ANTIMICROBIAL ACTIVITY OF EXTRACTS ISOLATED FROM MEDICINAL PLANT AJUGA TURKESTANICA

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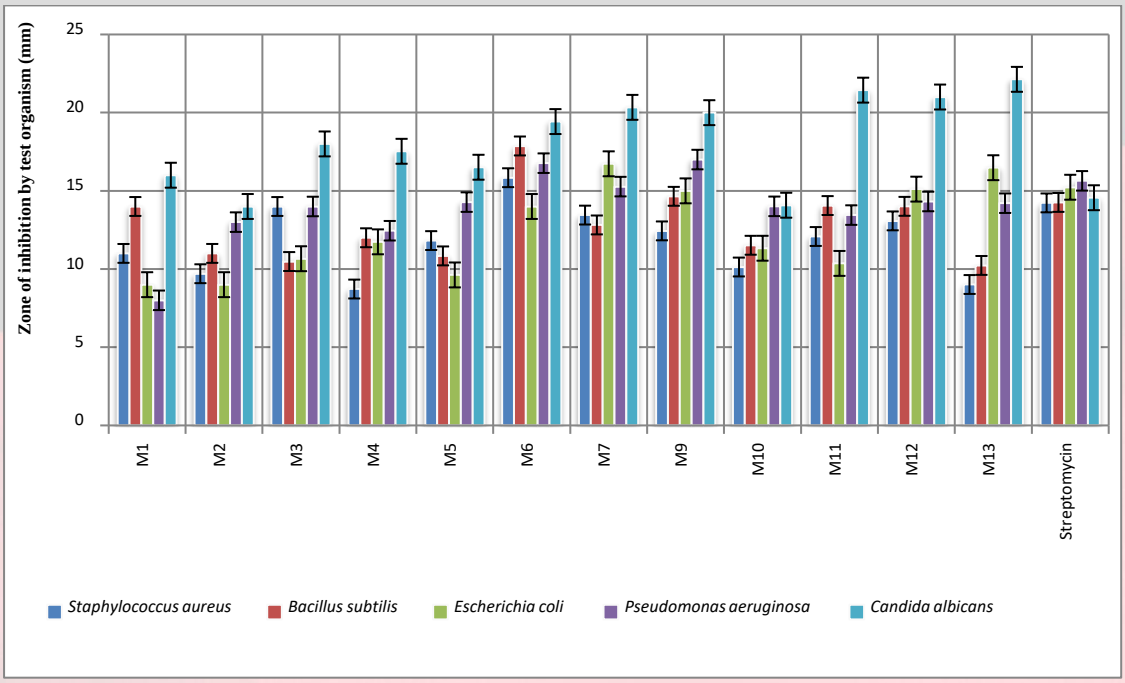
The Ajuga turkestanica extract is a whole plant of the dicotyledonous medicinal plant Lamiaceae, which has the effects of clearing heat and cooling blood, reducing fever and swelling, and treating symptoms such as pulmonary heat hemoptysis, and reducing fever and swelling.

Ajuga turkestanica has been used there to enhance muscular strength and physical endurance under harsh, exhaustingconditions. It is anabolic agent designed to increase muscle mass and decrease bodyfat. It can improve muscle for bodybuilder and athlete. Add it in the capsules of your brand sports supplements, and earn more fame for your brand.

The plant material is cut into small pieces (0.5-1cm in length) in a sterile petriplate using forceps and scissors under laminar hood to ensure aseptic condition. The cut plant material is subjected to surface sterilization technique using 70% ethanol (1min), 4 % sodium hypochlorite (5 min) 75% ethanol (30 sec) and double distilled water (30 sec). These plant segments are now placed in Soybean casein digest agar medium and incubated for 7-10 days at 28°C for the growth of the bacteria. In total, twelve isolates (M1-M13) of endophytic bacteria were isolated from the plant. The endophytic bacteria growing out of the plant explants are sub cultured periodically on separate Soybean casein digest agar plates at room temperature and stored at 4˚C for further experiments. Endophytic bacterial cultures were separately inoculated in the broth medium within the flasks. Flasks were then incubated at 28°C for 96 h in incubator shaker at 120 rpm.

The broth culture was centrifuged at 5000 rpm for 30 min. The extraction of the supernatant/filtrate with different solvents (Chloroform: Ethyl acetate) in 1:1 ratio and left for 15-30 minutes. The solvent phase containing the extracted secondary metabolites was separated using separating funnel. The extract was evaporated using a rotary evaporator at 40 °C with 90 rpm to yield the crude metabolites. The crude extract was then dissolved in methanol at 1 mg/mL of concentration and kept at 4º C. Antimicrobial activity of secondary metabolites extracted from endophytic bacteria was screened against gram-positive bacteria Staphylococcus aureus, Bacillus subtilis, gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa microscopic fungi pathogenic strains of Candida albicans using agar disk diffusion method (Figure 1).

To evaluate the antibacterial activity, bacteria were grown in liquid LB medium for 24 h, adjusted at a concentration of 10^6 cells/ml and spread (100 µl) on Petri dishes containing solid LB medium. In each dish were placed, equidistantly, four disks of sterile filter paper Whatman №1 (6 mm) inoculated with 10 µl of extracts. As negative controls, paper disks were inoculated with autoclaved distilled water and absolute methanol. As positive control, streptomycin (50 µg.ml-1) was used. The antibacterial activity was evaluated by the formation of inhibition halos.



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