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Antioxidant Activity of *Centella asiatica* (Linn.) Urban: Impact of Extraction Solvent Polarity

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In vitro antioxidant activity of *Centella asiatica* (Linn.) and the impact of extraction solvent polarity on the antioxidant potential were investigated in the present study. 100% ethanol, 50% ethanol and water were chosen as extraction solvent due to arithmetic progression of their polarity. Total polyphenol, flavonoid, β-carotene, tannin and vitamin C content of these three extracts were determined while their antioxidant potentials were assayed by total reducing power assay and 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activity. 50% ethanol extract of *C. asiatica* contained significantly higher amount of polyphenol, flavonoid while moderate amount of carotene and tannin but the lowest amount of vitamin C compared to 100% ethanol and water extract. All the phytochemicals showed solvent polarity specific extraction pattern. Total reducing power and DPPH-radical scavenging activity of 50% ethanol extract also were significantly higher when compared to those of the 100% ethanol and water extracts. Significant variations of antioxidant potentials of *C. asiatica* due to differences in the extraction solvent polarity were demonstrated in this study

Keyword: Antioxidant, Flavoniod, *Centella asiatica*, DPPH, Polarity Index.

1. Introduction:

Oxidative stress occurs when the generation of free radicals or reactive oxygen species (ROS) exceeds the antioxidant capacity of a biological system[1]. Excess free radicals and ROS attack biological molecules such as lipids, proteins and nucleic acids that lead to tissue or cellular injury[2,3]. Oxidative stress has already been implicated in atherosclerosis, cancer, diabetes, arthritis, reperfusion damage and inflammation[4]. Antioxidants are free-radical scavengers that provide protection to living organisms from damage caused by ROS. Although almost all organisms possess antioxidant defense and repair systems but these systems are insufficient to cope over entire damage. So, dietary antioxidant supplementation is a promising mean to strengthen the antioxidant defense and repair systems. However, antioxidants from natural source are of great value as most commonly used synthetic antioxidants (e.g. butylatedhydoxyanisole, butylatedhydoxytoluene and propylgallate) have health hazardous side effects like liver damage and carcinogenesis[5].

Centella asiatica (Linn.) Urban is a prostrate stoloniferous plant that belongs to the family Apiaceae and endogenous to Bangladesh[6]. The therapeutic use of C. *asiatica* with its wide range of application has been documented in South East Asia and Bangladesh for centuries. C. asiatica is effectively being used in the treatment of fever, jaundice, dysentery, diarrhea, mental illness

within the frame of traditional medicine of Bangladesh[7]. Though several investigations showed antioxidant activity of C. *asiatica* but none of these explained the role of extraction solvent polarity on antioxidant potential[8,9,10]. We, therefore, investigated in vitro antioxidant activity of C. *asiatica* and the impact of the extraction solvent polarity on antioxidant potential in this present study.

2. Materials and Methods

2.1 Collection of Plant Materials and Extraction

Wild *Centella asiatica* was collected from the Jabra, Manikgang. The plant was identified and authenticated by Department of Botany Jahangirnagar University, Savar, Dhaka (NoJUBD: 1206). The fresh leaves with petioles were then air dried in shadow and grinded by mechanical grinder. Fine plant powder was then used for the exhausted extraction by Soxhlet apparatus for four repeated cycle using 100% ethanol, 50% ethanol and water as extraction solvent. During extraction solute-solvent ratio was 10:1 and extraction temperature was 45º±2ºC. The extracts were then filtered, evaporated using oven to a thick residue at 45°C and stored at 4°C. This crude extract was used for further analyses.

2.2 Total Polyphenol Content

Total polyphenol content of extracts was determined following Amin *et al.* (2006) using pyrogallol as standard[11]. The concentration of total phenol compounds in extracts was determined as pyrogallol equivalents (µg of PE/mg of extract).

2.3 Total Flavonoid Content

Total flavonoid content of extracts was estimated following aluminum chloride colorimetric assay described by Chang *et al.* (2001)[12]. Quercetin was used as standard. The concentration of total flavonoid in the extract was determined as quercetin equivalents (µg of QE/mg of extract).

2.4 β-carotene Content

ß-carotene content of extracts was determined by the method described by Nagata and Yamashita (1992) with slight modification[13]. The dried extract of *C. asiatica* (100 mg) was vigorously shaken with 10 ml of acetone - hexane (4:6) for 1 min and filtered through filter paper (Whatman No. 4). The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm spectrophotometrically. ß-carotene content was calculated according to the following equations:

$$\beta\text{-carotene (mg/100 ml)} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

The concentration of ß-carotene in the extracts was expressed as µg of ß-carotene /mg of extract.

2.5 Total Tannin Content

Total tannin content of extracts was determined by the Folin-Ciocalteu's method using tannic acid as standard[14]. The concentration of total tannin in extracts was expressed as tannic acid equivalents (µg of TE/mg of extract).

2.6 Vitamin-C content

Vitamin C content of extracts was estimated by the method of Omaye *et al.* (1994)[15]. Vitamin C was used as standard. The vitamin C content of extract was calculated as ascorbate equivalents (µg of AE/mg of extract).

2.7 Total Reducing Potential

The reducing power of extracts was estimated following Oyaizu (1986) using vitamin C as standard[16]. The reducing power of extract was calculated as ascorbic acid equivalents (µg of AE/mg of extract).

2.8 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Scavenging Activity

DPPH-free radical scavenging activity of extracts was measured following Braca *et al.* (2002)[17]. DPPH-free radical scavenging activity of *C. asiatica* was calculated as % of radical inhibition by following equation:

% Radical Inhibition = {(Control OD – Sample OD)/ Control OD)} X 100

DPPH-free radical scavenging activity was calculated and expressed as IC_{50} that is the concentration of *C. asiatica* required to scavenge 50% of DPPH used.

2.9 Statistical Analysis

The results are expressed as mean ± SEM (Standard error of mean). The statistical programs used were StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA) and GRAPHPAD PRISM® (version 4.00; GraphPad Software Inc., San Diego, CA, USA). Intergroup variation was analyzed by one way ANOVA followed by Tukey's least square differences test for post hoc comparisons. A level of $P<0.05$ was considered statistically significant.

3. Results and Discussion

Antioxidant activity of a plant extract is a complex attribute of its phytoconstituents. In the present study we have estimated total polyphenol, total flavonoid, β-carotene, tannin and vitamin C content in three different extracts namely 100% ethanol extract, 50% ethanol extract and water extract of *C. asiatica*. The results of the phytoconstituents content of these three extracts have been summarized in Table 1.

Table 1. Antioxidant phytoconstituents of 100% ethanol extract of *C. asiatica* (100% E Ex), 50% ethanol extract of *C. asiatica* (50% E Ex) and water extract of *C. asiatica* (H_2O Ex).

Antioxidant Phytoconstituents	Content (µg/mg of extract)		
	100% E Ex	**50% E Ex**	**H_2O Ex**
Polyphenols (PE)	21.1 ± 0.1^a	45.2 ± 0.3^b	35.6 ± 0.5^c
Flavonoids (QE)	9.3 ± 0.3^a	14.6 ± 0.2^b	11.7 ± 0.2^c
ß-Carotene	1.1 ± 0.4^a	0.7 ± 0.1^a	0.2 ± 0.1^a
Tannin (TE)	85.7 ± 3.3^a	59.7 ± 0.9^b	60.7 ± 1.8^b
Vitamin C (AE)	12.5 ± 0.7^a	9.5 ± 0.2^b	13.3 ± 0.4^a

Results are mean±SEM (n=3). PE, Pyrogallol Equivalent; QE, Quercetin Equivalent; TE, Tannic acid Equivalent; AE, Ascorbic acid Equivalent. Intergroup variation was analyzed by one-way ANOVA followed by Tukey's least square differences test for post hoc comparisons. Values in the same row with different subscription are significantly different at P< 0.05.

Polyphenols are available plant secondary metabolites and a critical index for determining the antioxidant capacity[18]. The antioxidant activity of polyphenols are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators[19]. The mechanisms of action of flavonoids are exerted through scavenging or chelating process[20]. Vitamin C directly interacts with a broad spectrum of ROS and terminates chain reaction initiated by these free radicals through electron transfer while involved in the regeneration of vitamin E[21]. ß-carotene is an excellent scavenger of singlet oxygen[22].

In the present investigation, total reducing potential assay and the DPPH scavenging activity were performed to evaluate *in vitro* antioxidant potential of three different extracts of *C. asiatica* including 100% ethanol extract, 50% ethanol extract and water extract. Total reducing power of 50% ethanol extract of *C. asiatica* (63.4±1.7 µg of AE/ml) was significantly higher than total reducing power of 100% of ethanol extract of *C. asiatica* (40.4±0.7 µg of AE/ml) and water extract of *C. asiatica* (56.3±0.6 µg of AE/ml) (Table 2). DPPH is a relatively stable nitrogen centered free

radical that easily accepts an electron or hydrogen when reacts with suitable reducing agents as results of which the electrons become paired off and the solution losses color depending on the number of electrons taken up[23]. In case of DPPH-scavenging activity, IC_{50} values of 100% ethanol extract of *C. asiatica*, 50% ethanol extract of *C. asiatica* and water extract of *C. asiatica* were 35.6±1.3 µg/ml , 7.1±1.5 µg/ml and 10.3±1.2 µg/ml respectively. IC_{50} values of 50% ethanol extract of *C. asiatica* and water extract of *C. asiatica* were nearly similar and not significantly (P>0.05) different. But, IC_{50} value of 50% ethanol extract of *C. asiatica* was significantly higher than that of 100% ethanol extract and water extract (Table 2).

Table 2. Comparative *in vitro* antioxidant activity of 100% ethanol extract of *C. asiatica* (100% E Ex), 50% ethanol extract of *C. asiatica* (50% E Ex) and water extract of *C. asiatica* (H_2O Ex).

Extracts	100% E Ex	50% E Ex	H_2O Ex
Reducing power (AE)	40.37±0.73^a	63.4±1.72^b	56.3±0.57^c
DPPH scavenging Activity (IC_{50})	35.56±1.24^a	7.08±1.54^b	10.23±1.20^b

Results are mean ± SEM, n= 3. AE, Ascorbic acid Equivalent; IC50 =Concentration required to inhibit 50% of DPPH radical. Intergroup variation was analyzed by one way ANOVA followed by Tukey's least square differences test for post hoc comparisons. Values in the same row with different subscription are significantly (P<0.05) different.

The presences of reductive phytoconstituents are reflected by reducing potential and DPPH-scavenging activity of corresponding extract[24]. We therefore, speculated that observed total reducing power and DPPH scavenging activity of the different extracts may be the contribution of one or more antioxidant phytoconstituents.

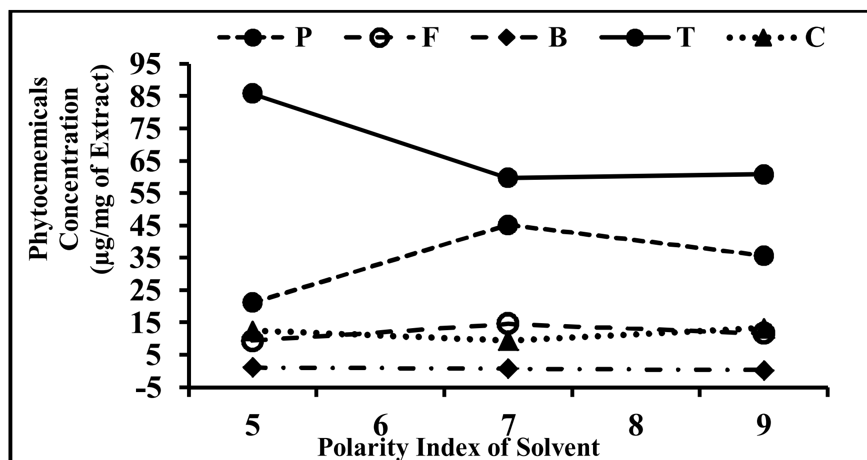

Fig 1: Impact of solvent polarity on the phytochemicals extracted. Here, P= total polyphenols content; F= total flavonoids content; B= ß-Carotene content; T= tannin content and C=vitamin C content. The polarity index value for 100% ethanol, 50% ethanol and water are 5.2, 7.1 and 9 respectively.

We chose 100% ethanol, 50% ethanol and water as extraction solvent in the present study. The polarity index value for 100% ethanol, 50% ethanol and water are 5.2, 7.1 and 9, respectively[25]. We took the arithmetic progression pattern (9-7.1=1.9; 7.1-5.2=1.9) of polarity as advantage to select the solvent of interest. Maximum extraction of both polyphenols and flavonoids occurs within a selective polarity range. Any deviation from that range either to higher polarity or to lower polarity decreases the extraction yields. The extraction yield for tannin was observed to decrease up to a steady state with the increase of polarity. ß-carotene showed a gradual decrease in extraction yield with the increase of polarity. Interestingly, maximum extraction of vitamin C was found in both higher and lower solvent polarity (Fig1). During extraction, organic solvents diffuse into the solid material and solubilize the compound with similar polarity. The nature of the solvent used will determine the type of chemicals likely extracted from plant materials[26]. Polarity is the relative ability of a molecule to engage in strong interactions with other polar molecules[27]. Polarity therefore represents the ability of a molecule to enter into interactions of all kinds. Polar solvents have property of dipole interaction forces, particularly hydrogen-bond formation for which solvating molecules become soluble and leads to the solubility of the compound[28]. Most of the bioactive components of plant matrices are medium-sized molecules. Due to the presence of aromatic delocalized μ-electrons, the molecules are highly polarizable[29]. Therefore, difference in the polarizability makes the phytochemicals liable to a variety of specific interactions with polar solvents that lead to polarity dependent extraction yield variation. This can explain the present observation though further studies are essential to explain the phenomenon minutely.

4. Conclusion

Besides the food value C. *asiatica* is used in a wide range of pharmacological activity within the ethnbotanic frame worldwide. Emphasis should be paid to the extraction solvent property as variation of pharmacological activity might be attributed through extraction solvent differences. Thus, significant variation of antioxidant potential of C. *asiatica* due to extraction solvent polarity difference was demonstrated in this study.

5. Conflict of Interests

Authors have declared that no competing interests exist.

6. References

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