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Research Article

QUANTIFICATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *PHASEOLUS SEMIERECTUS*

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ABSTRACT

The present study investigates the quantification of antioxidant and antimicrobial activities of the methanolic extract of *Phaseolus semierectus*. The antioxidant capacity was evaluated using established in vitro assays, demonstrating significant free radical scavenging activity. Antimicrobial efficacy was tested against common pathogenic bacteria, including *Staphylococcus aureus* (S.a), *Escherichia coli (*E.c), *Pseudomonas aeruginosa* (P.a), and *Bacillus subtilis* (B.s), employing the disc diffusion method. The extract exhibited notable inhibitory effects on all tested bacterial strains, with pronounced activity against *Staphylococcus aureus* and *Bacillus subtilis.* These findings suggest that the methanolic extract of Phaseolus semierectus possesses considerable antioxidant and broad-spectrum antimicrobial properties, indicating its potential application in the pharmaceutical and food industries.

Keywords: *Phaseolus semierectus*, Methanolic extract, Antioxidant activity, Antimicrobial activity, *Escherichia coli.*

INTRODUCTION

Antioxidants are molecules that have a central part in shielding biological systems against oxidative injury by reactive oxygen species (ROS) and free radicals. They are highly reactive molecules produced as a byproduct of regular cellular metabolism especially in the mitochondria or due to exposure to different environmental factors like pollution, ultraviolet light, and tobacco smoke (Valko *et al*., 2007; Birben *et al*., 2012). Although the roles of ROS play critical physiological roles, such as in cell signaling and immune response, when overproduced or not removed appropriately, they result in a condition termed as oxidative stress (Sies, 2015). Oxidative stress has been suggested to play a role in the pathogenesis of many chronic disorders, such as cardiovascular disease, cancer, diabetes, and neurodegenerative diseases such as Alzheimer's disease, and Parkinson disease (Uttara *et al*., 2009; Lobo *et al*., 2010). The body has an intricate antioxidant defense mechanism that includes enzymatic antioxidants which include superoxide dismutase (SOD), catalase, and glutathione peroxidase and non-enzymatic molecules which include vitamin C and E, carotenoids and polyphenols (Halliwell and Gutteridge, 2015)

Antioxidant-rich foods, including fruits, vegetables, nuts and whole grains, have been linked to decreased oxidative damage and decreased risk of chronic disease through dietary intake (Dauchet *et al*., 2006). Besides the exogenous antioxidants which are acquired in the diet, endogenous antioxidants which are produced in the body are important in the maintenance of cellular homeostasis (Jones, 2006). Recent studies have majored on identifying new antioxidants in natural products and their effectiveness in disease prevention and in disease treatment. Phytochemicals are a group of substances located in abundant amounts in vegetable food including flavonoids, phenolic acids, and tannins which have strong antioxidant and potentially synergistic effects with other substances (Pandey and Rizvi 2009) Additionally, the new developments in the field of analytical methods have now made it possible to look in more depth into the mechanisms by which antioxidants act in their protective effects on a more fundamental level (Apak *et al*., 2016) .Regardless of the increased evidence to support the health advantages of

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antioxidants, there are difficulties in applying the research results into practical dietary and treatment interventions. Research should proceed to better understand the best intake, bioavailability, and interplay of various antioxidants in complicated biological systems.

MATERIALS AND METHODS

1, 1- diphenyl-2-picrylhydrazyl (Sigma Chemical Company, St. Louis, USA), All other chemicals and reagents used were of analytical grade, methanolic extract, 4% dimethyl sulphoxide (DMSO) respectively. Determination of 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. 4 mg of DPPH was dissolved in 100 ml of methanol and kept it overnight in dark place for the generation of DPPH radical (Braca *et al*., 2002). The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*., 2003. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated as

$[(A_0-A_1)/A_0] \times 100$.

where A_0 was the absorbance of the control, and A_1 was the absorbance of the plant extract/ ascorbic acid.

Determination of zone of inhibition by cup plate method

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 5 mm diameter in the agar medium spread with the micro-organisms and 50µl of inoculums (Sukanya *et al*., 2009). These cups were spread on the agar plate by spread plate technique. Accurately measured (50 µl) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

The methanolic extract of *Phaseolus semierectus* demonstrated significant antioxidant potential in various in vitro assays, including DPPH radical scavenging, antioxidant activity. The extract exhibited concentration-dependent scavenging of DPPH, with IC50 values comparable to those of standard antioxidants such as ascorbic acid. The assay further confirmed the reducing power of the extract, indicating the presence of bioactive phytochemicals such as flavonoids, phenolic acids, and tannins. These findings suggest that *Phaseolus semierectus* is a rich source of natural antioxidants, capable of neutralizing free radicals and reducing oxidative stress, which is consistent with previous reports on leguminous plants. The methanolic extract of *Phaseolus semierectus* was found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC_{50} values for DPPH radical of Methanolic extract of was found to be 367 µg. The mean IC_{50} value of ascorbic acid was found to be 80µg (Table 1& 2), (Figure 1).

Table 1. Concentration dependent percent inhibition of DPPH radical by Different extracts of *Phaseolus semierectus* and Ascorbic acid in *In-vitro* studies.

Concentration (µg/mL)	Ascorbic acid (µg)	Methanol extract (µg)
20	27.87 ± 0.87	4.22± 0.88***
40	42.76 ± 1.00	9.55± 0.88***
60	58.32±24	20.44±2.0*
80	73.79 ± 1.26	32.16± 1.00***
100	80.96 ± 1.45	40.28± 0.87***
120	90.61 ± 0.96	48.16± 0.87***

Table 2. *In-vitro* 50% inhibition concentration (IC_{50}) of methanolic extract and Ascorbic acid on DPPH.

Sample taken	IC_{50} value (µg)
Met .OH.ext.	367
Ascorbic acid	80

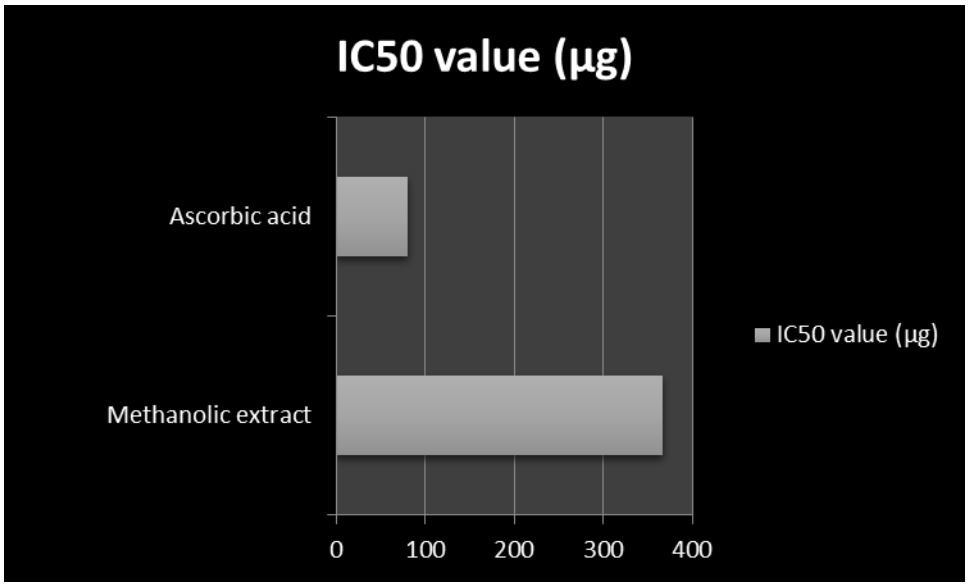

Figure 1. IC_{50} of methanolic extract and Ascorbic acid on DPPH.

The antimicrobial efficacy of the methanolic extract was evaluated against four clinically relevant bacterial strains: *Staphylococcus aureus* (S.a), *Escherichia coli* (E.c), *Pseudomonas aeruginosa* (P.a), and *Bacillus subtilis* (B.s), using the disc diffusion method. The extract showed pronounced inhibitory activity against all tested strains, with the largest zones of inhibition observed for *Staphylococcus aureus* and *Bacillus subtilis*. Moderate activity was noted against Escherichia coli and Pseudomonas aeruginosa. These results indicate a broad-spectrum antimicrobial property, likely attributable to the presence of secondary metabolites such as polyphenols and flavonoids. A sterile borer was used to prepare four cups of 5 mm diameter in the agar medium spread with the micro-organisms and 50µl of inoculum. Methanolic extract of *Phaseolus semierectus* plant at a concentration of 50 µg, 100 µg, 200 and 400 µg per each cup does not exhibit significant antibacterial activity against tested bacterial species (gram +ve and gram –ve) (Table 3).

Table 3. Antibacterial activity of *Phaseolus semierectus* extract.

Treatments	**Dose (µg/cup)**	**Zone of growth inhibition**			
		gram (+) ve		**Gram (-)ve**	
		B.s	***S.a***	***E.c***	***P.a***
Methanolic extract	**50**	07	08	08	09
	100	11	09	09	11
	200	11	11	11	12
	400	12	12	11	13
Rifampicin	**50**	**24**	**22**	**19**	**24**
DMSO		-	-	-	-
Water		-	-	-	-

S.a=Staphylococcus aureaus; *E.c=Escherichia coli*; *P.a=Pseudomonas aeruginosa*; *B.s= bacillus substilis*.

The observed antioxidant and antimicrobial activities of the methanolic extract of *Phaseolus semierectus* underline its potential as a natural source of bioactive compounds for pharmaceutical and food industry applications. The strong antioxidant capacity can be linked to the high content of phenolic and flavonoid compounds, which are known to scavenge free radicals and inhibit oxidative damage. The broad-spectrum antimicrobial activity, particularly against Gram-positive bacteria, suggests utility in combating bacterial pathogens and possibly addressing antibiotic resistance challenges.

CONCLUSION

Phaseolus semierectus methanolic extract showed good antioxidant as well as antimicrobial effects, which confirmed its suitability as a natural source of bioactive compounds. The extract displayed excellent free radical

scavenging ability in DPPH assay, which is a reflection that the extract has phenolic and flavonoid elements lending to its anti-oxidant activity. Moreover, the extract also demonstrated significant antimicrobial activity against Gram-positive and Gram-negative bacterial strains, implying a wide-spectrum antimicrobial activity. These results contribute to the ethnopharmacological topicality of *P. semierectus* and indicate that this plant can be used when creating antioxidant and antimicrobial products of plant origin. Phytochemical profiling and in vivo are further suggested isolating, characterizing and assessing the bioactive constituents that mediate these activities.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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