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Original Article

Anti-diarrheal and antioxidant activity of methanolic leaf extract of *Glycosmis pentaphylla* Retz.

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ABSTRACT

The aim of the present study was designed to evaluate the antidiarrheal and antioxidant effects methanolic extract of *Glycosmis pentaphylla*. The methanolic extract of *G. pentaphylla* showed significant reduction in diarrhea frequencies which were 26.99% and 57.67% at doses 200mg/kg and 400mg/kg b.w. respectively compared to loperamide 61.59%. In antioxidant activity, the ranges of total phenolic content of different partitionates were from 0.94 to 3.54 mg of GAE/gm, where the amount of phenolic content decreased in the order PESF (3.54mg)>DCMSF (2.07mg)>AOSF (1.44mg)>MESF (1.33mg)>CSF (0.94mg). Compare to BHT (5.6µg/ml) the IC_{50} value was significant for the PESF (6.82µg/ml), while AQSF, CSF, DCM and MESF were 7.22µg/ml, 8.0µg/ml, 19.88µg/ml and 60.27µg/ml respectively. It is found that the leaf of *G. pentaphylla* showed promising antidiarrheal and antioxidant properties and further study is required to ascertain its use in ethno medicine.

Keyword: *Glycosmis pentaphylla*, Daton, anti-diarrheal activity, antioxidant activity

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INTRODUCTION

For last few decades the uses of herbal medicines have increased significantly in both developing and developed countries due to its less side effects and readily availability [1,2]. To treat numerous diseases these plants are being used by human in a variety of forms, such as entire form, powders, pastes, juices, infusions and decoctions [3]. It has been reported that around 80% of world people mostly depends on traditional and herbal medicine to meet their primary healthcare needs [4].

Glycosmis pentaphylla Retz., an evergreen shrub or small flowering plant under Rutaceae family, is locally known as Daton. It is mainly found in Bangladesh, India, Sri Lanka, Thailand, Southern China and Indo-China, Malaysia, Indonesia and Philippines Islands [5]. Previously some major compounds have already been identified in *G. pentaphylla* for instance flavonoids, coumarins, arborine, alkaloids, terpenoids, amides and imides [6,7,8,9,10,11].

Traditionally different parts of *G. pentaphylla* (Rutaceae) are used to cure boils, chest pain, hook worm infestation, ureterolithiasis, cough, rheumatism, anaemia, bleeding, bone fracture and jaundice [12,13,1]. Some folk practitioners are used the juice and paste of leaves to treat fever, eczema, skin affections, liver complications and other bowel disorder. However, the wood of this plant has also traditionally been used to treat snakebite or to increase appetite for women after childbirth and in some district this plant is used for the prevention of all forms of cancer [14,15].

Diarrheal is a water-borne diseases and it's responsible for the several million deaths in the world annually [16]. Mostly death occur in less than 1 year children, and infants who are not exclusively breast-fed are faces risk can be 2 - 3 times higher [17]. Malnutrition is a major contribution of diarrhea, and also causes rapid dehydration in infant and elderly people, if treatment is not given accordingly than the patient may die [18].

Antioxidants are man-made or natural substances that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Phenolic compounds which may be defined as effective antioxidants that neutralize the free radicals and causes for oxidative stress. During the last 3 decades, the complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have been treated by the antioxidant based drugs and formulation [19,20]. Plants and their secondary metabolites are the important source of new medicinal value & pharmaceutical food additives respectively.

We, here in, investigated the anti-diarrheal activity and antioxidant activity of the methanol extract of *G. pentaphylla* leaves and their petroleum ether (PESF), Dichloromethane (DCMSF), chloroform (CSF) and aqueous (AQSF) fractions are evaluated. The objectives of the study were to investigate the anti-diarrheal activity of methanol extract of leaves of *G. pentaphylla* for the first time; methanol extract and its different organic soluble partitionates were evaluated for antioxidant properties.

MATERIAL AND METHODS

Plant Materials: In the middle of June, 2016 the sample of fresh leaves of *G. pentaphylla* Retz. were collected from Rajshahi, Bangladesh then the leaves were taxonomically identified and authenticated by the National Herbarium of Bangladesh, where a voucher specimen has been deposited in the herbarium with the Accession Number DACB 46513. Before air dried for several days, the leaves were washed properly and cut into small pieces. Then the dried leaves were powdered using high capacity grinding machine in Phytochemical Research Laboratory, Pharmacy Faculty, University of Dhaka.

Extraction of plant material by methanol: Firstly, 250gm powder of leaves of *G. pentaphylla* was soaked in 2.5 L methanol into a fresh 5 Lround bottomed flask and sealed with foil. After that it was kept for a period of 15 days accompanying occasional shaking and stirring. Then the fresh cotton plug followed by Whitman No.1 filters paper was used to filter the crude solution and with the help of Buchii Rota evaporator 10gm concentrate crude extract was obtained. With the help of modified Kupchan method petroleum ether (PESF), dichloromethane (DCMSF) and chloroform (CLSF) soluble fraction were partitioned [21]

Drugs and chemicals: Loperamide, *tert*-butyl-1-hydroxytoluene and normal saline (0.9% NaCl) were collected from Square Pharmaceutical Limited. All other reagents and solvents were analytical grade and received from Phytochemical research lab in State University of Bangladesh.

Animals: Swiss albino mice (25-30g) of either sex, aged 4-5 weeks, were used for anti-diarrheal activity evaluation, collected from the Animal Resource Branch of the International Centre (ICDDR, B). Mice are very sensitive to environmental transformation, so they were kept in the setting for at least 3-4 days ahead of the

experiment and fed ICDDR, B formulated food and remain fasted overnight prior to the experiments.

Antidiarrheal activity: Castor oil induced diarrheal method was used to evaluate the anti-diarrheal activity in mice [22,23]. Before the commencement of our experiments, Swiss albino mice were randomly divided into four groups (A, B, C and D) of 4 mice per group and each group received particular treatment. As negative and positive control group A and B were fed 1% Tween 80 in normal saline (10 ml/kg b.w.) and loperamide (50 mg/kg b.w.), whereas 200 mg/kg b.w. and 400mg/kg b.w. methanolic extract of *G. pentaphylla* were orally administered to group C and D respectively. After one hour of treatment, each animal of all groups received 1ml Castrol oil through feeding needle and kept in observation for 4 hours. The total number of fecal output was counted in every hour and the below formula was applied to calculate the percent of inhibition of defecation in mice:

Antioxidant activity: FC assay: Total phenolic content of *G. pentaphylla* were evaluated as the method used by Skerget *et al*., 2005 [24]. Standard curve was prepared by the Gallic acid solution (100 µg/ml to 0 µg/ml) using 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution. The mixture was incubated for 15 minutes at room temperature and the absorbance of mixture was measured at 760 nm. The total phenols content of the sample was measured and expressed as mg of GAE (Gallic acid equivalent) / gm of the extract.

Antioxidant activity: DPPH assay: The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.,* 1995 [25].Ascorbic acid (ASA) and tert-butyl-1-hydroxytoluene (BHT) both was used as positive control and dissolved in distilled water to make the different concentration (500.0 to 0.977 µg /ml). This solution was mixed with 3.0 ml of DPPH solution (20 µg/ml) and prepared through serial dilution then it kept in dark place at the room temperature for the 30 min. After 30 min the absorbance was measured at 517 nm by UV spectrophotometer against methanol as blank.

Inhibition of free radical DPPH in percent *(I%)* was calculated as follows:

$$\mathbf{(I\%) = (1 - A_{sample}/A_{blank}) \; X \; 100}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

RESULT

Antidiarrheal activity: Diarrhea was induced by the use of Castrol oil. It is well documented that castor oil produces diarrhea due to its most active metabolite, ricinoleic acid by hypersecretory response, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa [26].

Table 01: Effect of methanolic extract of leaves of *Glycosmis pentaphylla* Retz. on castor oil (1ml/mice) induced diarrhea in mice.

Group	Dose	Number of diarrheal faeces (Mean±SEM)	%Reduction of diarrhea
CTL (1% Tween 80 in normal saline)	10ml/kg b.w.	17.00 ±1.15	
STD (Loperamide)	5mg/kg b.w.	2.00±0.88	61.59*
MESF 200	200mg/kg b.w.	4.53±1.76	26.99
MESF 400	400mg/kg b.w.	4.00±1.054	57.67*

All values are expressed as mean ± SEM; n = 4, * $p < 0.05$ indicates significant compared to control.
Table 01 demonstrated that the methanolic extract of leaves of *G. pentaphylla* (400 mg/kg) exhibited statistically significant anti-diarrheal activity with a 57.67% reduction of diarrhea compared to the standard loperamide 61.59%.

Antioxidant activity

FC assay: The methanolic extract soluble fraction (MESF) of *G. pentaphylla* leaves and its different partitions petroleum ether (PESF), Dichloromethane (DCMSF), chloroform (CSF) and aqueous (AQSF) were subjected to total phenolic content determination. The ranges of total phenolic content of different partitionates were from 0.94 to 3.54 mg of GAE/g where in PESF highest amount of phenolic content was present.

DPPS assay: The absorbance of methanolic extract and its different partitions were measured at 517 nm and determined the inhibition concentration at 50% (IC_{50}). Among all, the IC_{50} value was significant for the PESF (6.82µg/ml), while AQSF, CSF, DCM and MESF were 7.22µg/ml, 8.0µg/ml, 19.88µg/ml and 60.27µg/ml respectively compared to standard BHT 5.6µg/ml.

Table 2: Test samples for determination of total phenolic content and IC_{50} values of the standard and partitionates of leaves of *G. pentaphylla* Retz.

Sample code	Name of Test Sample	IC_{50} (µg/ml)	Total phenolic content (mg of GAE / gm of extractives
BHT	*tert*-butyl-1-hydroxytoluene	5.6 ±0.30	-
MESF	Methanolic soluble fraction	60.27±0.73	1.33±0.34
PESF	Petroleum ether soluble fraction	6.82±0.30	3.54±0.26
DCMSF	Dichloromethane soluble fraction	19.88±0.35	2.07±0.52
CSF	Chloroform soluble fraction	8.0±0.58	0.94±0.26
AOSF	Aqueous soluble fraction	7.22±0.40	1.44±0.34

DISCUSSION

Antidiarrheal activity: Castor oil contains the active component ricinoleic acid which is induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhea [27]. Table 1 showed that methanolic extract of leaves of *G. pentaphylla* reduced castor oil induced diarrhea of the test animals at a dose of 400 mg/kg dose and the result was statistically significant ($P<0.05$). Due to the presence of denature proteins forming protein tannates as an antidiarrheal activity of the extract to reduce secretion and more resistant the intestinal mucosa [28]. The properties of antidysenteric and antidiarrheal of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars [29].

Antioxidant activity

FC assay: Plants are main source of the most antioxidant activity metabolites of phenolic compounds. Phenolic compounds have the ability to donate hydrogen or electrons and it is form stable radical intermediates. As shown in table 2, Pet ether, fraction of methanolic extract of *G.pentaphylla* presented the highest amounts of total phenolic (3.54 mg of GAE/g), whereas chloroform soluble fractions presented the minimum amounts of total phenolic (0.94 mg of GAE/g). The total phenolic contents in different fraction of *G. pentaphylla*demonstrated as a rich source of polyphenolic compounds.

DPPH: DPPH test is a direct and reliable method for determining radical scavenging action and DPPH is a stable free radical, to decolorize in the presence of antioxidants. Redox properties play an important role for the antioxidant activity of phenolic compounds and are considered for the adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [30]. Tert-butyl-1-hydroxytoluene (BHT) was considered as a reference antioxidant for the test. All partitions of methanolic extract of *G. pentaphylla* have been furnished in the Table 2 with their IC_{50} values. Pet ether soluble fraction was showed the highest scavenging with an IC_{50} value of 6.82µg/ml as opposed to the IC_{50} value 5.6 µg/ml of BHT. At the same time, chloroform and aqueous fractions both was exhibited moderate antioxidant activity 7.22µg/ml, 8.0µg/ml but dichloromethane and methanolic soluble fraction did not show significant activity.

Conclusion

The crude methanolic leaf extract and its different partitions of *G. pentaphylla* were involved in antidiarrheal & antioxidant activities. From the above study, it is found that the leaf of *G. pentaphylla* showed promising anti diarrheal and antioxidant properties. Further study is required to ascertain its use in ethno medicine.

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