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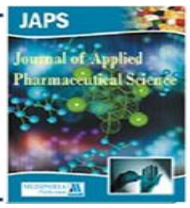

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Antimicrobial, antioxidant and cytotoxic effects of methanolic extracts of leaves and stems of *Glycosmis pentaphylla* (Retz.)

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ABSTRACT

The aim of the present study was to evaluate and compare the antioxidant, antimicrobial and cytotoxic effects of the methanolic extracts of *Glycosmis pentaphylla* stems and leaves. Efforts have been given to identify the activities by using highest grade solvent for preparing the extracts. A survey of the antioxidant effect was performed using DPPH method. The methanolic extract of *Glycosmis pentaphylla* stems showed moderate antioxidant property (103.35 µg/ml) where the leaves showed very little (337.62 µg/ml). This antioxidant activity may be due to some polyphenolic compounds identified from this plant. Both the extracts showed moderate antimicrobial activity with the highest zone of inhibition for *E. coli* (23.67±0.76 mm) and *Salmonella paratyphi* (15.33±0.76 mm) among the tested micro-organisms. This inhibition may legit the traditional implication of the stems of *G. pentaphylla* as toothbrush. The cytotoxic activity of stems extract of *G. pentaphylla* was found to be highly potent (5.53 µg/ml, 95% CI, 7.27-4.21) where as the leaves also showed significant activity (47.34 µg/ml, 95% CI, 50.77-44.15). Several antitumor alkaloids were identified from this plant in the laboratory. The study results also indicate that *Glycosmis pentaphylla* is a good source of medicinally important compounds.

KEYWORDS: *Glycosmis pentaphylla*, Antioxidant activity, Antimicrobial activity, Cytotoxic activity, DPPH.

INTRODUCTION

Medicinal plants possess various medicinal properties; have been serving as the major sources of therapeutic agents for maintenance of human health. These medicinal plants were used by the early human beings, as are done now, in a variety of forms, such as in the entire form, and as powders, pastes, juices, infusions and decoctions for the treatment of their various diseases and ailments. These various converted forms of the medicinal plants may therefore conveniently and genuinely called medicinal preparations or medicaments. This way, the medicinal plants formed an integral part of the health management practices and constituted important items of medicines used in the treatment of diseases from the very early days of human civilization. *Glycosmis pentaphylla* (Retz.) DC belongs to the family Rutaceae. The genus *Glycosmis* of the family Rutaceae is represented by nearly 11 species. *Glycosmis pentaphylla* (Retz.) DC, is a shrub or small (1.5–5.0 m) tree widely distributed from India, Malaysia and Southern China to the Philippine Islands where it occurs in tropical forests at low altitudes (Wang et al., 2006).

It is traditionally used for the treatment of fever, liver complaints and certain other diseases. The stems are widely used as a brush for cleaning the teeth (Quader et al., 1999). Phytochemical researches of this species were mainly focused on hydrophobic alkaloids, including those of the quinolone, quinazoline, acridone and carbazole types of leaves, root and stems bark (Quader et al., 1999).

The objective of this research work was to investigate the antimicrobial, antioxidant and cytotoxic activities of crude methanolic extracts of *Glycosmis pentaphylla* leaves and stems.

PLANT MATERIAL

Glycosmis pentaphylla Retz. DC., syn. *Glycosmis arborea* Roxb. DC. (Rutaceae) a small shrub locally known as 'Bonojbir gass' collected in November 2008 from Kodda, Brahmanbaria, Bangladesh and authenticated at Bangladesh National Herberium, where a voucher specimen has been deposited, Accession Number DACB 33540.

EXTRACTION AND ISOLATION

The air-dried material (1 kg) was finely pulverized and extracted by percolation with methanol (Merck, Germany) for one month at room temperature. The combined extracts were filtered and concentrated under vacuum using rotary evaporator (IKA, Germany) to obtain a crude extract (50g).

MATERIALS AND METHODS

Antimicrobial activity- disc diffusion method

The disc diffusion method (Bauer et al., 1966; Rahman and Rashid, 2008) was used to test antimicrobial activity of the extracts against twelve bacterias collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. Among the test micro-organisms the number of gram negative and gram positive bacteria were eight and four respectively.

Mother solution of sample was prepared by dissolving 8 mg of sample in 200 µl of methanol. The solution was vortexed (Gemmy Inc, Taiwan) for 5 minutes for proper dissolution. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with 10 µl of the test substances using micropipette (Eppendorf, Germany) and the residual solvents were completely evaporated. Discs containing the test materials were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc (Oxoid, UK) of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4^{o}C) for 24 hours to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37^{o}C for 24 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken.

Cytotoxic activity- brine shrimp lethality bioassay

Brine shrimp lethality bioassay (Meyer et al., 1982) technique was applied for the determination of general toxic property of the stems and leaves extracts of *Glycosmis pentaphylla*. Here, *in-vitro* lethality test has been carried out using brine shrimp nauplii eggs i.e. *Artemia salina*. Eggs were placed in one side of a locally fabricated small tank divided by a net containing 3.8% NaCl solution for hatching. In the other side of the tank a light source was placed to attract the nauplii. After 2 days of hatching period the nauplii were ready for the experiment.

4 mg of the complexes was accurately measured and dissolved in Dimethyl sulfoxide (DMSO) to get a solution of varying concentrations 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml. Thirty brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. Vincristine sulphate was used as positive control.

Mortality was observed and recorded after 24 hour of incubation, using a magnifying glass and the number of survivors in each vial were counted and noted. From these data, the percentage of mortality of the nauplii was calculated for each concentration. Mortality was corrected using Abbott's (1925) formula. The mortality data was subjected to Probit analysis according to Finney (1947) and Busvine (1971). The LC_{50} value with its 95% confidence interval was calculated using Microsoft Office Excel 2007.

Antioxidant activity

The antioxidant activity (free radical scavenging activity) of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Brand-Williams (1995). In the experiment, 2.0 mg of each of the extracts were dissolved in methanol. Solution of varying concentrations such as 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml were obtained by serial dilution technique. 2 ml of a methanol solution of the extract of each concentration was mixed with 3 ml of a DPPH-methanol solution (20 µg/ml) and was allowed to stand in the dark for 20 minutes for the reaction to occur. Then the absorbance was determined at 517 nm by UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) and from these values the corresponding percentage of inhibitions were calculated by using the following equation:

$$\% \text{ Inhibition} = [\,1 - (\frac{ABS_{sample}}{ABS_{control}})] \times 100$$

Ascorbic acid, a potential antioxidant, was used as positive control. A blank absorbance was taken using the entire reagent except sample and following the above mentioned procedure. Then %

inhibitions were plotted against respective concentrations used and from the graph IC_{50} was determined.

Presentation of data

The data presented here is expressed as Mean±SD. Probit analysis was used in determining LC_{50} value of methanolic extracts of *Glycosmis pentaphylla* leaves and stems.

RESULT AND DISCUSSION

Anti-microbial study

The methanolic extracts of leaves and stems of *Glycosmis pentaphylla* were screened against 12 test bacterias. Several were found to be insensitive to the extracts. However, the methanolic extract of stems showed significant antimicrobial activity against *E. coli* with the zone of inhibition 23.67 mm (Table 1).

Table 1 Antimicrobial activity of methanolic extracts of *Glycosmis pentaphylla* leaves (GPL), *Glycosmis pentaphylla* stems (GPS) and positive control kanamycin (KM).

Name of Bacteria	GPL	GPS	KM
	Diameter of zone of inhibition Mean (mm) ± SD		
Gram-positive bacteria			
Bacillusmegaterium	7.50±0.50	8.10±0.10	37.27±0.25
Bacillussubtilis	7.23±0.25	7.13±0.12	40.27±0.25
Sarcina lutea	-	-	30.23±0.25
Staphylococus aereus	-	8.50±0.50	34.17±0.29
Gram-negative bacteria			
Escherichia coli	9.00±0.50	23.67±0.76	32.53±1.76
Psedomonas aeruginosa	10.50±0.62	8.17±0.15	30.27±0.31
Salmonella paratyphi	14.50±0.50	15.33±0.76	36.50±1.04
Salmonella typhi	10.83±0.76	8.27±0.31	37.23±0.25
Shigella boydii	7.17±0.15	9.17±0.15	35.50±2.77
Shigella dysenteriae	7.97±0.15	9.27±0.31	42.60±0.52
Vibrio minicus	9.17±0.15	-	37.33±0.31
Vibrio parahemolyticus	-	10.40±0.53	30.10±0.10

The values are expressed as mean of 3 repetitions and standard deviations (SD).
"-" Indicates no zone of inhibition.

Table 2 LC_{50} data of test samples of leaves (GPL) and stems (GPS) of *Glycosmis pentaphylla* and Vincristine sulfate (VS).

Samples	Regression equation	R^2	LC_{50} (µg/ml)	95% Confidence interval	
				Upper limit	Lower limit
GPS	y = 0.4504x + 4.6652	0.9514	5.53	7.27	4.21
GPL	y = 0.7548x + 3.7355	0.9235	47.34	50.77	44.15
VC	y = 1.0221x + 4.54	0.9509	2.81	4.01	1.97

The finding of zone of inhibition was found higher than a study reported by other researchers (Jeeshna et al., 2009), in which the zone of inhibition was found to be 15 & 14 mm, at 50 & 100 µg/disc concentration. As in the current study 400 µg extract per disc was used, the result should be tantamount with the previous study. Local use of stems part of *Glycosmis pentaphylla* as a toothbrush (supplement of toothpaste) seems to be legit and it may be due to its ability to suppress the growth of *E. Coli*, which is one of the five main microorganisms responsible for plaque formation in teeth (Queiroz et al., 2009). Significant inhibition of bacterial growth was also seen in case of *Salmonella paratyphi* which indicates presence of potential antibacterial agents.

Cytotoxic activity study

In case of brine shrimp lethality bioassay, the lethality of the methanolic extracts of the stems and leaves of *Glycosmis pentaphylla* were evaluated against *A. salina*. Table 2 shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC_{50} were found to be 5.51, 47.63 and 2.81 µg/ml for stems, leaves and vincristine sulfate respectively (Table 2). In comparison with vincristine sulphate, the cytotoxicity exhibited by the methanolic extracts of stems of *G. pentaphylla* was highly potent whereas the activity of the methanolic extracts of leaves was significant. Arborinine, an acridone alkaloid obtained from *Glycosmis pentaphylla*, exhibited significant inhibition of crown gall tumors produced by *Agrobacterium tumefaciens* in a potato disc bioassay (Quader et al., 1999). This clearly indicates the presence of potent bioactive principles in these extractives, which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents.

Antioxidant study

Reactive oxygenated species (ROS) *in vivo* include super oxide radical, hydrogen peroxide and hypochlorous acid (Deore et al., 2009). The DPPH antioxidant assay is based on the ability of DPPH to decolorize in the presence of antioxidants. In case of antioxidant screening (Table 3), the methanolic extract of stems part showed the highest antioxidant activity with IC_{50} value of 103.35 µg/ml. At the same time, the methanolic extract of leaves also exhibited moderate antioxidant activity (IC_{50}=337.62 µg/ml), where standard ascorbic acid showed free radical scavenging with the IC_{50} value 22.78 µg/ml. The levels of scavenging effects on DPPH radicals were achieved by the application of the series of different concentrations of methanolic extracts of leaves and stems of *Glycosmis pentaphylla*. Though the antioxidant activity of a plant extracts depends on the type and the polarity of the extracting solvent, the extracting technique, the purity of the active principle, the antioxidant test, the substrate used and the structural requirement (a number of phenolic and hydroxyl groups on ring structures) (Potchoo et al., 2008). These results denote the presence of antioxidant principles in the extractives.

Table 3 IC_{50} values of stems and leaves of *Glycosmis pentaphylla* extractives and ascorbic acid.

Samples	IC_{50} (µg/ml)
Ascorbic Acid	22.78
Methanolic extract of stems of *Glycosmis pentaphylla*	103.35
Methanolic extract of leaves of *Glycosmis pentaphylla*	337.62

CONCLUSION

The methanolic extracts of leaves and stems were found to inhibit several microbial species growth in agar media. Most of the

significant inhibitions were shown to *E. coli* and *Salmonella paratyphi*. The antimicrobial activity could justify its traditional use as toothbrush. The napulii were found to be very much susceptive to the extracts of leaves and stems of *Glycosmis pentaphylla*. This result suggests that, there might be some potent bioactive compounds present which can inhibit cell growth. The stems extracts of *Glycosmis pentaphylla* showed moderate antioxidants property which indicates the presence of poly phenolic compounds.

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