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Antibacterial screening of *Ajuga bracteosa wall ex. Benth* leaves found in Swat, Kyber Paktunkhwa, Pakistan

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ABSTRACT

The present study was focused on the anti-bacterial potential of *Ajuga bracteosa*, found in northern areas of Pakistan. Three different extracts of the selected plant species were used during the experiment. The anti-bacterial study was carried out against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus epidermidis*. Agar pore diffusion tests indicated that all of the conducted extracts i.e., Methanolic, Ethanol and Ethyl Acetate extracts have unique antibacterial sensitivities. The susceptibility of the tested bacteria varied between species and strains. *Staphylococcus aureus* showed highest zone of inhibition (24 mm) in ethanolic extract while least (20 mm) in Ethyl Acetate. *Pseudomonas aeruginosa* showed highest zone (18 mm) in Methanolic while least (2mm) in Ethanolic Extract. *Bacillus subtilis* was resistant to Methanol and Ethanol extracts exhibiting 3 mm and 2 mm inhibition while susceptible to Ethyl Acetate extract leaving an inhibition area of 15mm. For *E. coli,* methanolic and ethanolic extract showed 2 mm and 15 mm respectively while Ethyl Acetate showed 14 mm. In case of *S. Epidermidis*, the highest inhibition was observed in Ethyl Acetate (20 mm) while lowest inhibition (12 mm) in methanolic extract.

Key words: *Ajuga bracteosa*, Anti-bacterial, Inhibition Zones, *E. coli*, *Pseudomonas aeruginosa*

1. INTRODUCTION

Ajuga bracteosa, commonly known as 'Bungle' belongs to Lamiaceae family. The plant is commonly called as ''Jan-e-Adam'' and ''Kori Booti'' due to its vicious taste [1]. In rural areas, medicinal plants are the easiest and cheapest way of medication. Approximately 65-80% of the world population in developing countries primarily focus on the medicinal plants for their basic health care as they lack the facilities of modern medicines [2]. *A. bracteosa* is a quality medicinal, aromatic and soft herb ranging from 10–30 cm in height. The habitat of the plant lies in temperate and subtropical region having elevation of 1300 to 2400 m [3]. The genus *Ajuga* is comprised of 50

species which is found cosmopolitan [4]. *Ajuga* species are widely used for the inflammatory and metabolic diseases including diabetes [5], malaria [6], hypertension etc. [7]. Medicinal plants are also important due to presence of antimicrobial compounds. Many infectious diseases are cured by plants extracts due to their potential microbial activity. There are numerous plants which consist of bioactive compounds and which need to be explored [8]. Numerous other Phyto-active compounds like glycosides, diterpenoids, ergosterols are also present. Apart from this, *Ajuga* is a rich in sitosterol (β and γ-sitosterol), palmitic acids, arabinose, glucoside and anthocyanidin [9].

The current research is carried mainly to find out the antibacterial analysis of *Ajuga Bracteosa* found locally in the area of Swat, Pakistan.

2. METHOD AND MATERIALS

2.1. Sample collection

In order to carry out the research, a proper survey was carried out in District Swat followed by collection of *A. bracteosa* plants in a zip lock bag. The samples were brought to the labs of Institute of Biological Sciences Peshawar, Sarhad University of Sciences and Information Technology, Peshawar.

2.2. Preparation of Extract

Leaves of the sample plant were air dried and crushed in pestle and mortal. Plant extracts were prepared in 1:10 for different mediums i.e., Ethyl Acetate, Methanol and Ethanol. Samples were gently mixed and kept in a closed container in cool place for 72 hours. The solution was then passed through filter paper. The extract were further stored in temperature ranging from 2 °C - 8 °C for antibacterial activity testing.

2.3. Bacterial strains

Gram-positive bacteria comprising *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633), *Staphylococcus epidermidis* (ATCC 12228) and gram-negative bacteria such as *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 25823) were prepared during the entire research.

2.4. Culturing of bacterial species

The identified bacterial strains were cultured in nutrient broth agar. For this purpose, 30 ml of the peripheral water were thoroughly mixed and sterilized in autoclave. It was then transferred to a glass test tube. To each test tube 5ml water was added followed by 10 µL of bacteria.

2.5. Agar diffusion test

Around 25-30 ml of nutrient Agar medium was then poured into a 14 cm diameter sterile Petri dish. The media was kept for solidification at room temperature. Inoculation of all the microbes was done over agar using a sterile swab. Small holes (6-8 mm) were punched with an antiseptic cork stopper and 100 µL of antimicrobial extract solutions (methanol, ethanol and ethyl acetate) were added to the holes. The agar plates were then incubated at 37 °C for 24-48 hours.

3. RESULTS AND DISCUSSION

The present study was designed to determine the antibacterial effect of *Ajuga bracteosa* against five selected species i.e., *Staphylococcus aureus, Araginosea streptococcus, Bacillus subtilis, E. coli* and *Staphylococcus epidermidis*.

Results have shown that *Ajuga* has an inhibitory effect on these specific pathogenic bacteria. The susceptibility of the tested bacteria varied between species and strains.

The Methanolic extract of *Staphylococcus aureus* showed low significant activity i.e., 16 mm inhibitory zone, while the Ethanolic extract and Ethyl acetate extract showed high activity i.e., 20 mm and 24mm. The results are in

Table 1. Shows antibacterial activity of *Ajuga bracteosa* leaves extract

Extract	*S. aureus*	*B. subtilus*	*P. aeruginosa*	*E. coli*	*S. epidermidis*
Ethanolic	24mm	3mm	2mm	15mm	15mm
Methanolic	16mm	2mm	18mm	2mm	12mm
Ethyl acetate	20mm	20mm	14mm	18mm	20mm

line with other studies who also concluded the same result of *Ajuga* against five types of bacteria [10].

In case for *Bacillus subtilis*, methanolic and ethanolic extracts were not effective and were in the suppression zones of 02 mm - 03 mm, respectively, but the antibacterial potential of the ethyl acetate extract showed a good activity against *Bacillus subtilis* with a suppression zone of 15mm. The results are found similar with previous studies the antimicrobial effect against *Bacillus* strains with a zone of inhibition (15mm) [11-12]. In the case of *Pseudomonas aeruginosa*, the ethanolic extract showed 02 mm, while the ethyl acetate extract and the methanolic extract showed inhibitory area as 14 mm -18 mm respectively. The given results are consistent with using a multidrug resistant bacterial strain against methanol leaf extract of *Ajuga spicata* [11]. The findings for methanolic and ethyl acetate extracts of *Ajuga bracteosa* was also similar to studies which determined the *in vitro* action separately against multi bacterial strains and which exhibited good effect i.e., 15 – 27 mm zone of inhibition. Lowest inhibition zone was observed for the methanolic extract for *E. coli* i.e., 02mm inhibition zone while the ethanolic extract and ethyl acetate extract were 15 mm - 14 mm, respectively. The results were in-line other studies on low MIC against *E. coli*. For *S. epidermidis*, methanolic extract showed activity with a suppression zone of 12 mm. The inhibitory ranges of ethanolic extract and ethyl acetate extract were 15 mm and 20 mm. Among all of the extract, Ethyl Acetate had a durable inhibition effect for the candidate bacterial strains. The effect was followed by ethanol and methanol extracts. It was observed that the inhibitory activity for ethyl acetate was stronger on all Gram-negative pathogens compared to others.

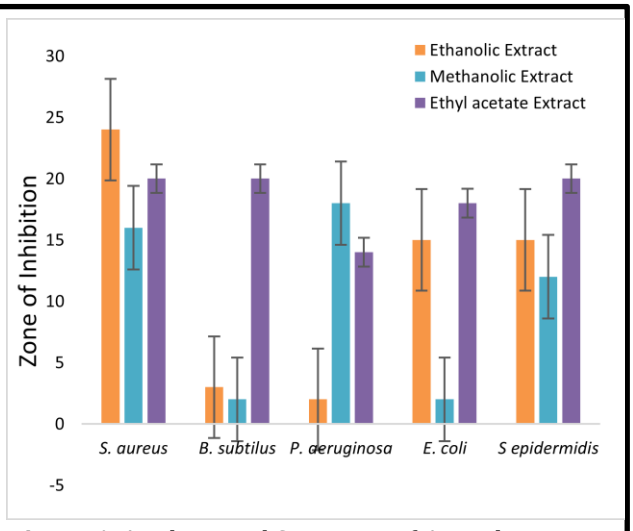

Figure 1. Antibacterial Screening of *Ajuga bracteosa*

4. CONCLUSION

It was concluded that *Ajuga* extract is a significant (inhibition area ≥18mm) antibacterial agent. A strong anti-bacterial effect was observed for ethyl acetate against *Staphylococcus aureus, Staphylococcus epidermidis,* and *E. coli* compared to methanolic extract which showed insignificant resistance to *Staphylococcus aureus* and *Bacillus subtilis*.

5. ACKNOWLEDGEMENT

NA

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

NA

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