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Phytochemical Screening, Estimation of Total Phenol and Flavonoid Content of the Leaves of *Smilax perfoliata* Lour.

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ABSTRACT

Plants are always part of traditional practice and have been a source of medicine since time immemorial. Based on such documents, researchers study their potential as medicine applying scientific knowledge. Such an effort was made to find out the possibility of biological activity through detecting the presence of phenols and flavonoids in the leaves of the plant Smilax *perfoliata* Lour. (Smilacaceae). The objective of the present study was phytochemical screening and quantitative estimation of total phenol and flavonoid contents in the leaf extracts of *Smilax perfoliata* Lour. using methnol and ethylacetate as menstrums. Standard biochemical and spectrophotometric methods were employed to study the phytochemical contents of the leaves of *Smilax perfoliata* Lour. Total phenol and flavonoid content of the extracts were spectrophotometrically determind. Phytochemical analysis of leaves of *Smilax perfoliata* Lour. showed the presence of Tannins, Flavonoids, Glycosides, Steroids, Alkaloids. Total phenol and flavonoid contents were found to be 38± 0.09 μgml^{-1} and 56 ± 0.24μgml^{-1}. Present study showed the presence of subtantial amount of phenolic and flavonoid components in the leaves of *Smilax perfoliata* Lour., which can be considered as good source for medicinal and food application.

Key words: *Smilax perfoliata,* leaves, phenol, flavonoid, Assam

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INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction which transfers electrons or hydrogen from a substance to an oxidising agent and produce free radicals. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer; coronary heart disease and even altitude sickness. Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline. Among the phytochemicals, flavonoids and other phenolic compounds are potential antioxidants [1, 2].

Medicinal plants are abundantly found in North-east India. Some of these are used not just for the treatment of specific diseases, but also for maintaining general health. There are several reports of such traditional medicinal uses of plants [3-6], phytochemical screening [7-8] and estimation of total phenol and flavonoid contents [8-10]. These types of plants have attracted much attention of researchers for their potential antioxidant activities [11]. The objectives of the present study were to determine the total phenol and flavonoid content and phytochemical screening of the plant *Smilax perfoliata* Lour. (Smilacaceae), used as vegetable by the local people of Dibrugarh district of Assam, India.

The genus *Smilax* L. (Smilacaceae) consists of more than 300 species, distributed all over the world, out of which 24 are found in India [12]. Four species viz. *Smilax aspera* Linn., *Smilax perfoliata* Lour., *Smila xwightii* A. DC. and *Smilax zeylanica* L. (Smilacaceae) occur in the forests and hills of South India [12-13].

MATERIALS AND METHODS

Collection and identification of Plant

The plant was collected and herbarium was prepared with conventional herbarium technique (No. BTMK/HKS/C-0162) and preserved. The plant was authenticated by Botanical Survey of India, Eastern Circle Shillong, Meghalaya. Leaves of *Smilax perfoliata* Lour. were collected from Dibrugarh district, washed and air dried at room temperature.

Chemicals

The chemicals used in this study were of analytical grade, procured commercially and used without testing and purification.

Sample preparation

Leaves were taken, thoroughly washed and air dried under shade for about 10-15 days separately. Dried leaves were ground with a pestle and mortar to obtain coarse powder and kept it in air tight container for one hour. The Plant materials were kept away from sunlight to avoid chemical reaction from ultra violet radiation .The coarse powder of plant was then

subjected to cold maceration and successive solvent extraction such as petroleum ether and methanol in Soxhlet apparatus respectively. The extracts were concentrated under reduced pressure with the help of a rotary evaporator and were filtered through Whatman No. 1 filter paper. Each extract was prepared just before the analysis for prevention of any degradation.

Phytochemical analysis

The crude extract was subjected to preliminary phytochemical screening for the detection of major chemical groups namely Carbohydrates, Protein, Amino acid , Steroids, Alkaloids, Glycosides, Flavonoids, Tannins and phenolic compounds according to standard methods [14-16].

Determination of total flavonoids (TFC)

The flavonoid content was determined by Aluminium-chloride method [9, 16]. The calibration curve was prepared by preparing querecetin solutions at different concentrations (20.0 to 100.0 $mg.l^{-1}$). A 1.0 ml aliquot of reaction mixture (3.0 ml) comprised of 1.0 ml of extracts, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml of potassium acetate (120.0 mM) is incubated at room temperature for 30 minutes. Absorbance of the mixture was determined at 510 nm versus the prepared water as blank. The value was calculated from the relationship obtained from the standard curve ($y = 0.0054x – 0.0434$; $R^2 = 0.9964$). The amount of flavonoids in the plant extracts were expressed in mg querecetin equivalents per g dry weight of the plant extract.

Determination of total phenols (TPC)

Total phenolic content in the methanolic extracts of leaves was determined by colorimetric assay using Folin-Ciocalteu reagent [10, 13]. Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent (FCR) in alkaline medium to produce a blue coloured complex known as Molybdenum-blue complex. To 1.5 ml of a dilute extract of plant, 5.0 ml of 10% diluted FCR and 4.0 ml of aqueous sodium carbonate (7.5%) were added and the resulting mixture was allowed to stand for 30 minutes. The total phenolic content was determined using a UV-Visible spectrophotometer at 760 nm. The value was calculated from the relationship obtained from the standard curve ($y = 0.3896x – 0.3183$; $R^2 = 0.9931$). The average of triplicate measurements was used to calculate the phenolic content as mg Gallic acid equivalents (GAE) per g dry weight of the plant extract.

Statistical Analysis

All analyses were performed in triplicate; data were recorded as means ± standard deviations (SD). Correlation coefficients (R) to determine the relationship between two variables (between radical scavenging activities and TPC and TFC) were calculated using MS Excel Software.

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RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening of the methanol extracts of *Smilax perfoilata* Lour. are enlisted in Table 1. The result exhibited the presence of Carbohydrate, Reducing Sugar, Steroides, alkaloids, Glycosides, Flavonoides, Tannins and Phenolic compounds.

Table 1: Result of phytochemical screening of the methanolic leaves extract of Smilax perfoilata Lour.

Phytochemical parameters	Present (+); Absent (-)
Carbohydrates	+
Protein	–
Amino acid	–
Steroids	+
Alkaloids	+
Glycosides	+
Fllavonoids	+
Tannins	+
Phenolic compounds	+
Reducing Sugar	+
Non reducing Sugar	–

Total Flavonoid and Phenolic contents

The results for estimation of Total Phenols (TPC) and Total Flavonoids (TFC) are presented in Table 2. Total phenolic content was calculated by using the standard curve of Gallic acid on the other hand, flavonoid content was calculated taking Querectin as standard. The co-relation coefficient (R^2) in both the calibration was found to be 0.9964 and 0.9931 exhibiting linearity in both the cases. Among the two extracts, methanolic extract showed higher value in comparison to ethylacetate extract. The difference in the phenolic and flavonoid contents was due to the difference in the polarity of the menstrums used for extraction.

Table 2: Total phenol and Flavonoid contents of the leaves extracts of Smilax perfoilata Lour.

Parameters	Methanolic extract (mean ± SD; n=3)	Ethylacetate extract (mean ± SD; n=3)
Phenolic content (Gallic Acid equivalent: $mg.g^{-1}$ of extract)	13.8± 0.09	3.5±0.05
Flavonoid content (Quercetin equivalent: $mg.g^{-1}$ of extract)	15.6± 0.24	2.5±0.12

CONCLUSION

The results of phytochemical screening demonstrated the presence of phenolic and flavonoid compounds along with other phytoconstituents in the leaf extracts of *S. perfoliata*

Lour. The methanol extracts of the leaves were found to be rich in phenolic and flavonoid compounds than that of ethylacetate extract. This can further be evaluated for their antioxidant properties with *in vitro* and *in vivo* models.

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