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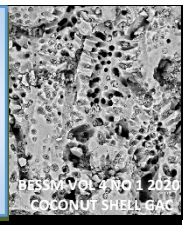

Preliminary Phytochemical Screening, Quantitative Analysis of Flavonoids from the Stem Bark Extract of *Commiphora africana* (Burseraceae)

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ABSTRACT

The use of medicinal plants as a fundamental component of the traditional African health care system is perhaps the oldest, sustainable and most diverse of all therapeutic methods. Traditional medicinal herbal healers are, in many parts of rural Africa, the most readily accessible and inexpensive health resource available to the local population and, at times, the only surviving treatment. The Phytochemical analysis of *Commiphora africana* was carried out in three different organic solvents n-hexane, methanol and aqueous. Preliminary qualitative analysis revealed the presence of all the Phytochemicals analysed except anthraquinone and steroids. Quantitative phytochemical analysis showed that n-hexane fraction of *Commiphora africana* contains more phenolics and glycosides (6.2 ± 0.02, and 7.2 ± 0.1) respectively as compared to flavonoid (4.1± 0.03). High performance liquid chromatography (HPLC) of n-hexane extract of the plant using three standard flavonoids rutin, quercitin and gallic acid showed comparable RT values. From the calibration curve, it was compared and revealed the presence of more quercitin (60%) followed by gallic acid in a negligible amount. Quercetin has specific biological properties, which can enhance mental / physical efficiency and reduce the risk of infection.

INTRODUCTION

The use of conventional medicines and medicinal plants as therapeutic agents for the preservation of good health has been commonly documented in most developed countries [1]. Traditional medicine is the sum total of expertise, skills and procedures based on ideas, values and experiences common to various cultures that are used to preserve health, as well as to prevent, diagnose, improve or manage physical and mental illness. Modern medicine adopted by other communities (outside of its indigenous culture) is sometimes referred to as complementary or alternative medicine (CAM) [2]. The World Health Organization (WHO) has estimated that 80 per cent of the developing world population relies on conventional medicine for treatment. In the past decades, the developed world has also witnessed an increasing trend in the use of CAM, especially herbal remedies [2,3]. Herbal medicines include spices, herbal materials, herbal preparations and finished herbal products containing parts of plants or other plant materials as active ingredients. Although 90 % of the population in Ethiopia uses herbal remedies for their primary health care, studies in developed countries such as Germany and Canada continue to indicate that at least 70% of their population has tried CAM at least once [3].

Commiphora africana (A. Rich.) is a genus of the Burseraceae family. The plant is named "dashi" in Hausa, "badadi" in Fulfude and "kabi" in the languages of Kanuri in Nigeria[4]. It is a bush shrub or small tree found mostly in the savannah forest and dry areas of tropical Africa. It is commonly used for the diagnosis of a variety of diseases including typhoid, wound healing, pain relief, dysentery, heartburn, snakebite, antimalaria, plaster and spasm [5]. Despite the medicinal applications of *Commiphora africana*, there is lack of information on the active phytochemical component responsible the medicinal properties. This study, therefore, is aimed at

extracting and studying the properties of the most active component of the aqueous extract of the stem bark of *Commiphora africana*.

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[6] system with minor modifications. Organic solvents were extracted under reduced pressure in the rotary evaporator system.

Phytochemical screening

The plant extracts and n-hexane and methanolic were assessed for the existence of the Phytochemical analysis by using the following standard methods

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: 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: 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: 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.
Anthracyanine: 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 color indicates the presence of anthocyanin.

Quantitative Determination of Flavonoids

Estimation of Flavonoids: The total flavonoid content of the sample was measured using the[7] test. A concentration of 0.25 ml of the sample was diluted to 1.25 ml of distilled water. 75 µl of 5 percent sodium nitrite was added and 0.1 5 ml of aluminum chloride solution was added after 6 minutes. 0.5 ml of 0.1 M NaOH was added after 5 min to make up to 2.5 ml of distilled water. The solution was well balanced, and the absorbance was read at 510 nm along with the normal quercetin at 5-25 µg concentration. The findings are expressed as mg flavonoids as a quercetin equivalent / gm of dried sample.

Determination of flavonoids by HPLC Method: HPLC was con**ducted by** [8] [9] For the purpose of the determination of flavonoids. The coloumn used is C18 fitted with a pump (LC-10AT VP1), a SIL-6A automatic injector and a detector (SPD-10AVP) of 370 nm. The sample extract was injected into the loop and the temperature was held at 40 °C and the mobile process consisted of 50 ml of methanol, 1 ml of water and 50 ml of phosphoric acid (100:100:1) with a flow rate of approximately 1.5 mL / min. Flavonoids have been described as mg / g of fresh weight.

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[5]. 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[10].

The quantitative analysis of *commiphora Africana* shows that it contains more phenolics and Glycosides (6.2 ± 0.02, and 7.2 ± 0.1) respectively as compared to Flavonoid (4.1± 0.03). The HPLC analysis of the n-hexane extract using three flavonoids standard i.e Quecetin, rutin and gallic acid was carriedout and the RT values of the extract correlate with that of the standard flavonoids. From the calibration curve, it was compared and revealed the presence of more Quercitin (60%) followed by gallic acid in a negligible amount.
Quercitin is a flavonoid that was found mainly in the glycoside form. Quercetin, has specific biological properties, which can enhance mental / physical efficiency and reduce the risk of infection [11]. it is used to reduce the risk of cardiovascular disease , cancer and also protects against osteoporosis [12]. Such features are the basis of potential advantage for overall resistance to health and disease, including antiviral, anti-inflammatory, antiviral, antioxidant and psychostimulating activities, and capacity to inhibit lipid peroxidation [13].

CONCLUSION

The present study showed that n-Hexane extract of *Commiphora africana* is an important medicinal plant rich in basic nutrients. Qualitative phytochemical analysis showed that it is abundant in phytochemicals such as alkaloids, saponins, flavonoids, phenols, proteins, tannins, terpenoids and glycosides especially it was found in high amount in n-hexane extract than other extracts. Quantitative analysis showed that n-hexane extract contains higher amounts than other two extracts (aqueous and methanol). From our findings of the study it may be concluded that the plant extract contains quercetin flavonoids, evidence of the use of the plants stem bark for various medicinal values especially cardiovascular.

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