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QUALITATIVE, QUANTITATIVE PHYTOCHEMICAL SCREENING AND ANTIOXIDANT STUDY OF LEAF AND STEM BARK EXTRACTS OF *Lannea microcarpa* and *Terminalia aviccinniodes*

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ABSTRACT
Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. They continue to provide natural product chemists and pharmacologists with invaluable compounds as starting points for the development of new drugs. Stem bark and leaves of the two plants were extracted with ethanol, screened qualitatively and quantitatively for the presence of secondary metabolites using standard methods. Quantitative determination of total phenolics, total flavonoids, and in vitro antioxidant activity (DPPH) of the extracts was carried out using colorimetric methods. The Phytochemical screening result showed the distribution of secondary metabolites in the extracts with the leaf extract of L. microcarpa having the highest amount of total phenolics (884.40±1.09 mg/g GAE) and total flavonoids (60.24±0.34 mg/g QE) respectively. The antioxidant potential of the extracts recorded significant IC_{50} values in µg/ml ranging from 10.12 to 23.50 when compared to ascorbic acid (0.07) and butylated hydroxytoluene (0.27).
Key words: Antioxidant, phenolics, flavonoids, colorimetric, extracts

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Samuelsson, 2004). Besides, there are a lot of antioxidant substances present in plants and the free radical scavenging molecules present in them are in the form of phenolics compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites (Govindarajan *et al*., 2005; Naruthapata and Supaporn, 2009). Antioxidants are chemical substances that the inhibit oxidation process by preventing the formation of free radicals that cause damage to healthy cells, thus treating and managing chronic diseases such as cardiovascular diseases, diabetes, obesity, and some forms of cancers (Benbrook, 2005; Lillioja *et al*., 2013).

Lannea microcarpa Engl. and Krause (Family: Anacardiaceae) commonly known as African grape, wild grape or "Faaru" in Hausa is a tropical deciduous tree that grows up to about 16 m tall and 70 cm in diameter (Abubakar *et al*., 2007). It is located in most regions of West Africa and largely distributed in the dry forest re-growth areas (Nacoulma, 1996). The plant parts are used to treat stomach pain, rheumatism, gonorrhea, diarrhea, rachitis, chest pain, gastric ulcer, wounds, and skin and respiratory tract diseases. They are also applied to treat mouth blisters, sore throat, and dysentery, as a cathartic and as a dressing on boils (Kone *et al*., 2006). Several studies have shown the presence of anthocyanins and tannins in extracts of the fruit epicarp of *Lannea microcarpa*. 4'-methoxy-myricetin 3-O-α-L-rhamnopyranoside, myricetin 3-O-α-L-rhamnopyranoside, myricetin 3-O-α-L-glucopyranoside, vitexin, isovitexin, gallic acid and epi-catechin have been identified in the leaves of *Lannea microcarpa* (Hilou *et al*., 2017). The presence of cyanidin 3-0-(2-0-β-D-xylopyranosyl) β-D-galactopyranoside and cyanidin 3-0-β-D-galactopyranoside was also reported in the dry fruit epicarp (Ajiboye *et al*., 2013). Compounds such as γ-tocopherol, α-tocopherol and δ-tocopherol have also been found in seed oils (Bazongo *et al*., 2014).

Terminalia aviccenniodes Guill and Perr., (Combretaceae) is a tree plant widely distributed and commonly growing in the Savannah region of West Africa (Burkill, 1985).

Terminalia aviccenniodes is locally referred to as "baushe" among the Hausa (Atawodi *et al*., 2011), 'Kpace" in Nupe, "Kpayi" in Gwari and "Idi" among the Yoruba (Mann *et al*., 2008). It is well represented in West Africa and has been used for various medicinal purposes. The plant parts from *T. aviccenniodes* is used in Ivory Coast for anemia and severe jaundice, in Senegal for sores and ulcer (Kerharo and Bouquet, 1950), while in Nigeria, the decoction is used for gastrointestinal disorders (Abdullahi *et al*., 2001) as well as for syphilis by the Jukuns. Several bioactive hydrolysable tannin compounds including ellagic acid, punicalagin, flavogallonic acid and terchebulin (Shuaibu *et al*., 2007) have been isolated from this plant. An extensive array of triterpenes namely, arjunolic acid, α-amyrin and 2,3,2,3-trihydroxylolean-12-ene have been also isolated from the root of *T. avicennioides* (Mann, 2012).

However, the two plants having a lot of information about their medicinal values, the present study is designed to explore the phytochemical profile and antioxidant activity of the leaf and stem bark extracts of *L. microcarpa* and *T. aviccenniodes.*

MATERIALS AND METHODS

Plant collection

Fresh plant materials of *Lannea microcarpa* and *Terminalia avicennioides* were collected from Gwarzo and Kumbotso Local Government areas of Kano State respectively. They are identified and authenticated at the Herbarium unit of Plant Biology Department, Bayero University, Kano, with Herbarium Accession Number BUKHAN 280 and 609, respectively (Table 1).

Table 1. Names (scientific and local), parts and herbarium number of plants under investigations

Plant name	Family	Local Name(s)	Part(s) used	Herbarium Voucher #
Lannea microcarpa	Anacardiaceae	Faruu (H) Nago oku (Y)	Leaves and stem bark	BUKHAN 280
Terminalia avicennioides	Combretaceae	Baushe (H) Kpace (N) Kpayi (G) Idi (Y)	Leaves and stem bark	BUKHAN 609

Key: H- Hausa, N-Nupe, G- Gwari, Y-Yoruba, K-Kanuri

Extraction of Plant Materials

The plant materials were air dried and ground using mortar and pestle. 1kg of each was percolated with 96% ethanol (2.5L) for two weeks (Fatope *et al*., 1993). The percolate was filtered and evaporated to dryness using a rotary evaporator (R200) at 40^0C. The ethanol crude residues obtained from the leaves and stem bark of *L. microcarpa* were labeled as F01 and F02 respectively. While the crude leaf and stem bark extracts of *T. avicennioides* were referred to as `F01 and `F02, respectively. The extracts were kept in a desiccator for further analysis.

Qualitative Phytochemical Analysis of the Crude Extracts

The extracts were subjected to Phytochemical screening, to determine the classes of secondary metabolites present in the plant materials according to standard methods of (Sofowora, 1993). These include alkaloids, saponins, carbohydrates, tannins, resins, flavonoids, phenolics, saponins, glycosides, terpenoids, steroids, coumarins, quinones, antraquinones, and phlobotanins.

Quantitative Phytochemical Analysis of crude extracts

Sample preparation

About 10–50 mg of the extract was dissolved in 5 cm^3 methanol and sonicated for 45 minutes at 40^0C followed by centrifugation at 1,000 rpm for 10 min. The clear supernatant was collected and stored in an amber bottle for analysis. Sample and standard readings were made using a spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) at 765 nm against the reagent blank (Chandra *et al*., 2014).

Total phenolic content

The total phenolics of the extracts were determined using the Folin-Ciocalteu reagent, following the method described by (Chandra *et al*., 2014). The test sample (0.2 cm^3) was mixed with 0.6 cm^3 of water and 0.2 cm^3 of Folin-Ciocalteu's phenol reagent (1:1). After 5 min, 1 cm^3 of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3 cm^3 with distilled water. The reaction was kept in the dark for 30 min and after centrifuging the absorbance of blue colour from different samples was measured at 765 nm. The phenolic content was calculated as gallic acid equivalents GAE/g of dry plant material on the

basis of a standard curve of gallic acid (5–500 mg/L, $Y = 0.0027x - 0.0055$, $R^2 = 0.9999$). All determinations were carried out in triplicate

Total flavonoids content

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 cm^3 methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (200-5 $\mu g/cm^3$). An amount of 0.6 cm^3 diluted standard quercetin solutions or extracts was separately mixed with 0.6 cm^3 of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm. The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.0162x + 0.0044$, $R^2 = 0.999$) and expressed as mg quercetin equivalent (QE)/g of dried plant material (Chandra *et al.*, 2014). All the determinations were carried out in triplicate.

Determination of Antioxidant Activity.

Preparation of extract concentration

The extracts were dissolved in methanol to make a stock solution of 20mg/ml. The antioxidant activity of the extracts was measured at a serial concentration of 1000 μg/ml - 7.81μg/ml.

Radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

In vitro antioxidant activities of the extracts were determined using the DPPH free radical scavenging assay described by (Nithianantham *et al.*, 2011) with some modifications. DPPH in oxidized form gives a deep purple colour in methanol. An antioxidant compound donates protons to the stable DPPH radical, thus neutralizing the nitrogen radical which results in colour changes from deep purple to yellow. DPPH solutions show a strong absorbance at 517 nm appearing as deep purple colour. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidant potentials of the test samples.

Preparation of DPPH solution (0.1 M)

DPPH solution (0.1 M) was prepared by dissolving 0.39 mg of DPPH in a volumetric flask, dissolved in methanol, and the final volume was made to 100 ml. DPPH free radical solution was stored in a covered amber bottle at −20°C for further use.

DPPH Assay

The assay was performed in 96-well plates. The reaction mixture, containing 200 μL of 100µM DPPH solution and 100 μL of the diluted test sample, were incubated in dark at room temperature for 30min. The absorbance was measured at 517 nm. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as a positive control.

Percent DPPH radical scavenging activity was calculated as follows:

Percent radical scavenging activity = {1 − (AS − AB) (AC− AB)} × 100.

AS= Absorbance of sample

AC= Absorbance of control

AB= Absorbance of blank

RESULTS

Phytochemical screening of ethanol extracts of *T. avicennioides* and *L. microcarpa* shows the distribution of secondary metabolites as shown in Table 2. The leaf extract (F01) of *L. microcarpa* shows the presence of all the tested metabolites except anthraquinones while the stem bark extract (F02) shows a negative result in tannins and coumarins. However, *T. aviccinniodes* stem bark extract (`F02) revealed the presence of all the secondary metabolites while tannins, alkaloids and anthraquinones are absent in the leaf extract (`F01). The quantitative analysis carried out on the crude extracts of the plants materials (Table 3, Fig 1) showed that, the highest amounts of flavonoids is seen in leaf extracts of *Lannea microcarpa*, with a total content 60.24±0.34 (mg/g QE) while lowest concentrations is seen in the Stem bark extracts with a value of 54.68±0.89 (mg/g QE). Extracts of *T. aviccinniodes* showed the least amount of total flavonoids with a total of 37.97±0.14 (mg/g QE) and 7.60±0.15 (mg/g QE) for the leaf and stem bark when compared with compared with extracts of *L. microcarpa*. Total phenolics contents of the plant materials (Table 3, Fig 2) showed high amounts in the leaf extracts of *L. microcarpa* and *T. aviccinniodes* with a value of 884.40±1.09 (mg/g QE) and 743.58±1.09 (mg/g QE) respectively. Low contents of phenolics is seen in the stem bark extracts of *L. microcarpa* and *T. aviccinniodes* with a value of 614.60±8.46 (mg/g GAE) and 743.58±1.09 (mg/g GAE). Antioxidant activity of the plant materials shows lowest IC_{50} values of 25.30 and 16.30 in the stem bark extract of *T. aviccinniodes* and *L. microcarpa*. However, the highest IC_{50} value of 10.12 and 13.21 is seen in leaf extract of *L. microcarpa* and *T. aviccinniodes* respectively.

Table 2 Qualitative phytochemical screening of the extracts

Metabolites	F01	F02	`F01	`F02
Alkaloids	+	+	-	+
Tannins	+	-	-	+
Steroids	+	+	+	+
Sugars	+	+	+	+
Saponins	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Terpenoides	+	+	+	+
Glycosides	+	+	+	+
Quinones	+	+	+	+
Coumarins	+	-	+	+
Resins	+	+	+	+
Phlobotannins	+	+	+	+
Carbohydrates	+	+	+	+
Anthraquinones	-	+	-	+

Key: + = positive; - = Negative

Table 3 Total flavonoids content and total phenolic content from the stem-bark and leaves of *L. microcarpa* and *T. avicennioides*

Extract code	Total Phenolics (mg/g GAE)	Total flavonoids (mg/g QE)	Antioxidant activity (IC_{50} µg/ml)
F01	884.40±1.09	60.24±0.34	10.12
F02	614.60±8.46	54.68±0.89	16.30
`F01	743.58±1.09	37.97±0.14	13.21
`F02	481.79±2.83	7.60±0.15	23.50
AA	-	-	0.07
BHT	-	-	0.27

Key: F01: Ethanol leaf extract of *L. microcarpa,* F02: Ethanol extract of *L. microcarpa* stem bark, F01: Ethanol leaf extract of *T. avicennioides,* `F02: Ethanol extract of *T. avicennioides* stem bark, AA: Ascorbic acid and BHT: Butylatedhydroxytoluene

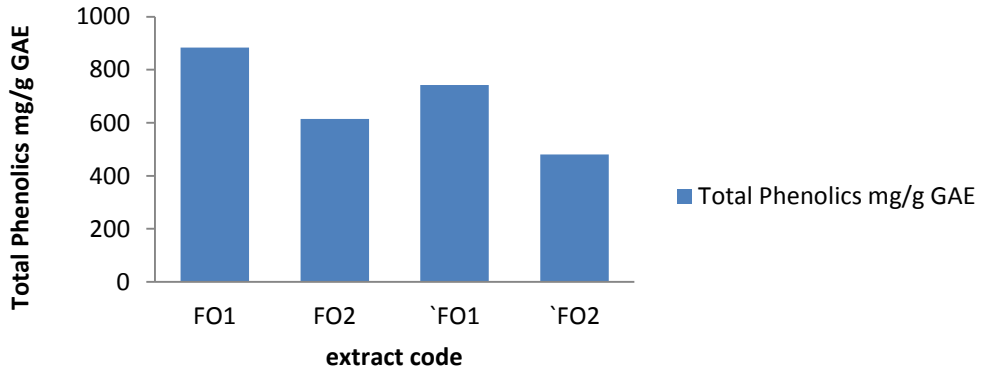

Fig 1: Results of total phenolics contents of *L. microcarpa* and *T. aviccinniodes*

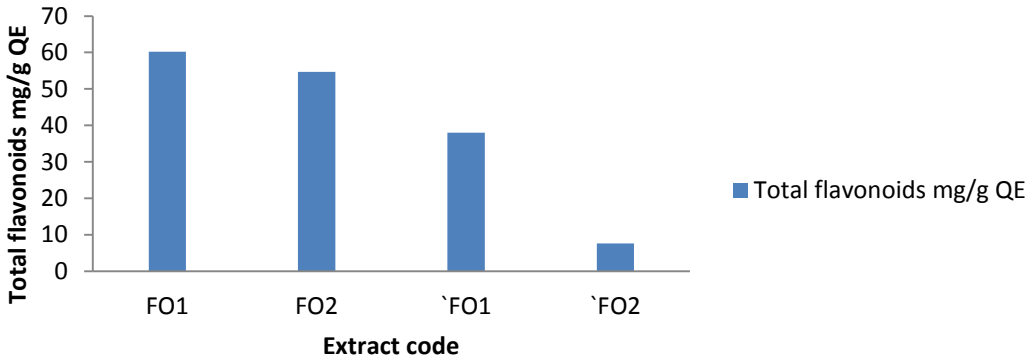

Fig: 2 Results of total flavonoids contents of *L. microcarpa* and *T. aviccinniodes*

DISCUSSION

The presence of secondary metabolites in plants produces some biological activity in man and animals and it is responsible for their use as herbs (Sofowora, 1986). Hence, the Phytochemical analysis is very much important to evaluate the possible medicinal values of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for guiding isolation of compounds and to perform more precise investigations. Extraction of a phytochemical from the plant material is mainly dependent on the type of solvent used. Previous work carried out on extracts of *Lannea microcarpa* has revealed certain phytochemical groups and molecules. The presence of triterpene sterols, anthracenosides, steroid and triterpene glycosides, coumarin derivatives, saponosides, reducing sugars, anthocyanins and phenolic compounds (tannins) have been demonstrated in the stem bark and root bark of *Lannea microcarpa* and *T. avicennioides* respectively (Nacoulma, 1996; Ouédraogo *et al*., 2010; Mann, 2012).

The Phytochemical composition of the stem-bark extract of *L. microcarpa* shows the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, and phenols. However, terpenoids and steroids were not detected in the plant materials (Sani *et al*., 2019). In addition, the results obtained are in agreement with the studies associated with other workers that show various distribution of metabolites tested (Mann *et al*., 2008; Salau *et al*., 2013; Musa *et al*., 2016; Lamien-Meda *et al*., 2008; Batiano *et al*., 2012; Ouedraogo *et al*., 2021).

The quantitative analysis carried out on the crude extracts of the plants materials showed that, the highest amounts of flavonoids and phenolics are seen in leaf extracts of *Lannea microcarpa* and *T. avicinnioides* while lowest concentrations in the Stem bark extracts. Polyphenolic compounds are some of the most widespread molecules among plant secondary metabolites. They are known to act as natural antioxidants. Results from Ouattara *et al*., 2010 reports the total phenolics content and total flavonoids content of the stem bark extract of *L. microcarpa* to be 40.07±0.05 GAE/100g lyophilized extract and 6.45±0.18 QE/100G lyophilized extract respectively. Similarly, Majumder *et al*., (2013), reports a value of 969.61±0.18 GAE mg/g for the total phenolics and 313.98±1.91 QEmg/g for total flavonoids of the aqueous stem bark extract of *L. microcarpa*. Ouattara *et al*., (2010) reported the total extractable phenolic contents of methanol extract of *Lannea microcarpa* stem bark extract to be 40.55mgGAE/g plant extract. In addition, Souley *et al*., (2018) have reported the stem bark extract to have a value of 36.61±0.4 (mgEqAG/g) for the total phenolics and 3.12±0.02 (mgEqQuer/g) for total flavonoids content.

Plant chemical substances such as flavonoids, tannins, have been shown to scavenge free radicals and therefore are viewed as promising therapeutic drugs for free radical's pathologies (Satoshi *et al*., 2018). According to Akanbi, (2013), *T. avicennioides* extracts have tendency to boost antioxidant level in organism because of the elicit significant increase in serum and liver catalase (CAT) and superoxide dismutase (SOD) levels in laboratory rats. Furthermore, the antioxidant effect of methanol extract of this plant in treated infected mice was also reported by (Omonkhua *et al*., 2013). The effects observed were listed to include an increase in antioxidant enzymes SOD and CAT activities, and a decrease in malondialdehyde (MDA) concentration. Balemnaba *et al*., (2019) reported that the LAMIC (freeze dried aqueous extract) extract exhibit better inhibitory activity against DPPH radical than ABTS radical with respective IC_{50} values of 45.38±3.21 µg/mL and 66.45±18.76 µg/mL, while FRAP assay exhibit antioxidant activity of 211.34±15.92 mmol EAA/g. The reducing power observed ranges from 0.40±0.02 to 0.76± 0.02 mg AAE/100 mg for fruit extract and its fractions, and 3.78±0.09 to 9.46±0.26 for leaves extract and its fractions, while a weak reducing power of the ethyl acetate and n-butanol fractions compared to that of the hydro-acetonic extract as reported by Balemnaba *et al*., (2019). In 2008, Lamien-Meda *et al*., showed that the antioxidant activity of *Lannea microcarpa* fruits was correlated with the content of phenolics and flavonoids. The antioxidant activity attributed to polyphenols is partly explained by their ability to capture free radicals and complex metals (Bahorun *et al*., 2004).

CONCLUSION

The distribution of several metabolites within different parts of the plant materials and their several reported bioactivities justify their potential as natural medicinal source for treatment of several ailments. The antioxidant results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity as the IC_{50} values of radical scavenging activity of the extracts and the contents of phenolics or flavonoids exhibited significant correlation.

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