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Potential health dangers of new invasive species similar to indigenous plants that are used as food or medicine--an example from Bangladesh

By

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Abstract

About 20,000 herbal products are currently available on the global market, and medicinal plants' annual trade turnover is approximately US $ 4 billion in the United States alone. As climate change starts increasingly interfering with monoculture grown crops--and this will likely be more serious more quickly in tropical areas--wild foods may become increasingly lifesaving. But at the same time, invasive plants will likely also spread more rapidly. *Centella asiatica* (L.) Urb, (Indian Pennywort) has been widely used from the wild (also cultivated and marketed in Bangladesh, China, Southeast Asia, India, Sri Lanka), the leaves eaten as a component of mixed green vegetable, pot herb and is also an important item in the traditional medicine systems. In Bangladesh it is widely used as a health food and in the folk and traditional system of medicine for improving memory and for the treatment of a variety of ailments. Market surveys have detected two different species, *C, erecta* and *C. verticillata,* wrongly identified as the exotic species, Indian Pennywort because of their morphological similarity. Comparison of taxonomic and pharmaceutical characteristics among these species indicated a wide difference and this misidentification might pose a health risk to the consumers, the edibility and safety of the two exotic species being unknown. Public health nutritionists need to work with experts in plant taxonomy to identify and attempt to reduce the risk of invasive species of plants that may be poisonous and are similar in appearance to indigenous plants that are used for food or medicine.

Key words: Medicinal plants, Identification, *Centella,* Indian Pennywort, Bangladesh

Introduction

About 20,000 herbal products are currently available on the global market, and medicinal plants' annual trade turnover is approximately US $ 4 billion in the United States alone (Buck and Michel, 2000). Wild greens which have been proved palatable and apparently safe down through history are consumed globally, particularly in the poverty prone areas and during food shortages. Many of these are also used medicinally to treat a wide variety of ailments and as sources of minerals and vitamins. However, some of these species may possess minimal toxicity, and some contain toxic ingredients that may not yet be identified.

Centella asiatica, a popular ingredient in various herbal products on the global market is known to contain pentacyclic triterpene glycosides in abundance. One ingredient, Asiaticoside, is considered to contribute in the pharmaceutical effects of *C. asiatica* and is widely used for wound healing (Gohil,et al,2010; Orhan, 2012; Bylka et al.,2014), as a "blood purifier" as well as for treating high blood pressure, for memory enhancement and promoting longevity (Ponnusamy et al., 2008; Hussin et al.,2009). The plant is also important for its high content of medicinally important triterpenoids and beneficial carotenoids, vitamins B and C, proteins, important minerals, and phytonutrients such as flavonoids, volatile oils, tannins, and polyphenol (Randriamampionona et al, 2007; Gamage et al, 2015). The immature leaves are commonly eaten raw as a salad, cooked as a soup, or mixed with other wild green vegetables (Reddy et al, 2007; Rahman, 2013; Khatun et al, 2013; Thongam et al, 2016), and the juice of fresh leaves is prescribed in traditional medicine (Gohil et al, 2010; Bhawmik et al., 2016).

Centella is known to occur in distinct 'morphotypes' with differences in morphological characteristics, eg, leaf shape, the length of lamina and petiole as well as inflorescence structure and have been shown to differ genetically (Schubert and van Wyk, 1997; Prasad et al, 2014). Interestingly, in the local markets around Dhaka (capital city of Bangladesh), a number of samples were seen to be sold as *C. asiatica* even though they did not appear to be so. Samples collected from Mirpur, Savar, Ganda, and Islampur markets and the GK Savar Campus were examined morphologically and taxonomically to verify their correct taxonomic identity. Of the three 'morphotypes' shown in Fig 1, Samples 1b and 1c were only available in markets and nurseries but were not found in the wild like the Sample (1a) which was identified as *C. asiatica*, suggesting that the other two (Fig 1 b and c) were not indigenous to Bangladesh.

As morphological characters, genetic identity and chemical contents can play important roles in the accurate identification, proper safety and quality evaluation of medicinal plants (Bhattacharyya et al., 2015), this report aimed at providing evidence that these two are not *C. asiatica*.

Materials and methods

Plant samples were collected from local markets and from fields around Savar, Dhaka and were used for morphological/anatomical examination using standard methods and equipment and standard pharmaceutical analysis.

Results and Discussion

Morphological differences

Centella asiatica is a clonal, perennial herbaceous creeper commonly found throughout Bangladesh growing in crop fields, fallow lands and moist places (Fig 1a), with small fan-shaped green leaves and white or light purple-to-pink flowers and small oval fruits. While *C. verticillata*, also a perennial, glabrous, creeping plant with slender stems (Fig. 1b); round leaves and cream-colored flowers was noted to be native to North and South America (Mathias, 1936; Wagner et al., 1990). There has been one report of this species now growing in Bangladesh (Khatun et al 2010). The third sample, *Centella erecta*, a very closely related species, is also found in North and South America (Fig 1c). The stolon and stem of *C. erecta* were deeply embedded in the soil, whereas the stems of *C. asiatica* and *C. verticillata,* grow near the surface. Other morphological features of the leaf (leaf size, surface area, number of dentate or crenate and length of leaf petiole) were helpful in differentiating these species (Table 1).



Fig 1 a *Centella asiatica*



Fig 1 b *Centella verticillata* Fig 1 c *Centella erecta*

Fig 1 Morphological characters of the three species of *Centella*

The stem of *C. erecta* displayed a thick and hard texture compared to *C. asiatica* and *C. verticillata,* which were thin and fragile. The leaves of *C. asiatica* were smaller in size (11.90–16.92, cm^2) than *Centella verticillata or C. erecta* (22.40–42.20, cm^2) and had a cordate blade shape which was more dentate or crenate in leaf margin, while *C. erecta* had reniform-shaped leaves with smooth-glossy texture and little dentate in the margin. Measurements of leaf characters (leaf area, blade length, petiole length) are presented in Table 1.

Table 1. Comparisons of leaf characteristics of the three species of *Centella.*

Species	*Leaf surface area(cm^2)*	*Leaf blade length (cm)*	*Length of leaf petiole (cm)*
C. asiatica	6.78 - 7.11	1.79 - 1.99	12.70 - 16.20
C. verticillata	15.58 - 21.43	2.98 - 4.05	18.09 - 20.65
C. erecta	30.09 - 34.24	4.92 - 5.45	23.99 - 25.42

Pharmaceutical comparisons

For pharmaceutical comparisons, enough plant materials were obtained for only two species, *C. asiatica and C. verticillata*. The freshly prepared crude extract was qualitatively tested for the identification of chemical constituents, such as, alkaloids, flavonoids, steroids, glycosides, saponins, terpenoids, gums and tannins.

Table 2 Results of Qualitative analysis of Phytochemicals for Centella species

Test	Methanol Extracts	
	Centella asiatica	*Centella venticillata*
Alkaloids	-	-
Carbohydrate	-	-
Flavonoids	-	-
Glycosides	+	-
Phenolic compounds	+	-
Protein	+	+
Quinones	+	-
Saponins	+	+
Terpenoide	-	-
Tanin	+	+

+ indicates high and - indicates low levels

Differences found between the two *Centella* species were that glycosides, quinones, and phenolic compounds were present only in C*entella asiatica.*

A DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay **(**Mazumder and Rahman, 2008) was carried out, results given in Table 3.

Table 3. DPPH Free Radical Scavenging Assay of the two Centella species

Name of sample	IC_{50} (µg/ml)
Ascorbic Acid Standard	70.3
C. asiatica	617.6
C. verticillata	482.3

The IC_{50} values of the methanolic extracts of *C. asiatica, C. verticillata were* 617.6µg/ml and 482.3 µg/ml respectively which were much higher than the ascorbic acid standard (70.3) showing a difference between the two species. The percentage Scavenging Activity of methanolic extract of C. *asiatica* and *C. verticellata* given in Table 4 show that the methanol extract of the plant samples exhibited good antioxidant potency (IC_{50}= 6.67µg/ml and IC_{50}=25.5µg/ml) respectively,

which was close to the antioxidant effect of ascorbic acid (IC_{50}= 7.5 µg/ml). The presence of phenols and flavonoid in the plant extracts may be a reason for this DPPH-scavenging activity.

Table 4. Percentage Scavenging Activity of the two *Centella* species

Concentration	% Scavenging of *C. asiatica*	% Scavenging of *C. verticillata*	% Scavenging of Ascorbic Acid
25	31.01	32.21	36.43
50	32.04	34.45	45.22
100	32.90	34.80	54.09
200	35.49	41.60	62.88
400	41.69	49.10	71.66
800	55.73	62.27	89.06

Total phenolic contents of the methanolic extracts of *C. asiatica and C. verticillata* were determined by using the Folin-Ciocalteu reagent and were expressed as Gallic Acid Equivalents (GAE) per gram of plant extract. Phenolics might be important components as anti-oxidants. Methanolic extracts contained considerable amounts of phenolic compounds (40.5 µg/ml in *C. asiatica* and 23.0 µg/ml in *C.verticillata).*

There are widely reported genetic and pharmaceutical differences too, the genetic diversity parameters calculated based on the Inter Simple Sequence Repeat (ISSR) polymorphism and Thin Layer Chromatography saponin profiles showed *C. asiatica* is distinctly different from *C. erecta* (Ali et al, 2017). Also, *C. erecta* exhibited lower total phenolic content than *C. asiatica* ($p < 0.05$) and the overall results showed that the *C. erecta* methanolic extract had significantly lower total saponin content than *C. asiatica* (Ali et al. 2017). The High-Performance Liquid Chromatography (HPLC) quantification of the target triterpene and phenolic compounds indicated that average content of madecassoside, total triterpenes and chlorogenic acid in *C. erecta* were significantly lower than *C. asiatica*. Also kaempferol and its derivatives were reported in *C. asiatica* (Maulidiani et al., 2012, 2014) but there is no reports of these compounds in *C. erecta* (Ali et al 2017). The colorimetric determination and the total content of the four triterpene glycosides and aglycones (asiaticoside, madecassoside, asiatic acid and madecassic

acid) obtained by HPLC- photodiode array (PDA) analysis indicated that *C. erecta* samples had the lowest content of total saponins and sapogenins compared to *C. asiatica* (Ali et al 2017)

Discussion

Though herbal and traditional medicines are often known to have fewer side effects in comparison with synthetic drugs, certain medicinal plants may cause acute poisoning incidents in children, sick or elderly (Wojcikowsk et al, 2004; Gill and Rieder, 2008; Phua et al. 2009; Asif, 2012; Malangu, 2014: Ghorani-Azam et al.2018; Philip Stark et al 2018). In a review, Malangu (2014) screened a total of 127 articles with 1453 intoxicated cases showing that some medicinal plants can cause acute poisoning and complications.

The morphological and pharmaceutical evidence suggests that *C. erecta* and *C.verticillata* are different from *C.asiatica* - a plant species used in many countries as a source of nutrition and medical cure. As the edibility of *C, verticillata* and *C. erecta* is unknown, a search in the PubMed Website, using the botanical names of the two plants landed with 626 hits for *C. asiatica* but only 4 hits for *C. verticillata* (Nature Weekly 2017). Though *C.erecta* has been included in the Guide book 'WILD EDIBLE PLANTS OF TEXAS' (https://medivetus.com/botanic/wild-edible-plants-of-texas-a-pocket-guide-to-the-identification-collection-preparation-and-use-of-60-wild-plants-of-the-lone-star-state/), there is no authentic report on its safe edibility.

The fact that the two species, *C, erecta* and *C. verticillata* are not indigenous (apparently being introduced mistakenly as *C. asiatica*) to South and Southeast Asia, may also have long-term ecological risks (Pacific Island Ecosystems at Risk, 2013a, b). Many of the *Centella* species have been proved to become a potential threat to indigenous species because of their invasiveness and vigorous vegetative as well as sexual reproduction. Confusioni with *C. asiatica* is probably the reason for the widespread planting of *C, verticillata* in Singapre (Reuben et al, 2014). A Weed Risk Assessment was performed, and a score of 20 was obtained for *C, verticillata* (the high-risk threshold for aquatic plants is 19), indicating it as a high-risk species. It was already recommended for Invasive Alien Species status in Singapore with a high risk of naturalising and also recommended more stringent measures for the cultivation of this species in that region (Reuben et al, 2014). For Bangladesh the same risk exits.

Centella asiatica is one of the most prominent marketed food supplements and medicinal herbs in the traditional systems of medicine in Indian, African and Chinese medicine (Brinkhaus et al 2000). The need for quality control has been envisaged by many international regulatory agencies and reflected in their guidelines to ensure safe use of natural products, food supplements, or botanical medicines (WHO, 2007; Speijers et al, 2010). However, chances of wrong identification remain and may create serious problems for food safety.
Public health nutritionists need to work with experts in the agricultural field to identify and attempt to reduce the risk of invasive species of plants that may be poisonous and are similar in appearance to indigenous plants that are used for food or medicine.

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