Research Article

Preliminary Phytochemical Estimation and Antioxidant Properties of Phoebe Goalparensis Hutch. - A Medicinal Plant Used by the Tribal Practitioners of Assam in Northeast Region of India

***Merina Narah[1], Moushumi Das[2], Jogen Chandra Kalita[3], Jibon Kotoky[4]**
[1,2,3]Animal Physiology and Biochemistry laboratory, Dept. of Zoology, Gauhati University, Guwahati, Assam, India.
[4]Division of Life Sciences, IASST, Paschim Boragaon, Guwahati, Assam, India.
***Corresponding author's E-mail:** anownarah@gmail.com

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ABSTRACT

Phoebe goalparensis Hutch., belonging to the family- *Lauraceae*, is traditionally used in Assam to treat diseases which are related to cancer. The bark of the plant was collected, cleaned, shade dried and extracted with methanol for 72 hours in an orbital shaker. Preliminary phytochemical estimation of *P. goalparensis* showed the presence of important phytochemicals like flavonoids, phenols, tannins, saponins and steroids. Quantitative estimation of total flavonoids and phenols in methanolic bark extract of *P. goalparensis* was found to be 489±0.70 µg of Quercetin Equivalent (QE)/g extract and 2728.5±5.30 µg of Gallic Acid Equivalent (GAE)/g extract. Scavenging activity revealed maximum 91 % inhibition of free radicals which was similar to that of the activity shown by the highest concentration of ascorbic acid with 92% inhibition. The calculated IC_{50} value was found to be 4.03 µg/ml for methanolic bark extract and 3.79 µg/ml for ascorbic acid. In the estimation of reducing power activity of methanolic bark extract of *P. goalparensis*, it was found that the activity increased with the increasing concentration of the extract. These results give the inference of important phytoconstituents present in the bark and its antioxidant properties which might act as a potent source against diseases.

Keywords: *Phoebe goalparensis* Hutch., phytochemicals, scavenging activity, reducing power.

INTRODUCTION

Phoebe goalparensis Hutch. belongs to the family *Lauraceae*, which is particularly used as a medicinal plant within the tribal practitioners of Assam in North Eastern region of India. It is commonly known as Assam teak, Bonsum, Nikahi etc. in different districts of Assam. Most specifically *P. goalparensis* is used as a timber yielding plant and an ingredient for versatile construction and carpentry. *P. goalparensis* occurs in moist forest and it is abundantly distributed in northeast India in the foothills of eastern Himalaya up to an altitude of 1650 m.[1] The family *lauraceae* is the abode of medicinally important plants namely *Cinnamom zeylanicum, Persia americana, Licaria canella and Phoebe grandis*, which are mostly characterised by their antioxidant, antimicrobial, anti-diabetic and anticancer properties.[2-5]

North eastern region of India, famously known as the cradle of flowering plants is a source to numerous medicinally active plants against dreadful diseases viz. diabetes, diarrhoea, malaria, hypertension, jaundice, gynaecological problems and cancer.[6] Assam, one of the states of biodiversity hotspot regions of the world, is a repository of potent indigenous medicinal plants incorporating *Vinca rosea*, *Artemisin annua, Centella asiatica, Curcuma longa* etc. World Health organisations, has depicted that almost 80 % of the total population of the world firmly depend upon plant derivatives. Interestingly, it has been estimated that people seek medical help from rural traditional practitioners as their first hand treatment against diseases. In case of *P. goalparensis*, local healers particularly amongst the Mising tribes of Assam specifically gathered the stem bark of the plant, minched it into small pieces, mixed with water and topically applied on the affected area for curing skin diseases or lesions.

Phytoconstituents like polyphenols, flavonoids, terpenoids etc. guard the body's immune system through different pathways.[7] Secondary metabolites consistently embolden antioxidant, anti-inflammatory and antitumor activities accelerating the defence mechanism against dreadful diseases. Free radicals and other reactive oxygen species by-products produced in the body due to physiological and biochemical processes are filtered by plant constituents. When required, these phytochemicals escalate antioxidant efficacy by scavenging free radicals in the host organism.

Based on the data generated from traditional practitioners, *P. goalparensis* bark extract was undertaken to investigate the presence of different phytochemicals along with its antioxidant efficacy.

MATERIALS AND METHODS

Plant sample

Plant material (bark of the tree) was collected from the Oyan region of Assam- Arunachal Pradesh border in the month of October in 2012, with the help of local traditional healers. The plant sample was identified by the taxonomist of Botanical survey of India, Shillong NER, India and the herbarium of the identified plant was submitted in the Dept. Of Botany, Gauhati University with the allotted herbarium accession No. 17780. The bark

material was washed carefully without any disturbances to the actual texture and rubbed with clean linen cloth for drying. Later, small pieces were properly dried under shade. After completely drying out the plant material, it was finely ground and the powder material obtained was sealed packed and stored at 4°C for future studies.

Chemicals

Chemicals used in the experiment such as - 1, 1-Diphenyl-β-picryl-hydrazyl (DPPH), Gallic acid, Quercetin and ascorbic acid were purchased from HiMedia (HiMedia Laboratories Pvt, Ltd., Mumbai, India). Other chemicals and reagents used in different experiments were procured from Merck India and Rankem (India).

Plant extract preparation

Powdered bark meterial (100 gm) was soaked in methanol (500 ml) and kept for 72 hours in an orbital shaker for total extraction. Filtration of the extract was processed with Whatman filter paper bearing pore size of 11µm. The filtrate was concentrated in a rotary vacuum evaporator under reduced pressure at 40°C and finally the yield obtained was transferred to a glass vial for longer storage period at 4°C in a refrigerator.

Investigation of preliminary phytochemical constituents

Standard test procedures as described by Trease and Evans, Harborne, Sofowara and Edeoga were followed for phytochemical estimation with slight modifications. Both powdered plant material and extract were taken for the investigation.[8-11] The following test procedures were performed for estimating the phytochemicals present in the bark of *P. goalparensis*.

Steroids

About 0.5 g of extract was dissolved in 3ml of chloroform and filtered. Few drops of conc. H_2SO_4 were carefully added to the filtrate to form a lower layer. A reddish brown colouration at the interface was taken as positive for steroid ring formation.

Tannins

0.5 g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ were added. Formation of brownish green or a blue-black colouration indicated the presence of tannins.

Flavonoids

a) 0.5 g of the powdered bark material was heated with 10 ml ethyl acetate over a steam bath for 3 minutes. This was further filtered and 4 ml of the filtrate was mixed with dilute ammonium solution. Formation of yellow colour was observed indicating a positive inference for flavonoids. b) 200 mg of dried powder material was soaked in 10 ml of ethanol and then filtered. 2 ml of the filtrate was added with a few drops of conc. HCl and some strips of magnesium ribbon. Inference showing pink-tomato red colour indicated the presence of flavonoids.

Alkaloids

200 mg of dry plant powder was extracted with 10 ml of methanol and filtered. A few drops of 1 % conc. HCl was added to 2 ml of the filtrate and steamed in a water bath. Then 1 ml of the filtrate was taken to which 6 drops of Mayer's reagent/Wagner's reagent or Dragendroff's reagent was added. Appearance of creamish precipitate/brownish-red precipitate or orange precipitate indicated the presence of different alkaloids.

Phenols

To 1 ml of the above extracted filtrate, 2 ml of distilled water was added followed by few drops of 10% $FeCl_3$. Formation of greenish colour indicated the presence of phenols.

Cardiac glycosides (Keller–Killani Test)

5 ml of the extracted filtrate was treated with 2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution. This was underlayed with 1 ml of conc. H_2SO_4. A brown ring at the interface indicated a deoxy sugar characteristic of cardenolides.

Saponins

About 200 mg of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously, then observed for a stable persistent froth. Foamy froth appearance assured the presence of saponin.

Determination of Total phenolics content

Total phenolic content in methanolic bark extract of *P. goalparensis* (MBPG) was determined by Folin-Ciocalteau reagent (FCR) method as described by earlier scientists with slight modification.[12] A stock solution of 1mg/ml aliquot was prepared for the estimation. Aliquots containing 0.5 ml of different concentrations (10 µg/ml to 60 µg/ml) of gallic acid as standard were taken. To it 2.5 ml of 10% (1:9 dilutions) FCR was added and incubated for 2 minutes at room temperature. 2 ml of $NaCO_3$ (7.5% w/v) was added to the above mixtures. These were allowed to stand for 30 minutes at room temperature. Later the absorbance was taken at 765 nm. Here, the experiment was done in triplicates and the absorbance was measured in UV spectrophotometer Specord 50 plus analytikjena.

Determination of total flavonoid content by $AlCl_3$ method

$AlCl_3$ method as previously described was used for the determination of total flavonoid content.[13] An aliquot of 1mg/ml stock solution was prepared both for the standard quercetin and methanolic bark extract of *P. goalparensis*. Five different concentrations (20 µg/ml to 100 µg/ml) were taken for the assay. 0.5 ml of sample was taken in each test tube. 2 ml of distilled water (dw) was added to it. After this, 0.15 ml of 5% $NaNO_2$ prepared

in dw was added and incubated for 5 minutes. At the 6th minute 0.15 ml of 10% $AlCl_3$ was added, which was followed by the addition of 1 ml of 1 M NaOH. Later the volume was made upto 5 ml by the addition of 1.2 ml of dw. The experiment was done in triplicates and the absorbance was taken at 510 nm in UV spectrophotometer Specord 50 plus analytikjena.

Determination of antioxidant property by DPPH scavenging activity

Scavenging assay was performed by the procedure as described by earlier workers or scientists.[14-18] An aliquot of 1mg/ml was prepared in methanol as stock solution both for the sample and the standard and allowed to dissolve completely for DPPH scavenging assay. Five different concentrations (2 µg/ml to 30 µg/ml) of MBPG were considered for the assay. Ascorbic acid was taken as the standard. 2 ml of the sample solution was taken in each test tube and an equal volume of DPPH (0.1mM) was added to keep the ratio, sample: DPPH, in 1:1 The reaction was allowed to stand for 30 minutes in the dark, completion of which signified the end point of the reaction and the absorbance was taken at 517 nm in UV-VIS spectrophotometer (Specord 50 plus analytikjena UV-VIS). All the readings were done in triplicates. Scavenging activity was calculated using the following formula:

$[(A_s - A_i)/ A_s] \times 100$ (Where, A_s = absorbance of DPPH alone and A_i = absorbance of DPPH in presence of the plant extract)

Reducing power assay

Reducing power assay was performed by the methods as described by Oyaizu, Yildirim and Shahriar.[19-21] Eight different concentrations (0, 10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 60 µg/ml and 70 µg/ml) were taken for the assay. 1mg/ml aliquot of stock solution was prepared. 1 ml of test samples were taken, to which 2.5 ml of 0.2 M phosphate buffer (pH=6.6) was mixed. Later, 2.5 ml of potassium ferricyanide was added. These mixtures were then incubated for 20 min at 50°C. After incubation, samples were allowed to cool for some minutes and 2.5 ml of trichloroacetic acid was added and centrifuged at 3000 rpm for 30 minutes. 2.5 ml of the supernatant solution was collected and then mixed with 2.5 ml of distilled water. Lastly, 0.5 ml of $FeCl_3$ was added to all the test samples. After proper mixing, the absorbance taken in triplicates was measured at 700 nm in UV-VIS spectrophotometer (Specord 50 plus analytikjena). Increased reducing power of the extract was measured based on increasing absorbance of the reaction mixture.

RESULTS AND DISCUSSION

Preliminary phytochemical estimation of methanolic bark extract of *Phoebe goalparensis* showed the presence of important phytoconstituents in table 1, such as flavonoids, phenols, steroids, tannins and saponins. Presence of phenolic compounds deciphered antioxidant properties which execute crucial role in maintaining normal health requirements. It can diminish free radicals that are sometimes being produced in the body due to certain metabolic activities.[22] Most of the compounds that act as secondary metabolites against dreadful diseases fall in the groups of polyphenols. Both flavonoids and polyphenols act as scavengers for free radicals. It was found that quantitatively 2728.5±5.30 µg of GAE/g extract of phenols and 489±0.70 µg of QE/g extract of flavonoids are present in MBPG (Table.2).

Table 1: Presence of phytochemicals in the bark of *Phoebe goalparensis* Hutch.

Phytochemicals	*Inference*
Steroids	+ +
Tannins	+ +
Flavonoids	+ +
Alkaloids	-
Polyphenols	+ +
Cardiac Glycosides	-
Saponin	+ +

+ + = appreciable amount, - = completely absent

Table 2: Total amount of phenol and flavonoid present in *P. goalparensis*

Sample name	Total phenol	Total flavonoid
MBPG (Methanolic bark extract of *P. goalparensis*)	2728.5±5.30 µg of GAE/g extract	489±0.70 µg of QE/g extract

DPPH free radical scavenging activity showed that MBPG could mimic the efficacy generated by ascorbic acid. It was found that ascorbic acid at concentrations 2 µg/ml, 5 µg/ml and 10 µg/ml could inhibit the free radical of DPPH at a percentage of 16%, 23% and 41% respectively (Fig.1). Approximately similar inhibitory percentage was also visible in case of MBPG which exhibited scavenging activity of 18%, 21% and 39 %. At 20 µg/ml and 30 µg/ml concentrations MBPG inhibitory percentage upto 72 % and 91 % whereas ascorbic acid showed 92% inhibition in the above mentioned concentrations. IC_{50} (inhibitory concentration at which 50 % scavenging occurs) values were 4.03 µg/ml and 3.79 µg/ml for MBPG and ascorbic acid respectively (Table 3). DPPH is a stable free radical characterized by the presence of delocalized spare electrons, which on mixing with a substance that can donate a hydrogen atom, gives rise to the reduced form with the loss of violet colour. It means that MBPG acts as a free radical scavengers or hydrogen donors evaluating its antioxidant properties.[23]

Reducing power capacity is one of the important assays for estimating antioxidant capacity of a medicinal plant.[24] Different concentrations of MBPG i.e. 0, 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 60 µg/ml and 70 µg/ml exhibited reducing power activity consequently inhibiting the affect of reduction potential of ferric ions. In reducing power assay of the extract it has been found

that at lower concentrations 10 µg/ml, 20 µg/ml and 30 µg/ml of MBPG showed more potent activity than the concentrations of the reference. However, higher concentrations of ascorbic acid (50 µg/ml to 70 µg/ml) produced more activity than MBPG (Fig. 2).

Based on the above *in vitro* antioxidant assays- DPPH scavenging assay and reducing power assay, it could be denoted that the methanolic bark extract of *P. goalparensis* has significantly shown its scavenging activity and reductive capacity incorporating the efficacy of the reference ascorbic acid.

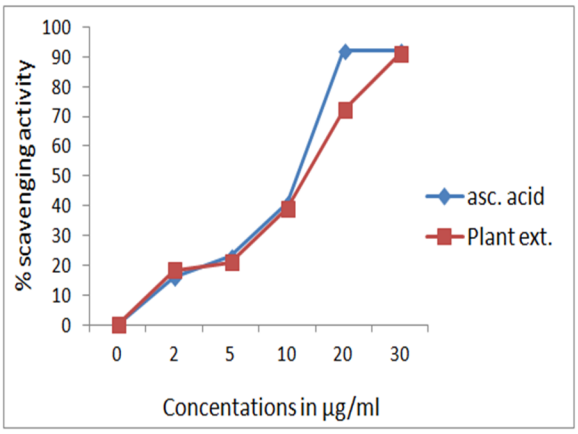

Figure 1: DPPH scavenging activity of methanolic bark extract

Table 3: IC_{50} values of *P. goalparensis* and ascorbic acid in DPPH assay

Sample	IC_{50} values DPPH (µg/ml)
MBPG	4.03 µg/ml
Ascorbic acid	3.79 µg/ml

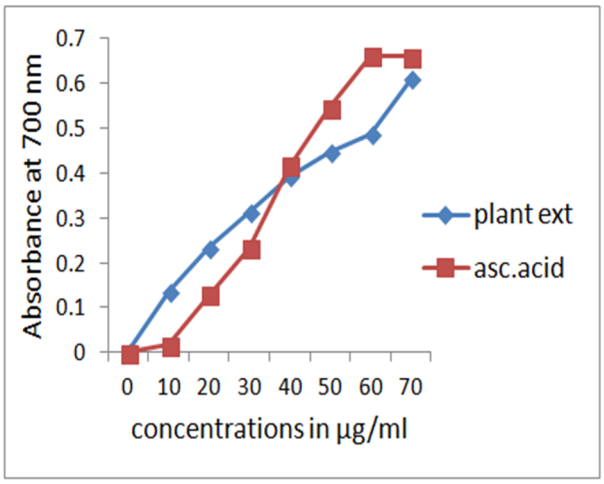

Figure 2: Reducing power activity of *P.goalparensis* and asc. acid at different concentrations

CONCLUSION

Phoebe goalparensis Hutch. used as a medicinal plant by the tribal practitioners of Assam has shown the presence of phytochemicals performing crucial role in scavenging and removing free radicals. As mentioned earlier, possession of flavonoids and polyphenols in the methanolic bark extract of the plant could be a defined data regarding its high antioxidant activities despite being a timber yielding plant. In accordance to these respective activities, further experiment has been processed out to elucidate the proper vitality of *P. goalparensis* for future health care perspectives.

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