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Evaluation of Antioxidant Activity and Phytochemicals Properties of Methanol Extract *Centella asiatica* Leaves

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Abstract

Centella asiatica is an herbaceous plant commonly known as Gotu Kola and belongs to Apiaceae family. It is found in most tropical and subtropical countries growing in swampy areas. It is a tasteless, odourless plant and it is traditionally used for the treatment of a wide variety of disorders. Its leaves and roots are used as vegetables and for medicinal purposes. Knowledge of their contributions to human nutrition and contents of bioactive components is lacking and has limited their use. Therefore, this study evaluated the Nutrients content and phytochemical composition of Centella asiatica leaves using standard methods. The result of proximate composition revealed moisture (13.10 ± 1.07%), ash (16.5 ± 0.45%), protein (8.35 ± 1.28%), lipid (1.20 ± 0.10%), fiber (17.00 ± 1.87%) and carbohydrate (43.81 ± 0.70%) contents. Physicochemical results revealed Saponification value of 238.43 mg/KOH. Fatty acid composition revealed a high concentration of palmitic acid (55.70%) as saturated and Linoleic acid (17.50%) as unsaturated fatty acids; while amino acid composition showed high level of glutamate (13.389 g/100 g) as nonessential and Histidine (11.64 g/100 g) as essential amino acids respectively. The phytochemical composition revealed the presence of bioactive compounds such as; Proanthocyanin (11.964 µg/g), Rutin (11.8883 µg/g), Nanngenin (3.0122 µg/g),Quinine (10.4490 µg/g), Flav-3-ol(2.5900 µg/g), Spartein (3.0122 µg/g), Phenol (18.8713 µg/g), Flavonones (2.1836 µg/g), Steroids (18.8974 µg/g), Kaempferol (0.7273 µg/g), Phytate (1.6851 µg/g), Naringenin (2.7523 µg/g), Resveratol (10.8596 µg/g), Tannin (4.4377 µg/g) and Ribalinidine (3.0500 µg/g). The presence of these nutrients and bioactive phytochemicals in Centella asiatica leaves makes them useful in pharmaceutical and food industries.

Keywords: Nutrients; Phytochemical Composition; Centella Asiatica; Leaves

Introduction

The growing global demand for functional foods and plant-based therapeutics has intensified scientific interest in medicinal herbs possessing both nutritional and pharmacological properties. Among these, Centella asiatica has received considerable attention due to its extensive traditional use and diverse biological activities [1]. This perennial herb belonging to the Apiaceae family is widely distributed throughout Asia, including India, Bangladesh, Sri Lanka, China, and Madagascar, where it has long been utilized in traditional medicine for the management of wound healing, inflammation,

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venous disorders, skin diseases, and neurological conditions [2]. Medicinal plants are recognized as rich sources of essential nutrients and bioactive phytochemicals that contribute significantly to human health. In recent years, there has been increasing emphasis on identifying natural alternatives to synthetic food additives and pharmaceutical agents because plant-derived compounds are generally considered safer, biocompatible, and environmentally sustainable [3]. Furthermore, populations in many developing regions continue to rely heavily on carbohydrate-based diets, often resulting in nutritional deficiencies. Exploration of underutilized edible herbs such as C. asiatica may therefore provide valuable nutritional supplementation while simultaneously offering therapeutic benefits [4]. The medicinal potential of C. asiatica is largely attributed to its diverse phytochemical composition, including phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids, and sterols. These secondary metabolites play critical protective roles in plants and exhibit a wide range of pharmacological activities in humans, including antioxidants, antimicrobial, anti-inflammatory, antidiabetic, and neuroprotective effects [5]. Phytochemicals, particularly polyphenolic compounds, have attracted substantial scientific interest because of their ability to neutralize reactive oxygen species (ROS) and reduce oxidative stress associated with chronic diseases such as cancer, diabetes mellitus, cardiovascular disorders, and neurodegenerative diseases [6]. Oxidative stress results from an imbalance between the production of free radicals and the antioxidant defense system of the body, leading to cellular and molecular damage. Antioxidants are compounds capable of delaying or inhibiting oxidative reactions by scavenging free radicals and preventing chain reactions that damage biomolecules such as DNA, proteins, and lipids [7]. Natural antioxidants derived from medicinal plants have therefore emerged as promising candidates for disease prevention and health promotion. Vitamins, flavonoids, carotenoids, and phenolic compounds present in medicinal herbs contribute significantly to antioxidant defense mechanisms and may reduce the risk of aging-related and metabolic disorders [8]. Despite the extensive traditional use of C. asiatica, comprehensive scientific data regarding its nutritional composition, phytochemical profile, and antioxidant potential remain limited, particularly in relation to regional varieties and extraction methods [9]. Therefore, the present study aimed to evaluate the phytochemical constituents and antioxidant activity of Centella asiatica leaves to provide scientific evidence supporting its potential application as a functional food ingredient and natural therapeutic resource [10]. The aim of this study was to evaluate the phytochemical composition and antioxidant activity of methanolic extract of Centella asiatica leaves using the DPPH assay.

Materials and Methods

Plant Collection and Identification

Fresh leaves of Centella asiatica were collected from Nagpur, Tangail, Bangladesh. The plant sample was authenticated by the Bangladesh National Herbarium (BNH), Dhaka, and a voucher specimen (DACB-94829) was preserved for future reference.

Preparation of Methanolic Extract

The collected leaves were washed, shade-dried, and further dried in an oven at 40°C. The dried material was ground into fine powder, and approximately 1.1 kg powder was macerated in methanol for 72 h with intermittent shaking at room temperature. The extract was filtered using Whatman No. 1 filter paper, and the extraction process was repeated three times to ensure maximum recovery of phytoconstituents. The combined filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 40°C, followed by freeze-drying to obtain the crude methanolic extract. The dried extract was stored in an airtight container at 4°C until further analysis.

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the methanolic extract was carried out using standard qualitative methods to detect the presence of major bioactive compounds, including tannins, saponins, alkaloids, flavonoids, phenolics, steroids, and terpenoids. Appropriate colour changes or precipitate formation after the addition of specific reagents were considered indicative of positive results.

DPPH Radical Scavenging Assay

The antioxidant activity of the methanolic extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Different concentrations of the extract were prepared in methanol and mixed with 0.1 mM DPPH solution. The reaction mixtures were incubated in the dark for 30 min at room temperature, and absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference standard. The percentage of radical scavenging activity was calculated using the following equation [11]:

I%=[Ablank-Asample)/Ablank]100

where AblankA_{blank}_Ablank represents the absorbance of the control and AsampleA_{sample}Asample represents the absorbance of the test sample. The IC_{50} value was determined from the concentration-response curve, where a lower IC_{50} value indicated stronger antioxidant activity.

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Results

Phytochemical property

The screening for the search of different secondary metabolites (phytochemical) present in the methanol extract of Centella asiatica leaves was carried out using standard chemical test method. Result obtained for qualitative screening of phytochemicals present in methanol extract of Centella asiatica leaves. The obtained result showed that flavonoids are the major component in the extract. No trace of Alkaloids, Tannin, Steroids, Saponins, Phenols, Terpenoids was found.

Table 1: Results of phytochemical screening of methanol extract of Centella asiatica leaves.

Phytochemicals	Results	Test
Alkaloids	-	Hegar's Test
Tannin	-	Ferric-chloride solution test
Saponins	-	Froth test
Flavonoids	+	Mg ribbon test
Phenols	-	Potassium ferrocyanide solution test
Steroids	-	Salkowski test
Terpenoids	-	Salkowski test

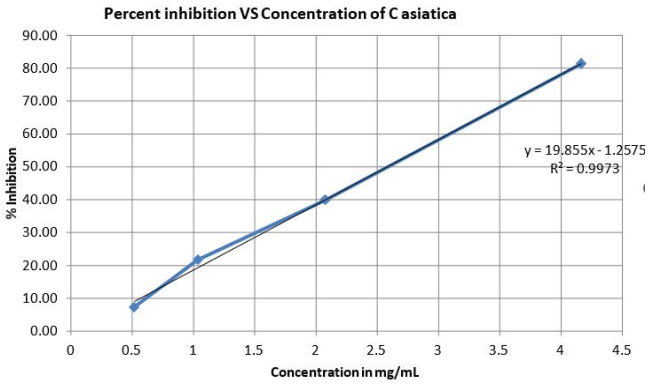

Figure 1 : Absorbance, % Inhibition and IC50 Centella asiatica leaves following DPPH method (517nm) by UV Vis-Spectophotometer.

The results of phytochemical screening of methanol extracts of Centella asiatica leaves revealed the presence of Flavonoids, but Alkaloids, Tannin, Steroids, Saponins, Phenols, Terpenoids were not observed in the extract respectively.

Antioxidant Activity

The methanolic extract of Centella asiatica leaves demonstrated notable antioxidant activity in the DPPH radical scavenging assay. The extract exhibited an IC_{50} value of 2.455 mg/mL, indicating moderate free radical scavenging potential. The ascorbic acid equivalent antioxidant capacity (AEAC) was calculated as 0.39 g/100 g, suggesting the presence of bioactive antioxidant phytochemicals in the extract.

Discussion

The proximate analysis of Centella asiatica leaves demonstrated considerable amounts of carbohydrate, crude fiber, and ash, indicating significant nutritional value. Carbohydrates were identified as the predominant nutrient component, which supports its potential role as an important energy source for cellular metabolism and physiological functions. Similar findings have previously been reported, where carbohydrate content ranged from 42.9-52.0% and ash content ranged from 13.5-16.4% in different C. asiatica species. Comparable carbohydrate contents of 38.48% and 20.0-66.8% have also been documented in other leafy vegetables [12]. The high ash content suggests that C. asiatica leaves may serve as a valuable source of essential minerals for human nutrition. In addition, the substantial fiber content indicates potential benefits in maintaining digestive health through improving bowel movement, reducing cholesterol absorption, and preventing excessive intake of starchy foods. Variations observed in fiber, ash, protein, and moisture contents compared with previous studies may be associated with environmental conditions, geographical variation, and analytical procedures [13]. The physicochemical properties of C. asiatica oil revealed relatively high saponification, iodine, and peroxide values. The saponification value was higher than those reported for Ipomoea involucrata leaf oil (196.50 mg KOH/g) and Canarium indicum nut oil (175.47 mg KOH/g), but comparable to coconut oil (246.28 mg KOH/g). Such a high saponification value suggests possible industrial application in soap and cosmetic production. The iodine value below 100 I_2/100 g classified the oil as a non-drying oil, reflecting moderate unsaturation and stability [14]. The low peroxide and free fatty acid values indicate good oxidative stability and low lipid degradation during storage. These parameters are important indicators of oil quality and shelf stability. Furthermore, refractive index analysis demonstrated the purity and optical characteristics of the oil, which may help detect adulteration. Fatty acid analysis showed that palmitic acid and lauric acid were the major saturated fatty acids, while linoleic acid followed by linolenic acid represented the predominant unsaturated fatty acids. Palmitic acid was found in higher concentration compared with previous reports on other leafy plants. Although palmitic acid has been associated with increased cholesterol levels, its adverse effects may be reduced in the presence of sufficient unsaturated fatty acids. Lauric acid possesses beneficial antimicrobial properties and may contribute to oral health, whereas linoleic acid (omega-6 fatty acid) is known to reduce cardiovascular risk, lower low-density lipoprotein (LDL) cholesterol, and improve lipid metabolism [15]. The presence of amino acids further highlights the nutritional significance

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of C. asiatica leaves, as amino acids are essential for protein synthesis, nutrient transport, tissue repair, immune function, and growth. Deficiency of essential amino acids may result in impaired immunity, digestive disorders, and delayed growth. Overall, the nutritional and physicochemical profile of C. asiatica suggests its potential application as a functional food and a valuable source of bioactive compounds.

Conclusion

According to the present finding, it may be concluded that methanol extract of C. asiatica has moderate antioxidant activity and it could be attributed to the presence of flavonoids. The constituents of C. asiatica can be used as an accessible source of natural antioxidants in different ailments with oxidative damage.

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