Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANT ACTIVITY AND NUTRIENT CONTENT OF *CENTELLA ASIATICA* (L.) URBAN LEAVES FROM DIFFERENT LOCALITIES OF ASSAM

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ABSTRACT

Leaves of *Centella asiatica* collected from three districts (considered different samples) of Assam were analysed for phytochemical constituents, antioxidant activity and nutrient content. Data showed that saponin, tannin, phenol, flavonoid, trapezoid, cardiac glycoside, alkaloid and reducing sugar were present in all the samples. The order of total phenolic content and antioxidant activity of the samples were sample 2 > sample 3 > sample 1 while the order of nutrient content was sample 1> sample 3> sample 2. The results revealed that both primary and secondary metabolite content of plants varies with variation of growing localities.

KEY WORDS: ***Centella asiatica*, phenolics, antioxidant activity, nutritive value.**

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INTRODUCTION

Many plants have great nutritive value and some of them are also the major source of medicine which plays a vital role in the human history. Studies on phytochemistry of plants have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules which act as antioxidants and antimicrobial agents [1-4]. There is an upsurge in demand of plant materials containing phenolics as they retard oxidative degradation of lipids and thereby improving quality and nutritional value of food [4-6]. *Centella asiatica* (L)Urban. (Apiaceae) is a small herb and grows wild in wet places. The herb is used for treatment of bronchitis, asthma, gastric, kidney troubles, leprosy, skin diseases, cough, cold, fever, to improve memory, measles, diarrhoea, dysentery, constipation, leucorrhoea, jaundice, dysmemorrhoea and applied on rheumatism. The plant has a good number of phytochemicals and high antioxidant activity [7]. The phenolic content and antioxidant activity of *C. asiatica* have been studied in different parts of the world and reported differently [8-10].

Synthesis of secondary metabolites including phenolic compounds in plants may be stimulated by the action of different parameters like environmental factors, use of precursors of the targeted molecules, use of elicitors and genetic transformation of the plants [11]. There has been little focus on investigating the effect of climate conditions on production of secondary metabolite in medicinal plants [12]. On the above context, a study was carried out to evaluate the phytochemicals, antioxidant activity and nutrient content of the leaves of *C. asiatica* collected from different localities of Assam.

MATERIALS AND METHODS

Leaves of *C. asiatica* were collected from three districts of Assam viz. Dibrugarh located at 120-13-MSL, 27°17'0"N and 94°47'15"E with soil p^H 4.7-5.0 (sample 1), Jorhat located at 85-95 MSL, 26°35'50" N and 94°15'40"E with soil p^H 4.6-6.5 (sample 2) and Tinsukia located at 140-150MSL, 27°29'19"N and 95°21'45"E with soil p^H .9-5.4 (sample 3). The average annual rainfall recorded at 2820mm in Dibrugarh and Tinsukia while it was 1950mm in Jorhat and the maximum and minimum temperatures recorded 30°C and 10°C respectively during the growing period of the plant i. e. from October to April. The samples were shade dried and powdered. The powder was macerated with 80% ethanol for 48 hours and filtered using Whatman filter paper No. 1. The filtrate was then evaporated at a constant temperature of 72°C until a very concentrated extract was obtained. The crude extract was dissolved in DMSO to make the final concentration, which kept in refrigerator till used.

Phytochemical analysis

Test for tannin: i) To 0.5 ml extract solution, added 1 ml distilled water and 1-2 drops of ferric chloride solution to it and observed for blue black coloration which indicates presence of tannin ii) 10% lead acetate solution was added to 0.5 ml extract solution and observed for white precipitation which indicates presence of tannin. Test for saponin: 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing shows the presence of saponin. Test for flavonoid: 0.2 g of the extract was dissolved in 10% NaOH solution, yellow coloration indicates the presence of flavonoid. Test for phenol: To 2ml of extract solution, added 2ml of alcohol and few drops of ferric chloride solution and observed for coloration. Test for cardiac glycoside: 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of conc. Sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycoside. (A violet ring may appear below the ring while in the acetic acid layer a greenish ring may for me). Test for alkaloid: 0.5 g extract was boiled with conc. HCl and filtered. 0.5ml of picric acid and Mayer's

reagent was added separately to about 1ml of the filtrate in a different test tube and observed for coloured precipitate or turbidity. Test for anthraquinone: To 0.2g of extract, added 5ml of chloroform and 5ml of 105 ammonia solution. The presence of bright pink colour in the aqueous layer indicated the presence of anthraquinone. Test for trapezoid and steroid: 5ml of extract solution was mixed in 2ml of chloroform, and 3ml of conc. sulphuric acid was added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenism. Red colour at the lower surface indicates presence of steroid. Test for reducing sugar: To 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitation.

Antioxidant activity

DPPH radical scavenging activity [13]

Antioxidants react with 1, 1- diphenyl - 2-picryl-hydrazyl (DPPH) radical and convert it to 1, 1- diphenyl -2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. 0.2 ml aliquots of extract solutions (1mg/ml) were taken and made up the volume to 3ml with methanol. Added to this, 0.15ml of freshly prepared DPPH solution stirred, and left to stand at room temperature for 30 minutes in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbance was noted at 517 nm using UV-Vis spectrophotometer. The capacity of scavenging free radicals was calculated as scavenging activity (%) = $[(Abs_{control}-Abs_{sample}\ /Abs_{control}.]$ x 100 where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

ABTS radical scavenging assay

For ABTS assay, the method of Re et al. [14] was adopted. The stock solution included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 $\pm$ 0.001 units at 734 nm using the spectrophotometer. ABTS solution was freshly prepared for each assay. 1 ml extracts (1mg/ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of ascorbic acid and calculated the percentage inhibition ABTS radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample}\ /Abs_{control}.]$ x 100 where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract/standard.

Determination of total phenol

Total phenol content of the extracts was determined according to the method described by Malik and Singh [15]. Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. The tubes with solution were warmed for 1 minute, and then cooled. A blue colour was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue coloured complex. Absorbance was measured at 760 nm. A standard calibration plot was generated at 760 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Determination of total flavonoids

The aluminium chloride method was used for the determination of the total flavonoid content of the extracts [16]. Aliquots of extract solutions were taken and made up the volume to 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The

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solution mixture was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Determination of Nutrient Content

Nutritive value is determined by the following formula (after Indrayan et al. [17])

Nutritive value = 4 x percentage of protein + 9 x percentage of fat + 4 x percentage of Carbohydrate.

Ash Content

For determination of ash content, 5g of sample was weighed in a silica crucible. The crucible was heated first over a low flame till the material completely charred, followed by heating in a muffle furnace for about hour at 300^0C. It was cooled in desiccator and weighed. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant. Weight of ash gave the ash content.

Moisture content

For determination of moisture content, 2g of each sample was weighed in flat bottom dish and kept overnight in a hot air over at 100 to 110^0 C and weighed. The loss in weight was regarded as a measure of moisture content.

Fat content

Crude fat was determined by extracting 5g of sample with petroleum ether in a soxhlet extractor heating the flask for about 6 hours till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether, the residual petroleum ether was filtered using Whatman number 40 filter paper and the filtrate was evaporated in a pre weighed beaker. Increase in weight of beaker gave crude fat.

Crude Protein

Nitrogen content was determined first by kjeldahl method and crude protein was determined then multiplying kjeldahl nitrogen by 6.25. Thus Protein = % of nitrogen x 6.25

Carbohydrate

Percentage of Carbohydrate was determined by the following formula – % Carbohydrate = 100 – (% of ash + % of moisture + % of fat + % of protein)

RESULTS AND DISCUSSION

The quantitative tests for phytoconstituents of the samples are summarized in Table 1. Presence of alkaloids, tannins, saponin, trapezoid, flavonoid, phenol, cardiac glycoside and absence of anthraquinone and steroid are confirmed in all the samples. Total phenol content in terms of catechol equivalent (the standard curve equation: Y= .002x + .034, r2 = .998) were between 199.5 and 277 mg/g dry material while total flavonoid content (the standard curve equation: Y= .002x + .207, r2 = .934) were between 18 and 144.5 mg/g dry wt (Table 2). In case of antioxidant activity, ethanoic extract of the samples showed effective scavengers of DPPH and ABTS radical (Table 2) and this activity was comparable to that of ascorbic acid. The percentage inhibition in case of DPPH was 73.7%, 86.2%, 83% and 88.2% for sample 1, 2, 3 and ascorbic acid respectively. On the other hand it was 69.4, 80.5, 76.5% and 83% for sample 1, 2, 3 and ascorbic acid in case of ABTS. Highest phenolic content and antioxidant activity was noted in sample 2. The highest nutritive value (349.06 cal/100g) was obtained in sample 1 followed by sample 3 and sample 2. Tan et al 9 found phenolic compounds of C. asiatica with a value of 967.2 mg/GAE/100g DW and 908.3 mg CE/100g DW and exhibit high antioxidant capacities with a value of 0.8133mμ and 2.0945mμ. Out of 4 accessions of Centella asiatica namely CA01, CA05, CA08 and CA11 studied by Zainol et al. 18, the antioxidant activity of roots, leaf and petiole showed higher antioxidant activity in CA01 and CA05, also the result showed that both the leafs and roots of Centella asiatica had high antioxidant activity which was as good

as α-tocopherol. An aqueous extract of C. asiatica leaves studied by Pittella et al 11 showed phenolic and flavonoid compounds were 2.86g/100 g and 0.36 g/100 g, DPPH scavenging activity with an IC50 value of 31.25 μg/ml.

It is observed that phenolic content, antioxidant activity and nutrient content of the plant differ among themselves in respect to their localities. Similar results were obtained by Singh & Sharma 19 in case of different market samples of Terminalia chebula. It indicates that growing condition affects the secondary metabolite as well as nutrient content of plants. Primary products such as carbohydrates, lipids, proteins, etc are common to all plants and are involved in primary metabolic processes 20-21. On the other hand, secondary metabolites may appear with respect to growing condition of a plant. Recognition of important climatic factor(s) in relation to secondary metabolite production is required for understanding the biology of secondary metabolites in plants and to increase yields in artificial growth medium [22]. There is a well established positive relationship between the intensity of solar radiation and the quantity of phenolics produced by plants. It can be seen at the intra-individual level by comparing plant part(s) exposed to different amounts of light [23]. In the present study, the highest content of phenolics and antioxidant activity were found in sample 2, while the lowest in sample 1. In contrast, nutrient content (as primary metabolite) was highest in sample 1 while the lowest was in sample 3. The results also represent that increase in the content of phenolics and antioxidant leads to decrease in the content of nutrient. The results showed correlation between antioxidant activity and phenolics content which was not in accordance with the earlier report [24] that stated no correlation between antioxidant activity and phenolics content in certain medicinal plants. Oloumi and Hassibi [22] stated that temperature and soil factors are the most important factors affecting secondary metabolite content in roots of *G. glabra* plants. Works of Hou etal.[25] also have shown that some special environmental conditions like low light intensity affect the accumulation of primary and secondary metabolites in *Glycyrrhiza uralensis*. Jovancevic et al. [12] in the study of wild population of bilberry gathered from different localities proposed the effect of habitat including altitude and sun shining on the content of phenolic compounds including flavonoids and anthocyanins. The effect of habitat parameters on secondary metabolites profile of *Lychnophora ericoides* were also investigated on different localities of Brazil by Gobbo-Neto et al. [26] and stated that leaf extracts from different localities show different metabolites profile. Variation in qualitative and quantitative phytochemical characteristics in the samples of *C. asiatica* from different localities is of great importance as a good number of active ingredients have been extracted from this herb, which are used in both medicine and cosmetics. Thus, the production of secondary metabolite of a plant may vary both qualitatively and quantitatively with respect to its growing conditions. In fact, this issue needs to be properly addressed in case of medicinal plants and is of great importance in quality control of crude drugs.

The present study shows phenolic content, antioxidant activity and good amount of nutritive value in the samples of C. asiatica. The study also provides scientific basis of the analysis of those plants belonging to same species collected from different localities.

Table 1
Phytochemical screening of extracts of C. asiatica leaves from 3different localities

Constituents	Sample 1	Sample 2	Sample 3
Tannin	+	+	+
Saponin	+	+	+
Phenol	+	+	+
Flavonoid	+	+	+
Cardiac glycoside	+	+	+
Anthraquinone	-	-	-
Alkaloid	+	+	+
Trapezoid	+	+	+
Steroid	-	-	-
Reducing Sugar	+	+	+

'+' indicates presence of constituents and '_' indicate absence of constituents

Table 2
Total Phenolic content and antioxidant activities of Centella asiatica leaves from 3 different localities*

Sample	Phenol (mg catechol) equivalent/g dry material)	Flavonoid (mg quercetin equivalent/g dry material)	Antioxidant activity (% inhibition in mg/ml)	
			DPPH radical scavenging activity	ABTS radical scavenging activity
Sample 1	199.5	18	73.7	69.4
Sample 2	277	144.5	86.2	80.0
Sample 3	231	30	83.0	76.5

Values tabulated are average of triplicate samples of each category.

Table 3
Nutritive value of Centella asiatica leaves from 3different Localities*

Sample	Ash (%)	Moisture content (%)	Crude fat (%)	Protein (%)	Carbohydrate (%)	Nutritive value (cal/100g)
Sample 1	8.06	7.15	1.98	32.55	50.26	349.06
Sample 2	11.6	12.3	2.74	30.97	42.39	318.1
Sample 3	8.62%	9.7	2.28	32.03	47.38	338.12

Values tabulated are average of triplicate samples of each category.

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