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ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF OTOSTEGIA LIMBATA L., AND AJUGA BRACTEOSA L., AGAINST PATHOGENIC MICROORGANISMS

Sarwat Iftikhar[1], Amjad ur Rahman[1], Yasir Ihtesham[2], Abdul Shakoor[1*],Gul Zaib[3]

[1]Department of Plant Sciences, Quaid-i-Azam University, Islamabad-Pakistan

[2] Department of Botany, Hazara University, Mansehra-Pakistan

[3] Department of Microbiology, Quaid-i-Azam University, Islamabad-Pakistan

Quaid-i-Azam University, Islamabad, Pakistan abdul_shakoor954@yahoo.com

Abstract- *Ajuga bracteosa* L., and *Otostegia limbata* L., are two plant species of family Lamiaceae. The crude methanol leaves extracts of these two medicinal plants were examined for their antibacterial, antifungal and antioxidant (radical scavenging) activity using 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical against clinically important species of bacteria and fungi were examined. Leaves extracts of these two plants were screened against six strains of bacteria (two were gram positive i.e. *Bacillus subtilis* and *Staphylococcus aureus* and four were gram negative i.e. *Vibrio cholerae, Enterobacter aerogenes, Escherichia coli* and *Klebsiella pneumonia*) and two strains of fungi (*Aspergillus niger* and *Aspergillus fumigatus*). Eight concentrations (15 mg/ml, 12.5 mg/ml, 10 mg/ml, 7.5 mg/ml, 5 mg/ml, 3 mg/ml, 2 mg/ml and 1 mg/ml) were used to check the antimicrobial activity of plant extracts. Maximum inhibitory zone 30 mm was observed in *Otostegia limbata* and 25 mm in *Ajuga bracteosa* at 15 mg/ml mm for antibacterial activity. *Ajuga bracteosa* and *Otostegia limbata* gave response against both *Aspergillus niger* and *Aspergillus fumigatus*. This study establishes the effective ethnomedicinal use of these plants in the treatment of various infectious diseases. There is high potential for the exploitation of the plants for development of novel antimicrobial agents.

Key words- Antibacterial, antifungal, antioxidant, methanol extracts, pathogenic.

I. INTRODUCTION

Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world [1]. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes [2]. More than 1,000 plants species are being reported to carry medicinal values [3]. These medicinal plants are used by marginal communities to cure various diseases [4] The plants of genus *Ajuga* are evergreen, clump-forming rhizomatous annual or perennial herbaceous flowering species in the mint family, Lamiaceae, with most plants native to Europe, Asia, and Africa, but also growing in Australia and North America [5]. Other reported activities of *Ajuga* plants include antibacterial [6]. Except *Otostegia persica*, no previous antimicrobial investigation of this species has been reported. Aim of the present work was to determine the antibacterial, antifungal and antioxidant activities of selected plant species of family Lamiaceae., antifungal and antioxidant activity.

II. MATERIALS AND METHODS

The present research work was carried out in the Department of Plant Sciences, Quaid-i-Azam University Islamabad-Pakistan. Brief accounts of materials as well as procedures used in it are described below.

Samples collection

Two plants of family Lamiaceae i.e. *Otostegia limbata* L., and *Ajuga bracteosa* L., were collected from Islamabad. The plants were identified and voucher specimens were deposited in Herbarium of Quaid-i-Azam University, Islamabad.

Extraction

Fresh medicinal plant parts plant namely; *Otostegia limbata* L., *and Ajuga bracteosa* L ., were taken rinsed with distilled water and kept under shade till drying. Extraction from plant parts were carried out by simple maceration process. The plant parts were taken and grounded in methanol using kitchen blender. This mixture was kept for two weeks at room temperature 25^0 C in extraction bottle. After two weeks mixture was filtered twice, using Whatman-41 filter paper. Methanol was then completely evaporated by rotary evaporator to obtain the extract.

Preparation of Samples

The extract (15 mg) was dissolved in 10 ml of DMSO. This stock solution 15 mg/10ml was again diluted, thus 8 concentrations of the extract were prepared i.e. 15 mg/ml, 12.50mg/ml, 10 mg/ml, 7.5 mg/ml, 5 mg/ml, 3 mg/ml, 2 mg/ml, 1 mg/ml. Along with these solutions of Standard antibiotic (2 mg/ml of the DOX) was also prepared. The solutions of extracts are used for test. Standard antibiotics and pure DMSO were used for positive and negative control.

Bacterial strains used

Six strains of bacteria were used. Two were grampositive i.e. *Bacillus subtilis* and *staphylococcus aureus* while four were gram-negative; *Escherichia coli*, *vibrio*

cholera, Enterobacter aerogenes and Klebsiella pneumoniae. The organisms were maintained on nutrient agar medium at 4^0C.

Antifungal assay

The agar tube dilution method is used for antifungal activity of extract. **Fungal strains used**

The following fungal strains were used in this study;

Fungal strains

a) *Aspergillus nigar*
b) *Aspergillus fumigatus*

Antioxidant activity (DPPH free radical scavenging activity)

The free radical scavenging activity of methanolic extracts of *Otostegia limbata* L., *and Ajuga bracteosa* L., was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).The test extracts were prepared in methanol therefore the DPPH was also prepared in methanol. 3.96 mg (x 4) of DPPH was dissolved in 20 ml of methanol solvent to get stock solution. With 0.5 ml of sample solution was added to 1 ml of DPPH solution separately. These solution mixtures were kept in dark for 30 min (incubation period) at room temperature. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All tests were carried out in triplicate and finally the radical scavenging activity was calculated as percentage of DPPH discoloration using the equation;

% scavenging DPPH free radical = 100 × (1-AS/AD)

Where AS is absorbance of the solution, when extract has been added at a particular level and AD is the absorbance of the DPPH solution with nothing added (blank, without extract).

III. RESULTS

Antibacterial study of *Lamiaceae* plants

When the methanolic plant extract of *Otostegia limbata* L. was tested against *Bacillus subtilis*, it exhibited the 15 mm inhibition zone at the concentration of 15 mg/ml. While minimum inhibition concentration value was nil. Further it was found that at 12.50 mg/ml, 10 mg/ml and 7.50 mg/ml concentrations showed 15mm, 12mm and 10mm inhibitory zones. At the concentration of 5 mg/ml the zone of inhibition was 10mm. While at the concentrations of 3 mg/ml and 2 mg/ml inhibitory zones were 10mm and 7mm. At concentration of 1 mg/ml showed 8mm inhibitory zone. Standard antibiotic DOX showed 20mm inhibitory zones.

The methanolic plant extract of *Otostegia limbata* L. was screened against *Enterobacter aerogenes* it was found that the concentration of 15 mg/ml gave 15 mm inhibition zone. While at the concentration of 12.50 mg/ml it showed 14 mm zone of inhibition. Whereas 10 mg/ml, 7.50 mg/ml and 5 mg/ml concentrations of plant extracts exhibited 15 mm, 15mm, 16mm and 15mm inhibitory zones respectively. At the concentration of 3 mg/ml the zone of inhibition was 13 mm while 12mm inhibitory zone was shown at the concentration of 2 mg/ml. MIC is 1 mg/ml and the zone shown by MIC was 12 mm. Standard antibiotic DOX showed 39mm inhibitory zone. The methanolic plant extract of *Otostegia limbata* L. at the concentrations of 15 mg/ml, 12.50 mg/ml, 10 mg/ml, 7.50 mg/ml and 5 mg/ml, inhibitory zones were 12 mm when tested against *Vibrio cholerae*. The inhibitory zone showed at 3 mg/ml concentration was 9 mm and at the concentration of 2 mg/ml zone of inhibition was noted as 7 mm. MIC showed 4 mm inhibition zone. The DOX showed 40mm zone of inhibition

At the concentrations of 15 mg/ml and 12.50 mg/ml, the inhibitory zones were the same i.e. 30 mm each when the methanolic plant extract of *Otostegia limbata* L. was tested against the *Staphylococcus aureus* at the concentration of 10 mg/ml the inhibitory zone was 30 mm. Inhibitory zone of 30 mm was shown at the concentrations of 7.50 mg/ml inhibitory zone of 25mm was shown at the concentration 5 mg/ml. At the concentration of 3 mg/ml, the inhibitory zone was shown as 20 mm and 18 mm zone of inhibition was shown at the concentration of 2 mg/ml. MIC which was 1 mg/ml, showed the 15 mm zone of inhibition. Zone of inhibition showed by the standard antibiotic DOX was 40mm. Methanolic plant extract of *Otostegia limbata* L. was tested against *Escherichia coli.* It was found that at the concentration of 15 mg/ml it showed 15mm inhibition zone. At 12.50 mg/ml inhibitory zone was 11 mm, while at the concentration of 10 mg/ml zone of inhibition was 17 mm. At the concentrations of 7.50 mg/ml, the inhibitory zone was 16 mm. 14 mm zones of inhibition were exhibited at the concentrations of 5 mg/ml, 3 mg/ml and 2 mg/ml. MIC showed 15 mm inhibitory zone while standard antibiotic DOX indicated 40 mm zone of inhibition.

The methanolic plant extract of *Otostegia limbata* L. was screened against *Klebsiella pneumoniae* it was found that the concentration of 15 mg/ml gave 13 mm inhibition zone While at the concentration of 12.50 mg/ml it showed 14 mm zone of inhibition. Whereas 10 mg/ml, 7.50 mg/ml and 5 mg/ml concentrations of plant extracts exhibited 12 mm inhibitory zone and at 5mg/ml concentration of plant extract exhibited 10mm. At the concentration of 3 mg/ml the zone of inhibition was 9 mm while 13mm inhibitory zone was shown at the concentration of 2 mg/ml. MIC is 1 mg/ml and the zone shown by MIC was 19 mm. Standard antibiotic DOX showed 7mm inhibitory zone at the concentration of 27mg/ml . *Ajuga bracteosa* L. methanolic plant extract showed 25 mm inhibitory zones against *Bacillus subtilis* at the concentrations of 15mg/ml, 12.50 mg/ml and 10 mg/ml. It showed 15 mm inhibition zone at the concentration of 7.50 mg/ml. At the concentrations of 5 mg/ml and 3 mg/ml zones of inhibition were measured as 13 mm. It indicated 9 mm clear inhibitory zone at the concentration of 2 mg/ml. MIC which was 1 mg/ml showed 6 mm zone of inhibition. While standard antibiotic DOX exhibited 30 mm clear zone of inhibition. When the methanolic plant extract of *Ajuga bracteosa* L., was tested against *Staphylococcus aureus*, it gave 20 mm inhibitory zones at the concentrations of 15 mg/ml. 19 mm was exhibited at concentration of 12.50 mg/ml. At concentration 10 mg/ml and 7.50 mg/ml, zone of inhibition was 16 mm. At the concentrations of 5 mg/ml, 3 mg/ml and 2 mg/ml zones of inhibition were 10 mm where as MIC i.e. 1 mg/ml indicated 5 mm inhibitory zone. Standard antibiotic DOX exhibited 35 mm zone of inhibition. *Ajuga bracteosa* L. methanolic plant extract yielded 20 mm inhibition zone at the concentration of 15 mg/ml against *Vibrio cholerae*. 18 mm and

15 mm zones of inhibition were showed at the concentrations of 12.50 mg/ml and 10 mg/ml respectively. At the concentrations of 7.50 mg/ml and 5 mg/ml, the zones of inhibition were showed as 14 mm each. While 3 mg/ml, 2 mg/ml and 1 mg/ml concentrations showed 10mm zones of inhibition. Thus here MIC was 5 mg/ml. DOX yielded 40 mm inhibitory zone

Methanolic plant extract was obtained from *Ajuga bracteosa* L., and tested against *Enterobacter aerogenes.* At the concentration of 15 mg/ml it gave clear zone of inhibition of 18 mm. Zones of inhibition i.e. 15 mm, 15 mm and 10mm were exhibited at the concentrations of 12.50 mg/ml, 10 mg/ml and 7.50 mg/ml respectively. It showed 12 mm inhibition zone at concentration of 5 mg/ml while 13 mm zones of inhibition were indicated at the concentrations of 3 mg/ml and 2 mg/ml each. MIC i.e. 1 mg/ml gave 10 mm zone of inhibition. DOX exhibited 35mm inhibitory zone. Methanolic plant extract of *Ajuga bracteosa* L., at the concentrations of 15 mg/ml, 12.50 mg/ml and 10 mg/ml exhibited 25 mm inhibition zones against *Escherichia coli*. Whereas at the concentrations of 7.50 mg/ml, 5 mg/ml and 3 mg/ml, zones of inhibition were 23 mm.20 mm zone of inhibition was exhibited at the concentration of 2 mg/ml while MIC which was 1 mg/ml, showed 18 mm inhibitory zone. At the concentration of 2 mg/ml, standard antibiotic DOX indicated 35 mm zone of inhibition.

Ajuga bracteosa L. methanolic plant extract yielded 25 mm inhibition zone at the concentration of 15 mg/ml against *Klebsiella pneumonia*. 18 mm and 16 mm zones of inhibition were showed at the concentrations of 12.50 mg/ml and 10 mg/ml respectively. At the concentrations of 7.50 mg/ml and 5 mg/ml, the zones of inhibition were showed as 10 mm each. While 3 mg/ml, 2 mg/ml and 1 mg/ml concentrations showed 7 mm zones of inhibition. Thus here
MIC was 5 mg/ml. DOX yielded 30 mm inhibitory zone
(Table 3).

Antifungal activity of *Otostegia limbata* L. and *Ajuga bracteosa* L.

Methanolic extract of *Otostegia limbata* L. showed 38.4 % and 50 mm growth inhibition against *Aspergillus nigar* at the concentration of 15 mg/10ml. The methanolic plant extract of the same plant at the same concentration gave 9.09 % and 90 mm growth inhibition against *Aspergillus fumigatus*. *Ajuga bracteosa* L. exhibited 23.01 % and 70 mm growth inhibition against the fungal strain i.e. *Aspergillus nigar*, at the concentration of 15 mg/10ml. At the same concentration the methanolic extract of the plant showed 18.18 % and 80 mm growth inhibition against *Aspergillus fumigatus*.

Antioxidant activity of Lamiaceae plants

In this study an attempt was made to check out the antioxidant activity of selected plants of family Lamiaceae. The free radical scavenging activity of the plants was determined through DPPH. All the samples showed good results which are discussed as follow.

% RSA of *Otostegia limbata* L. and *Ajuga bracteosa* L. One ml (1 ml) from extract stock solution and two ml (2 ml) from DPPH stock solution was taken and placed in cuvette for study. Three readings were taken and the mean of these readings was obtained as 0.22629. After applying the formula, % radical scavenging activity of *Otostegia limbata* L. was noted as 68.96 %. The same procedure was followed for
Ajuga bracteosa also. Three readings were taken and the mean was noted as 0.48687. After applying the formula, % RSA was obtained as 33.22 % .

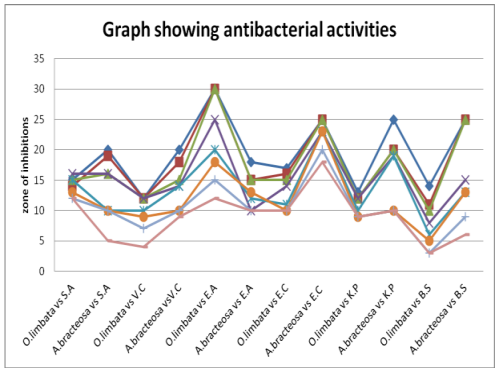


Figure 1. Antibacterial activities at different concentration produced by *Otostegia limbata* L. and *Ajuga bracteosa* L.

Where
S.A= *Staphylococcus aureus*
V.C= *Vibrio cholerae*
E.A= *Enterobacter aerogenes*
E.C= *Escherichia coli* **K.P**= *Klebsiella pneumoniae.*
B.S= *Bacillus subtilis*

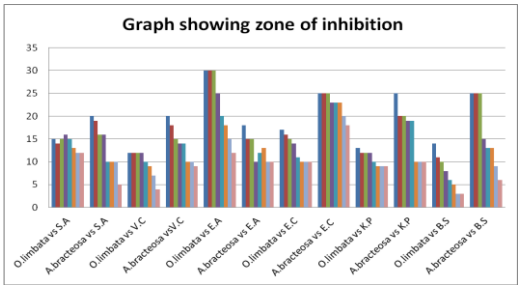


Figure 2. Zone of inhibition (mm) produced by *Otostegia limbata* L and *Ajuga bracteosa* L.

Where
S.A= *Staphylococcus aureus*
V.C= *Vibrio cholerae*
E.A= *Enterobacter aerogenes*
E.C= *Escherichia coli*

K.P= *Klebsiella pneumoniae.*
B.S= *Bacillus subtilis*

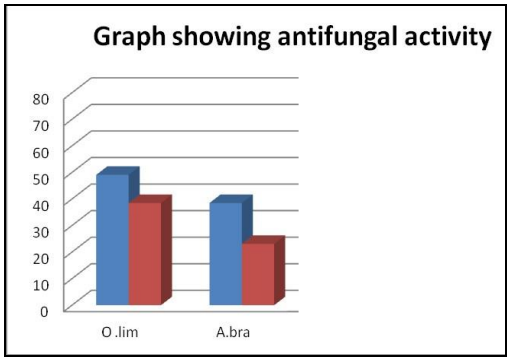


Figure 3. Antifungal activity of *Otostegia limbata* L., and *Ajuga bracteosa* L., against *Aspergillus niger* and *Aspergillus fumigatus*.

Where
O.lim = *Otostegia limbata* L.
A.bra = *Ajuga bracteosa* L.

IV. Discussion

The present work was undertaken to explore medicinal value of two different plant species i.e. *Otostegia limbata* L., and *Ajuga bracteosa* L., belonging to family Lamiaceae collected from different location of Islamabad, Pakistan. Among the selected bacterial strains, *Bacillus subtilis* was the most resistant while *Staphylococcus aureus* was the most sensitive at all concentrations. The findings are in line with [7] and [8], who reported the antibacterial activity of *Otostegia limbata* L. against these gram-positive and gramnegative bacterial strains.

Other member of family *Lamiaceae* i.e. *Ajuga bracteosa* L. was also screened for the antibacterial activity against gram-positive and gram-negative bacteria strain at different concentrations. *Staphylococcus aureus* was found to be sensitive at maximum concentration i.e. 15mg/ml and showed inhibition zone of 20 mm while minimum concentration gave 5mm. When the plant extract was applied against *Enterobacter aerogenes* 15mg/ml gave 18mm where as concentration 1mg/ml showed 10mm zone of inhibition. Methanolic plant extract of *Ajuga bracteosa* L.was used against *Vibrio cholerae*. At maximum concentration i.e. 15 mg/ml it gave 20mm zone of inhibition. On the contrary, at minimum concentration i.e. 1mg/ml it was 9mm.

Susceptibility of the pathogenic bacterial strains to methanolic extract of *Ajuga bracteosa* L. were varies with concentration of plant extract.

Methanolic extract of *Otostegia limbata* L and *Ajuga bracteosa* L. showed the best antibacterial activity, which support the taxonomic relation of these plants to family Lamiaceae. The activity against all the six pathogenic bacterial strains also indicates the presence of broad spectrum antibacterial compounds in the members of Lamiaceae. Both of plant species showed resistance against gram-negative bacteria and susceptibility towards gram-positive bacteria. The reason for higher sensitivity of the Gram-positive bacteria compared to negative bacteria could be ascribed to the differences between their cell wall compositions. The Grampositive bacteria contains an outer peptidoglycon layer which is an ineffective permeability barrier ,whereas in the case of Gram-negative bacteria, outer phospholipidic membrane makes the cell wall impermeable to lipophilic solutes and porins constitute a selective barrier to the hydrophilic solutes (Burt, 2004). [9]

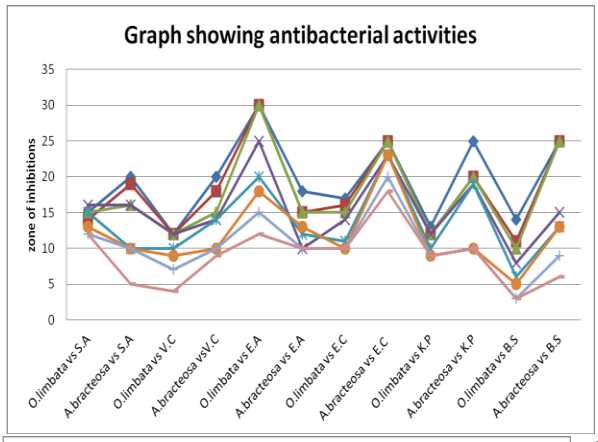
Ajuga bracteosa L. showed the same result against *Aspergillus fumigatus* as shown by *Otostegia limbata* against the same fungus. Against *Aspergillus niger* the plant extract exhibited the strongest activity i.e. 49.09 % in the present work. It can be concluded from the antifungal assay that *Otostegia limbata* L. has strongest antifungal activity against *Aspergillus niger* same as *Ajuga bracteosa* L. while against *Aspergillus fumigatus* plant species showed minimum activity that was a clear evidence for the relation of these species with family Lamiaceae which support their use as traditional medicine against various fungal disorders.

Antioxidant assay was also under taken to explore antioxidant activity of studied plant species of family Lamiaceae. As a result of this study, the confirmation of considerable antioxidant potential in the Lamiaceae plants proved the information that extends the knowledge of possible mechanism that underlie their traditional uses. Antioxidant activity of *Otostegia limbata* L and Ajuga *bracteosa* L. were determined by DPPH assay. DPPH values showed the strength of examined plant to neutralize free radical that can cause cancer. *Otostegia limbata* L. showed 68.96% presence of antioxidants. These are the chemical compounds that can inhibit oxidation carrying healthy attributes were found to be abundantly present in *Otostegia limbata* L. found to be an indication for supporting cardiovascular health. This is the first report of the antibacterial potency of *Otostegia limbata.* The findings provide the evidence that *Otostegia limbata* as a good medicinal plant for further investigations.

V. Conclusion

The present studies support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with biological properties that can be further explored for antimicrobial and antioxidant activity. This biological study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

VI. LIST OF FIGURES



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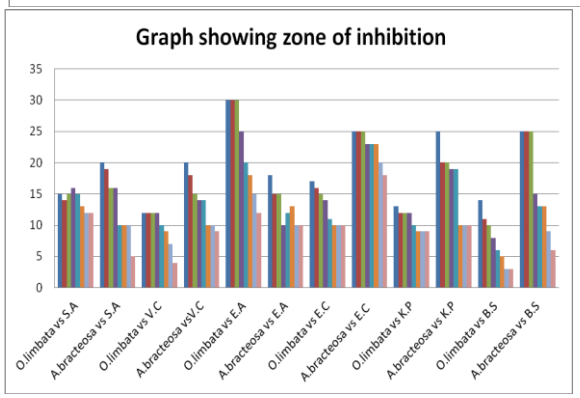

Figure 2.

Zone of inhibition (mm) produced by *Otostegia limbata* L and *Ajuga bracteosa* L.

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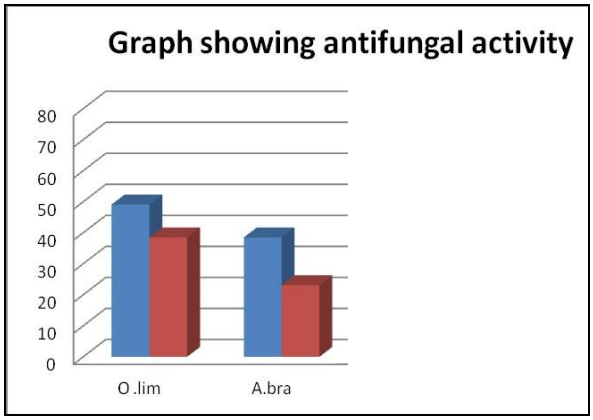

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Where
O.lim = *Otostegia limbata* L.

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A.bra = *Ajuga bracteosa* L.

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