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PHYTOCHEMICAL SCREENING OF THE EXTRACT OF THE FLAKING BARK OF *Commiphora kerstingii* AND ITS GC-MS ANALYSIS

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ABSTRACT

The crude methanol extract of Commiphora kerstingii, used traditionally for the treatment of tuberculosis was phytochemically screened. The phytoconstituents were qualitatively and quantitatively determined. The crude extract was partitioned into four fractions namely ethyl acetate, n-hexane, n-butanol and aqueous methanol soluble fractions. These solvent soluble fractions were also phytochemically screened. The crude extract and its fraction were subjected to thin layer chromatography analysis. The study revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, anthraquinones and flavonoids in the crude extract. Alkaloids, tannins, saponins and flavonoids were quantified. The distribution of phytoconstituents in the fractions was revealed. GC-MS analysis of the methanol soluble fraction revealed fifteen constituents. Two of these phytocompounds were found to be the cardiac glycoside, methyl-beta-D-glucopyranoside and the anthraquinone, 6-acetyl-5-hydroxy-2, 7- dimethyl-1, 4-naphthoquinone.

INTRODUCTION

The use of natural products for therapeutic purposes is a practise that has persisted since prehistoric times. Mankind has depended on both herbal and non-herbal traditional medicines for curative and prophylactic purposes [1]. The medicinal properties and pharmacological activities of any natural product is a direct consequence of its chemical content. The presence of secondary metabolites (for example tannins and saponins), have been found to impact certain pharmacological capabilities on the natural product, which brings about needed physiological changes in the consumer, for the improvement of certain health conditions[2].

Commiphora kerstingii is a shrub, the flaking bark of which has been used for ages among Nupe and Gbagyi ethnic groups of Central Nigeria, for the treatment of tuberculosis [3]. A number of phytoconstituents have been associated with antitubercular activity. These include alkaloids, cardiac glycosides, tannins, anthraquinones and saponins [4, 5]. This study aims to identify the metabolites associated with the antitubercular activity.

MATERIALS AND METHODS

The plant material, *Commiphora kerstingii* was identified by Plant Taxonomist, Umar S. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, and Voucher specimen deposited for future reference. The flaking barks of *Commiphora kerstingii* was collected by carefully peeling the flakes from the stem found in a residential garden at Gidan Kwano near the permanent site of the Federal University of Technology, Minna, Niger State, Nigeria. The flaking barks were kept in polythene bags and kept in dry cardboard prior to analyses.

Preliminary Preparation

The flaking barks were then cut into small pieces, air-dried and then oven-dried at 60°C. The dried bark sample was ground into powder (Excella Electric Grinder). The powder was stored in an air tight container [6]. The powder was then extracted using 70% aqueous methanol. The extraction was carried out thrice successively, each lasting for 72 hours in 2 litres of 70% methanol [7]. The cloudy mixture was then filtered using whatman filter paper No. 1, held in a glass funnel. The methanol solution was then concentrated using a rotary evaporator. The dark brown concentrate was then air dried [8].

Qualitative Phytochemical Screening of the Crude Methanol Extract

The crude methanol extract was screened for the presence of secondary metabolites including alkaloids, saponins, tannins, flavonoids, cardiac glycosides and anthraquinones, using standard procedures [9-12].

Quantitative Phytochemical Screening of the Crude Methanol Extract

The crude methanol extract was quantitatively screened to determine its percentage composition of alkaloids, saponins, tannins and flavonoids, using standard procedures [12-15].

Fractionation of the Crude Methanol Extract

The crude methanol extract was dissolved in70% methanol. It was then partitioned successively with n-hexane (100ml x 3), ethyl acetate (100ml x 3), *n*-butanol (100ml x 3) to give four fractions (i.e *n*-hexane, ethyl acetate, n-butanol and methanol soluble fractions). The four solvent soluble fractions were concentrated

separately using a rotary evaporator. The concentrates were evaporated to dryness over hot water bath and air dried to constant weight [16-18].

Qualitative Phytochemical Screening of the Solvent Soluble Fractions

The solvent soluble fractions were screened for the presence of secondary metabolites including alkaloids, saponins, tannins, flavonoids, cardiac glycosides and anthraquinones, using standard procedures [9-12].

Thin Layer Chromatography (TLC) Analysis of the Crude Extract and its Fractions

Samples were dissolved in 10mls of 70% methanol and filtered. Standard Whatman TLC plates (LK6D Silica Gel 60A) was washed with acetone in a regular solvent tank for one hour and then dried in a fumehood before use. A drop was placed on the TLC plate an inch from the bottom. Different solvent mixtures were used to develop the plates. A mixture of ethyl acetate and 70% methanol (2:1) was used for the total crude extract. Ethyl acetate and 70% methanol (1:2) was used for the methanol soluble fraction. A mixture of n-hexane and ethyl acetate (1:3) was used for the n-hexane soluble fraction. Ethyl acetate and 70% methanol (4:1) was used for the ethyl acetate soluble fraction, while the *n*-butanol soluble fraction was developed in a mixture of ethyl acetate and 70% methanol (3:1). The number of spots was observed and the R_f values calculated [7].

Gas Chromatography – Mass Spectrometric Analysis of the Methanol Soluble Fraction

The GC-MS analysis involved a GCMS-QP2010 Plus Model. The injection temperature was 220°C. The carrier gas inlet pressure was 100.2 KPa. The oven temperature was programmed at 15°C /min from 60°C (2mins) to 270°C (3mins). Mass spectrometry involved a positive ion Chemical Ionization (CI). The ion source temperature was 200°C. The interface temperature was 250°C. The solvent cut time was 2.5 minutes. 8µl of the sample was injected and the analysis carried out. The Gas Chromatogram and Mass Spectrum representing the constituents were given by the computer [16].

RESULTS AND DISCUSSION

Percentage Yield of the Extract

In the preparation of the crude methanol extract of the flaking bark of *Commiphora kerstingii*, a yield of 10.2% was obtained.

Quantitative Analysis of the Crude Methanol Extract

Table 1 shows the results of the quantitative phytochemical analysis of the crude methanol extract of the flaking bark of *Commiphora kerstingii*. The results revealed that there were more of tannins and flavonoids than there were saponins, while alkaloids constituted a small portion of the total crude extract.

Table 1: Quantitative Analysis of the Crude Methanol Extract

Metabolites	Amount Extracted (g) Per 100g Crude
Alkaloids	2.9
Tannins	12.5
Saponins	7.4
Flavonoids	11.2

Qualitative Phytochemical Screening

Table 2 shows the result of the qualitative phytochemical screening of the crude methanol extract and the solvent soluble fractions of the flaking bark of *Commiphora kerstingii*. The result revealed the presence of tannins, cardiac glycosides, anthraquinones, saponins, flavonoids and alkaloids in the total crude extract, the ethyl acetate soluble fraction and the n-butanol soluble fraction. The *n*-hexane soluble fraction contained saponins, and there were cardiac glycosides, anthraquinones and saponins in the methanol soluble fraction. The presence of saponins, cardiac glycosides, tannins, alkaloids, flavonoids, and anthraquinones in the crude methanol extract of the flaking bark of *Commiphora kerstingii* is in agreement with previous reports by other researchers [8]. These constituents have been known to exhibit medicinal properties as well as physiological activities [2]. Antitubercular activity has been attributed to a wide range of phytoconstituents including alkaloids,

glycosides, tannins and anthraquinones [4]. Antitubercular activity has also been attributed to the presence of saponins [5].

Table 2: Phytochemical Screening of the Crude Extract and its Fractions

	Crude Methanol Extract	n-Hexane Soluble Fraction	Ethyl Acetate Soluble Fraction	n-Butanol Soluble Fraction	Methanol Soluble Fraction
Tannins	+	-	+	+	-
Cardiac glycosides	+	-	+	+	+
Anthraquinones	+	-	+	+	+
Saponins	+	+	+	+	+
Flavonoids	+	-	+	+	-
Alkaloids	+	-	+	+	-

+ = Present; - = Absent

Yield of the Fractionation Process

The crude methanol extract was subjected to fractionation, which gave four fractions namely the *n*-hexane soluble fraction, the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the methanol soluble fraction. Table 3 shows the yield of the fractionation process. It shows that the process yielded more of the ethyl acetate and *n*-butanol soluble fractions and less of methanol and *n*-hexane soluble fractions.

Table 3: Yield of the Fractionation Process

Solvent Soluble Fraction(g)	Yield
Ethyl acetate soluble fraction	8.6
Methanol soluble fraction	4.2
n-Butanol soluble fraction	7.4
n-Hexane soluble fraction	3.4

TLC Analysis of the Crude Extract and its Fractions

Table 4 shows the TLC behaviours of the constituents of the crude methanol extract and that of its fractions. This analysis reveals the relative abundance of constituents in the total crude extract and each solvent soluble fraction.

Table 4: TLC Analysis of the Crude Extract and its Fractions

Sample	Number of Spots	R_f Values
Total Crude Extract	9	0.15, 0.2, 0.3, 0.4, 0.55, 0.6, 0.65, 0.75, 0.8
Methanol Soluble Fraction	3	0.2, 0.4 and 0.7
n-Butanol Soluble Fraction	3	0.3, 0.6 and 0.75
Ethyl Acetate Soluble Fraction	4	0.3, 0.4, 0.7 and 0.8
n-Hexane Soluble Fraction	2	0.4 and 0.6

Interpretation of the GC-MS Chromatogram

The methanol soluble fraction was subjected to GC-MS analysis. The analysis revealed fifteen constituents. Five major constituents are shown in table 5. The result of the analysis suggested that a cardiac glycoside, methyl-beta-D-glucopyranoside and an anthraquinone, 6-acetyl-5-hydroxyl-2, 7-dimethyl-1, 4- naphthoquinone are present.

Table 5: Molecular Masses, Percentage Areas and Major Peaks of Some of the Constituents of the Methanol Soluble Fraction

Line	Compound	Molecular Mass	Area (%)	Peaks (m/z)
4	(p-Hydroxybenzoyl) hydrazine	152	1.34	(121), 93, 92
6	Methyl-beta-D- glucopyranoside	194	18.97	(60), 131, 61, 97
8	1-Pentadecanecarboxylic acid	256	9.58	(73), 213, 199, 185, 171
10	6-Acetyl-5-hydroxy-2,7-dimethyl naphtoquinone	244	3.14	(229), 69
13	Octadecanoic acid	284	10.06	(57), 241,227, 185, 73

( ) Represents the base peak

CONCLUSION

In conclusion, the quantitative and qualitative phytochemical screening of *Commiphora kerstingii* revealed the presence of saponins, alkaloids, tannins, cardiac glycosides, anthraquinones and flavonoids and determined the content of saponins, flavonoids, tannins and alkaloids. The wide variety of secondary metabolites present gives sufficient drive for further investigations into its antitubercular activity.

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