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Unique Research Journals
Unique Research Journal of Medicine and Medical Sciences Vol. 4(5), pp. 029-034, September, 2016
Available online@http://www.uniqueresearchjournals.com/URJMMS
ISSN 2333-6935 ©2016 Unique Research Journals
Unique Research Journal of Medicine and Medical Sciences

Full Length Research Paper

Phytochemical screening and proximate analysis of young *Cola acuminata* leaves

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Accepted 30 September, 2016

Oxidative stress and impaired antioxidant system have been implicated in the pathophysiology of diverse disease states. Phytochemical screening and proximate analysis of dried young cola nitida leaves used in folklore medicine were carried out. The result revealed the presence of bioactive components comprising of alkaloids, phenolic acid, carotenoids, and flavonoid. Quantitative phytochemical analyses showed that alkaloid have the highest concentration. Proximate analysis of the sample showed the moisture content of the sample is (23.10±0.12), carbohydrate (36.80±0.25), crude fibre (19.20±0.1), crude protein (13.50±0.10), and ash content (6.70±0.07). The presence of secondary metabolites in this plant is indicative that if well researched, novel bioactive compounds can be discovered in it.

Key words: *Cola nitida* leaves, alkaloids, phenolic acid, carotenoids, flavonoid.

INTRODUCTION

The study of medicinal herbs in the treatment and prevention of diseases have attracted the attention of scientist worldwide (Sofowora, 1982). This is corroborated by world health organization (WHO) in its quest to bring primary health care to people. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants (Gamaliel, 2000). The prevalence of bioactive components such as flavonoids, alkaloids, carotenoids, phenolic acids etc. gives credence to continuous search for bioactive and active ingredients extracted from plants (Newall et al., 1996).

Cola, a tropical African genus of the family steruliaceae, comprises about one hundred and twenty five species. Cola species are evergreen. C*ola acuminata* have the greatest economic importance of all the species. The leaves of cola species are very simple, entire and narrowed or rounded towards the base. The arrangement of the leaves on the stem is alternate in some species and verticulate in whorls of three or more in others (Ayensu, 1975).

Proximate analysis, also known as Weende analysis is a chemical method of assessing and expressing the nutritional value of a sample, which reports the moisture, ash (minerals), crude fibre, crude fat and crude protein (total nitrogen) present in a sample as a percentage of dry weight. Carbohydrate was determined by difference. The proximate analyses

give the overall nutritional composition of the sample.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interplay with nutrients and dietary fiber to protect them. Recent researches demonstrate that they can protect humans against diseases as well as, in risk reduction for a variety of chronic or inflammatory conditions.

Research work has been done on the fruits of both *Cola nitida* and *C. acuminata* but little has been done on the leaves of these economically important plants. Traditionally, the leaves, trigs, flowers, fruits follicles and the bark of both *C. nitida* and *C. acuminata* were used to prepare a tonic as remedy for dysentery, coughs, diarrhea, and vomiting; though the traditional usage has not been authenticated by scientific research.

MATERIALS AND METHODS

Fresh young leaves of *C. acuminata* were obtained from Igede-Ekiti in Irepodun/Ifelodun local government area of Ekiti State, Nigeria. The plant was authenticated in the Department of Botany, Ekiti State University, Ado-Ekiti. The leaves were washed with distilled water and air-dried at room temperature before it was milled into a powder.

Qualitative phytochemical analyses

Extraction process was carried out by using Soxhelt extraction method. 50 g of pulverized *C. acumunata* leaves was weighed into a reflux apparatus containing 300 ml of distilled water. Electronic hot plate was set to the boiling point of water. The extraction was carried out between 8-10 hours until extraction was completed. The extract was then kept in an air tight container and kept in a fridge for preservation. The presence of alkaloid and flavomoids were determined by the method of Harborne (1973), saponin was determined by the presence of Trease and Evans (1996).

Quantitative phytochemical analysis

Phytochemical analysis was carried out quantitatively to determine the quantity of secondary metabolites in the young *C. acuminata* leaves. Alkaloids, carotenoids, flavonoids and phenolic acid were extracted and quantitatively determined using gas chromatography.

Alkaloid extraction

Alkaloid extraction was carried out by the modified method of Ngounou et al. (1995). 5.0 g of the pulverized sample was soaked in 25 ml of hexane for about 72 h. The extract was filtered and the residue was air-dried and later treated with 10% aqueous NH_3 and macerated in chloroform for 24 h. It was filtered and the filtrate was treated with 5% HCl. The aqueous phase was made alkaline with aqueous NH_3 and extracted thrice with chloroform. The chloroform fraction was washed with water and concentrated using a rotary evaporator. The concentrated extract was dried of water by using anhydrous sodium sulphate before gas chromatography analysis.

Carotenoid extraction

Carotenoid extraction was carried out by the modified method of Shigeaki (1985). 5.0 g of pulverized sample was soaked in acetone and kept at room temperature for 1 h in the dark before filtration. Extraction was repeated thrice with the same volume of acetone. The extracts were combined and the residue was re-extracted by a mixture of diethyl ether and petroleum ether in equal ratio. The extract was concentrated using a rotary evaporator. Analysis was done using gas chromatography

Flavonoids/phenolic extraction

Two stage extraction procedures were followed for the effective removal of the polyphenols/phenolic compounds.

Stage 1: 5.0 g of the sample was extracted with 25 ml of 1 M NaOH for 16 h on a shaker as described by Kelly et al.

(1994) and Provan et al. (1994). The sample was centrifuged at 5000 × g. the supernatant was placed in a glass test tube and heated at 90°C for two hours to release conjugated phenolic compounds as supported by Whitehead et al. (1983). The heated extract was cooled, titrated with 4 M HCl to pH <2.0 and diluted to 10 ml with deionized water. The diluted extract was centrifuged to remove the precipitate, the supernatant was saved for subsequent purification and the residue was extracted further in stage 2

Stage 2: The residue from stage 1 was extracted with 4 M NaOH, heated to 160°C as described by Provan et al. (1994). It was cooled and filtered. The supernatant was collected and the residue was washed with water. The supernatants from stage 1 and 2 were combined and adjusted to pH <2 with 4 M HCl.

Derivatisation

Following the extraction steps, the concentrated extract of about 2ml in the gas chromatography vial was derivatized by adding 20 µL of bis (trimethylsilyl) trifluoroaceamide (BSTFA). The silicon septum corked vial was lowered into the water bath with hanger to stand upright in the water bath with a magnetic stirrer at 45°C for 10 min.

Proximate analysis

Crude fat, crude fibre, ash and moisture content of *C. nitida* leaves were determined according to the methods of association of Official Analytical Chemists (AOAC, 1997); Nitrogen was determined by Kjeldal method (Pearson, 1976). Protein value was obtained by multiplying the nitrogen value by 6.25. Carbohydrate was determined by the difference between the parameters

RESULTS AND DISCUSSION

The result of qualitative phytochemical analysis showed the presence of secondary metabolites. These secondary metabolites are responsible for the pharmacological potentials of medicinal plants. The presence of these phytochemicals proves that young leaves of *C. acuminata* can be used in the treatments of various diseases. Phenols are well known for their antioxidant activities, high content of phenols in this plant shows it will be able to eliminate free radicals that are liable to cause various diseases in the body (Table 1).

Table 1. Qualitative phytochemical analysis of young *C. nitida* leaves.

Constituents	Presence
Phenols	Positive
Alkaloids	Positive
Tannins	Positive
Saponins	Positive
Flavonoids	Positive
Carotenoids	Positive

Phytochemicals are chemical compounds that occur naturally in plants, some are responsible for color and other organoleptic properties. Phytochemicals have been shown to possess free radical scavenging potential. Saponins are hypoglyceamic, antifungal and antihyperlipideamic agents in animals (Sapna et al., 2009). They play a prominent role in ensuring hormonal balance and synthesis of sex hormones (Okwu, 2003) (Table 2).

Quantitative analysis of phenolic acid content of young cola acuminta leaves revealed high presence of chlorogenic acid, caffeic acid and quinic acid. Phenolic acid plays a protective role against oxidative damage diseases. Epidemiological studies have suggested that diets rich in phenolic compound may have preventive effects on the development of dementia or Alzheimer's disease (AD) (Dai et al., 2006) (Table 3).

The alkaloids level of young *C. acuminata* leaves shows that the sample can be used as a CNS stimulant and as a powerful pain reliever (Stray, 1998). The high concentration of caffeine detected in this plant shows it can be used as a good source of caffeine especially for some flavoured drinks (Table 4).

Table 2. Quantitative analysis of phenolic acid component.

Constituent	Quantity (mg/100 g)
Chlorogenic acid	229.49
caffeic acid	111.61
Quinic acid	80.30
Syringic acid	0.19
Protocatechinic acid	0.07
Ellargic acid	0.05
P-coumaric acid	0.03
Rosmarinio acid	0.01

Table 3. Quantitative analysis of alkaloid content of young *C. nitida* leaves.

Constituent	Quantity (mg/100 g)
Caffeine	350.60
Theobromine	84.42
Theophylline	46.39
Nitidine	40.05
Akuammidine	6.47
Trigonelline	4.71
Vocangine	2.00
Echitammidine	0.05

Table 4. Quantitative analysis of flavonoid content of young *C. nitida* leaves.

Constituent	Quantity (mg/100 g)
Catechin	10.54
Epicatechin	5.62
Isoquercetin	2.51
Isohamnetin	2.51
Quercetin	2.17
Kaemferol	1.30
Luteolin	0.48
Apigenin	0.35

Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage; they also have a strong anti-cancer activity (Okwu, 2006) Flavonoids modulate lipid peroxidation which is a major factor in oxidative damage (Shi et al., 2006) (Table 5).

Table 5. Quantitative analysis of carotenoid content of young *C. nitida* leaves.

Constituent	Quantity (mg/100 g)
Lutein	18.62
Viola-Xanthin	6.28
Beta-carotene	4.28
Neo-xanthin	1.03
Malvidin	0.01
Beta-Cryptoxanthin	0.01

Carotenoids are organic pigments found in the chloroplasts ad chromoplasts of plants. Ampids and spider mites are the only animals known to produce carotenoids; they have been shown to have protective effects against cancer (Vieira et al., 2016). They also have antioxidant properties, lutein acts directly to absorb damaging ultraviolet light in order to protect the retina (Kidd and Parris, 2011) (Table 6).

Table 6. Proximate analysis of young *C. nitida* leaves.

Carbohydrate	**36.80±0.25**
Moisture content	23.10±0.12
Crude fibre	19.20±0.11
Crude protein	13.50± 0.10
Ash	6.70±0.07
Crude fat	0.70±0.02

Proximate analysis of the young leaves of *C. acuminata* showed some diaparity with some earlier research on the seed of *C. acuminata.* Dewole et al. (2013) proximate analysis revealed a higher crude fat (3.02±0.01), protein (19.14±0.25) and carbohydrate (58.09±0.89) but a lower moisture content (9.73±0.02), ash (2.27±0.01) and crude fibre (7.30±0.02). The result obtained in this research work is however in agreement with the work of Ajai et al. (2012), Moisture (20.62), ash (2.50), fat (0.80), protein (8.65), crude fibre (3.38) and carbohydrate (64.05). The high protein, carbohydrate and ash content of the leaves could be used as a supplement to complement the body need for these compounds. The leaves will also be a good component in the formulation of feeds for livestock.

DISCUSSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Qualitative analysis showed that the leaves of *C. acuminata* contain bioactive components; it shows a high concentration of alkaloid and phenolic acid in the young leaves. Flavonoids and carotenoids are in a smaller concentration when compared with the alkaloid and phenolic acid. Quantitative analysis of the various components revealed different bioactive components and their composition. The medicinal values of this plant may be related to their constituent phytochemicals because the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value.

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