

## Nutrient and Phytochemical Composition of *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 result 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), Nannigenin (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

In recent years, there has been an increased effort to find food and beverages with high nutrients and health-promoting properties. Herbs, traditionally used in folk medicine, have attracted consumer interest because of their long historical consumption and ready acceptability [1]. Plants are known to provide food, clothing, shelter and medicine [2]. *Centella asiatica* commonly called Gotu Kola or pennywort is a herbaceous, frost tender perennial plant in the flowering plants family called Apiaceae. It is native to India and other parts of Asia such as China, Sri Lanka, Nepal and Madagascar and has been utilized in folk medicine in different countries [3]. *Centella asiatica* has

been reported as being used for the treatment of varicose veins, chronic venous insufficiency, psoriasis, minor wounds, analgesic and anti-inflammatory agent [4]. *Centella asiatica* is widely available in Madagascar and is commonly used as tea, soft drink and syrup [5].

It is important to note that natural food and food ingredients from plants have been proved to be safer and healthier than synthetic ones [6]. Living things generally, require a number of organic compounds for growth and development. Plant materials contain most of the needed micro and macro nutrients in addition to water [7]. Most regions in Africa depend on starch-based foods; thus protein deficiency is generally found among the population. To alleviate the situation, efforts are being made

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to explore the lesser known wild edible plants as sources of nutrient supplements. Increasing the utilization of wild edible herbs in our diet may increase the nutrients as well as dietary fiber and can be a food-based approach to ensuring the intake of these nutrients [6]. Gupta et al. [8] have emphasized that analyzing such plants for various nutrients would enable identification of unconventional food resources.

The medicinal properties of plants like antidiabetic, antimicrobial, antidiuretic, antioxidants, etc. are mainly dependent on the type, nature and concentration of secondary metabolites present. Reports have shown that some of the most important bioactive compounds are phytochemicals such as alkaloids, saponins, flavonoids, tannins, sterols and phenolic compounds [9,10]. These phytochemicals are plant derived metabolites naturally occurring in medicinal plant leaves, stem and roots where they are used as defence mechanism to protect the plant from various diseases. The phytochemicals are secondary metabolites that exhibit various important pharmacological activities [10]. *Centella asiatica* is utilized traditionally as medicine in the treatment of diverse ailments. However, the scientific knowledge on its nutrient content and chemical constituents is grossly inadequate. Hence, this research investigated the nutrient and phytochemical composition of the leaves of *Centella asiatica*.

## MATERIALS AND METHODS

### Collection of plant material and identification

*Centella asiatica* (Gotu kola) leaves were collected from the surroundings of Bonny Island in Rivers State, Nigeria. The plant material was identified in the Department of Plant science and Biotechnology in the University of Port-Harcourt by Dr. Chimezie Ekeke.

### Preparation of plant sample

The leaves were sorted, cleaned and dried at room temperature for 14 days. The dried leaves were pulverized with electric blender, transferred into an airtight container and labelled for future use.

### Determination of proximate composition

The proximate composition (moisture, ash, protein, fat, fiber and carbohydrate) of the *Centella asiatica* leaves was performed using Association of Official Analytical Chemists methods [11]. Moisture content of the sample was determined by air drying at room temperature (25-28°C) for 14 days. The crude protein was determined using micro-Kjeldahl method. Crude lipid was determined by Soxhlet extraction method. The ash content was determined using muffle furnace set at 550°C for 4 hours until constant weight of ash was obtained. Crude fiber was determined according to the method of Saura-Calixto [12]. The carbohydrate content was obtained by difference.

### Determination of physicochemical properties

Physicochemical parameters of *Centella asiatica* leaves such as refractive index, density, viscosity, acid value, peroxide value,

iodine value, saponification value, free fatty acid and thiobarbituric acid were estimated by AOAC method [13].

### Determination of fatty acid composition

The fatty acid composition was determined by GC/MS. Powdered sample (10g) was subjected to soxhlet extraction with 300 mL of n-hexane for 24 hours, and then evaporated to dryness using a rotary evaporator at 40°C. Sample was prepared by dissolving 1 mL of filtered residue in 50 mL chloroform and evaporated at room temperature; followed by the addition of 1 mL of reagent (benzene and methanol 20: 55 Vol %) and sample heated for 30 minutes at 40°C. Organic sample was extracted with hexane and taken for GC analysis.

### Determination of amino acid composition

Sample weighing 0.1 g was hydrolysed with 6N HCl at 110°C for 24 hours. After hydrolysis, the contents were vacuum filtered (using Whatman #541, Maidstone, England). Sample derivatization procedure was done by a modification of the method of Elkin et al. [14] by adding 30 µL of methanol-water-phenyl isocyanate (2: 2: 1 v/v) agitating and then removing the solvents under nitrogen stream. Chromatographic procedure was evaluated using gradient elution: Eluent A was aqueous buffer of 0.5 mL/L triethylamine and 0.14 M sodium acetate titrated with glacial acetic acid to pH 6.20 while Eluent B was acetonitrile-water (60: 40 v/v).

### GC-FID Identification and quantification of phytochemical constituents

For the GC-FID analysis, 1g of the powdered *Centella asiatica* sample was weighed and transferred into a test tube. Exactly 15 mL of ethanol and 10 mL of 50% w/v potassium hydroxide were added to the sample in the test tube. The test tube was allowed to stand in a water bath at 60°C for 60 minutes. Then the content of the test tube was carefully transferred into a separatory funnel and the tube rinsed into the same funnel with 10 mL of cold water, 10 mL of hot water, 20 mL of ethanol and 3 mL of hexane. The extract in the test tube was washed three times with 10 mL of 10% v/v ethanol solution. The extract solution was then dried with anhydrous sodium sulphate and the solvent was evaporated. A sample of the extract was then made soluble in 100 µL of pyridine of which 20 µL was transferred into a vial on the Gas Chromatography machine for phytochemical analysis. The GC-FID phytochemical analysis was performed on a BUCK M910 Gas Chromatograph (GC) (BUCK Scientific, USA), equipped with a flame ionization detector (FID) [15].

## STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 22 was used to process and analyze the data obtained. Values were expressed as means  $\pm$  standard error mean (SEM).

## RESULTS

Results of the proximate composition of *Centella asiatica* leaves are contained in Table 1. Carbohydrate (43.81  $\pm$  0.70%) was

highest followed by crude fiber (17.00 ± 1.87%) and ash (16.55 ± 0.45%) contents.

**Table 1:** Proximate composition of *Centella asiatica* leaves.

Proximate Compositions	Concentration (%)
Moisture	13.10 ± 1.07
Ash	16.55 ± 0.45
Protein	8.35 ± 1.28
Lipid	1.20 ± 0.10
Fiber	17.00 ± 1.87
Carbohydrate	43.81 ± 0.70

Values are presented as mean ± standard error mean (SEM) of replicate determination

Results of the physicochemical properties of the *Centella asiatica* leaves are presented in Table 2. The saponification value was highest (238.43 mg/kOH) while the free fatty acid content had the least value (0.56%).

**Table 2:** Physicochemical composition of *Centella asiatica* leaves.

Parameters	Concentration
Saponification value (mg/KOH)	236.43 ± 2.13
Peroxide value (mEq/kg)	26.80 ± 0.13
Acid value (%)	1.12 ± 0.10
Free fatty acid (%)	0.56 ± 0.01
Iodine value (g I <sub>2</sub> /100 g)	62.60 ± 1.07
Refractive index(at 40°C)	1.42 ± 0.11
Viscosity (Pa.S)	0.99 ± 0.00
Density (g/mL)	0.97 ± 0.04
Thiobarbituric acid (mg/kg)	1.92 ± 0.05

Data presented as mean ± standard error mean (SEM) of replicate determination

The fatty acid composition of *Centella asiatica* leaves is shown in Table 3. The leaves contained a considerable amount of fatty acids with 78.48% saturated and 21.53% unsaturated. The predominant fatty acids are palmitic acid (55.70%), linoleic acid (17.50%) and lauric acid (13.73%). Whereas the highest saturated fatty acid was palmitic acid (55.70%), linolenic acid (17.50%) was the highest unsaturated fatty acid. Myristic acid was found in trace amount of 0.50%.

**Table 3:** Fatty acid composition of *Centella asiatica* leaves.

Fatty acid	Concentration (%)
<b>Saturated fatty acids</b>	
Myristic acid (C14)	0.50
Palmitic acid (C16)	55.70
Stearic acid (C18)	8.55
Lauric acid (C12)	13.73
Total	78.48
<b>Unsaturated fatty acids</b>	
Oleic acid (C18: 1)	ND
Arachidonic acid (C20: 4)	ND
Arachidic acid (C20)	ND
Linolenic acid (C18: 3)	4.03
Linoleic acid (C18: 2)	17.50
Total	21.53

The amino acid composition of *Centella asiatica* leaves is contained in Table 4. The results indicated high levels of the glutamate (13.389 g/100 g), Histidine (11.64 g/100 g), Lysine (9.72 g/100 g), Isoleucine (9.56 g/100 g), Aspartate (9.37 g/100 g) and phenylalanine (8.49 g/100 g). The results also showed higher amount of essential amino acids compared to the nonessential amino acids.

**Table 4:** Amino acid composition of *Centella asiatica* leaves.

Amino acid	Concentration (g/100 g)
Valine	4.48
Threonine	3.68
Isoleucine	9.56
Leucine	6.48
Lysine	9.72
Methionine	1.50
Tryptophan	1.12
Phenylalanine	8.49
Histidine	11.64
Glycine	3.79
Alanine	4.68

Serine	5.88
Proline	3.07
Aspartate	9.37
Tyrosine	7.79
Cysteine	1.42
Glutamate	13.39

Arginine	6.55
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Gas chromatogram of the ethanol extract of *Centella asiatica* leaves is presented in Figure 1. Results of the quantitative analysis revealed the presence of steroids (18.90 µg), phenols (18.87 µg), proanthocyanin (11.96 µg) and rutin (11.89 µg), while kampferol (0.73 µg) had the lowest value (Figure 2).

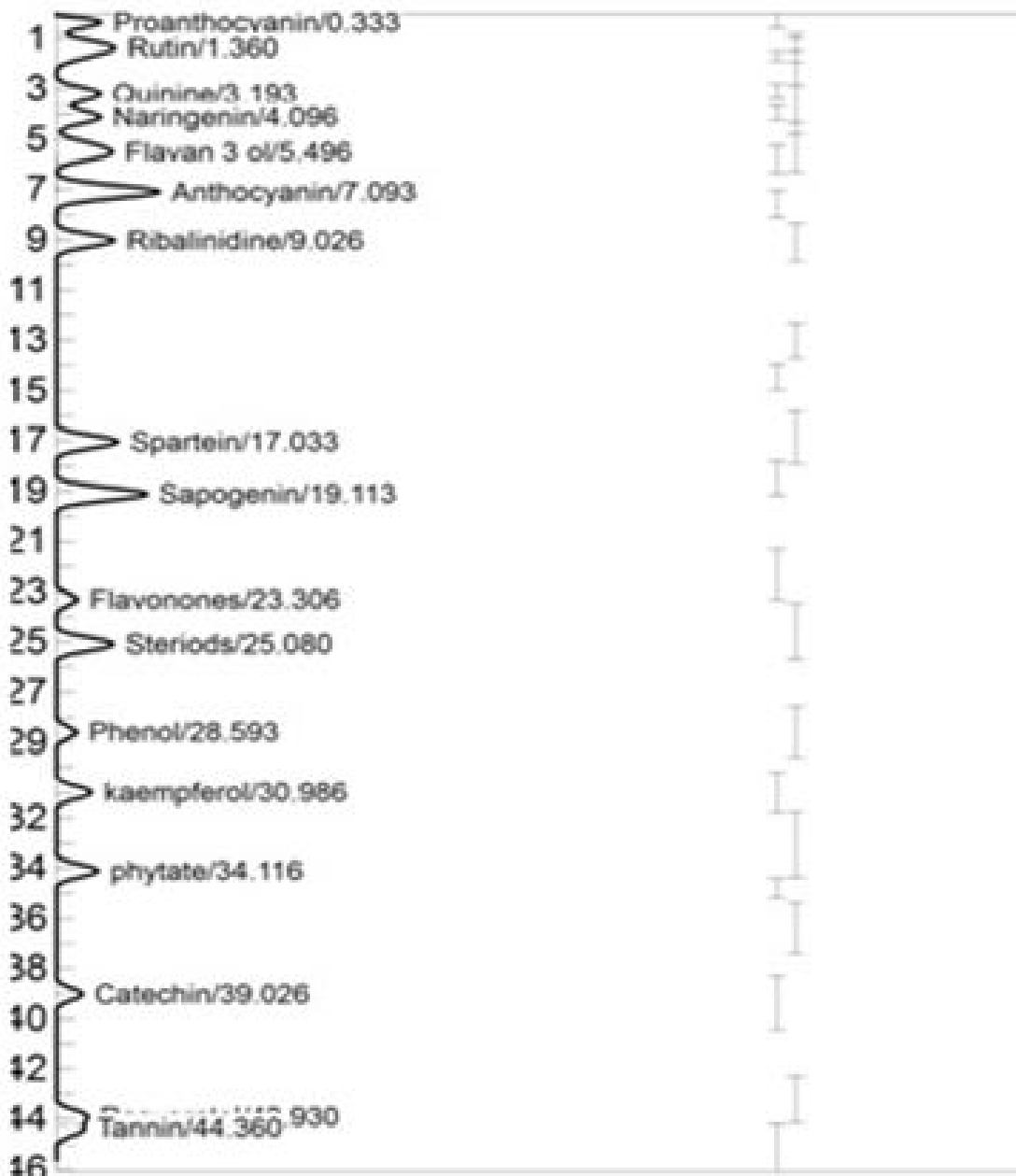


Figure 1: Chromatogram showing phytochemical constituents.

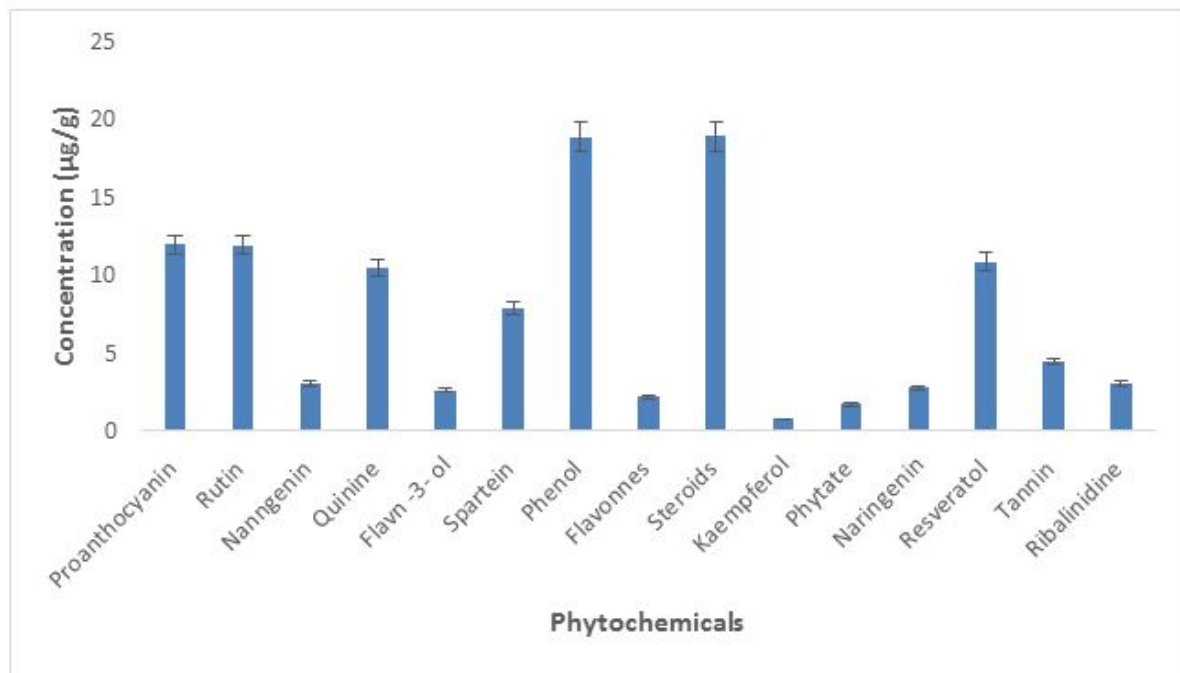


Figure 2: Phytochemical composition of *Centella asiatica* leaves.

## DISCUSSION

The proximate analysis data indicated that *C. asiatica* leaves contained an appreciable amount of carbohydrate, crude fiber and ash. The result suggests that carbohydrate is the major nutrient contained in the leaves. The high content of carbohydrate and ash recorded in this study agree with the report by Mertz et al. [16] on different species of *Centella asiatica* grown in Madagascar with carbohydrate content ranging from 42.9-52.0% and ash content ranging from 13.5-16.4%. Ogunka-Nnoka and Nwabueze, [17] and Gopalan et al. [18] reported carbohydrate content of 38.48% in *Jatropha tanjorensis* leaf-stalk and 20.0 to 66.8% carbohydrate in some conventional Indian leafy vegetables respectively. Carbohydrates provide the body with the necessary energy required to drive cellular metabolism as well as a raw material for many industries. The high ash content is an indication of good sources of minerals for human nutrition. The substantial amount of fiber shows that the leaves of *Centella asiatica* can help in keeping the digestive system healthy by removing potential toxicants from the body and prevents the absorption of excess cholesterol. It also adds bulk to the diet and prevents the intake of excess starchy foods [19]. There were variations in the amount of fiber, ash, protein and moisture contents of the present study when compared to the reports of Mertz et al. [16].

The physicochemical properties of the *Centella asiatica* leaves revealed high saponification, iodine and peroxide values. The saponification value in this study was higher than the value for *Ipomoea involucreta* leaf (196.50 mg KOH/g) and *Conarium indicum* nut oil (175.47 mg KOH/g) but comparable to coconut oil (246.28 mg KOH/g) as reported by Opene et al. [19], Rehman et al. [20] and Rahman et al. [21] respectively. Oils with this level of saponification value will serve useful purpose in the soap making industries [22]. The iodine value of oil is a measure

of its unsaturation and is a useful criterion for purity and identification. The oil from *Centella asiatica* is regarded as non-drying because the iodine value is below 100 I<sub>2</sub>/100 g [21]. The low peroxide value of *Centella asiatica* oil (Table 2) indicates that the oil is stable. The low free fatty acid content is indicative of the low enzymatic hydrolysis; this could be advantageous as oil high free fatty acid develops off flavour during storage. Free fatty acid and peroxide values are a valuable measure of the oil quality [23]. The refractive index of oils is an important optical parameter that analyzes the light rays traversing through materials medium and can be used as a tool to determine adulteration of oils [24].

The major saturated fatty acids in *Centella asiatica* oil were palmitic and lauric acids and the predominant unsaturated fatty acid was linoleic acid followed by linolenic acid. Palmitic acid was the most abundant saturated fatty acid found in the leaves of *Centella asiatica*, this corresponds to what was reported for leaf-stalk of *Jatropha tanjorensis* [17]; although the value reported for *Jatropha tanjorensis* was lower than that of the Present study.

Palmitic acid has been reported to increase cholesterol level [25]. On the contrary, Davis, [26] demonstrated that palmitic acid has no hypercholesterolemic effect if the intake of linolenic acid is greater than 4.5% of energy. Lauric acid as the next predominant saturated fatty acid is effective in preventing tooth decay [27]. Linoleic fatty acid (omega-6) is used for reducing the risk of heart disease, lowering total cholesterol levels, low density lipoprotein level, raising High density lipoprotein levels and reducing cancer risk in humans [28].

Amino acids are building blocks of proteins in muscle fibers and other structures in the body. They help to transport nutrients, prevent illness and perform other functions. Its deficiency can result in decreased immunity, digestive problems, depression, slowed growth in children and many other health issues [29].

The amino acid profile of the leaves of *Centella asiatica* showed ten essential amino acids including histidine and arginine and eight non-essential amino acids. The highest essential amino acid found was histidine followed by lysine, Isoleucine and phenylalanine; while glutamate and aspartate were recorded as highest in the nonessential amino acids category (Table 4). The results obtained in the present study were lower compared to the values obtained for these amino acids as reported by Mertz et al. [16] for the *Centella asiatica* from Madagascar; but higher than the values (except for glutamate) obtained by Ogunka-Nnoka and Nwabueze, [17] for leaf-stalk of *Jatropha tanjorensis*. Histidine facilitates growth, the creation of blood cells and tissue repair. It also helps to maintain the special covering over nerve cells, which is called the myelin sheath [29]. Lysine plays a vital role in building muscle, maintaining bone strength, aiding recovery from injury and regulating hormones, antibodies and enzymes. It may also have antiviral effects [30]. Isoleucine helps in wound healing, immunity, blood sugar regulation and hormone production [31]. Phenylalanine helps the body to use other amino acids as well as proteins and enzymes. It is needed in treating brain disorder and the normal functioning of the central nervous system. Its deficiency can lead to poor weight gain [32]. Glutamate as a nonessential amino acid plays critical roles in nutrition metabolism and signalling. Post-translational carboxylation of glutamyl residues increases their affinity for calcium and plays a major role in haemostasis [33]. Aspartate was found to be very important in the functioning of RNA and DNA, as well as in the production of immunoglobulin and antibody synthesis. It also helps the body to promote a robust metabolism and in the treatment of depression and fatigue [34]. The leaves of *Centella asiatica* may serve as nutritional supplement in addressing nutritional and health related issues.

Phytochemical analysis is very useful in the evaluation of active biological components of plants with medicinal value. Steroids, phenols, proanthocyanin and rutin were high in the *Centella asiatica* leaves compared to the other phytochemical components (Figure 2). Steroids are important in wound recovery; improve athlete's performance and other health promoting effects. Prolonged intake of steroids can lead to risk of liver and kidney diseases as well as prostate cancer, high blood pressure and elevated levels of cholesterol leading to heart disease [34]. Phenolic compounds can be a major determinant of antioxidant potentials of food plants and therefore, a natural source of antioxidants [35]. Due to its antioxidant property, phenols have the ability to scavenge free radicals and neutralise its effect thereby preventing cardiovascular diseases and cancer [36]. Rutin a flavonoid has been shown to have anti-ulcer, antibacterial, antiviral, and anti-thrombosis activities [34]. Kaempferol as an alkaloid has been shown to have anti-ulcer, anti-inflammatory, antiviral and anti-cancer activities. Alkaloids generally play some important metabolic role in living organisms, causing some physiological changes and are involved in protective function in animals [37]. The presence of these phytochemicals in *Centella asiatica* leaves shows that it could serve important physiological function with good nutritional and therapeutic value.

## CONCLUSION

The result of the present investigation revealed that *Centella asiatica* leaves are rich sources of nutrients such as carbohydrate, crude fiber, ash and proteins. Most of the amino acids found were essential amino acids while the physiochemical properties revealed high saponification value and stability to rancidity. The leaves are rich in bioactive components that possess wide range of biological activity and therapeutic value.

## DISCLOSURE

Authors have no conflict of interest.

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