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Solvent Extraction and its Effects on the Phytochemical Yield and Antioxidant Capacity of *Commiphora africana* (Burseraceae)

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ABSTRACT

The use of medicinal plants as a core component of the traditional African health care system is perhaps the oldest and most diverse of all therapeutic approaches. Traditional herbal healers are, in many parts of rural Africa, the most readily accessible and affordable health resource available to the local population and, at times, the only surviving treatment. Finding a suitable solvent is important to obtain a high yield of antioxidants in the phytochemicals present in *C. africana*. Among the solvents, methanol displayed the highest capacity in extracting glycosides (223.12±1.20 mg LE/g dry weight) phenolics (89.09±0.24 (mg GAE/g dry weight) and flavonoids (80.88±0.24 mg QE/g dry weight) from *C. africana* stem bark extract. Closely related trend was observed in glycosides, phenolics and flavonoids extracted with n-hexane. These findings suggest a high number of polar glycosides and phenolics in the stem extract of *C. africana*. These findings validate the pharmacological activities of the plant in Africa.

INTRODUCTION

Commiphora africana (A. Rich.) is a genus from the family of Burseraceae. The plant is known as "dashi" in Hausa, "badadi" in Fulfude, and "kabi" in the Kanuri languages of Nigeria [1]. It is a bush or small tree found mainly in the tropical African savannah forest and dry areas [2]. *Commiphora africana* is a savannah. The shrub is around 4 – 6m long, but can often be too tall developing to between 12 and 15 metres. Most of the bark *Commiphora africana* is papery and peels free, Papery flakes, exposing a green bark underneath. This is the leaves are entirely made up. The Fruit of *Commiphore africana* greatly improves the identification of an individual. The species when the fruit is ripe, it splits into half showing this is a brightly colored pseudo-aril. This fleshy little appendage in whole or in part, the seed as a part of a connection to part of the seed. The type Pseudo-aril varies from species to species [3].

Commiphora africana stem bark has been documented for the treatment of infected wounds, but it is typically chewed for the treatment of co inflammation, arthritis, obesity, microbial infection, wounds, pain, fractures, tumors and gastrointestinal diseases [5].
The selection of the best solvent for phytochemical extraction is important due to the presence of phytochemicals with various chemical structures and polarities that may affect their solubility in the chosen solvent. The optimal solution extraction will optimize the output of phytochemicals and antioxidants [2,6]. Water is commonly used in phytochemical mining with methanol, ethanol, acetone and a combination of these organic solvents with water [3]. The rise in solvent polarity between hexane and distillated water (hexane < ethnol < methanol < distilled water) further indicates that the solubility of phytochemical compounds is affected by solvent polarity.

MATERIAL AND METHOD

Plant materials

The stem barks of the plants were collected from a forest in Doko town, Garki Local Government, Jigawa state, Nigeria. The Plant sample was authenticated at the Biology section of Department of Science Laboratory Technology, Jigawa State polytechnic, Dutse. The plant materials were air dried in air under shade for four weeks and pounded to powder form. The stem powder was soaked in water for 24 h, filtered using Whatman Filter paper and stored refrigerated until use.

Extraction
Twenty-five grams of powder of the plant was successively fractionated with n-hexane and methanol by [4] system with minor modifications. Organic solvents were extracted under reduced pressure in the rotary evaporator system.

Phytochemical screening
The plant extracts and n-hexane, ethanol, water and methanol were assessed for the existence of the Phytochemical analysis by using the following standard methods [3-7].

Qualitative Phytochemical Screening

Test for Carbohydrates
The presence of carbohydrates was confirmed when 2 mL of extract was treated with 1 mL of Molisch's reagent and few drops of concentrated sulphuric acid which resulted in the formation of purple or reddish color.

Test for Tannins
To 1 mL of extract, 2 mL of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Flavonoids
To 2 mL of extract, 1 mL of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Glycosides
To 2 mL of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Quinones
To 1 mL of extract, 1 mL of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for Phenols
To 2 mL of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

Test for Terpenoids
0.5 mL of the extract was treated with 2 mL of chloroform and conc. sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.

Test for Cardiac Glycosides
To 0.5 mL of the extract, 2 mL of glacial acetic acid and few drops of ferric chloride were added. This was under layered with 1 mL of conc. sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Test for Saponins
To 2 mL of extract, 2 mL of distilled water were added and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that shows the presence of saponins.

Test for Alkaloids
To 2 mL of extract, 2 mL of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Ninhydrin Test
To 2 mL of the fruit extract few drops of 0.2% ninhydrin reagent was added and heated for 5 min. Formation of blue colour indicates the presence of amino acids.

Test for Coumarins
To 1 mL of 10% sodium hydroxide was added to 1ml of the extract. Formation of yellow colour indicates the presence of coumarins.

Anthraquinones
To 1 mL of fruit extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones

Steroids
To 1 mL of fruit extract equal volume of chloroform is added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Test for Phlobatannins
Few drops of 2% hydrochloric acid were added to 1ml of the extract. Appearance of red colour precipitate indicates the presence of phlobatannins.

Anthracyanin
To 1 mL of the extract was added 1 mL 2N sodium hydroxide and heated for 5 min at 100 °C. Formation of bluish green colour indicates the presence of anthocyanin.

Quantitative Determination of Flavonoids

Determining the content of Total Phenolics (TPC)
The TPC *Commiphora africana* extracts was calculated using the method of Folin-Ciocalteu as defined by [7]. Using a UV-Visible spectrophotometer, the absorbance was read at 765 nm. The TPC was expressed as mg per gram of dry weight (mg GAE/g DW) of gallic acid equivalents (GAE).

Total Flavonoid Quality Determination (TFC)
The TFC was calculated using the colorimetric assay of aluminum chloride as defined by [8]. Using a UV-Visible spectrophotometer, the absorbance was read at 510 nm. Quercetin equivalents (QE) per gram of dry weight (mg QE/g DW) is expressed as mg of TFC.

Absolute Glycosides Content Determination (TTC)
The TTC was measured using a calorimetric assay of sulfuric acid [9]. The UV-Visible spectrophotometer was used to read the absorbance at 538 nm. Linalool equivalent per gram (mg LE/g DW) of dry weight was expressed as the total terpenoid content.

Antioxidant Assay
Antioxidant activity was calculated using a 2,2-diphenyl-1-picryl-hydrazile (DPPH) free radical scavenging activity assay adapted from [10]. The initial absorbance of the DPPH solution was calculated at 517 nm without a sample. Approximately 0.2 mL of each sample extract was mixed with 3 mL of 0.1 mM of DPPH solution. The mixture was incubated at room temperature in the dark for 30 minutes. The improvement in absorbance was measured at 517 nm after 30 minutes of incubation using a UV-Visible spectrophotometer. The results obtained were measured and expressed as a percentage of DPPH free radical scavenging operation using the following formula.

DATA ANALYSIS

All data were analyzed using Excel for the estimation of mean and standard deviation. One-way ANOVA of GraphPad software was used to calculate the statistical difference and to obtain the graph and the correlation.

RESULT AND DISCUSSION

Phytochemical Screening

The phytochemical analysis of various fractions of *Commiphora africana* stem bark extract is shown in **Table 1.** All the phytochemicals analysed were virtually present in all the solvents with the exception of steroids and anthraquinones which was found to be absent in all the samples.

Table 1. Phytochemical Analysis of *Commiphora africana.*

s/no	Phytochemicals	n-hexane extracts	Methanol extracts	Aqueous extracts
1	Flavonoids	+++	++	+
2	Phenols	++	++	+
3	Saponins	+	+	+
4	Tannins	_	+	+
5	Steroids	-	-	-
6	Terpenoids	+	+	+
7	Anthraquinones	-	-	-
8	Amino acids	+	+	+
9	Alkaloids	+	++	+
10	Proteins	+	-	++
11	Glycosides	+	++	+

Note: ++ present in moderate; +++ present in more quantity; - Absent

The existence of phytochemicals in *Commiphora africana* has varying levels of protective antioxidants and antimicrobial molecules [11]. In addition, these phytochemicals serve as the best antioxidants and protect cells from free radical damage, e.g. carotenoids, polyphenols, etc. or to minimize the risk of cancer by inhibiting tumor development or hormonal stimulation and antibacterial activity [12].

Commiphora africana extract produce numerous phytochemicals [12]. The availability of the phytochemicals depends on the solubility of the compounds in the solvent. In our finding's different concentration of phenolics, glycosides and flavonoids extracted were presented in **Table 2**.

Table 2. Total flavonoid, glycosides and phenolic content of *Commiphora africana* stem bark extract (mg/g) in different extraction solvents.

Extraction solvent	flavonoids	glycosides	phenolics
n-hexane	65.88 ± 0.32	81.34±0.22	70.12 ± 0.22
methanol	80.88 ± 0.24	223.12±1.20	89.09±0.24
ethanol	55.83 ± 0.40	36.89±0.14	28.37 ± 0.42
water	33.25 ± 0.14	32.70± 0.14	65.00±0.29

Among the solvents, methanol displayed the highest capacity in extracting glycosides (223.12±1.20 mg LE/g dry weight) phenolics (89.09±0.24 (mg GAE/g dry weight) and flavonoids (80.88±0.24 mg QE/g dry weight) from *Commiphora africana* stem bark extract. Closely related trend was observed in glycosides, phenolics and flavonoids extracted with n-hexane. These findings suggest a high number of polar glycosides and phenolics in the stem extract of *C. africana.* That may be due to the formation of hydrogen bonds between hexane and methanol hydroxyl groups with electronegative oxygen. The hydrogen bond can also form between the methanol hydroxyl group and ethanol with an oxygen atom located in phenolic and glycoside structures.

The methanol and hexane were the best in extracting phenolics including flavonoids from other plants. The methanol extract also exhibits the highest DPPH scavenging activity (**Table 3**). The solubility of glycosides and phenols in extracting solvents depends on the functional groups attached to the main structure of these phytochemicals, the molecular size and the length of the hydrocarbons [13]. In addition, the solvation potential determines the solubility of phytochemicals. For example, methanol has a better solution of phenols and flavonoids than ethanol's due to the presence of shorter methyl radicals in methanol compared to long ethyl radicals in ethanol [14].

Table 3. DPPH scavenging activity of *Commiphora africana* stem bark extract in different extraction solvents.

	Percentage DPPH scavenging activities (%)
n-hexane	79.98 ± 1.40
methanol	85.19 ± 0.67
ethanol	51.67 ± 0.00
distilled water	66.11 ± 1.16

The presence of glycosides, flavonoids and phenolic compound in the stem extract of *C. africana* may be responsible for the high antioxidant properties in methanol extract. This could be due to the presence of polar antioxidant compounds. The substantial association between phenolics and biological activity was consistent with previous evidence suggesting the significant contribution of phenolics and flavonoids [4].

CONCLUSION

The presence study shows that hexane exhibited the highest capacity to extract phytochemicals from *C. africana* stem bark extract, and the methanolic extracts displayed the highest antioxidant activity compared to other solvents used.

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