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Effect of methanolic and ethyl acetate leaf extract of *Diospyros discolor* against gram positive and gram negative bacteria

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ABSTRACT

Secondary metabolites from plants like alkaloids, terpenoids, saponins and vitamins provide an excellent source of pharmaceutically active compounds. Majority of the FDA approved drugs are inspired from the small molecules obtained from the plant extract which leads to drug discovery even for the treatment of life threatening conditions. This study aimed to perform primary phytochemical analysis and antibacterial activity of methanolic and ethyl acetate leaf extracts of *Diospyros discolor*. Phytochemical analysis of methanol showed the presence of pharmaceutically important compounds like alkaloids, tannins, flavanoids and phenols whereas ethyl acetate extract showed an absence of alkaloid. Antibacterial assays were carried out using disc diffusion and well diffusion assay for both gram positive (*Bacillus cereus* and *Staphylococcus aeureus*) and gram negative (*Salmonella typhii* and *Eschericia coli*) bacteria. Methanol extract showed maximum antibacterial activity in both assays compared to ethyl acetate leaf extract. Antibacterial activities of the leaf extract of *Diospyros discolor* revealed potentiality of the metabolites against the bacteria.

Keywords: Antibacterial assay, well diffusion, disc diffusion, phytochemical analysis

INTRODUCTION

World Health Organisation (WHO) reports that more than 80% of the world's population relies on traditional medicine from plants for their primary health care needs. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds (Shihabudeen, 2010). The knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Abd-Alrahman, 2013).

Diospyros discolor belongs to the family Ebenaceae which is a medium-sized tree growing to a height of 20 m. Leathery leaves are oblong, up to 20 cm long, with a round base and acute tips. Different parts of *Diospyros discolor* is used as traditional medicines. Bark is used for fevers, dysentery, diarrhea, itch skin ailments and also decoction of bark for coughs. Juice of unripe fruit is used for wounds in some parts of Southeast Asia. Oil from seeds is used for diarrhea and dysentery. In Bangladesh, juice of bark and leaves are used for snakebites. Bark and leaves extract are used as eyewash (Das, 2010, Howlader, 2012). Secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin, 1985). Medicines obtained from plants are relatively safer than synthetic alternative (Akinnibosun, 2009). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Farnsworth, 1966). The objective of the present study was to investigate the antibacterial activity and also to carry out the preliminary phytochemical analysis of methanol and ethyl acetate extract of leaves of *Diospyros discolor*.

MATERIALS AND METHDOLOGY

Materials: All chemicals and reagents were of analytical grade and purchased from Hi-media Laboratories Pvt. Ltd., Mumbai, India, Sd-fine Chemicals. Ltd., Mumbai, India

Methodology:

Preparation of plant extract: The leaves of Diospyros discolor was collected from The College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The collected leaves were washed with tap water and shade dried for one week and then ground to coarse powder. The ground sample (10g) was subjected to soxhlet extraction using methanol and ethyl acetate (100 mL) as solvent for 3 hours (Maridass, 2008). The extract was poured into petriplates at room temperature and allowed to evaporate in open air.

Antibacterial Activity:

Disc Diffusion Assay: Kirby-Bauer method was followed for disc diffusion assay. The Muller Hinton Agar (MH) plates were prepared by pouring 20 ml of molten media into sterile petriplates. After solidification test microorganisms namely *Escheriachia coli*, *Salmonella typhii*, *Staphylococcus aureus* and *Bacillus cereus* were seeded into MH medium by spread plate method. The filter paper disc (6 mm in diameter) impregnated with the extract (100µg/disc) was placed on test organism-seeded plates and was allowed to diffuse for 5 minutes. It was then kept for incubation at 37°C for 24 h. Standard antibiotic (tetracycline 30T) discs and blank (sterile water) discs were used as positive and negative control respectively. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter (Shihabudeen, 2010).

Well Diffusion Assay: Sterilized Muller Hinton (MH) agar was poured into petri-dishes and allowed to set. Nutrient broth inoculated with the test bacteria namely *Escheriachia coli*, *Salmonella typhii*, *Staphylococcus aureus* and *Bacillus cereus* was seeded into the already set petriplates using spread plate method. 6mm diameter holes were punched in the agar. From the stock (0.1g/mL), dilutions were made to obtain 250µg/100µl, 500µg/100µl, 750µg/100µl and 1000µg/100µl concentrations which was then added to the wells and incubated for 24 hrs at 37°C. Erythromycin (0.05%) was used as positive control and sterile water as negative control. The active extracts had zones of inhibition which were measured to indicate the degree of sensitivity (Akinnibosun, 2009).

Phytochemical analysis: The methanol and ethyl acetate extracts was qualitatively tested for the presence of chemical constituents, by using Tiwari *et al.,* (2011) protocol.

RESULTS AND DISCUSSION

Antibacterial Activity: In disc diffusion methanolic leaf extract of *D. discolor* showed maximum inhibitory activity against *B. cereus* and *E. coli* when compared to that of ethyl acetate extract (Figure-1). The results of well diffusion method for methanol extract (Figure-2) showed highest inhibitory activity against *E. coli* and *S. aureus* at 1000µg/100µl concentration where as the ethyl acetate extract (Figure-3) showed maximum inhibitory activity against *E. coli* and *B. cereus* at the same concentration. The antimicrobial traits of the plant extract are attributed to the secondary metabolites synthesized by the plant. The components with phenolic structures were highly active against the microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents depending upon the concentration used (Nagesh, 2009).

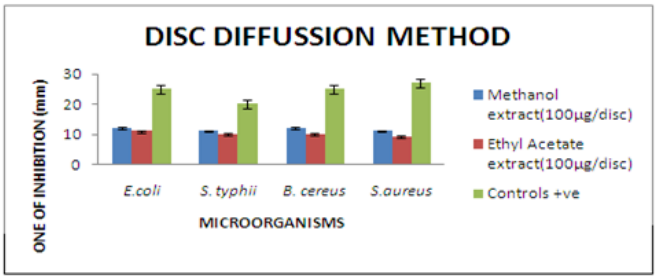

Figure 1: Comparison of zone of inhibitions (mm) of *E. coli, S. typhii, B. cereus* and *S. aureus* against methanol and ethyl acetate extracts of leaves of *D. discolor*

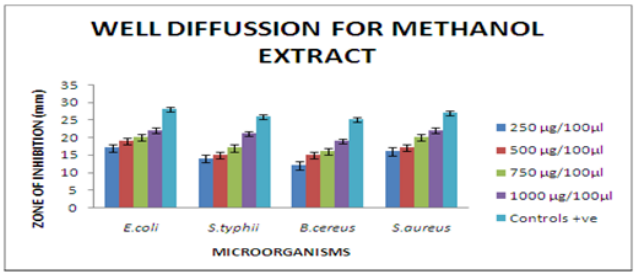

Figure 2: Comparison of zone of inhibitions (mm) of *E.coli, S.typhii, B.cereus* and *S.aureus* against each concentration of methanol extract of leaves of *D. discolor*

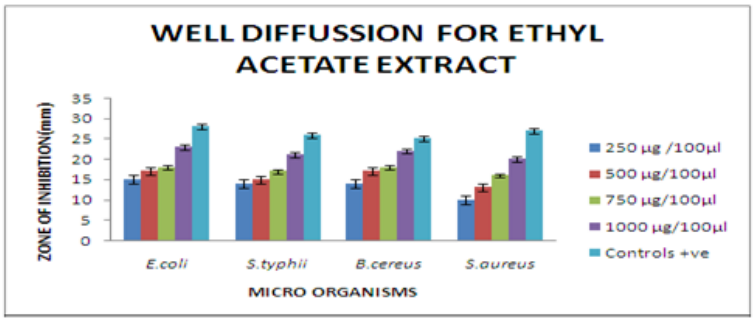

Figure 3: Comparison of zone of inhibitions (mm) of *E.coli, S.typhii, B.cereus* and *S.aureus* against each concentration of ethyl acetate extract of leaves of *D. discolor*

Phytochemical analysis: Preliminary phytochemical analysis (Table-1) was carried out for the methanolic and ethyl acetate extract of *D. discolor*. The methanolic extract showed the presence of alkaloids, tannins and flavanoids, whereas the ethyl acetate extract showed absence of alkaloids. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites

of plants that exhibits antibacterial activity (Shihabudeen, 2010). Tannins bind to proline rich proteins and interfere with the protein synthesis of microorganism (Shimada, 2006). Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999). Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Raquel, 2006).

Table.1.Phytochemical chemical screening of leaf extract of *D. discolor*

Phytochemicals	Methanol Extract	Ethyl Acetate Extract
Carbohydrate	--	--
Tannins	++	++
Flavanoids	++	++
Sapponins	--	--
Alkaloids	++	--
Glycosides	--	--
Phytosterols	++	--
Diterpenes	++	++
Proteins and Amino acids	++	++
Phenols	++	++

CONCLUSION

Alkaloids, tannins, phytosterols, flavonoid, diterpenes and phenols were the secondary metabolites present in leaf extracts of *D. discolor*. The methanol extract showed the presence of higher number of phytochemicals due to its highly polar nature. The phytochemical screening resulted in positive findings of the plant secondary metabolites which could serve as a bioactive compounds against the bacterial strains. This study has shown that the extracts of *D. discolor* have *in-vitro* anti bacterial activities and has also given information which could lead to further research in the area of isolation and characterization of active chemical compounds present in the plant.

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