Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(2): 187-188

Received: 25-01-2017
Accepted: 26-02-2017

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Investigation on phytochemical, cytotoxic and antimicrobial properties of ethanolic extracts of *Centella asiatica* (L.) Urban

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Abstract
Centella asiatica (L.) Urban. is known to exhibit certain therapeutic properties. In the present work, plant extract of *C. asiatica* was considered for qualitative assessment of its secondary metabolite contents such as alkaloids, flavonoids, sterols, tannins, glycosides and saponins. Phytochemical, cytotoxic and antibacterial properties of the ethanolic extract of the plant were also determined. Plant extract showed the presence of all aimed metabolite contents as well as the brine shrimp lethality with LC_{50} value 186.46 µg/ml and significant inhibition zone against *Escherichia coli*, *B. Subtilis*, *Vibrio cholerae*, *S. Sonnei*, *and Salmonella paratyphi* and non-inhibition against *Bacillus cereus*, *Staphylococcus aureus* and *Shigella dysenteriae.* The highest zone of inhibition record was 8 mm (at 100mg/ml) and the lowest was 5 mm (at 50mg/ml).

Keywords: therapeutic properties, secondary metabolite, brine shrimp lethality

1. Introduction
Centella asiatica (L.) Urban. Belongs to the family Apiaceae which is commonly found in the marshy areas of the countries of Asia including Bangladesh. In Ayurvedic system of medicine, *C. asiatica* is used as brain tonic, and to treat chronic diseases and mental disorders. It possesses a wide range of pharmacological effects, being used for wound healing, mental disorders, antibacterial, antioxidant and anticancer purposes. The plant is highly effective in ulcer- preventive (Cho, 1981) [8], anti-depressive sedative and ability to improve the venomous insufficiency (Zheng and Qin, 2007) [32]. The plant is found to improve the power concentration, general ability and behavior of mentally retarded in children (Appa Rao *et al.*, 1973) [4] and to treat rheumatic disorders (Howes and Houghton, 2003) [13]. Asiaticoside is one of the prime triterpene saponin found in leaves in large amount is utilized commercially as a wound healing agent due to its potent anti-inflammatory effect [21,27] (Pointel *et al.*, 1987; Shukla *et al.*, 1999) and showed the potential use as anti-gastric ulcers drugs (Cheng *et al.*, 2004) [7].

2. Materials and Method
Whole plant extract of *Centella asiatica* (L.) Urban. was used in the present work. For extraction, naturally grown plant samples were collected, cleaned, chopped, air dried at room temperature and finally ground into coarse powder. About 100 g powder was macerated with ethanol (1:5) in a sealed container for 5 days at room temperature with occasional shaking. Extract was filtered through Whatman No.1 filter paper and evaporated to dryness under vacuum below 50 ºC to get about 3 g blackish extract. The extract thus prepared was kept at 4 ºC for future use. Methods of Cromwell [9], Aplin and Cannon [3], Wall *et al.* [31], Farnsworth [11], Bhattacharjee and Das [6] were followed for qualitative assessment of different secondary metabolites. Alkaloid contents were assessed with the help of Dragendroff (D), Wagner (W), Mayer (M), Hager (H) and Tannic acid (T) reagents. The relative abundance of secondary metabolite contents in the extract was expressed by ─ or + signs, ranging up to 4+, signifying its abundance in degrees. Cytotoxicity of the leaf extract was determined according to Meyer *et al.* [17] using brine shrimp nauplii and antimicrobial activity was assessed by disc diffusion method described by Bauer *et al.* [5] using the ethanolic extract of plant. Each set of experiment was replicated thrice.

3. Result and Discussion

The curative value of medicinal herbs largely depends on their secondary metabolites, especially alkaloids, terpenoids and phenolic compounds (Alamgir *et al.* 2014) [1]. In the present investigation, (Table 1) all objective secondary metabolites were found to be present in the plant extract which seems be similar to Samy *et al.*, 2011 [25] who observed the presence of Alkaloids, Lignins, Phenols, Proteins, Saponins, Starch, Steroids and absence of Coumarins, Flavones, Sugar, Tanins, Triterpenoid while studying Hexane, Dichloromethane and Methanol extracts of *C. asiatica*. On the other hand, Rahman *et al.* 2012 [22] discovered the presence of Alkaloids, Reducing Sugar, Tannins, Flavonoids and Steroids and the absence of Saponins and Gums in the plants leaf extract.

Table 1: Qualitative assessment of different secondary metabolites in *C. asiatica plant extract*.

Secondary metabolites										
Alkaloids Reagents used					Other secondary metabolites					
D	W	M	H	T	Flavonoid	Glycoside	Resin	Saponin	Sterol	Tanin
3+	3+	3+	3+	3+	+	+	+	+	+	+

The occurrence of different secondary metabolites suggests a wide range of biological application of the plant (Tanrisever *et al.*, 1988, Tashiro *et al.*, 2000, Ramzi *et al.*, 2008) [23, 28, 29]. Alkaloids like berberine is useful as antibacterial agents **(**Sharma *et al.*, 2013) [26] According to Kunle and Egharevba, 2009 **[**15**]** the presence of flavonoids in a plant is the indication of its antioxidant, antiallergic, antiinflammatory, antimicrobial and anticancer properties. However, glycoside derivatives showed very promising activity *in vitro* and *in vivo (*Keller-Juslén *et al.*, 1971) **[**14**]**. Terpenoids and steroids are cytotoxic. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and antibacterial activities and can aid in wound healing and burns (Haslem *et al.*, 1989) **[**12**]**. Recent reports show that tannins may have potential value as cytotoxic and/or antineoplastic agents **(**Aguinaldo *et al.*, 2005) **[**2**]**. Some researchers have also reported that some saponins have antimicrobial and antidiarrheal properties **(**Kunle and Egharevba. 2009, Evans 2002) [10, 15]. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions **(**Oloyode, 2005) **[**19**]**

The presence of significant chemical constituents encouraged to explore its cytotoxic activity by using brine shrimp lethality assay. In the present work LC_{50} value of the plant extract was found to be 186.46 µg/ml with 95% confidence limit, where the lower and upper limits were 74.41 and 400.34 µg/ml which correlates the findings of Ullah *et al.* 2009 **[**30**]** who observed the significant cytotoxic potentials of the n-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions to exhibit LC_{50} value of 1.254, 0.826, 3.866 and 5.366µg/ml respectively in the brine shrimp lethality bioassay.

This assay has been considered as prescreening assay for antimicrobial, antitumor, antimalarial, antifungal, and insecticidal activities. It is suggested to be a convenient probe for the assessment of pharmacological potentialities of plant extracts (Mayerhof *et al.* 1991) [16]. Moreover, crude extracts resulting in LC_{50} value less than 250 µg/ml could be considered significantly active and potential for further investigation (Rieser *et al.* 1996) [24]. In the present work, the plant extract considered had shown LC_{50} value less than 250 µg/ml and the plant might be the potential source of lead compounds for new drugs. It has been found that antidiarrheal activity is associated with the antimicrobial (Otshudi *et al.* 2000) **[**20**]**. Hence the ethanolic extract of the plant was tested against some human pathogenic bacteria to check it's antibacterial properties.

Results (Table: 2) of the ethanolic extract of *C. asiatica* exhibited mild but significant activity against the selected human pathogenic bacteria. These pathogens cause a variety of diseases including diarrhea and gastroenteritis in human (Nawfor 2000) [18]. It had shown no inhibition against *B. cereus, S. dysenteriae* and *S. aureus* although there was high concentrations as much as 25mg/ml, 50mg/ml and 100mg/ml but showed inhibition zone against *E. coli* (7 mm), *V.* cholerae (6 mm) and *B. subtilis* (6 mm) only at 100mg/ml. *S. sonnei* and *S. paratyphi* had significant inhibition in case of both of the concentrations 50mg/ml and 100mg/ml.

Table 2: Antibacterial activity of plant ethanolic extract of *C. asiatica* against human pathogenic microorganisms

Test organisms	Diameter of zone of inhibition (mm)			
	2mg/ml	25mg/ml	50mg/ml	100mg/ml
Escherichia coli	-	-	-	7
Bacillus cereus	-	-	-	_
Bacillus subtilis	-	-	-	6
Vibrio cholerae	-	-	-	6
Shigella dysenteriae	-	-	-	-
Shigella sonnei	-	-	7	8
Staphylococcus aureus	-	-	-	-
Salmonella paratyphi	-	-	5	6

4. Conclusion

On the basis of the results evaluated as above, it may be concluded that, the plant *C asiatica* have promising medicinal properties. It possesses important phytochemical, cytotoxic and antibacterial activities which may be implied as the potential source for antidiarrheal drugs.

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