

Full Length Research Paper

Chemical characterization of bottlebrush leaves found in NISLT premises, Samonda, Ibadan, Nigeria.

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This study investigated the chemical composition of Bottlebrush leaves for its ascribed mythical medicinal uses. Bottlebrush leaves were collected from the Nigerian institute of science laboratory technology premises in Ibadan, Nigeria. Proximate analyses, phytochemical screening, elemental analysis and antioxidant analysis were carried out on the leaves extract. This study has provided a scientific justification that the leaves contained relevant phyto constituents such as Alkaloids, cardenolodes, tannins, cardiac glycosides, saponin and steroids while flavonoid, terpenoids, phenol, anthraquinones and resins are absent. The proximate analysis revealed an appreciable amount of carbohydrate (50.46%), ash content (6.6%), moisture content (8.95%), crude protein (12.54%), and fiber content (15.75%) in the leaf. The mineral analysis showed a massive amount of calcium (4931.29 mg/Kg), magnesium (4447.14 mg/Kg) and potassium (1714.41 mg/Kg, in the bottlebrush leaves while zinc (15.66 mg/Kg), iron (287.70 mg/Kg) and manganese (51.90 mg/Kg) were detected in minimal amount, chromium, sodium and copper were absent in the leaves. The results of inhibition of leaf extract of bottlebrush also indicated that it is a good source of antioxidant in moderate concentrations when compared with that of vitamin C. The presence of secondary metabolites in the leaf extract suggests that bottlebrush could be a good source of antioxidants, phyto-constituents and minerals.

Key words: Antioxidant, Bottlebrush, Phyto-constituent, Proximate analysis.

INTRODUCTION

Bottlebrush plant is an ornamental medicinal plant from the *Myrtaceae* family consisting of 132 genera and 5950 species (Christenhusz and Byng, 2016). It is commonly known as crimson bottlebrush, red bottlebrush or lemon bottlebrush. Bottlebrush plant originated from Australia and arrived in Europe in 1789. The genus *Callistemon* was described taxonomically for the first time in 1814. One of the most well-known *Callistemon* species is the crimson bottlebrush (*Callistemon citrinus*) an evergreen

plant that reaches up to 7 m high in its natural habitat in Australia. Bottlebrush plant also grow into stately bushes in Mediterranean regions. In temperate climates, on the other hand, they only reach up to about 3 m high. They grow as upright shrubs or small trees with overhanging branches and bloom continually throughout up to four flowering periods between May and September (Mohamed et al., 2017). Bottlebrush plant is found as a small tree or shrub distributed around the world

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particularly in tropical Asia, Australia, Sri Lanka, South America, and India (Ahmad and Athar, 2017) as well as cultivated in Egypt. It was planted as an ornamental plant, and as a source of essential oil (Shahenda et al., 2021). Essential oils from *C. citrinus* show higher anti fungi activity over few synthetic antimicrobials like miconazole and clotrimazole (Sales et al., 2017). *Callistemon viminalis* is traditionally used for treating skin infections, hemorrhoids, gastroenteritis, diarrhea, and respiratory conditions (Salem et al., 2017). Among the plant components, secondary metabolites like, phenolic compounds are of exceptional interest due to their noticeable health-related properties. From the chemical point of view, there are few reports about the phenolic compounds of *C. viminalis* which concentrates on the presence of flavonoids, tannins, and phenolic acid (Ahmed 2020). Australian Aborigines have been known to suck the nectar from flowers for the production of sweet drinks due to its refreshing flavor (Fayemi et al., 2017). Antimicrobial activity of lyophilized extracts from flowers and leaves of *C. citrinus* inhibits the growth of *Listeria monocytogenes* in beef burgers (Andrade et al., 2015). Either in dietary or non-dietary form, natural antioxidants can reduce oxidative stress and prevent oxidative rancidity in fat-based foods, meat and dairy products (Ortuno et al., 2017). Phytochemical screening is effective for predicting antioxidant activity, cytotoxicity, toxic dynamics and therapeutic properties of plants (Nyman et al., 2014). Various parts of this plant have shown numerous biological and pharmacological activities, including antioxidant, anti-inflammatory antithrombin, elastase-inhibitory, antibacterial, antifungal, molluscicidal, neuroprotective, hepatoprotective, cardioprotective and anticancer activities (Nazreen et al., 2019). The objective of this study is to determine the proximate contents, phytochemical constituents, elemental analysis and the antioxidant activities of the leaves of bottlebrush.

MATERIALS AND METHOD

Plant sample collection and analysis

Fresh bottle brush leaves were collected from the Nigerian institute of science laboratory technology premises in Ibadan, Nigeria. Identification and taxonomic studies was done by taking into account the anatomical properties using Fact Sheet ST-110 (Srivastava et al., 2003). The plant leaves were collected in September, 2022. Out of the 3 types of bottlebrush plants, the white species of bottlebrush plant (Figure 1) was harvested and air dried in our sample room for four weeks. The leaves were separated from the stems and grinded into powder form with the use of an industrial blender.

Phytochemical analysis

Qualitative phytochemical screening was done by the method of Chandran (2017). Leaf extracts of ethanol were subjected to various phytochemical analyses using standard methods. Alkaloids analysis

was detected by mixing the extract with 2 ml of Wagner's reagent and the formation of reddish brown colored precipitate indicated the presence of alkaloids. Steroids were tested by the reaction of Liebermann. 10 ml of ethanol extract was evaporated and the residue was dissolved in 0.5 ml of hot acetic anhydride and added 0.5 ml of chloroform. Then the mixture was treated with the reagent of Liebermann Burchardt. The appearance of blue-green ring at the interphase denotes a positive reaction. Foam test was performed to test the presence of saponins. To the 2 ml of the extract, 6 ml of water was added in a test tube and was shaken vigorously, then observed for the formation of persistent foam that confirms the presence of saponins. Alkaline reagent test was performed to test the presence of flavonoids. The extracts were mixed with 2 ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids. The extracts were mixed with 2 ml of 2% solution of FeCl₃. A blue green or black coloration indicated the presence of phenols and tannins.

Quantitative analysis

Estimation of alkaloids

Alkaloids were quantified by the method described by Agoreyo et al. (2012). Five grams sample was weighed into beaker and 200 ml of 10% acetic acid in ethanol were added. The mixture was covered and allowed to stand for four h. After that the mixture was filtered and the filtrate was concentrated on water bath at 100°C, to one-quarter of the original volume. Concentrated ammonium hydroxide was added to the extract in drops until precipitate was formed. The end product was filtered and the precipitate washed with dilute ammonium hydroxide and then dried and weighed.

Estimation of flavonoids

The method was described by Bharathidasan et al. (2013). 100 ml of 80% aqueous methanol was used to extract ten grams of the sample. The mixture was filtered using Whatman filter paper. The filtrate was evaporated until dried, and then weighed until constant weight was achieved.

Estimation of saponins

The method was described by Gupta (2013). 20 grams of plant sample was put into a conical flask and 100 ml of 20% ethanol was added. The solution was heated over water bath at 100°C for 4 h with continuous stirring at 55°C. The solution was then filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and was vigorously shaken. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated. 60 ml of n-butanol was added, the combined extract was washed twice with 10 ml of 5% NaCl. The remaining solution was heated in a water bath and after evaporation; the sample was dried in the oven at 105°C to a constant weight.

Estimation of tannins

The method was described by Fahal et al. (2018). 100 ml of distilled water was added to two grams of the sample. The solution was kept in water bath at 90° C for 1 h. The mixture was filtered using Whatman's paper and the residue was re-extracted again.



Figure 1. Bottlebrush plant found in NISLT premises, Ibadan.
Source: Authors

The two filtrates were collected together and allowed to cool down. Distilled water was added to the filtrates up to 500 ml volumetric flask. One hundred ml of the solution was transferred to a beaker, and then 10 ml of 40% formaldehyde and 5ml of concentrated sulphuric acid were added respectively. The whole mixture was refluxed for 30 minutes and was left to cool down. The mixture was filtered and the precipitate dried and weighed.

Estimation of phenols

The method was described by Gupta (2013). The quantity of phenols is determined using the spectrophotometer method. The plant sample was boiled with 50 ml of Pet spirit for 15 min. 5 ml of the boiled sample was then taken into 50 ml flask, and 10 ml of distilled water was added. After the addition of distilled water, 2 ml of NH_4OH solution and 5 ml of concentrated butanol was added to the mixture. The sample is made up to the mark and left for 30 min to react for colour development and measured at 505 nm wavelength using a spectrophotometer

Proximate analysis

Proximate analysis was done according to the procedure of Association of Official Determination of moisture content.

Moisture percentage of fresh plants was calculated by oven-dry method. The plant samples were kept in an oven at $102 \pm 2^\circ\text{C}$. The weight loss recorded represents the moisture%.

Moisture content%

$$= \frac{\text{Weight of sample} - \text{Weight of oven dried sample}}{\text{Weight of sample}} \times 100$$

Determination of crude protein

Micro kjeldahl method was used to determine the crude proteins in plant samples AOAC 2019. The percentage of nitrogen was determined by formula mentioned below:

$$\text{N}\% = \frac{(\text{S}-\text{B}) \times \text{N} \times 0.014 \times \text{D}}{\text{weight of sample}} \times 100$$

Where, D= Dilution factor

T= Titration value (S – B)

W= Weight of sample

0.014= Constant value

Crude protein% = $\text{N} \times 6.25$ (factor)

Determination of lipid

The lipid content was calculated by AOAC(2019) Soxhlet extraction technique. The lipid contents present in plants can be extracted by petroleum ether at a temperature of $40-60^\circ\text{C}$. Formula for lipid content is:

$$\text{Crude lipid (\%)} = \frac{\text{weight of lipid in sample}}{\text{weight of sample}} \times 100$$

Determination of ash content

Muffle furnace was used to calculate ash content in plants. The sample was kept at 600°C for 8 h in furnace according to methods of AOAC (2019).

$$\text{Ash (\%)} = \frac{\text{weight of Ash}}{\text{weight of sample}} \times 100$$

Determination of crude fiber

Dried plant sample was mixed with acetone ethanol mix and then AOAC methods were carried out to calculate fibers (AOAC, 2019).

$$\text{Fiber (\%)} = \frac{\text{Weight of residue} - \text{weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of carbohydrates

Difference method was used to calculate carbohydrates. The total of ash%, fibers%, proteins% and lipid% were subtracted from 100% according to AOAC methods (2019). The formula used is as follow.
Carbohydrates% = $100 - (\text{Ash}\% + \text{Fibers}\% + \text{Proteins}\% + \text{Crude lipid}\%)$



Figure 2. Proximate analysis of bottlebrush leaves.
Source: Authors

Elemental analysis

The elemental analyses was done according to Oloruntola and Ayodele (2022), Elements such as calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), sodium (Na), iron (Fe), manganese (Mn) and chromium (Cr) were estimated. 2g of the samples (bottlebrush leave) were weighed and digested with nitric acid. The sample was heated until complete digestion was achieved and filtered using Whatman filter paper. The filtrate was made up to 100ml in a volumetric flask with deionized water. Various working standards for each elements analyzed were prepared. The absorbance of the samples and the working standards were read directly on the Atomic Absorption Spectrophotometer (AAS Thermo Scientific, iCE 3000 seri).

Preparation of leaf extract for antioxidant potential determination

Antioxidant potential determination was done according to method by Chandran (2017). The powdered leaf samples (30 g) were suspended in 250 ml methanol and kept for 72 h. After 72 h, the extract was filtered using a what man No 1 filter paper and the filtrate was concentrated under reduced pressure using rotary evaporator (IKA RV 10 digital, Germany). The dried extracts were then stored at 4°C for further assays. The antioxidant activity was evaluated according to the scavenging activity of stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The DPPH free radical scavenging activity of methanolic leaf extracts at different concentrations were performed by using method based on the reduction of methanolic solution of colored free radical 2,2-Diphenyl-1-picrylhydrazyl by free radical scavenger.

Percentage inhibition (%) = $(A_0 - A_1) / A_0 \times 100$

Where: A₀ is the Absorbance of control and A₁ Absorbance of test,

RESULTS AND DISCUSSION

The result of the proximate composition of bottlebrush leaves is depicted in Figure 2. The leaves show an

appreciable amount of carbohydrate (50.46%), ash content (6.60%), moisture content (8.95%), protein content (12.54%), and fiber content (15.75%). Low moisture content indicates less chances of microbial degradation of the leaves during storage and the general requirement for moisture content in a crude drug is not more than 14% (Njoya et al., 2018), thus the values obtained from this study is within the accepted level. The lower moisture content of bottlebrush leaves, suggests that it may have a better quality and shelf life (Olugbenga et al., 2022). The ash content of bottlebrush leaves (6.60%) is lower than that of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%), indicating that the mineral content is preserved in the leaves of the plant (Abdulghani et al., 2016). The crude fiber content of bottlebrush leaves (15.75%) is high when compared to *Talinum triangulare* (6.20%), *Piper guineenses* (6.40%), *Corchorus solitorius* (7.0%), *Vernonia amygdalina* (6.5%) (Abdulghani et al., 2016). The presence of fiber in the leaves can be employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Dahl and Stewart, 2015). The composition of crude protein (12.54%), present in the leaves compared moderately with *Tapinanthus bangwensis* (15.32%). This is indicative of the potential benefit of bottle brush plant since proteins are essential for the synthesis of body tissues and regulatory substances such as enzymes and hormones (Reynolds et al., 2022). The result of quantitative and qualitative analyses is depicted in Tables 1 and 2. The quantitative analysis shows the proportion of the phyto-constituents in milligrams per gram, while qualitative analyses shows presence and absence of the phyto-constituents in bottlebrush leaves. Phyto-chemical screening of bottlebrush extract revealed the presence of Alkaloids, cardenoloids, tannins, cardiac glycosides

Table 1. Phyto-chemicals screening (quantification) of bottlebrush leaves found in NISLT premises, Ibadan.

Parameters (mg/g)	Bottlebrush leaves
Alkaloids	1.040
Saponins	0.230
Flavonoids	-
Tannins	1.216
Phenols	-

(-) Absent.

Source: Authors

Table 2. Phytochemicals screening (qualitative) of bottlebrush leaves found in NISLT premises, Ibadan.

Parameters	Presence/absence
Alkaloids	+
Saponins	+
Tannins	+
Phenols	-
Flavonoids	-
Cardiacglycoside	+
Antraquinones	-
Cardenolides	+
Resins	-
Terpenoids	-
Steroids	+

(+) = presence (-) = absent.

Source: Authors

Table 3. Elemental analysis of Bottlebrush leaves found in NISLT premises, Ibadan.

Parameters	Quantity (mg/Kg)
Manganese (Mn)	51.90
Potassium (K)	1714.41
Sodium (Na)	ND
Iron (Fe)	287.70
Zinc (Zn)	15.66
Copper (Cu)	ND
Magnesium (Mg)	4447.14
Calcium (Ca)	4931.29
Chromium (Cr)	ND

ND: no detected, below the detection limit of the equipment which is 0.001mg.

Source: Authors

saponin and steroids while flavonoid, terpenoids, phenol, antraquinones are absent. Alkaloid is one of the largest components produced by this plant, and they are

metabolic byproducts that are derived from the amino acids from this plant (Deeba, et al., 2015). Tannins are also detected in this plant. These are generally used in the tanning process and used as healing agents in inflammation, burn, piles, and gonorrhoea (Boroushaki et al., 2016). Tannins are good source of antioxidants especially in a condensed form (Soldado et al., 2021). Condensed tannins prevent lipid peroxidation by donating hydrogen atoms to reactive with oxygen species. Tannin is widely applied to a complex large biomolecule of polyphenol nature having sufficient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules (Agidew, 2022). This plant contains steroids, which has made it very useful to relieve swelling and inflammation, such as prednisone and cortisone; vitamin D; and some sex hormones, such as testosterone and estradiol as reported by Agidew (2022). Moreover, the presence of terpenoids in bottlebrush shows that it has a significant pharmacological activities, such as antiviral, antibacterial, antimalarial, anti-inflammatory, inhibition of cholesterol synthesis, and anti-cancer activities (Boroushaki et al., 2016).

The mineral element result from bottlebrush leaves is illustrated in Table 3. It shows that bottlebrush leaves contains high elemental value. In this study, the elements observed in the plant showed higher concentration of calcium (4931.29 mg/Kg), potassium (1714.41 mg/Kg) and magnesium (4447.14 mg/Kg). Calcium as an essential mineral was high in the leaves of bottle brush. This suggests that this leaf sample can produce a significant proportion of calcium and other essential minerals if consumed appropriately. It is important to humans because it contributes in blood clotting, muscle contraction, bone and teeth formation/repairs and in some enzymatic metabolic processes (Piste et al., 2015). Potassium found in the bottlebrush leaves can help to control body weight and improve water and electrolyte balance in the blood and tissues (Sur and Smohiuddin, 2022). The level of Magnesium present in the bottlebrush leaves is high, and by implication, the leaves can be used in managing cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal dystrophy, impaired spermatogenesis, congenital malformations and bleeding disorders (Ghasemian et al., 2016). Iron (287.70 mg/Kg) is moderately detected in the leaves of bottlebrush. It is an essential component in proteins and enzymes. As an essential trace element/metal, it plays numerous biochemical roles in the body including oxygen binding in hemoglobin and acting as important catalytic center in many enzymes activities (Al-Bazi et al., 2021). Bottlebrush leaves can therefore be recommended for inclusion in the diets of patients with iron deficiency anemia. Zinc moderately present in the bottlebrush leaves suggest that it can be used in the treatment of acute diarrhea (Ismail et al., 2016). The absence of

Table 4. Free radical scavenging activity in bottlebrush leaves found in NISLT Premises.

Concentration(mg/ml)	Bottlebrush % inhibition	Vitamin C % inhibition
0.5	66.63	93.29
0.25	44.36	93.07
0.125	30.84	92.51
0.0625	17.70	85.99
0.03125	8.37	56.32

Source: Authors

sodium in the leaves implies that the consumption of extract cannot aggravate hypertension in consumers.

The result of the free radical scavenging activity of the bottlebrush leaf is illustrated in Table 4. It shows the percentage inhibition of bottlebrush as against the standard of vitamin C. The results of phytochemicals quantitative analysis on bottlebrush leaves also showed that it contains tannins, saponins, and alkaloid that are good source of antioxidants. Much of the protective effects of herbal plants are attributed to their constituents. The results of inhibition of leave extract of bottlebrush also indicated that it is a good source of antioxidant in moderate concentrations when compared with that of vitamin C. The antioxidants properties (the percentage of inhibition) decreased with decrease in concentration. At a certain low concentration, the antioxidant capacity becomes minimal. This also supports the claim by Lingxi et al. (2020) that some antioxidants (tannins, saponins, and alkaloid) are more potent in condensed and hydrolysable form. The presence of these secondary metabolites in the leave extract suggests that bottlebrush could be a good source of antioxidants. They offer excellent antioxidant defense mechanism in preventing oxidative stress by scavenging free radicals and inhibiting lipid peroxidation (Bhattacharya, 2015). According to Kumar et al. (2020), tannins, alkaloid and saponins are all good antioxidants especially in hydrolyzed and condensed form. This was also confirmed in the study of Lingxi et al. (2020) in which condensed tannin was more potent.

Conclusion

The study has revealed the presence of many phytoconstituents such as tannin, saponins and alkaloids in bottlebrush. This justifies that bottlebrush is a good source of antioxidants and could be helpful in management of oxidative stress and some inflammatory disorders when consumed in moderate concentration. It was also observed from the study that bottlebrush contains many nutritionally and medicinally elements and compounds such as zinc, calcium, magnesium, manganese, potassium, crude proteins, crude fiber, lipid and carbohydrates, etc. which indicates the nutritional and medicinal values of this plant. From this study, it was surmised that bottlebrush can also be helpful in the

management of obesity, constipation, cardiomyopathy and heart disorders through nutritional therapy due to the presence of crude fiber, magnesium, tannins and cardiac glycoside in the bottlebrush.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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