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Antimicrobial Activity of Ajuga Bracteosa against Certain Multi Drug-Resistant Pathogens

Muhammad Yar

Department of Microbiology, Faculty of life Sciences, Abasyn University Peshawar

ABSTRACT:-The study was designed to determine the in vitro action of methanol extracts and ethyl acetate extracts of Ajuga bracteosa against certain multidrug resistant bacterial strains. The study was carried out against four multidrug resistant strains (MDR). Both methods were applied; disc diffusion and well diffusion to determine the interaction. Antimicrobial sensitivity showed; that Amikacin antibiotic was the most effective against all tested bacterial strains, with zone of inhibition: 15 – 27 mm, followed by Meropenem 26 – 29 mm, while Ciprofloxacin and Cefuroxime almost showed no effect to all strains with the exception of S. typhi and P. aeruginosa. Results were showed that; most of the bacterial strains were resistant to antibiotics .Methanol and ethyl acetate extract of Ajuga bracteosa was used separately against all bacterial strains and showed good effect. The methanol extract having high antimicrobial activity against all tested bacterial strains while ethyl acetate was found less effective as compare to methanol extract. The study concluded that plant is a natural product for natural therapies and the extract of plant could be useful against the emerging multidrug resistant bacterial strains.

Key words: Antibiotics, ethyl acetate, Bacterial strains

I. INTRODUCTION

Antimicrobial resistance is raised in Pakistan and all world to first line of antibiotics and hence numerous classes of antibiotics become less effective and resulted antibiotics resistance [1, 2]. (Oskay et al., 2009; Mustapha et al., 2009). This is due to improper and overuse of existing antibiotics. The resistance develops in bacteria either through mutation or acquisition of new genes through a process known as horizontal gene transfer [4, 4]. (Tenover., 2006; Otajevwo and Momoh., 2013).

The resistance to the first commercial antimicrobial agent (penicillin) was identified in 1948 and after that numerous classes of antibiotics resistance were occur day by day. To minimize antibiotic resistance various strategies are used. Appropriate antibiotic prescribing, hygiene and disinfection, the development of novel antibiotics, phage therapy etc [5]. (Lee et al., 2013). To minimize antibiotic resistance plants are also use in the present era. For this purpose plants secondary substances were used as a natural products in the past [6]. (Nascimento et al., 2000).

Ajuga bracteosa is an important medicinal plant which member of family Lamiaceae and tribe Ajugeae which is commonly known as "bungle" in English and "Kamargul" in Pushto [7]. (Jan et al., 2008. *Ajuga bracteosa* is an important medicinal plant having good activity against bacterial, fungal, parasitic, inflammatory, tumor and diabetes. Several studies were present of *Ajuga bracteosa* against different diseases like skin disease i-e Dermatitis, gastrointestinal disease i-e Diarrhea, parasitic disease i-e Malaria, water and food borne diseases i-e Typhoid fever and Cholera [8]. (Castro et al., 2015).

Therefore the study was conducted to investigate the antimicrobial activity of methanol and ethyl acetate extract of *Ajuga bracteosa* against MDR bacterial strains isolated from clinical samples.

II. MATERIALS AND METHODS

The plant *Ajuga bracteosa* was collected aseptically in sterile poly ethylene pouches from Lower Dir district of KPK Pakistan in March 2015. The plant was identified by a Taxonomist Mr. Ghulam Jilani department of Botany; University of Peshawar and a voucher of plant specimen were submitted for reference while processed in microbiology laboratory Abasyn University Peshawar. The roots, leaves and aerial part of *Ajuga bracteosa* used as plant sample for extraction preparation [9]. (Ahmed and Chaudhary., 2011). Plants were then shades dried and grind through grinder. The powder samples were then packed in clear poly ethylene pouches, which was then sealed and store at 4ºC [10]. (Pal and Pawar., 2011).

Extraction

The fine powder was ground in six flask containing methanol and ethyl acetate having different volume. 40g of root and aerial part was ground in four flask containing methanol and ethyl acetate having 500ml

volume. 150g of leaves was ground in two flask containing methanol and ethyl acetate having 700ml volume, and was vigorously shake until the powder mixed well in solvent [11].(Fekete et al., 2004; Shafi et al., 2004). The flasks containing fine powder solution was kept at room temperature for 24 hours. The crude solution was filtered through muslin cloth, and the filtrate evaporated to dryness in water bath at 100 ℃ [12].(Zerroug et al., 2011). The dried extract was then dissolved (1mg/ml solvent) in Dimethyl sulfoxide (DMSO) as solvent [13].(Shafi et al., 2004).

Antimicrobial sensitivity

Kirby-Baur method described by Benson [14].(2002) was used to study antimicrobial sensitivity tests. The organisms were tested against ten commonly used antibiotics. Used antibiotics were Ciprofloxacin (CIP) (30µg), Ceftriaxone (CRO) (30µg), Meropenem (MEM) (10µg), Cefoxitin (FOX) (30µg), Ampicillin (AMP) (10µg), Amikacin (AK) (30µg), Doxycyclin (DO) (30µg), Ceftazimide (CAZ) (30µg), Azithromycin (AZT) (10µg), Cefuroxime (CXM) (30µg).

III. RESULTS

Staphlococcus aureus (S. aureus) was isolated from pus wound samples, *Escherichia coli (E. coli)* and *Pseudomonus aeruginosa (P. aeruginosa)* was isolated from urine samples while *Salmonella typhi (S. typhi)* was isolated from blood samples. Four different bacteria were used for antimicrobial sensitivity profile against ten different antibiotics. Our results show that most of the tested bacterial strains showed resistance to antibiotics. *E.coli* was examine the most resistant bacterial strains (70%) to all tested antibiotics, *S. aureus* (60%), while *P. aeruginosa* and *S. typhi* strains was found (40%) resistant to all used antibiotics (Fig 4.1).

Among all used antibiotic AK (Amikacin) showed good activity against all bacterial strains with zone of inhibition against *S. typhi* (27mm) followed by *E.coli* (20mm) while *S.aureus* and *P. aeruginosa* (15mm). The most effective antibiotics were MEM (Meropenem), CRO (Ceftiaxone), CAZ (Ceftazimide) and AZT (Azithromycin) (Table 4.1).

Plant extracts activity

The extract of *Ajuga brateosa* methanol extract and ethyl acetate extract was used separately. The methanol extract of aerial part gave maximum zone against *E. coli* (23mm) followed by *S. aureus (*22mm), *S. typhi* (18mm) and *P. aeruginosa* (15mm) while ethyl acetate extract of aerial part gave maximum zone against *E. coli* (21mm) followed by *S. aureus (*19mm), *S. typhi* (16mm) and *P. aeruginosa* (12mm).

Methanolic extract of roots were examine with a zone against *E. coli* (27mm) followed by *S. aureus (*24mm), *S. typhi* (16mm) and *P. aeruginosa* (15mm) while extract of ethyl acetate of root were examine with a clear zone against *E. coli* (24mm) followed by *S. aureus (*20mm), *S. typhi* (15mm) and *P. aeruginosa* (14mm).

The methanol extract of leaves were examine with a clear zone against *E. coli* (20mm) followed by *S. aureus (*23mm), *S. typhi* (14mm) and *P. aeruginosa* (15mm) while ethyl acetate extract of leaves were examine with a clear zone against *E. coli* (20mm) followed by *S. aureus (*19mm), *S. typhi* (11mm) and *P. aeruginosa* (11mm) (Table 4.2).

All the MDR bacteria were found sensitive to plant extract, but *E. coli* was found (75%) sensitive, followed by *S. aureus* (69%), *S. typhi* (50%) and *P. aeruginosa* (44%) (Fig 4.2).

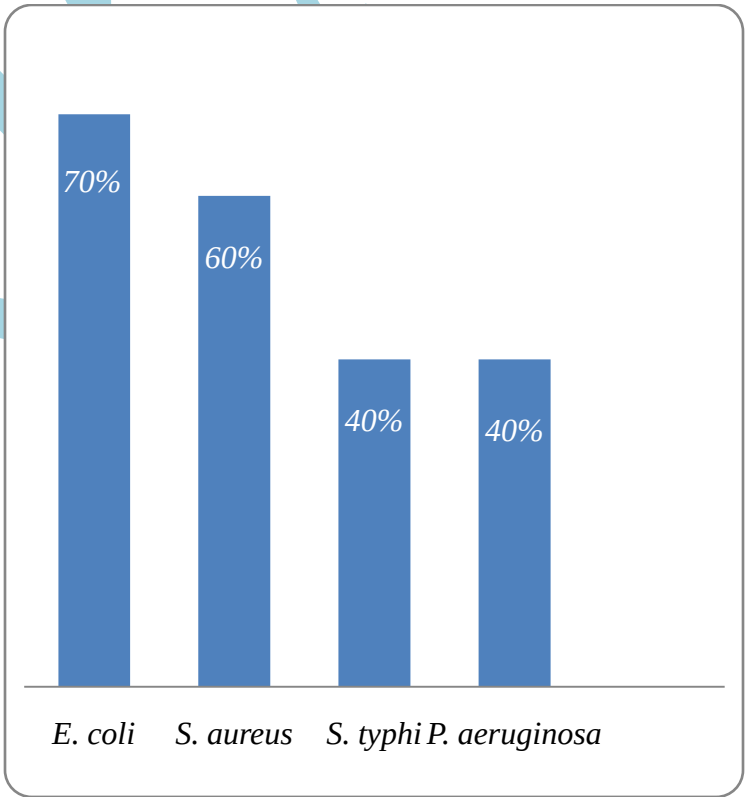

Fig.4.1: Drug resistance profile of tested organism

Table 4.1: MDR Strains and their antimicrobial sensitivity profile.

Bacterial strains	CIP	CRO	MEM	FOX	AMP	AK	DO	CAZ	AZT	CXM
E. coli	--	--	26	--	--	20	--	--	18	--
S. aureus	--	--	23	--	--	15	17	--	21	--
S. typhi	18	23	29	24	--	27	--	22	--	--
P. aeruginosa	--	19	--	--	25	15	30	29	--	25

Key:- ZI in: mm --: no ZI, CIP: (Ciprofloxacin), CRO: (Ceftriaxone), MEM: (Meropenem), FOX: (Cefoxitin), AMP: (Ampicilin), AK: (Amikacin), DO: (Doxycyclin), CAZ: (Ceftazimide), AZT: (Azithromycin), CXM: (Cefuroxime).

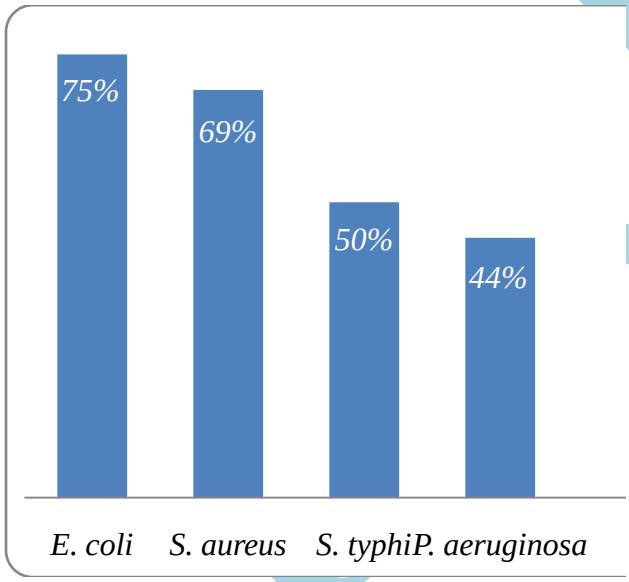

Fig.4.2: Plant extract sensitivity profile of tested organism

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