Phytochemical and Elemental Analysis on the Leaf of *Borreria Verticillata*(Rubiaceae)

Supported by tetfund

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Received: February 14, 2025, Accepted: April 28, 2025

Abstract: *Borreria verticillata* is a medicinal plant widely distributed in tropical and subtropical regions; it is a common herb in traditional medical practices in Nigeria. A decoction made from its leaf is used for management of skin ailments, skin itches, psoriasis, scabies and various infectious dermatitis. The plant grows as a weed in West Africa; it is a resistant weed and can grow on a number of lands types. The leaf was analyzed for phytochemicals, elements present and physico-chemical contents using standard laboratory techniques. Phytochemicals observed to be present includes; carbohydrates, cardiac glycosides tannins, flavonoids, saponins and steroid/triterpenes and while anthraquninones and alkaloids were absent. Elemental analysis showed the presence of Co, Na, Zn, Cd, Fe, Mg and Ca while Pb, Ni and Cu were found to be absent. Values of physico-chemical parameters (%w/w) obtained were moisture content (5.0), total ash (8.0), acid insoluble ash (1.5), water soluble ash (0.7), alcohol extractive (6.6) and water extractive value (3.3). The results obtained from this study may provide information that will be useful in establishing standards for the plant.

Keywords: *Borreria verticillata*, Phytochemical, Physico-chemical, Elemental analysis

Introduction

Borreria verticillata is a medicinal plant belonging to the family Rubiaceae. The genera *Borreria* comprises of about 280 species distributed in tropical and subtropical America, Africa, Asia, and Europe. The plant *Borreria verticillata* is a clambering and climbing yearly or perennial plant which is indigenous to the South Americas (Izuogu *et al*.,2020). This plant is dispersed broadly then irregularly through the Pacific, Africa, Australia and Asia. Stems sprawling, to 110 cm or more, glabrous or nearly so, usually standing straight and simple or thinly branched, often abundantly branched from the base, usually 40 cm high or less, the stems tetragonous. It is highly resistance hence can grow on a number of lands types but frequently needs some intrusion to establish (PIER, 2016 , Izuogu *et al*.,2020). In Nigeria, it is common to all vegetation, normally found in wastelands, in non-cultivated fields or within the vicinity of ponds, mainly in the rainy season (Murtala *et al*., 2015). It is commonly called shrubby false button weed or shrubby false button wood (Burkill, 2000) while in Nigerian indigenous language it is known as Karya garma in Hausa , Irawo-ile in Yoruba and Abia-ikana in Ibibio. *B. verticillata* grows as a weed in West Africa and is used widely in their traditional medicine. In West African region, its decoction (extraction from the upper part) is rub topically on the skin for management of skin related ailments such as Tinea versicolor (eczema), Tinea capitis (ring worm), pityriasis versicolor, skin itches, psoriasis, scabies and various infectious dermatitis (Balde *et al*., 1991, 2015). A blend from the roots is used as emetic and its broad leaves are employed as antidiarrheal, against hemorrhoids and erysipelas (Lorenzi and Matos, 2002).

The plant has been investigated by several authors for its antimicrobial property and reported to possess antimicrobial activity (Pioxoto neto *et al* 2002; Ushie and Adamu, 2010). Due to its established use in traditional medicine for a wide range of ailments particularly skin infections; this study aims to establish the phytochemicals and elements present in the leaves of the plant.

Materials and Methods

Collection , Identification and Preparation of Borreria verticillata

Fresh leaves of *B. verticillata* were collected from Malali, Kaduna State, Nigeria in the month of September 2023. The plant was identified and authenticated by Mallam U.S. Gallah of the Herbarium section of Department of Biology, Kaduna State University, Kaduna State and a voucher specimen number (KASU/BSH/982) was deposited.

The plant was carefully cleaned with water to remove any foreign matter, the leaves were carefully plucked from the whole plant, they were then air dried under shade for about two weeks, comminuted to powdered form using a pestle and mortar. The powdered leaf sample gotten was then stored in an airtight container for further use.

Preparation of plant extract

Extraction of the plant material was done according to the method described by Kokate (1994). Two hundred grams (200 g) of pulverized plant sample was extracted by cold maceration in aqueous-methanol mixture (methanol-water 90:10 v/v) for 72 hours with occasional shaking. The filtrate obtained was allowed to stand for evaporation of content; final evaporation to dryness of the extract was done via a rotary evaporator and then stored in a desiccator for experimental use.

Phytochemical analysis

The phytochemical analysis of the methanol leaf extract of *B. verticillata* was carried out to identify the phyto-constituents present in the extract, this test was carried out according to guidelines described by Sofowora (2008), Evans (2009), Edeoga *et al*. (2005) and Ushie and Adamu, (2010).

Test for alkaloids

0.5g of the extract was stirred in 5mL of 2 M aqueous hydrochloric acid on a steam bath. 1.0 mL of the filtrate was taken in three separate test tubes and treated with a few drops of Mayer's reagent and Dragendoffs' reagent respectively. The solutions were observed for colour change and recorded.

Test for tannins

0.5 g of the plant extract was boiled with distilled water (100 mL) for 5 min. To 2.0 mL of the cooled solution (filtrate) a few drops of ferric chloride was added. The observed colour change was recorded.

Test for saponins

The froth test and heamolysis test as described in Sofowora (2008) were used to determine the presence of saponins in the extract. 0.5g of the plant extract was added to distilled water (20 mL) in a 100 mL beaker, boiled and filtered and the filtrate used for the test; (a) Froth test: 5 ml of the filtrate was diluted with water (20 mL) and shaken vigorously and allowed to stand for 30 min. (b) Heamolysis test: To 2mL of filtrate above that gave positive for frothing, 5mL of distilled water was added and shaken ,1mL was taken in a test tube and 2-3 drops of blood in normal saline (0.9% solution of NaC1) was added and set aside for 3 hours. The solution was observed for haemolysis (i.e disintegration of the red blood cells in the blood sample).

Test for carbohydrates

0.5 g of the extract was dissolved in 5 mL of distilled water and filtered. To the filtrate, 3 drops of alcoholic α-naphthol solution followed by 1 ml of concentrated sulphuric acid down the side of the test tube was added. The observed colour change was recorded.

Test for anthraquinones

0.5 g of the plant extract was shaken with benzene (2.0 mL) and filtered. 4.0 mL of 10 % ammonia solution was added to the filtrate. The mixture obtained was shaken and observed for colour change.

Test for flavonoids

0.5 g of the plant extract was dissolved in water, filtered and then 2 ml of 10% aqueous sodium hydroxide solution was added and the solution observed, on addition of dilute hydrochloric acid. The resultant colour obtained was recorded.

Test for steroids/triterpenes

About 2 mL of acetic anhydride was added to 0.5g of the extract and mixed gently. Few drops of conc. sulphuric acid were added. The reaction obtained was observed and recorded.

Physicochemical parameters

Powdered sample of the dried plant was subjected to physicochemical analysis such as total ash, acid insoluble ash, water soluble ash ,moisture content, water and alcohol soluble extractives as outlined by Evans (2009).

Determination of elemental analysis

Elements such as manganese, cobalt, nickel, copper, zinc, cadmium, iron, lead, magnesium and calcium were qualitatively and quantitatively analysed using Atomic Absorption Spectrophotometry (AAS) (Rajurkar *et al*., 1997).

Results and Discussion

The result of preliminary phytochemical screening of methanol leaf extract of *B. verticillata* revealed the presence of carbohydrates, tannins, flavonoids, cardiac glycosides, steroid/triterpenes and saponins in the plant; this is as shown in Table 1. Preliminary phytochemical screening gives an insight into the qualitative nature of active phytochemical constituents present in plant extracts. The above result conforms to the report by Aremu and co-workers which also reported the presence of these secondary metabolites in the plant. These secondary metabolites are known to have various pharmacological effects. Their presence in plants has been known to give the plant anti-allergic, anti-inflammatory and antimicrobial properties (Cushnie and Lamb, 2005). They are also antioxidants and free radical scavengers which prevent cell damage, and have strong anticancer activity and protect the cell against carcinogenesis (Okwu, 2004; Aremu *et al* .,2016). Hence the presence of these phytochemicals in *B. verticillata* can be attributed to its use in traditional medicine for the treatment of various skin infections.

Table 1: Phytochemical constituents of methanol leaf extract of *Borreria verticillata*

Phytochemicals	Test	Inference
Carbohydrate	Molisch	+
Cardiac glycoside	Keller kiliani	+
Tannins	Ferric chloride	+
Flavonoids	Sodium hydroxide	+
Saponins	Frothing	+
	Heamolysis	+
Anthraquinones	Borntrager's	-
Steroids/triterpenes	Lieberman burchard	+
Alkaloids	Dragendroff's	-
	Mayer's	-

Key: (-) Absent; (+) Positive

Physicochemical constants of the powdered leaf of Borreria verticillata

The various physical constants determined for the leaf of *B. verticillata* were as follows: Moisture content was calculated to be 5.0% .The percentage total ash, water soluble ash and acid insoluble ash values were 8.0, 0.7 and 1.5% respectively. The percentage yield for the alcohol extract was 6.6 % while the percentage yield of the water extract was 3.3 % (Table 2).

Moisture content is a value that determines the moisture present in a given sample. Moisture content obtained was 5.0 % indicating the drug has low chances of undergoing microbial degradation during storage, also the general requirement of moisture content in crude drug is that, it should not be more than 14 % (B.H.P 1990), the value obtained in this study was within the accepted range. The values gotten for total ash, acid-insoluble ash and water-

soluble ash (8.0 %, 0.7 % and 1.5 % respectively) indicates low amounts of impurities such as carbonate ,silica and sand in the plant sample, The total ash value is used as a criteria to judge the identity and purity of drugs (WHO., 1996; Prasad *et al*., 2012).

Table 2: Physicochemical constants of *Borreria verticillata* leaf powder

Parameter	Values (%w/w) ± SEM*
Moisture content	5.0 ± 0.04
Total ash value	8.0 ± 0.35
Acid insoluble ash	1.5 ± 0.01
Water soluble ash	0.7 ± 0.01
Water extractive value	3.3 ± 0.05
Alcohol extractive value	6.6 ± 0.35

Elemental analysis

Ten elements were screened as shown in Table 3; of which Co, Ca, Zn, Ni, Fe, Na, Mg and Cu are classified as elements essential for humans while Cd and Pb are classified as highly toxic elements. Elements Ca, Na, Fe and Mg were obtained in high amounts with values of 3062.6, 913.6, 125.8 and 116.3 respectively; elements Cu and Ni were found to be absent. Toxic element Pb was found absent while Cd (0.1) was obtained in low amount.

Calcium is one of the most important nutritional elements, and is critical for healthy bones and many vital physiological functions. The element magnesium is the second most abundant intracellular element; it plays important roles in the physiological functions of cardiovascular and neurological systems and muscles (de Baaij *et al*., 2015). This element is a cofactor for several hundred enzymes and it acts as an activator for at least 200 enzymes (Jomova *et al*., 2022). High concentration of certain metals; Mg , Ca and Fe in plants are essential for proper growth and normal functioning of the plant (Underwood, 1971; Zafar *et al*.,2010).

B. verticillata has not been reported to be eaten as a vegetable but as an unwanted weed in well-tended farms as such the presence of these elements in high amounts in the plant can be beneficial to grazing animals in tackling elemental deficiencies. The level of toxic elements Pb and Cd is also important in determining the safe handling or/ removal of the plant. Lead is an environmental pollutant. Despite the low amounts absorbed, prolonged exposure to lead can accumulate in the human body system, resulting in lead poisoning or toxicity. (Engwa *et al*., 2019). Element Pb was found to be absent in the plant indicating it lead free at the area of collection.

Table 3: Elemental analysis of *B. verticillata*

Elements	Concentration (ppm)
Co	20.6
Ca	3062.6
Cu	0.0
Na	913.6
Zn	18.3
Cd	0.1
Fe	125.8
Pb	0.0
Mg	116.3
Ni	0.0

Conclusion

The result obtained in this study has provided information that may be useful in establishing standards for the plant and it also confirms its therapeutic effect as used in traditional medicine.

Declaration of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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