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IN VITRO ASSESSMENT OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF GROUNDNUT LEAF EXTRACTS

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Abstract

The antibacterial activity of both ethanol and methanol leaf extracts of groundnut against gram-positive and negative bacterial strains was evaluated through broth dilution and agar diffusion methods. Strong antibacterial activity was observed in a concentration dependent manner. The prepared leaf extracts showed maximum zone of inhibition and minimum inhibitory concentration against gram-negative bacteria *E. coli*. In contrast, greater inhibitory concentration and less antimicrobial activity were noticed in gram-positive bacteria *B. subtilis*. Tested leaf extracts also exhibited antioxidant activity which was determined by the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay method. In conclusion groundnut extracts investigated in this study demonstrated both antibacterial and antioxidant activities that warrant further study to identify the biologically active chemical constituents which are responsible for their activities.

Keywords: Antibacterial, Antioxidant, Disc Diffusion Method, DPPH Free Radical.

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INTRODUCTION

The situation of increasing resistance towards antibiotics in microbial organisms urges the necessity of finding novel effective plant constituents against pathogenic bacteria or virus [1]. Generally resistance develops due to long term exposure or misuse of the drug [2]. Mutations in the microbial genome and genes that assist survival also one of the reason for drug resistance [3]. Research on biologically active compounds from plant sources have always been of an area of great interest to researchers those who are working on infectious diseases. At present large number of researchers are working on natural products worldwide specifically to combat new diseases [4]. In this process, antimicrobial screening of plant extracts represent a beginning for drug discovery [5-7]. Most of the living organisms possess the defense systems to protect against free radical damage but these systems are not enough to prevent damage. It is well-known fact that the antioxidants are employed to form a cooperative defense system for normalizing the cell status and because of this reason only natural antioxidants are preferred in food applications. The existence of antioxidants such as phenolics, tannins, flavonoids etc. in plant cells may provide protection against a number of diseases [8]. Every plant has its own medicinal value, but scientists are giving paramount importance to certain species due to their antimicrobial as well antioxidant properties. In extent the demand for natural antioxidants is increasing day by day due to less side effects [9]. Very few studies were conducted on chemical constituents of groundnut and mainly focused on the dietary valuables of leaf extracts. In previous study, groundnut leaf extracts contains 42.2% carbohydrate, 18.0% protein, 8.8% fat, 25.4% ash, 4.6% moisture and 0.8% fiber on a dry basis [10]. However, lack of adequate knowledge on biologically active compounds present in groundnut encouraged us to screen the antibacterial and antioxidant activities of these leaf extracts.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

In the present study, local variety of groundnut was selected for evaluation of antibacterial and antioxidant activities.

PREPARATION OF CRUDE EXTRACT

The fresh leaves of groundnut were washed under the running tap water, shade-dried for 5 days and oven-dried at 55°C for 24 hrs. Later leaf sample was ground to a fine powder using an electrical mixer. 5 grams of leaf powder was suspended in 40 ml of solvent and kept on a rotatory

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shaker for continuous agitation for 24 hrs. Both ethanol and methanol extracts were filtered using whatman no.1 filter paper and the filtrate was dried at ambient temperature in a fume hood in dark until the remnants of solvent was evaporated.

DETERMINATION OF TOTAL PHENOLIC CONTENTS IN THE PLANT EXTRACTS

The total phenolic content present in the plant extracts was determined by regular spectroscopic method [11]. For this experiment, reaction mixture was prepared by adding 150 µl of crude extract (1 mg/ml) followed by 500 µl of 10% Folin-Ciocalteu's reagent and 400 µl of 7.5% Na_2CO_3. Blank sample was prepared without adding plant extract and used as control. Later the samples were incubated at 45°C for 30 min and later absorbance was measured at 735nm. All the samples were prepared in triplicate for each analysis and the mean value of absorbance was recorded regularly. The same procedure was repeated to prepare gallic acid standard solution and the calibration line was constructed. The concentration of phenolics was read (mg/ml) from the calibration line and the phenolic contents in extracts was expressed in terms of gallic acid equivalent (mg of GA/gm of extract).

ANTIBACTERIAL ACTIVITY

Agar well diffusion method [12] was used to evaluate the antibacterial activities of plant extracts. A stock solution of plant extracts was prepared and diluted to working concentrations of 0.5, 1.0 and 1.5 mg/ml respectively with dimethyl sulfoxide (DMSO). Fresh culture was used to prepare lawn on nutrient agar plates. Prepared extracts were loaded into the well on the swabbed plates and incubated at 37°C for 48 hrs. After the incubation, the zone of inhibition was measured in mm and compared with the standard antibiotic. Simultaneously minimum inhibitory concentration (MIC) was also determined by the broth dilution method using nutrient agar broth (NAB). A stock solution (10 mg/ml) of the extracts was prepared in DMSO and the dilutions of the stock solution containing 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml were prepared in NAB. 10 ml of each dilution was taken into the respective test tubes and 10 ml NAB used as control in the separate test tube. Loop full of broth culture was inoculated into tubes. Streptomycin was used as standard reference drug (100 µg). All the test tubes were incubated at 37°C for 18-24 hrs. The lowest dilutions that showed no growth were termed as minimum inhibitory activity.

ANTIOXIDANT ACTIVITY

The antioxidant activity of the extract was determined by DPPH assay [13]. DPPH solution was prepared by dissolving 2.5 mg in 100 ml 80% methanol. Reaction mixer was prepared by adding 200 µl of each extract (100-1000 µg/mL) to 3.8 ml DPPH solution and incubated in the dark at

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room temperature for 1 h. The absorbance of the mixture was then measured at 517nm. Ascorbic acid (vitamin C) was used as a positive control. All the experiments were performed in triplicate and the percentage of inhibition was calculated by the following equation.

Percentage of DPPH scavenging effect= $\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$

The antibacterial activities of both ethanol and methanol plant extracts was measured through zone of growth inhibition in mm. Similarly antioxidant activity was displayed as IC_{50}. In the present study experiments were conducted thrice and all the statistical work has been done using Excel program in personal computer.

RESULTS AND DISCUSSION

TOTAL PHENOLICS

The total phenolic contents in the selected plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: 0.0096x + 0.0957, r^2 = 0.9894). The values obtained for the concentration of total phenols are expressed as mg of GA/gm of extract. Considerably less content of phenols present in both the examined plant extracts. Ethanol extract contained 25.2±0.607 mg of GA/gm and methanol extract contained 23.5±0.721 mg of GA/gm. Data obtained in the average of the three replicates along with standard deviation.

ANTIBACTERIAL ACTIVITY

The antibacterial potential of both the leaf extracts were evaluated according to their zone of inhibition against gram positive and negative bacterial strains, *B. subtilis* and *E. coli* respectively and the results were compared with the activity of the standards. The results revealed that both the extracts have potent antibacterial activity against *E.coli* and showed minimum activity on *B. subtilis*. The results for the agar well diffusion were represented in Table-1. Ethanol and methanol extracts showed better MIC value of 0.5-1.0 mg/ml against *E.coli* where *B. subtilis* was less effective (Fig.1-4). Similar antibacterial tests were conducted by several researchers using different plants species [5,6].

Table 1: Antibacterial activity of ethanol and methanol leaf extracts against *E.coli* and *B. subtilis* (zone of inhibition in mm)

Name of the strain	Standard	Ethanol extract			Methanol extract		
	Streptomycin 0.1 mg	0.5mg	1.0mg	1.5mg	0.5mg	1.0mg	1.5mg
E.coli	19mm	9mm	14mm	17mm	8mm	14mm	18mm
B. subtilis	17mm	4mm	7mm	9mm	2mm	3mm	5mm

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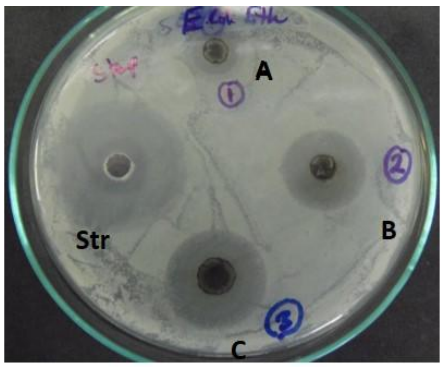

Fig. 1: *E. coli* treated with ethanol extract

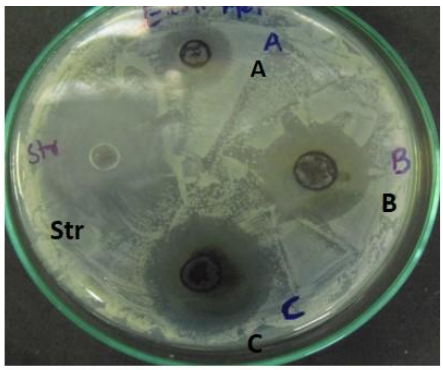

Fig. 2: *E. coli* treated with methanol extract

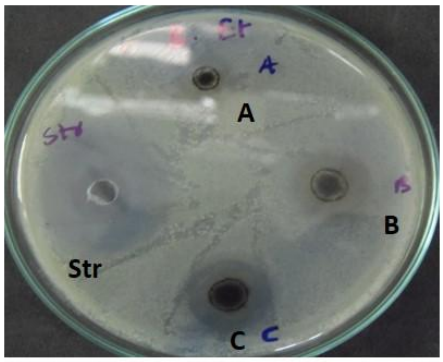

Fig. 3: *B. subtilis* treated with ethanol extract

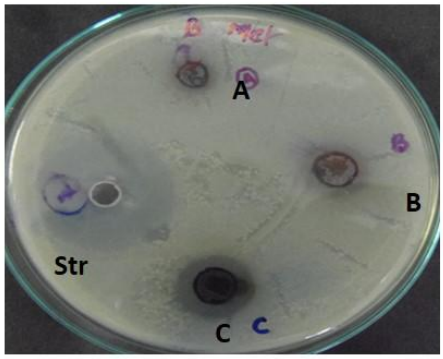

Fig. 4: *B. subtilis* treated with methanol extract

Fig.1-4: Antibacterial activity of groundnut leaf extracts. Str- Streptomycin (0.1mg/ml), A- plant extract (0.5mg/ml), B- plant extract (1.0mg/ml), C- plant extract (1.5mg/ml).

ANTIOXIDANT ACTIVITY

The experimental leaf extracts showed concentration dependent scavenging activity of DPPH radicals (Fig. 5). Antioxidant activity was expressed as IC_{50}, which was defined as the concentration of antioxidant desirable to trap 50% of DPPH. The IC_{50} of the ethanol extract was 0.55 mg/ml and methanol extract was 0.62 mg/ml, where the ascorbic acid, a well-known antioxidant was 0.31 mg/ml. So it is must to check the antioxidant levels in our food stuff for health safety [8].

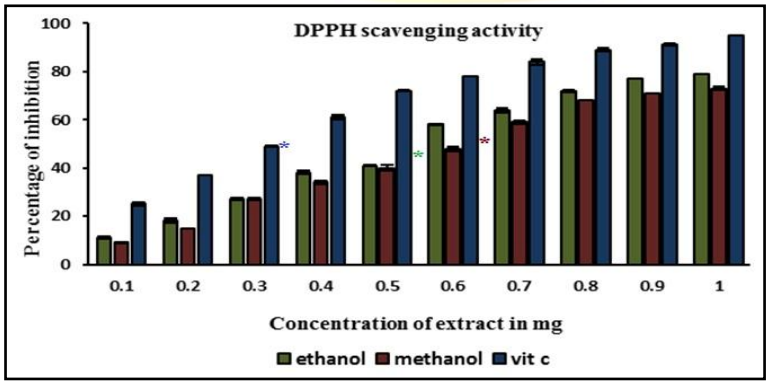

Fig.5: DPPH scavenging activity of groundnut ethanol and methanol leaf extract (* indicates the IC_{50} values for antioxidant activity)

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CONCLUSION

Both ethanol and methanol extracts showed antibacterial activity, which were less than standard antibiotic streptomycin activity. The percentage of antioxidant activity increased in concentration dependent manner. This activity is probably be due to the amount of phenolic compounds present in the plant leaf extracts, which would have acted as electron donors there by reducing free radical generation. The results of the present study indicate that both the leaf extracts of plant retain *in vitro* antibacterial and antioxidant activities. Overall the amount of phenolic compounds may correspond to their biological activity.

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