IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 5 issue 2 May, 2026 Research Article

Evaluation of Nutrients, Phytochemicals and Fungal Pathogens that Deteriorate Gotu kola (*Centella asiatica*)

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ABSTRACT

Centella asiatica is an under-exploited plant rich in nutrients and phytochemicals but are prone to fungal pathogens that cause spoilage and reduce the shelf-life of the vegetable. Therefore, evaluation of nutrients, phytochemicals and fungal pathogens that deteriorate Gotu kola (*Centella asiatica*) was conducted in the Department of Plant Science and Biotechnology and Food Science and Technology of Rivers State University Port Harcourt. Fresh leaves of *C. asiatica* were obtained from Igwuruta, Rivers State. Nutrient and phytochemical compositions were done in triplicates using standard methods. Statistical analysis was done using one-way Analysis of Variance (ANOVA). The result of proximate composition revealed the contents as; moisture (20.43± 1.05%), ash (5.13 ± 0.05%), protein (16.16 ± 0.28%), lipid (7.53± 0.05%), fiber (4.63 ± 0.05%) and carbohydrate (46.67 ± 1.15%). Mineral composition result showed potassium (125.33 ± 0.57mg/100g) to be the highest and least (5.03 ± 0.05) for Iron. Vitamin composition showed vitamin A (1.23 ± 0.05mg/100g) and thiamine (0.01± 0.00mg/100g). Phytochemical result revealed presence of glycoside, oxalate, tannin, carotenoid, polyphenol, flavonoid and lignan. This study also identified three fungal isolates namely *Fusarium* sp, *Collectrochium* sp, and *Guignardia* sp. *Centella asiatica* is rich in nutrients and bioactive phytochemicals and therefore should be incorporated into meals. Proper care should be taken during handling to reduce fungal contaminations.

Keywords: Gotu Kola, Nutrients, Proximate Composition, Phytochemicals, Fungal Pathogens.

Introduction

Centella asiatica, commonly called Gotu kola or pennywort is an herbaceous tender perennial plant of the family Apiaceae (Das, 2011, Brinkhaus *et al*., 2000). It originated from India and other parts of Asia such as China, Sri Lanka, Nepal and Madagascar and has been used in traditional medicines in different countries. It grows well in damp, marshy and wet places and it is abundant in tropical regions (Kokate *et al.,* 2001).

Gotu kola is a slender creeping plant with stems but are green to reddish green in colour, have long stalked, green leaves with rounded apices. The root stock consists of rhizomes which grow vertically down and are covered with root hairs (Wahundeniya, 2007). Gotu kola is cultivated through cuttings, roots and stolon and through seedlings, which are best started in the nursery flats (Jamil *et al*., 2007).

The crop matures after 90 days of planting and harvested through hand cutting when the leaves are fully grown (Brinkhaus *et al*., 2000). The plant is rich in essential minerals and contains a broad spectrum of phytonutrients that provide a range of beneficial effects (Chandrika *et al*., 2015). Major pests of gotu kola include aphids, whiteflies, caterpillars and spider mites and diseases include bacterial wilt (Sharma and Singh, 2018; Kumar and Singh, 2000), and Leaf spot (Verma *et al*., 2016; Kaur and Kaur, 2018).

The plant has been known to exhibit antioxidant properties (Jayashree *et al*., 2003; Jaswir *et al*., 2004 and Subathra *et al*., 2005), antidiabetic (Chauhan *et al*., 2010; Kabir *et al*., 2014), antimicrobial (Ullah *et al*., 2009); Seevaratnam *et al*., 2012), neuroprotective (Subathra *et al*., 2005, Lee *et al*., 2000; Soumyanath *et al*., 2005), cytotoxic and anti tumour (Bunpo *et al*., 2004).

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It is also anti-inflammatory (Guo *et al*., 2004), antiviral and antiprototozoa (Yoosok *et al*., 2000; Singh *et al*., 2010) and memory enhancing effect (Rao *et al*., 2005; Nasir *et al*., 2011).

Therefore, the aim of this research is to determine the nutrients, phytochemicals and fungal pathogens that deteriorate Gotu kola.

Materials and Methods

Collection of Samples

Fresh leaves of *Centella asiatica* (Gotu kola) were identified by Professor M.G Ajuru in the Department of Plant Science and Biotechnology, Rivers State University Port Harcourt. The plant was harvested around Igwruta town in Ikwerre Local Government Area in Rivers State, Nigeria. The plant leaves were divided into two, and a part was sent to Department of Food Science and Technology for Proximate analysis and Phytochemical Composition analysis while the other part was kept for isolation and identification of fungi.

Sterilization of Glass wares for Fungi Isolates

All the glass wares were first soaked and washed thoroughly with tap water and detergent solution and then rinsed with several changes of distilled water in order to completely remove traces of detergent. The glass ware were Air dried completely before sterilizing them in steam oven at temperature 121 degrees Celsius for 15 minutes and then allowed to cool down at room temperature before usage. The entire working surface was also disinfected with 95% according to the method of Wekhe *et al.,* (2022) to reduce contamination.

Isolation, Subculturing and Identification of Pathogens in *Centella asiatica* (Gotu kola)

Isolation of the fungi in Centella asiatica (Gotu kola) was carried out as described by Obire *et al*., (2016) and Chuku (2009). The infected Gotu kola leaves showing disease symptoms were first washed and surface sterilized with 10% ethanol using a cotton wool. Four small pieces from the margin of lesion of each sample were directly inoculated aseptically onto freshly prepared plates of Sabaraud Dextrose Agar (SDA) and incubated at 28ºC for 3 to 5 days. When fungal growth from the tissue was visible, fungi were sub-cultured onto SDA to obtain pure cultures for identification.

Pure cultures were safely refrigerated at 4ºC to prevent any fungal growth in the plates. Identification of pure fungal isolates was based on growth patterns, colour of mycellia and microscopic examination of vegetative and reproductive structures. For each microscopic examination a streak of fungal mycelium was placed on a clean grease free glass slide. One drop of lactophenol cotton blue was added and the cover slip placed on it. The slide was mounted on the microscope and observed at magnification of X10 and X40. Morphological characteristics of fungi isolated were determined and identified with the aid of identification guide (Giraldo and Crous, 2019).

Proximate Composition Determination

Proximate composition (moisture, ash, protein, lipid, crude fibre, and carbohydrate) of healthy leaf sample was determined using standard analytical methods AOAC (1990).

Determination of Minerals

Mineral analysis was carried out after wet digestion with a digestion mixture containing concentrated nitric acid and concentrated tetra-oxosulphate (VI) acid in a ratio of 3:1 (ASTM, 2004).

Determination of Vitamins

The vitamins in the species studied were determined by the official methods of the Association of Official Analytical Chemists (AOAC, 1990).

Determination of Phytochemicals

Phytochemical analyses were conducted to determine the presence of Glycoside, oxalate, tannin, carotenoid, polyphenol, flavonoid and lignan of Gotu kola. This was determined gravimetrically using the method described by AOAC, (2005).

Results

The result of Proximate Composition of Gotu kola in Table 1 shows a moisture content of 20.43±0.05%, Ash (5.13±0.05%), lipid (7.53±0.05), fibre (4.63±0.05%), carbohydrate (46.67±1.15%) and protein (16.16±0.28%). Moisture had the highest value, followed by carbohydrate and the least was fibre.

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Table 1: Proximate Composition of Gotu kola

Parameter	Composition (%)
Moisture	20.43±0.05
Ash	5.13±0.05
Lipids	7.53±0.05
Fibre	4.63±0.05
Carbohydrate	46.67±1.15
Protein	16.16±0.28

The result of mineral and vitamin composition of Gotu kola presented in Table 2 reveals the presence of calcium 36.60±0.55, iron 5.03±0.05, magnesium 31.33±0.57, phosphorus 25.00±0.00, potassium 125.33±0.57, sodium 24.33±0.57, vitamin A 1.23±0.05, thiamine 0.01±0.00mg/100g. Potassium recorded the highest value for minerals, while iron had the least.

Table 2: Mineral and Vitamin Composition of Gotu Kola

Parameter	Composition (mg/100g)
Calcium (Ca)	35.60±0.55
Magnesium (Mg)	31.33±0.57
Phosphorus (P)	25.00±0.00
Potassium (K)	125.33±0.57
Sodium (Na)	24.33±0.57
Iron (Fe)	5.03±0.57
Vitamin A	1.23±0.05
Thiamine	0.01±0.00
Chlorophyll	0.30±0.00

The result for Phytochemicals of Gotu kola in Table 3 shows glycosides 0.03±0.00%, oxalate 0.01±0.00%, tannin 0.03±0.00%, carotenoid 14.26±0.02%, polyphenol 7.82±0.02%, flavonoid 5.48±0.02%, lignan 8.50±0.00%.

Table 3: Phytochemical Composition of Gotu kola

Parameter	Composition (%)
Glycoside	0.03±0.00
Oxalate	0.01±0.00
Tannin	0.03±0.01
Carotenoid	14.26±0.02
Polyphenol	7.82±0.02
Flavonoid	5.48±0.02
Lignan	8.50±0.00

Fungal isolates of *C. asiatica*

The result of the fungal characterization of Gotu kola presented in Table 4 revealed *Fusarium* sp, *Collectrochium* sp and *Gingnardia* sp respectively.

Table 4: Fungal Characterization

Fungal Isolate	Macroscopic Examination	Microscopic Examination	Probable Organism
Isolate 1	White to cream cottony colonies	Septate and branched hyphae. Macroconidia septate, fusiform microconidia oval to kidney shape	*Fusarium* sp
Isolate 2	White to greyish in colour	Septate and branched hyphae with ovoid to cylindrical and single celled conidia	*Colletrotrichium* sp
Isolate 3	Slow growing white glucose aerial mycelium	Branched and septate hyphae with ovoid conidia	*Guignardia* sp

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Discussion

The result of the proximate composition of Gotu kola (*Centella asiatica*) in this study revealed that large amount of carbohydrate 46.67±1.15%, protein 16.16±0.28%, moisture 20.43±0.05%, were found in *Centella asiatica*. The result indicates that the key nutrient found in the plant was carbohydrate. The high carbohydrate content reported in this study agrees with the report by Mertz *et al,* (2019) on different species of

Centella asiatica grown in Madagascar with carbohydrate content ranging from 42.9-52 %. Ogunka-Nnoka and Nwabueze, (2019) 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 moisture content of this study recorded 20.43%, protein content of 16.16%, lipid 7.53%. This result is higher than 13.10% of moisture content, 1.20% lipid content and protein content 8.35% reported by Ogunka-Nnoka *et al,* (2020). The fiber content of this study recorded 4.63%. The result is closer to the fibre content (5.92%) reported by Joshi and Chaturvedi, (2013). *C. asiatica* is a good source of dietary fibers which is a significantly important nutrient component. Dietary fiber intake provides many health benefits. A generous intake of dietary fiber reduces risk of developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal disorders, and provides many other health benefits (Anderson *et al.,* 2009).

The current study revealed different mineral elements in Gotu kola, however the highest mineral value recorded was 125.33±0.57 mg/100g for potassium and least mineral element 5.03±0.05 was recorded for iron. This study reported lower mineral elements compared to the values obtained from Joshi and charverdi (2013). Chandrika *et al,* (2011) also reported the presence of mineral contents of different morphotypes of *C. asiatica*. In general, *C. asiatica* contains a high concentration of potassium and calcium. Potassium intake reduces the risk of stroke, kidney stones, renal damages, and many heart-related problems. Calcium is also an important structural component of bone. Adequate calcium intake throughout childhood and adolescence is needed to achieve maximum bone mass in young adulthood which is an important determinant of bone mineral status in later life. *C. asiatica* can be used as a non-expensive nutritional source of both potassium and calcium. (Joshi and Chaturvedi, 2013). The vitamin results revealed thiamine (0.01±0.05 mg/100g) while vitamin A, recorded (1.23±0.05mg/100g). Vitamin A obtained in this study was higher compared to (0.39mg/100g and 0.4mg/100g) by Ajayi *et al.,* (2020) and Hashim, (2011) respectively.

In the evaluation of active biological components of plants with medicinal value, phytochemical analysis is very useful. The phytochemical Composition of *Centella asiatica* is shown in Table 4.3. Glycoside recorded 0.03±0.00, oxalate 0.01±0.00, Saponin 0.00±0.00 (absent), tannin 0.03±0.01, carotenoid 14.26±0.02, polyphenol 7.28±0.02, flavonoid 5.48±0.02, lignan 8.50±0.00. Literatures have shown the occurrence of this Phytochemical in several plants (Wekhe *et al.,* 2020, 2021 and 2022). The presence of flavonoid in *Centella asiatica* supports the finding of Das (2011), who observed flavonoids derivative in *Centella asiatica* leaf. Chandrika *et al,* (2011) also reported the presence of carotenoid in *Centella asiatica.* He analyzed carotenoid composition of different morphotypes of *C. asiatica* leaves. Carotenoids are one of the most important phytonutrients found in *C. asiatica*. Carotenoids, the colorful plant pigment, of which the body can turn some into vitamin A, are powerful antioxidants that can help prevent some forms of cancer and heart disease and act to enhance your immune response to infections. Chandrika *et al.,* (2011). Phytochemical composition of *C. asiatica* is also comparable to the activity of rosemary and sage and has been identified with high potential to be explored as a source of natural antioxidants (Jaswir *et al,* 2004). According to Zainol *et al.,* (2003), *C. asiatica* leaves recorded the highest antioxidant activity which also contains highest phenolic contents, when compared to other plant parts. This result suggested that phenolic compounds are the major contributors to the antioxidative activities of *C. asiatica*. The presence of these phytochemicals in *Centella asiatica* suggests that, with good nutritional and therapeutic value, it could serve significant physiological function.

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The results of this study revealed three fungal organisms *(Fusarium* sp, *Collectrochium* sp., and *Guignardia* sp*)* to be responsible for the spoilage of Gotukola. This seems to be in line with the submission of Rakotoniriana *et al.,* (2008) who identified endophytic fungi from leaves of *Centella asiatica*. *Guignardia sp* is a genus of ascomycete fungi. They are found as endophtes of plants, but some species can also be pathogens. *Guignardia* species are responsible for a variety of diseases in plants including fruit rot and leaf spot. Fungal pathogens responsible for the deterioration of Gotukola can also affect the quality and appearance of Gotukola leaves (Rakotoniriana *et al.,* 2008, Wekhe *et al.,* 2026a and b).

Conclusion

In conclusion, this study revealed that Gotu kola (*Centella asiatica*) has nutritional value since it is a good source of carbohydrates and protein, as well as a great source of minerals. This study showed that *Centella asiatica* had high content in carotenoid, polyphenol and lignan. Moreso, the presence of these phytochemicals in the *Centella asiatica* leaves could be used in the chemical and pharmaceutical industries. However, it also faces the challenge of spoilage by prevailing fungal pathogens hence; it should be hygienically preserved to prevent microbial contaminations.

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