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 LC₅₀ 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 venous 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 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. The extract thus prepared was kept 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 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 t

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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 to 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, Tanins, Flavonoids and Steroids and the absence of Saponins and Gums in the plants leaf extract.

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 tanins 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 antidiarreal 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 LC50 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 LC50 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 potencialities of plant extracts (Mayerhof et al. 1991) [10]. Moreover, crude extracts resulting in LC50 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 LC50 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 antidiarreal activity is associated with the antimicrobial (Otshudi et al. 2000) [28]. 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.

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 antidiarreal drugs.

5. Reference