PHYTOCHEMICAL SCREENING AND DPPH FREE RADICAL SCAVENGING ACTIVITY OF *CENTELLA ASIATICA (L)*

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Abstract: *The present study was aimed at investigating the chemical profiles of the methanolic extract of leaves of Centella asiatica as well as to assess its antioxidative activity. In this study, a methanolic extract (50 g/L) was obtained by infusion followed by cold maceration for 24 h. The phytochemical screening of the plant extract demonstrated the presence of several classes of compounds like alkaloids, flavonoids, tannins and steroids. The concentration of the plant extract needed for 50% scavenging (IC_{50}) of DPPH was found to be 40.4 µg/ml whereas IC_{50}values for the positive control was found to be 16.34 µg/ml for BHT and 65.03 µg/ml for Ascorbic acid (AS).*

Keywords: *Centella asiatica, Phytochemical screening,* 1, 1-diphenyl-2-picrylhydrazyl (DPPH), *Free radical scavenging Activity.*

Introduction

Aging is a natural process which is related to several morphological and biochemical changes that happen from maturity to making the organism vulnerable to diseases and toxicity, and eventually leading to cellular death. According to the hypothesis of oxidative stress on aging, the loss of functional capacity associated to senescence comes from accumulation of molecular oxidative damages[1] brought by free radicals produced during normal breathing. Free radicals have previously been reported as being capable of damaging a lot of cellular components such as proteins, lipids and DNA[2].

To protect the cells from damages by oxidants, produced during oxygen methabolism, an antioxidant system is used by aerobic organisms. The main antioxidant agents such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione, ascorbic acid and tocopherol are important for cellular protection, due to their ability to eliminate free radicals, such as reactive oxygen species (ROS)[3]. Nowadays, there is an increasing interest in the biochemical functions of natural antioxidant extracts from vegetables, fruits, and medicinal plants, which can become candidates to prevent oxidative damage, promoting health. The phenolic constituents found in vegetables have received considerable attention for being the main components of antioxidant activity, in spite of not being the only ones. The antioxidant activity of phenolic constituents has been attributed to its oxide-reduction properties, which play an important role in the adsorption or neutralization of free radicals[4].

Medicinal plants have been a useful source for the research of new and biologically active compounds. Different approaches are used to select a plant for research, specially the ethno-medical data approach. Unfortunately, the ethno-medical data is not always completely reliable, since it is difficult to diagnose cancer as well. Apart from the medicinal effects of traditional herbs, exploratory researches have been made and a wide variety of new biological activities from traditional medicinal plants have recently been reported, including anticancer activity[5].

Centella a*siatica* (synonym: *Hydrocotyle asiatica* L.), Bengali name- thankuni pata, belonging to the family of Mackinlayaceae is native to most of the countries of Asia. It grows well in both tropical and sub-tropical countries. It is a popular herb that is either consumed fresh, or processed into tea or juice[6]. The plant has been claimed to exert many physiological effects and is traditionally used for various ailments including wound, bronchitis, asthma, diabetes, kidney troubles, urethritis, liver complaints, allergy, cancer and hypertension.[7]. In ulcer, depression and venomous insufficiency *Centella asiatica* is a potent drug[8,9]. The plant is also found to improve the general behavior and mental ability of retarded children[10]. The anti-diabetic property of *C. asiatica* has been known to the ancient people of Bangladesh for centuries especially in Ayurvedic system of medicines. Phytochemical screening of *C. asiatica* will lead to the rationalization of the use of this plant in various diseases as mentioned and will also lead tothe discovery of specific causative compound which have effective treatment roles in against specific diseases[11]. Although the plant is being used in our country for long time, a chemical and pharmacological stuies conducted with the plant in Bangladesh.

Materials and Methods

Collection of plant materials: Fresh leaves of *C. asiatica* were collected from Savar, Dhaka, Bangladesh in July, 2011 and the plant samples were identified and authenticated by Bangladesh National Herbarium. A voucher specimen with accession number DACB 33537 has been deposited there for further references.

Preparation of plant extract : The leaves were washed with running tap water without squeezing to remove debris and dust particles, dried at room temperature and pulverized into a coarse powder. The powdered material (1.2 kg) were macerated with 6.0 and 5.0 L of methanol, respectively at room temperature for 15 days with occasional shaking. The methanolic extracts of leaves were collected, filtered by cotton plug followed by whatman filter paper (no. 1) and evaporated to dryness (45°C) under reduced pressure by rotary evaporator. The obtained crude extract was stored in a refrigerator at 4°C until time of use. The percentage yield of the extract was calculated using the formula below:

% yield= (weight of the extract/ weight of plant material) ×100

Photochemical s**creening :** For preliminary phytochemical analysis the freshly prepared crude methanolic extract was tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, saponins, gums, steroids and alkaloids by using standard procedures[12].

DPPH Free radical scavenging assay : The free radical scavenging activitiy of the methanolic extracts of leaves of *C. asiatica* on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method developed by of Brand-Williams[13]. The percentages of inhibition was calculated by using the following equation:

$$\% \text{ inhibition} = [1- (A_{sample} / A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test material) and A_{sample} is the absorbance of the sample. Then percent inhibitions were plotted against respective concentrations and IC_{50} values were calculated. Tert-butyl-1-hydroxytoluene (BHT) and Ascorbic acid were used as positive controls. The experiments were performed thrice and the results was expressed as Mean ± Standard Error of Mean (SEM) in every case.

Result

Table-1: Test for phytocostituents in methanolic extract of *C. asiatica*

No.	Chemical constituents	Test	Result [+Present, -Absent]
1	Test for reducing sugar	Benedict's test	+
		Fehling's test	+
		Alpha napthol solution test	+
2	Test for tannins	Ferric chloride test	+
		Potassium dichromate test	+
3	Test for flavonoids	Hydrochloric acid test	+
4	Test for saponins	Foam test	-
5	Test for gums	Molisch's test	-
6	Test for steroids	Libermann-Burchard test	+
		Sulphuric acid test	+
7	Test for alkaloids	Meyer's test	+
		Wagner's test	+
		Dragendroff's test	+
		Hager's test	+

Discussion

Phytochemical screenings : Phytochemical screenings of the plant extract demonstrated the presence of several classes of compounds like alkaloids, flavonoids, tannins and steroids which could be responsible for the versatile medicinal properties of this plant (Table 1).

DPPH free radical scavenging activity : The antioxidant activity exhibited by the plant extract was statistically significant. It supports previous finding of the antioxidant activity of the methanolic extract of *C. asiatica* is due to the presence of substances with free hydroxyls[14]. The presence of several phenolic constituents such as flavonoids, tannins etc. which possess ideal structures for the scavenging of free radicals contributes to the

anti-oxidant activity of the plant extract. The concentration of the plant extract needed for 50% scavenging (IC_{50}) of DPPH was found to be 40.4 µg/ml. The IC_{50}values for the positive control were found to be 16.34 µg/ml for BHT and 65.03 µg/ml for Ascorbic acid (AS).

Conclusion

The methanol extract of leaves of *C. asiatica* displayed moderate DPPH free radical scavenging activity. However, the specific compound responsible for this antioxidant activity needs to be resolved. This might lead to the development of new drugs. Moreover, the present study supports the traditional use of the plants for the purposes mention above.

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