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ANTIBACTERIAL ACTIVITY OF *WOODFORDIA FLORIBUNDA* LEAF EXTRACTS

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Antibacterial activity of aqueous as well as ethanol leaf extracts of *Woodfordia floribunda* were tested on five bacteria *viz., Staphylococcus aureus , Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium*. Both aqueous and ethanol extracts inhibited growth of all bacteria. Comparatively *K. pneumoniae* was found to be more susceptible while *S. typhimurium* and *P. aeruginosa* less susceptible to the effect of leaf extracts. Ethanol extract is more effective than aqueous extracts.

Keywords: Antibacterial; Leaf extracts; *Woodfordia floribunda;* Zone of inhibition.

Introduction

Plants represent a rich source for secondary metabolites that have potential to inhibit the growth of multi drug resistant bacteria. The major classes of plant compounds which can inhibit the growth of bacteria include phenolics,terpenoids and essential oils, alkaloids, lectines and polypeptides and polyacelytenes[1]. These compounds with enormous therapeutic potential can form the base for the development of new medicines[2]. All plant parts are good source of antibacterial compounds[3]. Goyal *et al.*[4] reported greater antibacterial activity in leaves of *Catharanthus roseus* than in other parts of plant. Gram positive bacteria get inhibited more than Gram negative bacteria by plant extracts[5-6]. The extracts of different parts of plants have been reported to have antibacterial activity[7-14]. Thus in the present investigation, leaves of *Woodfordia floribunda Salisb* were extracted in water and ethanol and tested in vitro for their antibacterial effect *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella typhimurium.* The antibacterial activity in the flowers extract of *W.fruticosa* Kurz.was investigated[15].

Material and Methods

The leaves of the plants collected from the Kalsubai regions of Western Ghats were washed under running tap water and surface sterilized by 0.1% w/v $HgCl_2$, followed by rinsing twice in distilled water so as to remove the traces of $HgCl_2$. These leaves were then dried in shade at room temperature, homogenized to fine powder and stored in airtight bottles.

Aqueous extraction-Ten g of dry leaf powder was extracted in 100 ml of distilled water for 6 hrs on hot water bath. After every 2hrs, it was filtered through eight layers of muslin cloth and centrifuged at 5000g for 15min.The supernatant was collected and condensed in boiling water bath until the water was evaporated and the extract thus obtained was stored in brown bottle at 4°C for further use.

Solvent extraction-Ten g of dry leaf powder was extracted in 50 ml of ethanol on rotary shaker at 150 rpm for 24hrs.Therafter it was filtered through eight layers of muslin cloth and centrifuged at 5000g for 15min. The supernatant was collected and the solvent was allowed to evaporate and the residue was stored in brown bottle at 4°C for further experiment.

Bacterial cultures-Five strains of bacteria *viz., Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 23564 and *Staphylococcus aureus* ATCC 25923 obtained from the National Chemical Laboratory (NCL),Pune,India were subjected to susceptibility test. The stock cultures were maintained on Muller Hinton Agar slants at 4°C. The bacteria were revived on sterilized MHA medium in Petri plates at 37°C.

Inoculum preparation-Bacterial strains were grown to exponential phase in saline medium (0.85% NaCl) at 37°C for 24hrs and adjusted to final density of 10^4cfu / ml to obtain a turbidity visually compared to 0.5 McFarland standards[16].

Antibacterial activity testing-The antibacterial activity of leaves extract was evaluated by agar well diffusion assay[17]. Aqueous and ethanol leaves extracts were dissolved in distilled water and dimethyl sulfoxide (DMSO)

Table 1. Antibacterial activity of *Woodfordia floribunda* leaf extracts. Diameter of inhibition zone (mm).

Plant extract	Bacteria									
	E. coli		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*		*S. typhimurium*	
	Aq.ext	Eth.ext	Aq.ext	Eth.ext	Aq.ext	Eth.ext	Aq.ext	Eth.ext	Aq.ext	Eth.ext
Woodfordia floribunda	12.66*** ± 0.47	13.33*** ± 0.47	12.33* ± 0.47	14.33_{NS} ± 1.70	16.33_{NS} ± 0.47	20.33*** ± 0.47	6.00*** ± 0.81	10.33*** ± 0.47	8.67*** ± 0.47	9.70** ± 0.47
+ ve control Tetracycline (25 µg/ml)	7.5 ± 0.5		14.33 ± 0.94		15.33 ± 0.47		15.33 ± 0.47 #		12.66 ± 0.47	
-ve control DW / DMSO	-		-		-		-		-	

: Positive control for *P.aeruginosa* : 10mg / ml NS = Not significant. ** = P < 0.01. *** = P < 0.001

respectively to get a concentration of 10mg / ml. 100µl inoculum (10^4cfu / ml; 0.5 MacFarland standards) of each test bacterium was spread with the help of sterile glass spreader on sterile MHA medium in Petri plates so as to achieve a confluent growth. With the help of a sterile cork borer (8mm diameter) wells were made in the seeded agar plates. Each well was then filled with 50µl (10mg/ml concentration) of plant extract. The plates were allowed to stand for atleast half an hour for diffusion to take place and then incubated at 37°C for 24hrs. DMSO and sterile distilled water was used as a negative control for the ethanolic and aqueous extracts, respectively. Different concentrations of tetracycline were tested for inhibitory effect on bacteria. Lowest concentration of 10mg / ml and 25 µg/ ml of tetracycline that inhibited *P.aeruginosa* and other bacteria, respectively was used in positive control sets while for negative control DMSO and distilled water was used. Diameter of zone of inhibition of bacteria excluding the diameter of well is presented in Table 1.

Results and Discussion

Table 1 and Figs. 1-3, reveal that all the growth of all bacteria was inhibited by the aqueous as well as ethanol leaf extracts of *W.floribunda.* However, ethanol extract was more effective in inhibiting the bacterial growth than aqueous extracts which corroborates with earlier reports[10,14,18,19]. Comparatively *K. pneumoniae* was found to be more susceptible while *S. typhimurium* and *P. aeruginosa* less to the effect of leaf extracts. *Sailaja et al.*[20] reported greater inhibition of *P. aeruginosa* by the leaf extract of *O.sanctum.*

The metabolic compounds in plants can be used in the development of new antibacterial drugs to counter the emergence of drug resistant pathogenic bacteria. Many plants need to be screened for their antibacterial properties and there is wide scope for further research on this aspect.

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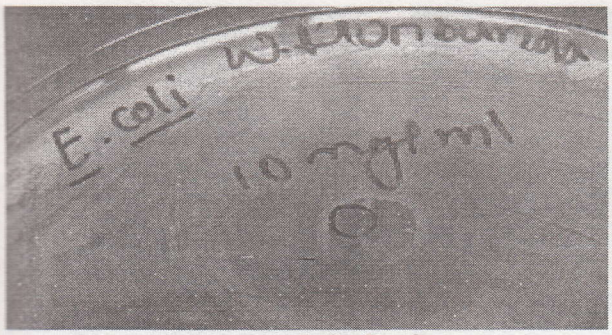
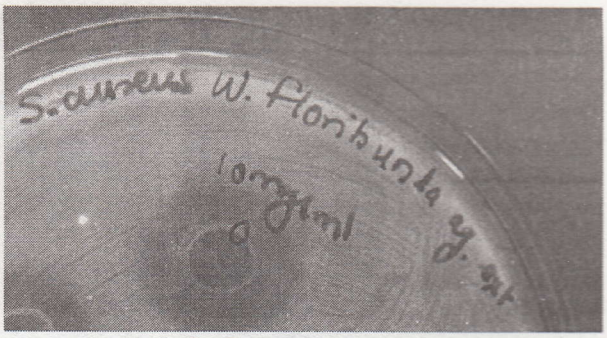
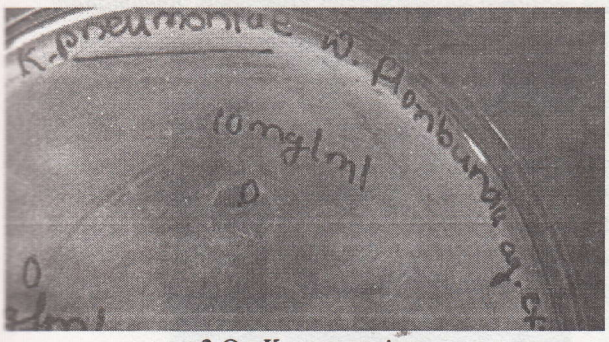


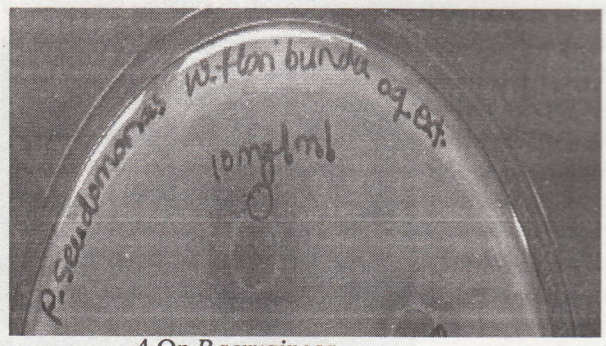
1. On *E.coli*



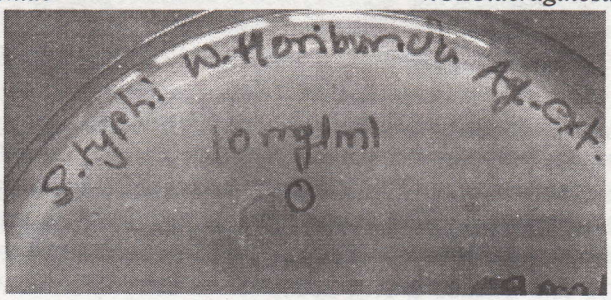
2. On *S.aureus*



3.On *K.pneumoniae*

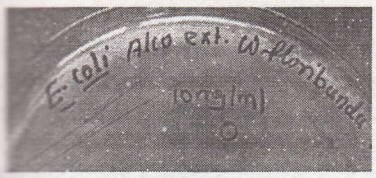


4.On *P.aeruginosa*



5.On *S. typhimurium*

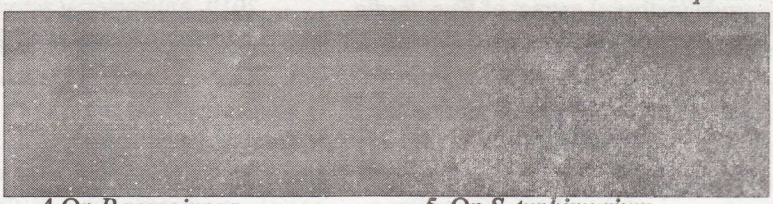
Fig. 1. Zone of inhibition of bacteria due to aqueous leaf extract of *Woodfordia floribunda.*



1.On *E.coli*

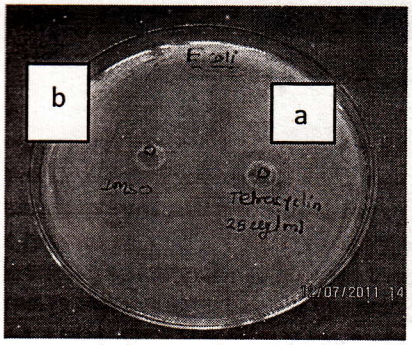
2. On *S.aureus*

3.On *K.pneumoniae*

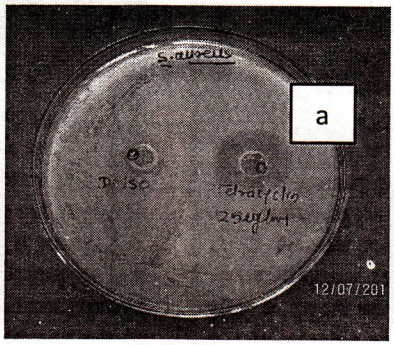


4.On *P.aeruginosa* 5. On *S. typhimurium*

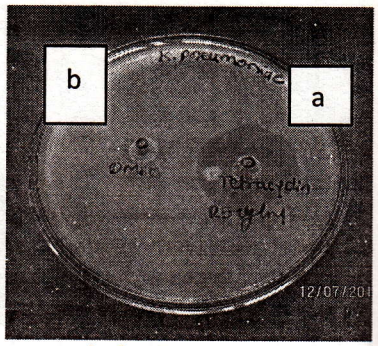
Fig.2. Zones of inhibition of bacteria due to ethanol leaf extract of *Woodfordia floribunda.*



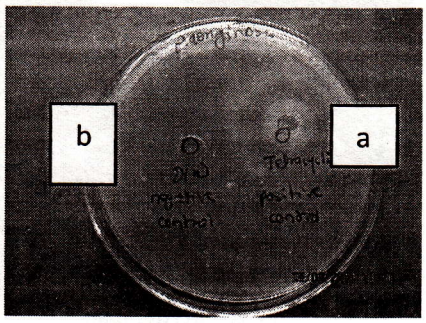
1. On *E. coli*



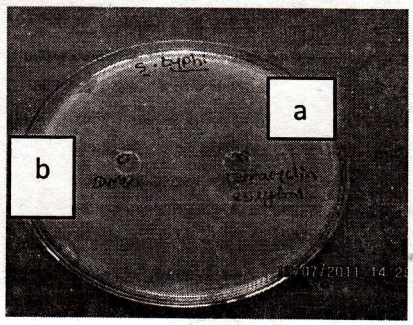
2. On *S. aureus*



3. On *K. pneumoniae*



4. On *P. aeuroginosa*



5. On *S. typhimurium*

Fig.3. Positive control by tetracycline and negative control by DMSO. (a: Tetracycline. b: DMSO).

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