelia calendulaceae.*

IV. CONCLUSION

Based on these results, it can be concluded that plant extracts have great potential antimicrobial compounds that can be used in treatment of infectious diseases caused by a range of resistant microorganisms

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