



Pharmacological studies on *Glycosmis pentaphylla* (corr.) whole plant

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Abstract

Glycosmis pentaphylla, a potential medicinal plant is a species of the family Rutaceae, which has been evaluated in folk medicine in Bangladesh for the estimation of several pharmacological activities. In the recent study ethanol extract of whole plant of *Glycosmis pentaphylla* was pharmacologically investigated to survey and assess the antioxidant, analgesic and antibacterial activities to afford a lead, for future new line of investigation. In scavenging assay by DPPH, a stable radical, the extract showed a significant inhibition of scavenging activity of DPPH radical in concentration dependent manner, where IC₅₀ value of the extract was 32 µg/mL which was comparable to the IC₅₀ value of the standard ascorbic acid, 16 µg/mL. In acetic acid-induced writhing test, the extract produced significant ($p < 0.001$) inhibition of writhing reflex (36.54 and 57.70% inhibition of writhing at the doses of 250 and 500 mg/kg body-weight, respectively) as compared to the standard drug diclofenac sodium which was in dose dependent manner. In antibacterial activity test performed by disc diffusion method, the extract showed activity against the bacterial strains including *S. aureus*, *S. dysenteriae*, *S. paratyphi* and *S. Typhi* at the dose of 250 µg/disc and 500µg/disc. The results suggest that the ethanol extract of *G. pentaphylla* could be a potential source of antioxidant, analgesic and antibacterial activity and demands for further pure compound isolation to identify the underlying mechanism.

Key words: *Glycosmis pentaphylla*, Rutaceae, antioxidant, 1,1,-diphenyl-2-picrylhydrazyl (DPPH) assay, writhing test, disc diffusion assay

Introduction

Materials and Methods

The plant *Glycosmis pentaphylla* Corr. belongs to the family Rutaceae. This plant is a small shrub having bitter stems and leaves (1). The plant is indigenous to south-eastern Asia and northeastern Australia. It is found in various states of India including Assam, Arunachal, Meghalaya, Nagaland and Mizoram (2). Six new isoflavone glycosides were isolated from the stems of *G. pentaphylla*, together with four known compounds. The known compounds were identified as 7-hydroxy-4'-methoxyisoflavone 7-O- β -D-apiofuranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (3), coromandelin (4), 4',5'-dihydroxy-6,7-dimethoxyisoflavone 4'-O- β -D-apiofuranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (5), and tectorigenin 7-O- β -D-apiofuranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (6).

This plant is well known as indigenous medicine for the treatment of cough, jaundice, inflammation, rheumatism, anemia and helminthic infestations (7). *Glycosmis pentaphylla* has also been established to possess antioxidant, galactagogue, immune stimulant, larvicidal activity, antipyretic and hepatoprotective activities (8). In folkmedicine, the bark of *Glycosmis pentaphylla* is useful for the management of diabetes and gonorrhoea (9). Paste of leaves is used externally in eczema and other skin affections. Root is beneficial in fever and fruits used in dysentery (1). Whole plant of *Glycosmis pentaphylla* was subjected to numerous pharmacological investigations to determine antioxidant, analgesic and antimicrobial activities upon literature survey and on the basis of traditional uses.

Plant material

The whole plant of *G. pentaphylla*, was collected from the Karamjol region, Sundarbans, Bangladesh. It was collected in September 2012 at the day time. During collection, any type of adulteration was firmly avoided.

The sample was identified and validated by the

experts at Forestry and Wood Technology Discipline (FWT), Khulna University, Khulna, Bangladesh and for further reference a voucher specimen (Accession number- 39570) has been submitted there.

Preparation of plant extract

In order to obtain plant extract, the whole plant was subjected to shade drying and grinded into a coarse powder with the help of a suitable grinder. Then the powder was kept in air tight container unless the extraction was commenced. To perform extraction, cold extraction method was operated in which 250 g of grinded powder was soaked in 800 mL of ethanol in a glass container for ten days and regular shaking and stirring were applied to accelerate extraction process. Filtration having clean, white cotton plug was performed to remove debris from the plant extract.

The residue was again soaked in 450 ml of ethanol for five days to obtain clear solution of the extract. Rotary evaporator was utilized to evaporate ethanol from the extract. After evaporation and drying deep purple gummy crude extract was obtained. The yield was 7.80 % of dried plant material. Extract was preserved in refrigerator at 4 °C for the protection from microbial attack and until experiment was conducted.

Experimental Animals

For the present studies, Swiss-Albino mice of male and female sex (3-4 weeks of age and 20-25 g of average weight) were purchased from International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The mice were stored in well ventilated cages in animal house of pharmacy Discipline, Khulna University, maintaining the temperature of 25 ± 0.5 °C and relative humidity of 56-60 % for adaptation. They were supplied with formulated rodent pellet food and water *ad libitum*. The present investigations in mice were conducted in accordance with the guidelines provided by

Institutional Animal Ethics Committee (10) and ethical guidelines of ICCDR, B.

Test Pathogens

Both Gram-positive and Gram-negative bacteria were collected from ICCDR, B and preserved in Microbiology Laboratory of Pharmacy Discipline, Khulna University. The pathogenic bacteria including *Staphylococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli* and *Shigella dysenteriae* were applied for antimicrobial evaluation.

Chemicals and Reagents

Ascorbic acid, acetic acid, 70 % Ethanol and dimethyl sulfoxide (DMSO) were purchased from Merck, Germany. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Tween-80 and castor oil were purchased from Loba Chemie Pvt Ltd, India all of which were in analytical grade.

Standard Drugs

Diclofenac sodium and loperamide were collected from Beximco Pharmaceuticals Ltd, Bangladesh.

Phytochemical Screening

The ethanol extract of whole plant of *G. pentaphylla* was subjected to numerous preliminary phytochemical tests to identify major phytochemical groups (11,12).

In Vitro Antioxidant Activity

Qualitative Analysis

To determine polar, non-polar and medium polar compounds; TLC plates were developed with solvent systems containing different polarities. The plates were sprayed with ethanol solution of DPPH (0.02%) by spray gun. Presence of strong yellow

spot on a purple background of the TLC plate by the resolved bands was observed for 30 min which led to the quantitative antioxidant activity.

DPPH Scavenging Assay

In quantitative assessment of antioxidant activity, DPPH (a stable free radical) assay was performed to determine scavenging activity of the ethanol extract (13). Serial dilution was carried out for the preparation of different concentrations of sample in ethanol including 256, 128, 64, 32, 16, 8, and 1 µg/mL. From each concentration, 1 mL of sample was mixed with 3 mL ethanol solution of DPPH (0.004%). For the completion of the reaction, it was subjected to an incubation period of 30 min in dark place at room temperature.

UV spectrophotometer was utilized to measure absorbance at 517 nm against blank. Ascorbic acid, standard free radical scavenger was used for the comparison of the activity of the extract. Activity of the sample was calculated using the following formula:

$$\text{Percent inhibition} = \frac{[(\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance}] \times 100}{100}$$

IC₅₀ value was determined from % inhibition versus concentration (µg/mL) graph. Here A_c is the absorbance of control and A_s is the absorbance of standard or sample.

Analgesic Activity

Acetic Acid Induced Writhing Test

Randomly selected mice were divided in four groups (group-I, group-II, group-III and group-IV) each group consisted of five mice. Each group (test groups with 250 and 500 mg/kg ethanol extract, positive control group with standard drug diclofenac sodium 25 mg/kg and control group with 1% tween-80 in distilled water 10 mL/kg in oral route by feeding needle) received a specific treatment. Then 30 minutes was allowed for proper absorption of

the administered agents. After that the pain inducing agent 0.6% v/v acetic acid solution was intraperitoneally administered at a dose of 10 mL/kg to each of the mice in all four groups. After a break of 5 minutes, the number of writhing was counted for each animal in duration of 10 minutes. To evaluate analgesic activity, percent writhing inhibition was likened to control and the standard drug diclofenac sodium.

Antibacterial Activity

Disc Diffusion Assay

Using disc diffusion method (14,15), the antibacterial activity of *G. pentaphylla* extract was evaluated. DMSO was used to prepare desired concentration of the extract. Using micropipette, sterile blank discs (BBL, Cocksville, USA) were impregnated with the test extract at the concentrations of 250 and 500 µg/disc. Then discs were dried. Using sterile forceps, standard antibiotic discs (Ciprofloxacin 5 µg/disc, Oxoid Ltd., UK), dried sample discs, and control discs containing DMSO were applied on nutrient agar medium in Petri dishes seeded with bacteria. After an incubation period of 16 h at 37 °C, zone of inhibition was measured using digital slide calipers from the Petri dishes.

Statistical Analysis

Results are expressed as Mean ± S.E.M. The difference between experimental and control group was determined by Student's t-test. The results were considered statistically significant when $P < 0.001$.

Results

Results of Phytochemical Screening

In phytochemical screening the ethanol extract of *G. pentaphylla* revealed the presence of alkaloids, glycosides, gums, saponins, flavonoids, and terpenoids (Table 1)

Phytochemical groups	Results
Reducing sugars	-
Alkaloids	+
Glycosides	+
Steroids	-
Gums	+
Saponins	+
Flavonoids	+
Tannins	-
Terpenoids	+

+ = Presence - = Absence

Table 1: Phytochemical screening of whole plant of *G. pentaphylla*

Activity in DPPH Scavenging Assay

Depending on the concentration the ethanol extract showed DPPH radical scavenging activity at IC₅₀ value of 32 µg/mL, while standard antioxidant, ascorbic acid showed IC₅₀ value of 16 µg/mL that was highly comparable.

see Figure 1.

Activity in Acetic Acid Induced Writhing Test

The ethanol extract of whole plant of *G. pentaphylla* showed dose relied inhibition of writhing. The extract produced 36.54% and 57.70% writhing inhibition at the doses of 250 and 500 mg/kg-body weight respectively. In contrast, the standard diclofenac sodium exhibited 86.54% inhibition of writhing. Thus the results of statistical analysis were significant.

see Table 2.

Activity in Disc Diffusion Assay

The ethanol extract of whole plant showed antibacterial activity with zone of inhibition ranging from 2 to 6 mm and 3 to 11 mm at the doses of 250 µg/disc and 500 µg/disc against the tested bacterial strains namely *S. aureus*, *S. dysenteriae*, *S. paratyphi* and *S. Typhi* whereas exhibited no activity against *S. pyogenes* and *E. Coli*.

see Table 3.

Discussion

Phytochemical screening was performed to get preliminary idea about the phytochemicals present in the extract which revealed the presence alkaloids, glycosides, gums, saponins, flavonoids and terpenoids.

In vitro antioxidant activity study of the extract using DPPH scavenging assay model the conversion of free radical, DPPH was occurred to stable DPPH-H by accepting electron, or hydrogen radical. As a result the deep violet colour of DPPH is converted to light yellow colour. Among the phytochemicals flavonoids and saponins are responsible for antioxidant properties (16, 17). At 517 nm against blank, DPPH radical containing an odd electron is detected by UV spectrophotometer and due to the development of its non-radical form, DPPH-H, upon reduction with an antioxidant this absorption decreases (18). The extract showed comparable concentration dependent DPPH radical scavenging activity.

From the sensitization of pain receptors by prostaglandins release analgesic activity of the extract was evaluated acetic acid-induced writhing method (19, 20). The active principle responsible for the analgesic activity of extract may be terpenoids, gums and flavonoids (21, 22, 23).

The extract showed potential analgesic activity exhibited by dose dependent inhibition of writhing as compared to control group. The probable mechanism may be the inhibition of prostaglandins (PGE₂ and PGE_{2α}) and bradykinin synthesis or anatomization the action of these substances (24).

In the antibacterial assay using disc diffusion method the extract showed activity against the bacterial strains namely *S. aureus*, *S. dysenteriae*, *S. paratyphi* and *S. typhi*. It showed no activity against *S. pyogenes* and *E. coli*. Among the chemical constituents investigated by phytochemical screening terpenoids, alkaloids and flavonoids (25, 26 and 27) may be responsible for antimicrobial properties.

Conclusion

Potential antioxidant, analgesic, and antibacterial activities were indicated by these results. The investigations were carried out using crude extract of the plant. Further investigations might be conducted using the pure compounds of extract.

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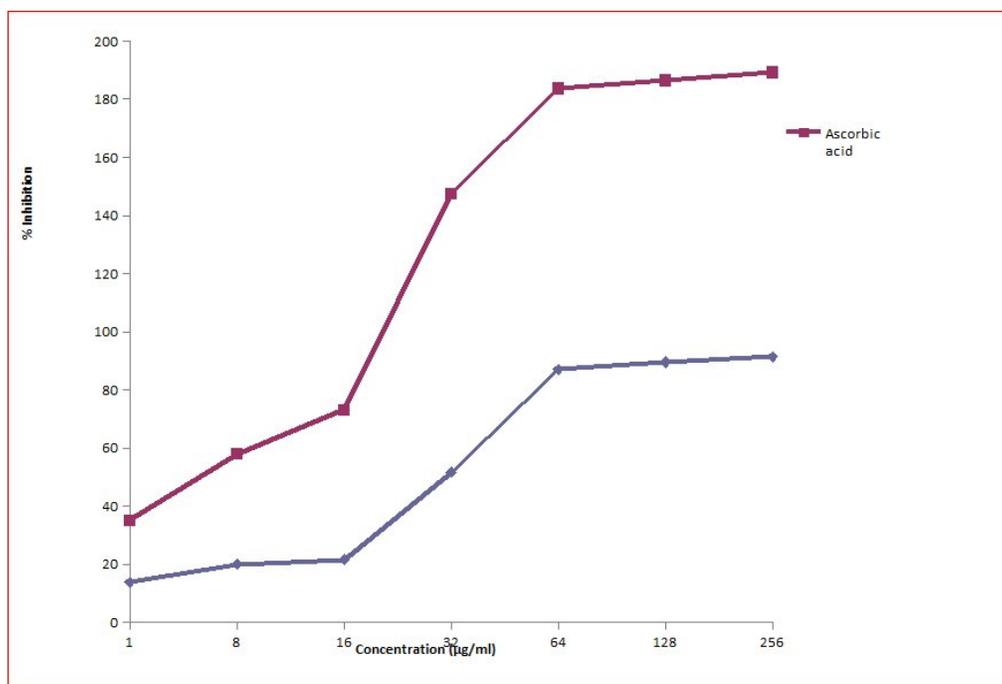


Figure 1: DPPH scavenging activity of whole plant of *G. pentaphylla*

Treatment <i>n</i> = 5	Dose (mg/kg)	No. of writhes	% Inhibition
Control	---	13±1.58	---
Diclofenac sodium	25	1.75±1.11*	86.54
Extract	250	8.25±2.25*	36.54
	500	5.5±1.44*	57.70

Table 2: Effect of whole plant *G. pentaphylla* on acetic acid induced writhing in mice
Results are expressed as mean ± SEM, SEM= Standard error of mean, **P* < 0.001 versus control, Student's *t*-test.

Bacterial strains	Type of bacteria	Diameter of zone of inhibition (mm)			
		Blank	Extract (250 µg/disc)	Extract (500 µg/disc)	Kanamycin (30 µg/disc)
<i>Staphylococcus pyogenes</i>	Gram (+)	-	0	0	32.00
<i>Staphylococcus aureus</i>	Gram (+)	-	6	11	29.00
<i>Salmonella typhi</i>	Gram (-)	-	3	7	31.00
<i>Salmonella paratyphi</i>	Gram (-)	-	2	3	30.00
<i>Escherichia coli</i>	Gram (-)	-	0	0	34.00
<i>Shigella dysenteriae</i>	Gram (-)	-	4	8	29.00

Table 3: Antibacterial activity of whole plant of *G. pentaphylla* in disk diffusion assay