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Quantitative Analysis of Phytochemical Compounds in Barks and Leaves of Okoubaka Aubrevillei Collected from Iwo, Southwestern Nigeria

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QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL COMPOUNDS IN BARKS AND LEAVES OF *OKOUBAKA AUBREVILLEI* COLLECTED FROM IWO, SOUTHWESTERN NIGERIA

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ABSTRACT

Okoubaka aubrevillei is an indigenous and sacred tropical tree in Africa. It is rare with allelopathic properties and has relatively little information available in terms of what is responsible for its usage for medicinal and traditional usage. The phytochemical screening and quantitative analysis of the compounds in the barks and leaves of *Okoubaka aubrevillei* was determined to ascertain and establish its earlier claimed usage as traditional and modern medicine. The most important classes of secondary metabolites (phytochemical compounds) specifically alkaloids, flavonoids, tannins, saponins, anthraquinnes, mucilages, oses, holosides, coumarin and glycosides were detected in both, the leaves and barks of the tree. Quantitative determination of the phytochemical compounds found in the barks and leaves of the tree revealed that the leaves of *O. aubreville* tree had significantly higher values of alkaloids, flavonoids and glycosides than barks. Saponins and anthraquinnes were found to be significantly more in barks than in leaves. However, there was no significant difference found in the values of tannins, mucilages, oses and holosides and coumarin in barks and leaves of *O. aubreville* trees.

Keywords: Phytochemical compounds, quantitative analysis, flavonoids, tannins and alkaloid.

INTRODUCTION

The resistance of bacteria to antibiotics and antimicrobials over the years has become a major challenge for the healthcare sector (Mbaeyi-Nwaoha and Onwuka, 2014). With the increase in occurrence of lethal and widespread infectious diseases, there is a need for urgent and continuous discovery of new antimicrobial compounds. Recently, the use of plants for medicinal purposes is gaining more attention due to the presence of secondary metabolites (phytochemicals). In the past, parts of plants such as herbs, leaves, root, bark of trees, vegetables and fruits etc. have been used for cure of various diseases.

Studies have shown that some plants have a diverse variety of bioactive compounds such as tannins, alkaloids, flavonoids, carbohydrates and steroids among others that can offer significant physiological actions in the human body (Choo et al., 2014; Dua et al.,2013; Farhat et al., 2013; Adefega and Oboh, 2012; Chlopicka et al., 2012).

Phytochemicals are naturally occurring chemical, biological and active compounds found in plants that are of benefit to human health apart from those that act as macronutrients and micronutrients (Jasiem, 2016). These compounds shield plants from disease, environmental damage such as contamination, stress, drought, pathogenic attack and contribute to fragrance, color and flavor (Kotche et al., 2016). The various compounds (phytochemicals) especially secondary metabolites such as flavonoids, phenols, alkaloids, glycosides, saponins, tannins, steroids, anthraquinnes,

mucilages, coumarin, oses and holosides found in plants have anticancer, antimicrobial, antiviral, anti-inflammatory, antitumor, analgesic and many other properties (Rahaman et al., 2017; Erum et al.,2015; Abdelwahab et al., 2010). The fundamental means of their usage for medicine is due to their bioactive properties which are used as substrates for biochemical and enzymatic reactions (Samell et al., 2018; Dillard and German, 2000).

Okoubaka aubrevillei, is an indigenous and sacred tree in Africa. It has allelopathic properties. It is a tropophilous plant tree that grows up to 40 meters high with trunk as wide as 3 meters (Borokini, 2014). The tree has a huge shaggy crown, cylindrical and straight bowl; and reddish-brown coarse bark surface (Borokini, 2014). Leaves of these trees usually do not wither, though they sometimes shed leaves into cobwebs. Samples of this plant can only be collected using the traditional ways of the locals. For this purpose, bark of the tree must be harvested during the day or at night and not when the sun is set or just rising and can only be harvested with a wooden batten, and no metal materials such as knife, cutlass and axe should be used on the tree (Field survey, 2019). The paucity in its native range and little or no information about its scientific authentication for the plant's medicinal usage draws the attention of this study. This work carried out a preliminary phytochemical screening and determination of their total content in barks and leaves of *Okoubaka aubrevillei* tree collected from Amera village near Iwo town in Osun state, Southwestern Nigeria.

MATERIAL AND METHODS

Geographical Location

Okoubaka aubrevillei is a tropical tree species majorly found within the Central and West African countries (Figure 1) (Borokinni, 2015; Poorter et al., 2004). The location of the studied tree is in Amere village near Iwo town, Osun State Southwestern Nigeria (Figure 2). The study area is geographically located on within 7º 43´ 0´´ N and 4º 13´ 0´´ E in Ola-Oluwa near Iwo town, Osun State.

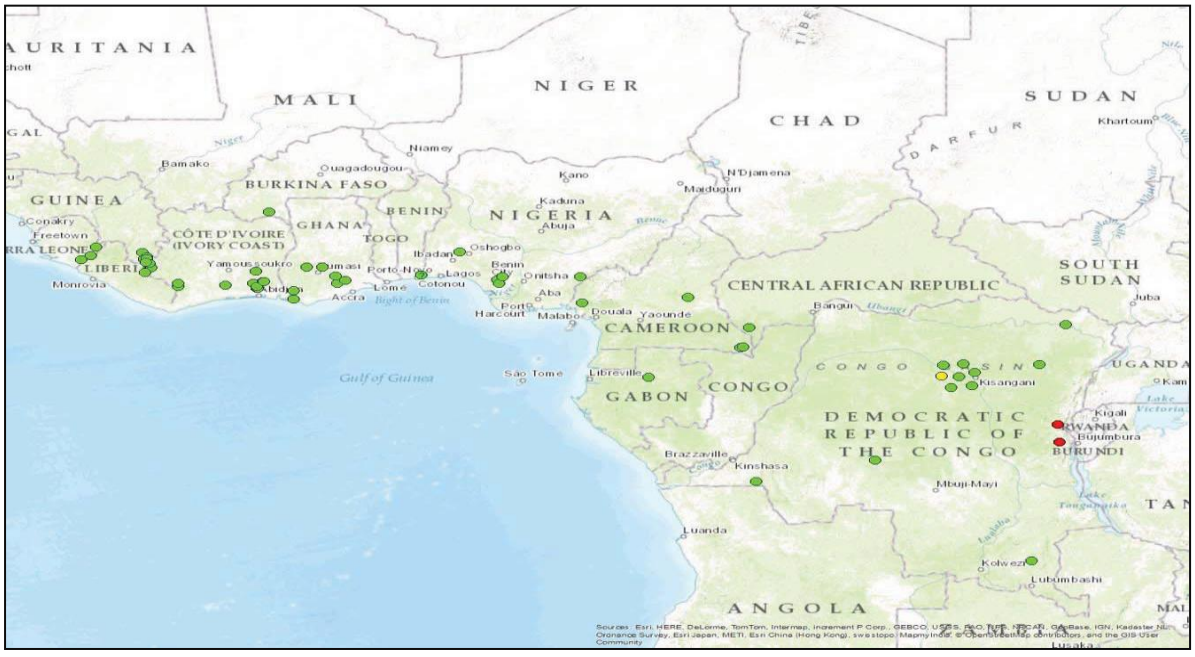

Figure 1. Map of distribution of Genus *Okoubaka* in West and Central Africa (Borokini, 2014)

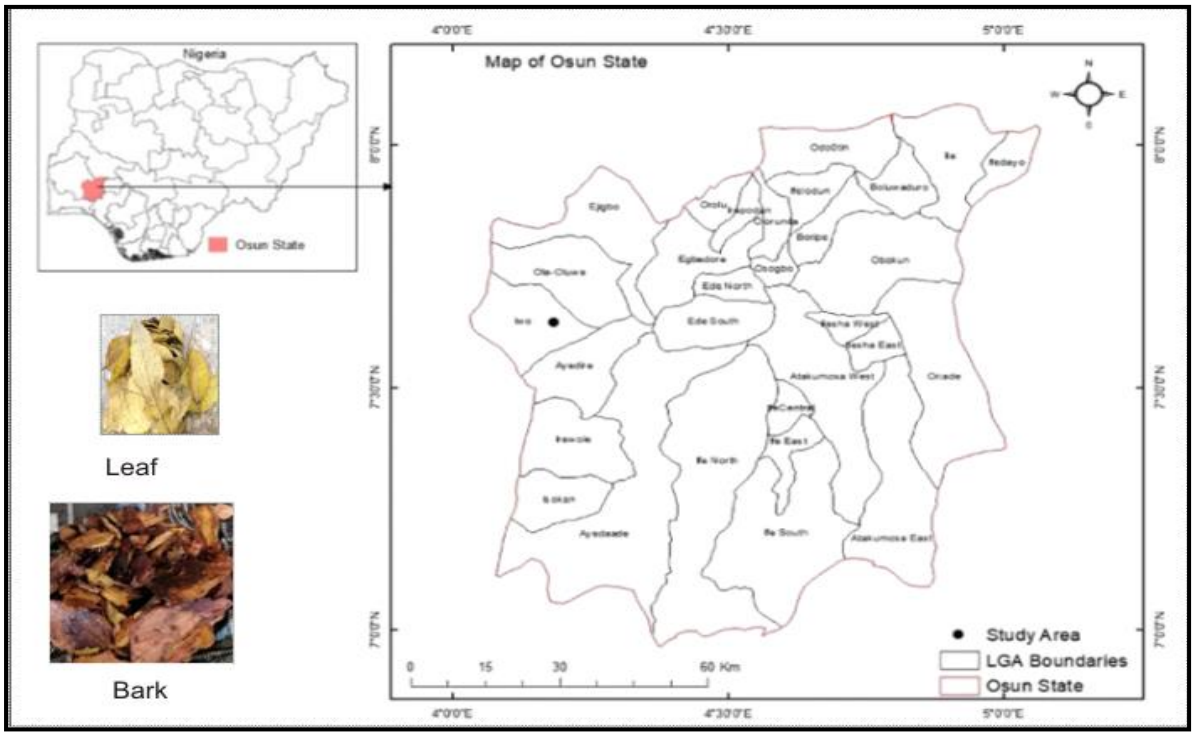

Figure 2. Location map of the Okoubaka *aubrevillei* tree with the sample of the leaf and tree

Sample Collection

The dried leaves on the ground floor of the trees were handpicked based on the traditional method for sample collection, while the harvested barks were collected from the tree. Both the leaf and the bark samples of the tree were labeled accordingly.

Sample Preparation

Barks and leaves of *O. aubrevillei* collected from tree worship place were rinsed with tap water followed by distilled water to remove the dirt on the surface. They were then air dried for 2 days and dried until a constant mass was obtained. The dried samples of the barks and leaves of *O. aubrevillei* were ground into fine powder and kept in desiccators for extraction. The extraction was carried out using the method described by Anowi et al. (2012). One kilogram of dried powder of leaves and barks were soaked separately in 200 ml of analytical ethanol and allowed to stand for 48 hours with sporadic shaking. The suspension was filtered with filter paper and the filtrate was exposed for evaporation in a soxhlet apparatus for 10 hours using absolute methanol. The solvent, the crude ethanolic extracts of leaf and bark of the tree recovered were kept in desiccators at 4 º C for further analyses.

Qualitative Determination of Phytochemical Compounds

The ethanolic extracts of bark and leaf of *O. aubrevillei* were tested for the presence of alkaloids, flavonoids, tannins, saponins, anthraquinnes, mucilages, oses and holosides, coumarin, glycosides and reducing sugar (Oloyed, 2005; Guessan et al., 2009; Roopashree et al., 2008; Sofowora, 1993; Trease and Evans, 1989). The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Quantitative Phytochemical Analysis of the Leaf and Bark Extracts

The total contents of alkaloids, flavonoids, tannins, saponins, anthraquinnes, mucilages, oses and holosides, coumarin, glycosides and reducing sugars in the barks and leaves of *O. aubrevillei* were analyzed using 6305 UV/Visible spectrophotometer (Wavelength range of 198 to 100 nm, Jenyway UK). The procedures used for the analysis are discussed as follows:

- ***Determination of Alkaloids***: This was determined using the method of Nbaeyi-Nwaoha and Onwuka (2014). One gram each of the grounded samples of leaf and bark of *O. aubrevillei* were dissolved with 20 ml of 20% H_2SO_4 in ethanol (1:1) and filtered. 1 ml of each of the filtrates (leaf and barks) was put into two different test tubes and 5 ml of 40% H_2SO_4 was further added and mixed thoroughly. The mixture was covered and allowed to settle for 4 hours before taking the measurement. The measurements were taken using spectrophotometer at 568 nm for the two (leaf and bark) samples. The procedure was repeated three times and values were recorded.

- ***Determination of Flavonoids***: This was determined using the method of Nbaeyi-Nwaoha and Onwuka (2014). One gram each of the grounded samples of leaf and bark of *O. aubrevillei* were dissolved in 200 ml of ethyl and filtered. 5 ml of each of the filtrates (leaf and barks) was put into two different test tubes; 5 ml of dilute ammonia was added, mixed vigorously and allowed to settle for some hours. After which, the absorbance was measured using spectrophotometer at 490 nm for the two (leaf and bark) samples (Nbaeyi-Nwaoha and Onwuka, 2014). The procedure was repeated three times and values were recorded.

- ***Determination of Tannins***: This was determined using the method of Nbaeyi-Nwaoha and Onwuka (2014). 10 ml of water was added to 1 gram each of the samples of grounded leaf and bark of *O. aubrevillei* put in separate conical flasks, shook for 30 minutes at an interval of 5 mins and filtered. A fixed quantity (2.5 ml) of each of the filtrates was put in two separate conical flasks, 1 ml of Follin-Denis reagent and Na_2CO_3 were added to it and mixed thoroughly. The mixture was left to settle for 90 minutes at room temperature and the absorbance was measured using spectrophotometer at 720 nm for the two (leaf and bark) samples. The procedure was repeated three times and values were recorded.

- ***Determination of Saponins***: Petroleum ether (10 ml) was added to 1g of each of the grounded samples of leaf and bark of *O. aubrevillei* in separate conical flasks using the method of Nbaeyi-Nwaoha and Onwuka (2014). This was further mixed with 10 ml of petroleum ether, drained and dried. Ethanol (5 ml) was added to the dried mixture and mixed thoroughly and about 2 ml of the mixture each of the samples was put in the two different test tubes allowed to settle for 30 minutes, after which the measurements were taken at 550 nm for the two (leaf and bark) samples. The procedure was repeated three times and the values were recorded.

- ***Determination of Anthraquinnes***: This was determined using the method of Kuster and Rocha (2014). Grounded samples (1 g each) of leaf and bark of *O. aubrevillei* were soaked with 100 ml benzene in separate conical flasks for 10 min and filtered. 1 ml of the filtrates was mixed with 5 ml of 70 % H_2SO_4 in test tubes and allowed to settle for some minutes. The absorbances of the two samples were measured using spectrophotometer at 284 nm. The

procedure was repeated three times and the values were recorded.

- ***Determination of Mucilage***: This was determined using the method of Fatemeh et al. (2017). Powder of barks and leaves (10g each) of the studied tree was mixed with distilled water for 6 h and boiled for 30 mins. The mixture was left for 1 hour and filtered through muslin cloth. Ethanol was added to the filtrate to allow mucilage precipitation. The mucilage was separated and dried at temperature of 50 ºC. The dried mucilage was then weighed and measured. The procedure was repeated three times and values were recorded.

- ***Determination of Oses and Holosides****:* This was determined using the method of Peixoto Sobrinho et al. (2008). Leaves and barks (5 g each) was put in separate conical flasks, mixed vigorously with 100 ml absolute ethanol, mixed and filtered. The filtrates were mixed gently with concentrated H_2SO_4 (2 ml) in test tubes for 5 minutes and allowed to settle for some minutes. The dissolved Bromohymol (in absolute ethanol) and 2 drops of concentrated H_2SO_4 was added to the mixture and allowed to settle for 15 minutes. The absorbances of the mixture were read using spectrophotometer at 510 nm wavelength. The procedure was repeated three times and values were recorded.

- ***Determination of coumarins*****:** This was determined using the method of Kuster and Rocha (2014). Distilled water (2 ml) and 0.5 ml of lead acetate solution were added to 0.5 ml extract of each of the samples in separate test tubes and thoroughly mixed. Hydrochloric acid (8 ml) was further added to the mixture and left for 30 minutes at room temperature. The absorbance was measured using spectrophotometer at 320 nm wavelength. The procedure was repeated three times and the values were recorded.

- ***Determination of Glycosides*****:** This was determined using the method of Nbaeyi-Nwaoha and Onwuka (2014). Fifteen percent lead acetate (2.5 ml) was mixed thoroughly with 1 g of each of the samples in test tubes and filtered. Chloroform (2 ml) was added to the filtrate, mixed thoroughly and allowed to settle. The lower portion was collected and evaporated to dryness. Glacial acetic acid (3 ml), 0.1ml of 5% ferric chloride and 0.25 ml concentrated H_2SO_4 was added to dried lower portion, mixed vigorously and left for 3 hours. The absorbance was measured using spectrophotometer at 568 nm. The procedure was repeated three times and the values were recorded.

Data Analyses

All the tests were conducted in triplicates and the data was analyzed using One-way analysis of variance (ANOVA). The significant differences were evaluated using Tukey's HSD test with a level of significance ($P<0.05$). ANOVA was performed with SPSS version 20.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemicals are secondary metabolites in plants that facilitate their ability to overcome the transitory or persistent coercion associated with their environment and they are of medical benefit to man (Molyneus et al., 2007). These secondary metabolites have been used as plant derived anti-cancerous drugs. The medicinal plants used for ethnomedicine have been broadly screened for the presence of phytochemicals (Philipson, 2001; Edeoga et al., 2005). Phytochemicals such as alkaloids, flavonoids, saponins, tannins etc. are protective compounds for treatment of persistent diseases such as cancers, hypertension, diabetes etc. (Craig, 1997; Samell et al., 2018)

In this study, the phytochemical screening of crude ethanolic extracts of leaf and bark samples of *O. aubrevillei* as indicated by positive sign (+) (Table 1) revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, anthraquinnes, mucilages, coumarins, oses and holosides.

Table 1. Phytochemical screening of leaf and bark extracts of *O. aubrevillei*.

Phytochemical Compounds	Barks	Leaves
Tannin	+	+
Saponin	+	+
Alkaloid	+	+
Flavonoids	+	+
Anthraquinnes	+	+
Mucilages	+	+
Oses and Holosides	+	+
Coumarin	+	+
Glycosides	+	+

They were detected in both the leaf and bark extracts of *O. aubrevillei*. The phytochemical compounds detected are known to have medicinal importance. The presence of these compounds could be responsible for antimicrobial and immunity stimulating properties of *O. aubrevillei* making it effective as a detoxifying, antibacterial and anti-inflammatory agent (Bagot, 2015).

Furthermore, the presence of the numerous phytochemical compounds in the barks and leaves of the *O. aubreville* tree may be responsible for its local (traditional) usage especially the bark as concoction to treat various skin diseases (effective for bruising), as antidote for food poison.

Previous studies state that phytochemicals act as best antioxidants and protect the cells from free radical damage such as carotenoids, polyphenols and reduce the risk of cancer by discouraging the production of tumour or hormonal stimulation and antibacterial activity (Devasayam et al., 2004; Mathew et al., 2012; Omoregie and Osagie, 2012).

Quantitative Determination of Phytochemical Compounds in O. aubrevillei

The mean contents of each of the phytochemical compounds in the ethanolic extract of *O. aubreville* leaf and bark are shown in Table 2. It was observed that the leaves of *O. aubreville* tree had significantly higher values of alkaloids (0.64 ± 0.03), flavonoids (0.35±0.02) and glycosides (11.32±0.001) than barks with alkaloids (0.47± 004), flavonoids (0.24±0.01) and glycosides (8.64±0.16) values. The significantly higher values of saponins (0.25± 0.01) and anthraquinnes (0.17±0.01) were found in barks than in leaves. However, there was no significant difference found in the values of tannins, mucilages, oses and holosides and coumarin in barks and leaves of *O. aubrevillei* trees as shown in Table 2.

Table 2. Total Mean Contents of Phytochemical Compounds of *O. aubrevillei*.

Phytochemical Compounds	Barks (mg/100g)	Leaves (mg/100g)
Tannin	0.29±0.02[a]	0.25±0.01[a]
Saponin	0.25± 0.0[a]	0.18±0.01[b]
Alkaloid	0.47± 004[b]	0.64±0.03[a]
Flavonoids	0.24±0.01[b]	0.35±0.02[a]
Anthraquinnes	0.17±0.01[a]	0.02±0.001[b]
Mucilage	0.02±0.001[a]	0.01±0.001[a]
Oses and Holosides	0.03±0.001[a]	0.02±0.001[a]
Coumarin	0.01±0.001[a]	0.01±0.001[a]
Glycosides	8.64±0.16[b]	11.32±0.001[a]

Values with different letters are significantly different from each other

Alkaloids, phytochemical compounds found in the bark and leaves of the studied tree have the most advantageous significance among plant chemical constituents and have analgesic, antibacterial and antispasmodic characteristics among others (Enujugha and Agbede, 2000; Harisaranraj et al., 2009; Uyo et al., 2013). These beneficial properties of the alkaloids justify the traditional and modern medicinal usage of the studied trees. Similarly, the presence of flavonoids in the bark and leaves of the tree is a good contributor to its medicinal usage. Flavonoids are useful antioxidants that take part in fighting against liver tumors, toxins, allergies, inflammation, viruses and other microorganisms (Harisaranraj et al., 2009; Uyo et al., 2013). It is a powerful and dominant compound in protecting blood vessels particularly those that are answerable to distribution and transportation of nutrients and oxygen to cells as well as delaying cataract enlargement in diabetic patients (Harisaranraj et al., 2009).

Glycosides found in the bark and leaves have been used in the treatment of congestive heart failure due to its diverse action which strengthens the force of myocardial contraction as well as its direct action on the smooth muscles (Braunwald et al., 1961). Its effects on neutral tissues and indirect effect on electrical activities of the heart and vascular resistance as well as capacitance has been reported (Chukwuma et al., 2016). Saponins, found in the bark and leaves of the tree have been described as phytochemical compounds that have the ability to coalesce with cholesterol, they impart a bitter taste and cause haemolytic activity in water solution (Sodipo et al., 2000). They can also be used for fungal infections (Sheikh et al., 2013). Anthraquinnes may also contribute to this plant's effective medicinal usage due to the laxative effects of anthraquinnes for relieving constipation (Portalatin and Winstead, 2012).

Tannins found in minute quantities are known to be anti-nutrient in diets as well as having some degree of astringency (Chikezie et al., 2008). This compound can intermingle with proteins to form insoluble complexes, thus lessening protein bioavailability (Enujugha and Agbede, 2000). Tannins also possess antiviral, antibacterial, and antitumor properties (Kakiuch et al., 1986; Khanbabaea and Ree, 2000). Thus, they are used for treating diarrhea, dysentery and urinary tract infections (Fahey, 2005; Akinpelu and Onakaoya, 2009). Mucilage, coumarins, oses and holosides found in

parts of the tree studied can also contribute to the traditional and medicinal usage of the tree. According to Somboro et al. (2011), mucilages are favourable for stool firmness and can also prevent sudden dehydration while coumarins as polyphenolic compounds have anti-inflammatory, antimicrobial and anticoagulant properties.

This plant has been used in the production of the herbal antimalaria drug (Maloff-Hb) in Nigeria (Ogunkunle et al., 2014). Internationally, the bark of the tree has been extensively used in Europe and America for production of homeopathic drugs and in veterinary medicine (Bagot, 2015; Borokini, 2015). Previous studies (Borokini, 2015) have shown ability of *Okoubaka* for stimulating body's defense mechanism against poisoning and the extract from its bark has shown to be effective for upset stomach, constipation, pesticide poisoning, lethargy, depression and all this can be attributed to the presence of the numerous and varying quantities of the phytochemical compounds found in this study. *Okoubaka* tree is listed as one of the top 300 herbal medicinal plants sold in United Kingdom while the bark of the trees is known to be used in pediatric medicine in Germany (Borokini, 2015; Kohlrausch, 2011). Based on the numerous phytochemicals in the bark and leaves of *O. aubreville* tree, the tree can be also termed as a protective health agent.

CONCLUSION

The most important classes of secondary metabolites (phytochemical compounds) specifically alkaloids, flavonoids, tannins, saponins, anthraquinnes, mucilages, oses and holosides, coumarin and glycosides were detected in both the leaves and bark of the tree. Quantitative determination of the phytochemical compounds revealed significantly higher values of alkaloids, flavonoids and glycosides in leaves than barks of *O. aubreville* tree. However, there was no significant difference found in the values of tannins, mucilages, oses and holosides and coumarin. The wide diversity and numerous secondary metabolites found in samples of this tree may be responsible for its copious medicinal and traditional usage. It is recommended that further studies should be carried out using other solvents such as propanolic extract of the leaves and bark of the tree among others.

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