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- **Short Communication**

Phytochemical contents, antioxidant and antibacterial activity of the ethanolic extracts of *Centella asiatica*(L.) Urb.leaf and stem

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Centella asiatica (L.) Urb. (locally known as thankuni) is a well-known traditional herb in the South Asian region especially in Bangladesh. It is available as a weed growing in the waste lands and in the river basins. *C. asiatica* has antimicrobial, antifungal, antidiabetic, antidiuretic and antioxidant activities due to the production of various bioactive secondary metabolites (Sekhar*et al*., 2014,Veeramuthu*et al*., 2006; Ghosh *et al*., 2008; Abubakar *et al*., 2011). It has many bioactive components that are necessary for the betterment of health conditions such as anti-inflammatory (George *et al*., 2009), antiulcer (Cheng *et al*., 2004), cardio protective (Gnanapragasam *et al*., 2004), cytotoxic and antitumor (Lee *et al*., 2002), and antiviral (Yoosook *et al*., 2000) activities. Pittella *et al*. (2009) reported that *C. asiatica* prevents the oxidative damage that takes place in neuropathological disorders including stroke, Parkinson's disease and Alzheimer's disease by improving the antioxidant neurological state related to aging.

Polyphenols, flavonoids, tannins, terpenoids, vitamin C, β-carotene and *p*-coumaric acid are present as secondary metabolites in different parts of the plants. These secondary metabolites play important role as antioxidants and have antimicrobial activities. They do bring about antioxidant activities through scavenging the reactive oxygen species (Hatano *et al*., 1989), inhibiting the generation of free radicals and chain breaking activities (Laranjinha *et al*., 1995) and chelating metal ions (van Acker *et al*., 1998).

However, a detailed study on the antioxidant and antimicrobial activities of *C. asiatica* leaf and stem extracts is yet to be performed. Herein, we have investigated and compared the phytochemical contents, *in vitro* antioxidant activityand antibacterial activity of the ethanolic extracts of *C. asiatica* leaf and stem against both the pathogenic (*i.e.*, *Salmonella typhi*) and nonpathogenic (*i.e.*, *Escherichia coli* and *Bacillus subtilis*) bacteria.

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Centella asiatica herbs were collected from the local market, Savar, Dhaka. The plant was identified and authenticated by a taxonomist of the department of Botany, Jahangirnagar University, Bangladesh.Leaves and stems were separated out and cleansed with tap water followed by a distilled water wash to remove any dirt,dried in the sun and were ground separately into fine powder using mortar and pestle.

The ethanolic extracts of *C. asiatica* leaf and stem were prepared according to the previously published protocol(Polash *et al.,* 2017, Mondal *et al.,* 2017).The plant parts were extracted separately using 70% ethanol upon gentle shaking at 120 rpm and 37°C for 72 hours. It was then filtered using Whatman No. 1 filter paper and the solvent was removed using rotary evaporator to get the dried extracts. Finally, 10 µg/µL of *C. asiatica* extract was prepared using saline solution (*i.e.*, 0.9% NaCl).

The presence of tannins and phenols were confirmed through ferric chloride test(Tiwari*et al.*, 2011). Flavonoids, and saponins were detected using alkaline reagent test(Tiwari *et al.*, 2011), and foam test(Cavalcanti *et al.,* 2014), respectively. Furthermore, Kellar Killani's test was used to reveal the presence of glycosides in the ethanolic extracts (Nagaraja *et al*., 2014).

The total phenol content (TPC) of the ethanolic extracts of *C. asiatica* leaf and stem was determined according to Terpinc *et al.,* 2012, with slight modifications. Briefly, diluted Folin-Ciocalteu's reagent (10% v/v) was taken into different test tubes to which 5µg/µLand 10 µg/µLconcentrationsof both the leaf and stem extracts were added. A chlorogenic acid (*i.e.*, gallic acid) was used as standard and the concentration of TPC in the samples was derived from the standard curve of gallic acid. Sodium carbonate (7.5 % w/v) was then added to all the test tubes and incubated at room temperature for 60 minutes before taking the absorbance at 765 nm using a UV-visible spectrophotometer (Optizen POP, Korea). Finally, the total phenol content of both the leaf and stem extracts was expressed as gallic acid equivalents (µg GAE/mL).

The antioxidant activity of the ethanolic extracts of *C. asiatica* leaf and stem was investigated by DPPH method according to Manzocco *et al.*, 1998 and Braca *et al*., 2001 with slight modifications. Briefly, three different concentrations (*i.e.*, 2.5, 5 and 10 µg/µL) of both the extracts were used to investigate their free radical scavenging activity. 50 µL of each concentration of the extracts and standard (*i.e.*, freshly prepared ascorbic acid) were taken in different test tubes and the final volume was adjusted to 500 µL using absolute methanol. Then 500 µL of the methanolic solution of 0.004% DPPH was added to all the test tubes and incubated at 4 °C in the dark for 30 minutes. Finally, the absorbance of the solution was measured at 517 nm using a UV-Vis Spectrophotometer (Optizen POP, Korea).

The free radical scavenging activity in percentage (%) was calculated from (Ab – As)/Ab x 100.

Here, Ab is the absorbance of the blank, and As is the absorbance of the standard or extract.

The antibacterial property of the ethanolic extract of *C. asiatica* leaf and stem was carried out by disc diffusion method (Bauer *et al*., 1996). A day old starter culture of both the nonpathogenic (*i.e.*, *Escherichia coli*, and *Bacillus subtilis*) and pathogenic (*i.e.*, *Salmonella typhi*) bacteria were uniformly spreaded on LB agar plate to prepare the test plates. At the same time, sterile blank antimicrobial discs (~6 mm in diameter, Oxoid, UK) were soaked with different amounts (i.e., 25, 50 and 100 µg) of the extracts and dried properly. Gentamycin (i.e., 100µg) was used as a positive control. The discs containing different amountsof extracts, and Gentamycin were then placed on the test LB agar plates having uniformly seeded bacteria and incubated at 37 °C for 24 hours. The extracts having antibacterial property prevented the growth of bacteria on LB agar media and showed a clear and transparent area surrounding the discs defined as the 'zone of inhibition'. The antibacterialactivities of the extracts were determined by measuring the diameter (in millimeter) of the zones of inhibition.

The results of phytochemical screening are qualitative data. *C. asiatica* leaf and stem extracts showed that various phytochemicals are present in the ethanolic extracts (Table 1). Both the ethanolic extracts showed the presence of several phytochemicals. The antioxidant activity of plant extracts depend on the flavonoid contents. Leaf extract showed greater (++) presence of flavonoid (Chahmi *et al*., 2015; Kumar *et al*., 2013) when compared to that of stem extract (+). However, the phenol content was higher in the stem extract when compared to that of the leaf extract. Ethanolic leaf extract showed the maximum amount (+++) of tannin, saponin, and glycoside. By contrast, there was no saponin present in the ethanolic stem extract of *C. asiatica*.

Table 1. Phytochemical tests of the ethanolic extracts of *C. asiatica* leaf and stem

Test	Leaf	Stem
Tannin	+++	++
Flavonoid	++	+
Saponin	+++	-
Phenol	++	+++
Glycoside	+++	++

N.B: +++ = Strong; ++ = Moderate; + = Weak; - = Negative.

Gallic acid was used as a standard to compare the total phenol content (TPC) of the ethanolic extracts of *C. asiatica* leaf and stem and the value of TPC was expressed in µg GAE/mL. The maximum TPC was found in the stem extract at both the concentrations (*i.e.*, 5 and 10 µg/µL) when compared to that of the leaf extract. The value of TPC increased as the concentration of both the extracts were increased (Fig. 1).

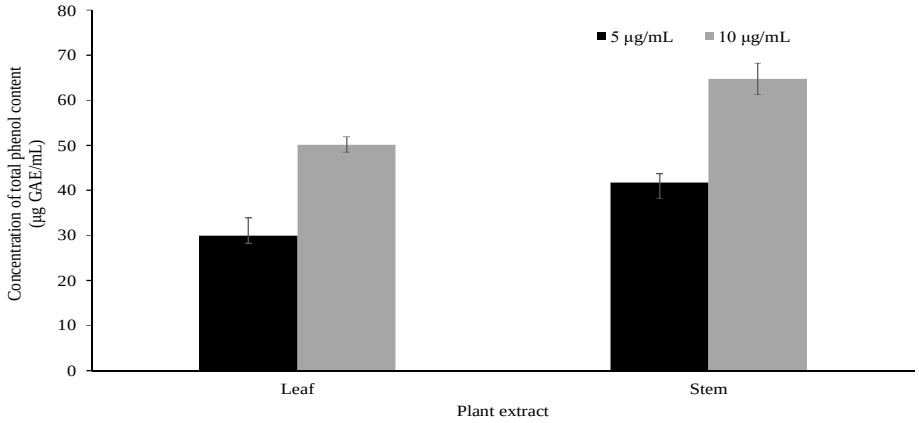

Fig. 1.Total phenol content (TPC) of the ethanolic extracts of *C. asiatica* leaf and stem. Two different concentrations (*i.e.*, 5 and 10 µg/mL) were used to determine the TPC of both the extracts. Testes were carried out three different times and the value of n was equal to 3

The antioxidant activity of the stem extract was slightly higher than that of the leaf extract at all the concentrations (Fig. 2). The greater free radical scavenging activity of the stem extract is directly proportional to its higher total phenol content (Spiridon *et al.*, 2011). The antioxidant activity was also increased as the concentration of the extracts were increased from 2.5 µg/mL to 10 µg/mL.

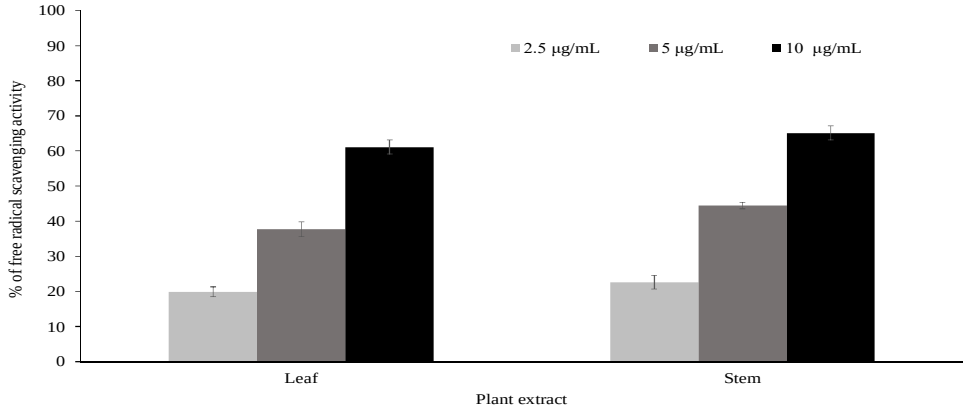

Fig. 2. Free radical scavenging activity of the ethanolic extracts of *C. asiatica* leaf and stem. Three different concentrations (*i.e.*, 2.5, 5 and 10 µg/mL) of the extracts were used to measure the free radical scavenging activity

The stem extract showed greater sensitivity against *E. coli* when compared to leaf extract. However, the antibacterial activity of leaf extract was more to *S. typhi* and *B. subtilis* than that of the extract obtained from the stem (Table 2). The zone of inhibition (in millimeter)

increased with the increased concentration of both the extracts against *S. typhi,E. coli* and *B. subtilis*. The leaf extract had the maximum zone of inhibition against *E. coli* (i.e., 10.33±0.98 mm) followed by *B. subtillis* (i.e., 9.8±0.36 mm) and *S. typhi (*i.e.,8.5±0.67 mm). Similarly, the stem extract also showed the maximum zone of inhibition against *E. coli* (i.e., 11.21±0.62 mm) followed by *B. subtillis* (i.e., 7.95±0.41 mm) and *S. typhi* (i.e., 7.5±0.33mm). This difference in the antibacterial activity of the extracts is due to the different cell wall composition of the gram negative (*i.e., E. coli*) and gram positive (*i.e.,B. subtilis*) bacteria (Elisha *et al.*, 2017). This difference may also be due to the presence of different phenolic and other secondary metabolite constituents in the stem and leaf extracts.

Table 2. Antibacterial activity of the ethanolic extracts of *C. asiatica* leaf and stem

Sample	Concentration (μg/μL)	Zone of inhibiton (mm)		
		Salmonella typhi	*Eschericia coli*	*Bacillus subtilis*
Leaf extract	2.5	6.6 ± 0.85	-	6.1 ± 0.23
	5	7.1 ± 0.33	8.67 ± 0.42	6.7 ± 0.17
	10	8.5 ± 0.67	10.33 ± 0.23	9.8 ± 0.36
Stem extract	2.5	-	6.49 ± 0.98	-
	5	6.5 ± 0.28	8.52 ± 0.73	7.2 ± 0.62
	10	7.5 ± 0.33	11.21 ± 0.62	7.95 ± 0.41
Positive control (Gentamycin)	10	24.7 ± 0.91	27.9 ± 0.29	32.33 ± 0.5

It can be concluded that the phytochemical constituents of the ethanolic extracts of *C. asiatica* leaf and stem are different.The stem extract showed greater*in vitro* free radical scavenging activity when compared to that of the leaf extracts. Both the extracts showed antibacterial activity against a wide range of bacteria such as *E. coli*, *Salmonella typhi*, and *Bacillus subtilis*. More specifically, the stem extract had the maximum antibacterial activity against *E. coli*, and the leaf extract had the maximum antibacterial activity against *B. subtillis*.

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