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Assay of Bioactive Constituents of *Baphia nitida*

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Abstract

The phytochemicals, minerals and vitamins compositions of *Baphia nitida* leaves were determined using standard methods of chemical analysis. The results of the determination revealed that *B. nitida* leaves contain high amount of phytochemicals which suggest that the plant leaves can be explored in drug discoveries and treatment of diseases in pharmaceutical industries. The results of the mineral compositions of the plant part showed that the plant is rich in minerals which helps in cellular metabolism. On the other hand, the plant part was found to contain high amount of Ascorbic acid and β-Carotene which as antioxidants, can help in the balancing of oxidative stress in the body.

Keywords: Bioactive, phytochemicals, minerals and vitamins.

INTRODUCTION

From time antiquity, the use of plant based materials in the treatment of diseases has been employed by man for his survival and continued existence. For decades, extracts from plants have been useful in the development of eco friendly substances used as herbicides, pesticides and pharmaceutical products (Bobbarala *et al*., 2009). The pharmacological potential of plants lie in their phytochemical (bioactive) constituents which shows various physiological effects on human body. These phytochemicals which include alkaloids, flavonoids, coumarins, tannins, terpenes, terpenoids, phenols, gums, polysaccharides, and glycosides have different physiological effects on human body such as anti-depressive, stimulative and psychoactive effects (Manjula *et al*., 2012).

These phytochemicals though produced by plants to help them resist fungi, bacteria and viral attacks as well as being eaten by predators, have found usefulness in the treatment and management of human diseases. Therefore, through phytochemical screening one could detect the various important compounds which may be used as the bases of modern drugs for curing various diseases (Sheikh, *et al*., 2013)

Medicinal plants are gaining immense interest in recent times in treating various types of diseases which are resistant to many drugs (Ajala *et al*., 2013). Traditional medicine practice plays an essential role in most developing countries' primary health care delivery system, including Nigeria. The local use of herbal medicine as a source of treatment in Nigeria cannot be disregarded due to the country's large population and low quality of our health care delivery system (Sade, 2002). Even the World Health Organization encourages the national government of its member countries to make use of their traditional system of medicine with procedures appropriate for their health care systems. As estimated by the World Health Organization, 80 % of the people that live in rural areas of developing countries depend on traditional medicine for their health needs (WHO, 2002).

Often, concortion made via the use of herbs are used for prevention and treatment of numerous diseases that are common among people living in the rural areas of developing nations. It is therefore paramount to screen medicinal plant extracts for their active principles and phytoconstituents with desirable pharmacological effects and consequently purify them as a preliminary step towards achieving drugs which may be useful in curing some of the dreaded diseases of mankind. Many drugs in the market today are of herbal origin. Exampls are; ergotamine, reserpine and vincristine which are used to treat migraine, high blood pressure and cancer, respectively (Ajaiyeoba, 2000). Among these valuable plants of therapeutic importance is *Baphia nitida* Lodd.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *Bahia nitida* were harvested from a farm in Nekede, Umudibia autonomous community in Owerri West Local Government area, Imo state and was taken to Dr Duru C. M, a taxonomist in the department of Biotechnology, Federal University of Technology Owerri for proper identification.

2.2 Sample Preparation

The collected leaves of *B. nitida* were properly washed in distilled water to remove dust particles and other dirt before air-drying for thirty days. The dried leave samples were ground into small powdery particles using a pulverizer. The weight of the ground sample determined to be 623 g.

2.3 Qualitative Phytochemical Screening

Five grams (5 g) of the crude extract was dissolved in 50 ml of 2 M HCl, boiled and filtered. The filtrate was subjected to the following phytochemical tests using the methods of Harbone (1973), Sofowora (1984), and Trease and Evans (1989);

2.3.1 Test for alkaloids (Mayer's test)

Five millilitre (5 ml) of 1 % aqueous HCl was added to 2 ml of the leaf extract and stirred in a water bath. Six drops of Mayer's reagent was then added to the filtrate. A cream coloured precipitate indicated the presence of alkaloids (Sofowora 1984).

2.3.2 Test for glycosides

One millilitre (1 ml) of the extract in a test tube was mixed with 2 ml of glacial acetic acid, after which 1 drop of 15 % ferric chloride and 1 ml of concentrated sulfuric acid were added to the mixture. A brown coloration formed at the interface indicated the presence of glycosides (Harbone, 1973).

2.3.3 Test for saponins

One millilitre (1 ml) of the extract in a test tube was mixed with 5 ml of distilled water. The mixture was shaken vigorously and observed for persistent froth which indicated the presence of saponins (Harbone, 1973).

2.3.4 Test for tannins

One millilitre (1 ml) of the extract in a test tube was heated for 5 minutes to boil. After which 2 drops of 15 % ferric chloride was added. A blue black coloration indicated the presence of tannins (Harbone, 1973).

2.3.5 Test for flavonoids

One millilitre (1 ml) of the extract was mixed with 5 ml of dilute ammonia in a test tube and 1 ml of concentrated sulfuric acid was added to the mixture. A yellow colour indicated the presence of flavonoids (Harbone, 1973).

2.3.6 Test for polyphenols

Two millilitre (2 ml) of the plant extract was treated with 5 ml of distilled water and heated in a water bath for 5 minutes. After which 1ml of 1% $FeCl_3$ was added to the solution. The formation of green-blue colouration indicated the presence of polyphenol (Trease and Evans, 1989).

2.3.7 Test for phlobatannins (aqueous hydrochloric acid test)

Two millilitre (2 ml) of the extract was boiled with 5 ml of 1% aqueous solution of HCl. Absence of red colour or precipitate indicated the absence of phlobatannins (Trease and Evans, 1989).

2.4 Determination of Vitamin Content

The vitamins (Ascobic acid, Niacin, Riboflavin, Thiamin) were determined using the methods described by Okwu and Ndu (2006).

3.4.1 Determination of ascorbic acid

Five grams of the sample was dispersed in 50 ml of EDTA/TCA (2:1) solution and homogenized. The homogenate was filtered using a filter paper and more of the extractant was used to wash the residue in the filter paper until 100 ml filtrate was obtained. A 20 ml portion of the filtrate was measured into a conical flask and 10 ml of 30 % potassium iodide solution was added to it, mixed well and then followed by four drops of 1 % starch solution. The mixture was titrated against 0.01 M $CuSO_4$ solution until a blue-black colour appeared. A reagent blank was also titrated using 20 ml of distilled water. The vitamin C content was calculated based on the relationship that 1 ml $CuSO_4$ = 0.88 mg vitamin C. Therefore,

$$\text{Vitamin C (mg/100g)} = \frac{100}{W} \times 0.88 \times (T-B) \times \frac{Vt}{Va} \quad \ldots\ldots\ldots\ldots (1)$$

Where,
W = weight of sample
T = titre value of sample
B = titre value of blank
Vt = total extract volume
Va = volume of extract titrated

3.4.2 Determination of niacin

Five grams of the sample was treated with 50 ml of 1 N sulphuric acid and shaken for 30 minutes. 3 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipetted into a 50 ml volumetric flask and 5 ml of potassium cyanide was added. This was acidified with 5 ml of 0.02 N H_2SO_4 and absorbance was read at 470 nm wavelength in an ultraviolet-visible spectrophotometer (Spectrumlab 752s). A standard niacin solution was prepared and treated as described above for the extract with the reagent blank at zero. The formula below was used to calculate niacin content;

$$\text{Niacin (mg/100g)} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va} \quad \ldots\ldots\ldots\ldots(2)$$

Where,
W = weight of sample
Au = absorbance of sample
As = absorbance of standard solution
C = concentration of standard solution
Vt = total volume of extract
Va = volume of extract analyzed.

3.4.3 Determination of riboflavin

Five grams of the sample was extracted with 100 ml of 50 % ethanol (by shaking for an hour and filtering). An aliquote of the filtrate (10 ml) was treated with equal volume of dilute potassium permanganate (5 %) and 10 ml of 30 % hydrogen peroxide solution. The mixture was heated for 30 minutes over a water bath before 2 ml of 40 % sodium sulphate solution was added to it. Meanwhile, dilute standard riboflavin solution was prepared and treated as described above for the extract. The absorbance of both the standard solution and the sample extract were read in an ultraviolet-visible spectrophotometer (Spectrumlab 752s) at a wavelength of 510 nm with the reagent blank at zero. The formula below was used to calculate the riboflavin content;

$$\text{Riboflavin (mg/100g)} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va} \quad \ldots\ldots\ldots\ldots\ldots(3)$$

Where,
W = weight of sample
Au = absorbance of sample
As = absorbance of standard solution
Vt = total volume of extract
Va = volume of extract analyzed.
C = concentration of standard solution

3.4.4 Determination of thiamin

Five grams of the sample was homogenized with 50 ml of ethanolic sodium hydroxide. It was filtered into a 100 ml flask. 10 ml of the filtrate was pipetted and the colour developed by the addition of 10 ml of potassium dichromate solution. A standard thiamin solution was prepared and treated as described above for the extract. Their respective absorbances were measured at 360 nm wavelength in an ultraviolet-visible spectrophotometer (Spectrumlab 752s) with a reagent blank at zero. The formula below was used;

$$\text{Thiamin (mg/100g)} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va} \times D \quad \ldots\ldots\ldots(4)$$

Where,
W = weight of sample
Au = absorbance of sample
As = absorbance of standard solution
C = concentration of standard solution
Vt = total volume of extract
Va = volume of extract analyzed.
D = dilution factor where applicable.

3.4.5 Determination of β-carotene

The method of AOAC (2006) as described by John (2014) was used. Ten grams of the sample was extracted in 100 ml of hot ethanol for 30 mins, with occasional shaking. The ethanol extract was put in a beaker and placed in a hot water bath, to increase the surface area. The extract was decanted and distilled water was added to bring the volume to 85 % ethanol. The warm ethanol extract was allowed to cool at room temperature. After cooling, it was shaken with 30 ml of petroleum spirit in a separating funnel, the bottom layer was run into separate beaker and the top petroleum spirit layer was put into another beaker. The bottom ethanol layer was returned back to the separating funnel and was re-extracted with petroleum spirit and this was done repeatedly

The petroleum spirit extract that contained the extracted carotenoids was returned to the separating funnel where 50 ml of the 85 % ethanol was added and shaken as in the first stage of extraction with the petroleum spirit. The lower layer of the mixture was run off to remove any xanthophylls that may have escaped into the petroleum spirit during the process. The procedure was carried out in triplicate and the extract was analyzed for β-carotene with ultraviolet-visible spectrophotometer (Spectrumlab 752s) at 480 nm. Concentration of β-carotene was gotten using the following equation;

$$\beta\text{-carotene } (\mu g/100g) = V_a \times V \times D \times 100 \times \frac{100}{W} \times Y \quad \ldots\ldots\ldots\ldots..(5)$$

Where;
V_a = Absorbance (480 nm)
V = Total volume of extract
D = Dilution factor
W = Sample weight
Y = Percentage dry matter content of the sample

RESULTS AND DISCUSSION

Table 1: Qualitative phytochemical screening of ethanol leaves extract of *B. nitida*

Parameter	Inference
Alkaloids	++
Polyphenols	+++
Flavonoids	++
Saponins	+++
Tannins	++
Phlobotannins	_
Glycosides	+

Key: + = present in small amount, ++ = present in moderate amount, +++ = present in heavy amount and - = absent

The table 1 above shows the results of the phytochemical screening of ethanol leaves extract of *B. nitida.* From the result, the polyphenol and saponins were found to be present in large amount, the Alkaloids, Flavonoids and Tannins were found to be present in

moderate amount. The Glycoside was only found to be present in small amount while phlobatannis was found to be absent in the plant leaf. These results are similar with the results obtained by Okon *et al.* (2013).

Table 2: Quantitative phytochemical determination of ethanol leaves extract of *B. nitida*

Phytochemicals	% composition
Alkaloids	5.34 ± 1.03
Saponins	8.27 ± 1.14
Tannins	3.34 ± 0.15
Flavonoids	3.74 ± 0.21
Polyphenols	7.45 ± 0.16
Glycosides	1.26 ± 0.12

The results of the phytochemical composition of B. .nitida as shown in table 2.0 above revealed that the plant part contains high amount of phytochemicals, especially, the saponnins and the polyphenols. Theses results suggest why B. nitida has been reported to have medicinal potentials because according to Sofowora (1983), the medicinal properties of plants are related to the phytochemicals present in them. This may suggest why B. nitida has been reported to show efficicay in the treatment of certain diseases in some rural areas. Phytochemicals such as saponins have anti-inflammatory effects (Vinha and Soares, 2012), haemolytic activity and cholesterol binding properties (Nyarko and Addy, 1990). Glycosides are known to lower blood pressure and also alleviate heart related diseases (Marinkovic and Vitale, 2008), and tannins exhibit anti-oxidant and antimicrobial effects. Alkaloids interfere with neurotransmission and block enzyme action (Sayyah and Hadida, 2004). Hence, their presence in the plant may be the reason for its analgesic activity and usage in herbal medicine as a pain reliever. These phytochemicals may account for the medicinal value attributed to the *B. nitida* leaves.

Table 3: Composition of vitamin in *B. nitida* leaf

Parameter	Quantity (mg/100g)
Thiamin	0.363 ± 0.014
Riboflavin	0.052 ± 0.001
Niacin	1.210 ± 0.035
Ascorbic Acid	6.410 ± 0.003
β-Carotene	3.440 ± 0.026

*Results are expressed as MEAN of three determinations ± SD

Table 3.0 shows the result of the vitamin composition of *B. nitida* leaf. From the results above, B. nitida contains 0.363 ± 0.014, 0.052 ± 0.001, 1.210 ± 0.035, 6.410 ± 0.003 and 3.440 ± 0.026 of Thiamin, Riboflavin, Niacin, Ascorbic Acid and β-Carotene respectively. The results revealed that the plant part is a good source of vitamin C and β-Carotene. While vitamin C is known for its antioxidative properties which plays a vital role in the balancing the oxidative stress in a human system, β-Carotene is primarily a precursor in the synthesis of vitamin A, which helps in vision. It also protects against viral diseases like hepatitis, measles and chicken pox (Akande *et al.*, 2010) Niacin has been reported to have cholesterol lowering effect, relief of arthritis, improve brain function, treat heart disease and impotency. Riboflavin helps in improving vision, skin beauty, digestive tract, blood cells as well as other vital organs (Gupta *et al*., 2014). Thiamin has been reported to play a vital role in carbohydrate and amino acid metabolism and gluconeogenesis. Hence, it plays a fundamental role in energy metabolism. Thiamin is considered very essential in optimal organs and tissues functioning because it helps in oxidative adenosine triphosphate (ATP) metabolism in living organisms.

Table 4: The mineral composition of *B. nitida* leaves

Parameter	Quantity (mg/100g)
Calcium	29.65 ± 1.12
Potassium	19.74 ± 0.01
Magnesium	24.70 ± 0.02
Zinc	9.61 ± 0.20
Sodium	22.12 ± 1.02
Iron	4.06 ± 1.10
Copper	0.04 ± 0.10
Phosphorus	18.67 ± 1.01
Nitrogen	7.63 ± 0.02

*Results are expressed as MEAN of three determinations ± SD

Table 4.0 shows the mineral composition of *B. nitida* leaves. The result shows that the plant part contains high amount of Calcium (Ca) which as an electrolyte, plays a vital role in the physiological and biochemical processes of organisms and cells. In signal transduction, pathways, calcium acts as a secondary messenger and it also serves as a cofactor in some enzymes. It is essential in the formation of teeth and bones and it is also found in small percentage in soft issues like heart and kidney where it is responsible for nerve impulses and muscle contractions (El Sohaimy *et al*., 2015).

Sodium content was the lowest among the macro elements determined. This observation agrees with many other reported results that have shown sodium to be present in low concentration particularly when compared to potassium. Among the micro elements found, iron was the most abundant, which is similar to the result obtained by Akande *et al*. (2010) from the ethanol extract of *B. nitida* leaves.

Iron is an essential micro element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of protein and fats (Adeyeye *et al*., 1996). Zinc is involved in normal functioning of the body. Calcium is essential in the formation of teeth and bones. It is also found in small percentage in soft issues like heart and kidney where it is responsible for nerve impulses and muscle contractions

(El Sohaimy *et al*., 2015). Potassium reduces blood pressure, and Sodium is an important source of electrolytes within the body. Magnesium is required in the plasma and extracellular fluid where it helps in maintaining osmotic equilibrium (Gupta *et al*., 2014). The concentrations of Ca, Mg and N obtained were higher than those reported in previous studies. That may be as a result of environmental or climatic factors like sunshine and nutrient contents of the soil where the plant was cultivated. The accumulation of elements in medicinal plants have been reported to depend on climatic factors, plant species, air pollution and other environmental factors (Sovljanski *et al*., 1989).

CONCLUSION

The results of this work revealed that *B. nitida* leaves contains high amount of phytochemicals which suggest that the plant part can play a major role in pharmaceutical industries. The vitamin composition of the plant also show that the plant is rich in vitamins which helps in nourishment of the body. This imply that the plant can be use as a source vitamin supplements, especially for those with Ascorbic acid and β-Carotene deficiency. On the hand, the results of this work also revealed that the plant is a good source of minerals which helps to improve cell metabolism.

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