PHYTOCONSTITUENTS DETERMINATION AND CHARACTERIZATION OF THE ROOT BARK EXTRACT OF *Maytenus senegalensis*

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ABSTRACT

Phytoconstituent analysis and characterization of the root bark extract of Maytenus senegalensis were determined using GC-MS analysis. The crude extract was partitioned using solvents of different polarities and the fractions obtained were tested for their phytoconstituents. The preliminary phytochemical screening indicates the presence of flavonoids, saponins, phenols and steroidal compounds. Anthraquinones and tannins were completely absent. Results obtained indicate that 70% methanolic fraction contains all the constituents mentioned above. This fraction was then subjected to thin layer and column chromatography as well as GC-MS analysis. The GC-MS spectrum reveals the presence of seven different compounds in which three of them may be associated with the ethnomedicinal properties of this plant.

Keywords: Phytoconstituents, *Maytenus senegalensis*, Methanolic, GC-MS Analysis.

INTRODUCTION

Many indigenous plants are major ingredients of traditional medicine [1]. Numerous studies have been conducted on traditional usage of plants which are often substantiated by scientific investigation that resulted in the isolation of bioactive compound for direct use in medicine [2]. The medicinal value of these plants lies with the chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are terpenes, alkaloids, tannins, flavonoids and phenolic compounds [3]. One of these medicinal plants is called *Maytenus senegalensis* which belongs to the Celastraceae family used by the Nupes in Niger State, Nigeria to manage diabetes [4, 5]. The present research work is to determine the phytoconstiteunts responsible for the antidiabetic activities associated with this plant. Previous works done on this plant showed some pharmacological activity, but the bioactive components responsible for these activities are yet to be identified.

MATERIALS AND METHODS

Plant materials

The root bark of *Maytenus senegalensis* was collected on January, 2012 from Patishin swasun forest, 5 km along Bida-Busu Road, Niger State, Nigeria. The root bark was air-dried under the shed at room temperature. The dried plant material was manually pulverized and the powder was kept in polyethylene bags until used. The plant was duly identified by Plant Taxonomist, Umar S Gallah and Voucher specimen deposited at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Preparation of extract

The powdered material (249 g) was macerated by soaking in 70% methanol at room temperature for 72 h. This was repeated thrice and the resultant extracts were combined and then concentrated *in vacuo* using rotary evaporator at 35°C to give the crude methanolic extract. It was then air dried and packed in a glass bottles with proper labeling and kept under refrigeration, and away from light by wrapping with aluminum foil prior to further processing.

Preliminary phytochemical screening

The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures [3, 6].

Partitioning of the crude extract

The crude methanolic extract (69g) was dissolved in 500ml of methanol. The resultant solution was partitioned with 250 ml thrice each in order of their increasing polarities (*n*-hexane, chloroform and butanol) [7]. All the four fractions obtained were filtered one after the other using Whatman No.1 filter paper sheets (Whatman Int. Ltd. Maidstone, England). Each extracts was then concentrated in water bath at 35°C by the evaporation of various solvents from the extracts after which phytochemical screening was conducted on each fraction and the methanolic extract which was having most of the metabolite was taken for further analysis.

Column Chromatography for the methanolic fraction

The methanol was mounted on the prepared column and eluted with the following gradient mixtures of n-hexane-EtOAc (100:0→95:5→20:80→0:100) and MeOH.

Eluents were collected in 50ml and were then monitored by TLC behavior [7].

Gas Chromatography – Mass Spectrometric (GC-MS) Analysis

GC-MS analysis was carried out on a GC-MS (QP2010 PLUS MODEL, SHIMADSU, JAPAN). 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 [8].

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). Spectrum of the unknown was compared with the spectrum of the known components stored in the NIST library with which the name, molecular weight and structure of the components of the test material were ascertained.

RESULTS AND DISCUSSION

Percent yield of the Extract

From the crude methanolic extract of the root bark of *M. senegalensis*, a yield of 27.30% was obtained.

Preliminary Phytochemical Screening

Phytochemical screening was done using colour forming and precipitating chemical reagents on both the crude and fractionated extract to generate preliminary data on the constituents of the plant extracts. The results obtained from the tests were summarized in Table 1 for both crude and fractionated extracts. The chemical tests reveals the presence flavonoids, alkaloids, saponins and phenolic compounds, which agree with the previous study by other researchers, indicated the presence of secondary metabolites like flavonoids, alkaloids and saponins [9].

Table 1: Phytochemical screening of the crude extract and soluble methanolic fraction of *M. senegalensis*

Phytoconstituents	Tests	Crude methanolic extract	Soluble methanolic extract
Alkaloids	Dragendorff's	++ +	++
	Mayer's Reagent	++ +	
Steroidal compounds	Acetic anhydride and conc. sulfuric acid	++	+
	Chloroform and conc. sulfuric acid	+++	+
Phenolic compounds	Ferric chloride and potassium ferrocyanide	++	+
Flavonoids	Ammoniacal silver nitrate	+++	
Saponins	Froth	++	+++
Tannins	Ferric chloride	-	+
			-
Anthraquinones	Borntrager's	-	
			-

Key: +++ → Very strong positive,++ →positive, + →Trace, - → Negative

Partitioning of the crude extract

The partitioned crude extract: n-hexane, chloroform, n-butanol and methanol soluble fraction as shown in Table 2 below.

Table 2: Fractions obtained during the partitioning

Fraction	Weight(g)
n-hexane	2.8
Chloroform	6.9
Butanol	12.13
Aqueous methanolic fraction	16.40

Column Chromatography analysis

The eluents obtained from the column using gradient mixtures of n-hexane-ethyl acetate and ethyl acetate-methanol gave series of fractions which were obtained which were monitored by TLC behavior. Based on the TLC analysis of the fractions, some of the fractions were pooled together as follows: F_{23}, F_{33}, F_{43}, F_{50}, F_{57}, F_{58}, F_{63}, F_{65}, F_{71}, F_{75}, F_{81}, and F_{84}. The Rf values ranges between 0.2-0.8. Most spots are of lower Rf values indicating that most of the component are polar. F_{23} and F_{50} show three spots indicating that there are three likely components in each fraction. The spot(s) of each fraction and their corresponding Rf values are shown in Table 3.

Table 3: The pooled fractions after TLC analysis

Fractions	Spots	Rf values
F_{23}	3	0.5, 0.3, 0.5
F_{33}	1	0.3
F_{43}	1	0.4
F_{50}	3	0.5, 0.6, 0.7
F_{57}	2	0.6, 0.6
F_{58}	2	0.6, 0.4
F_{63}	1	0.5
F_{65}	1	0.3
F_{71}	1	0.3
F_{75}	2	0.6, 0.8
F_{81}	1	0.5
F_{84}	2	0.6, 0.4

Phytochemical analysis for the TLC fractions

Fractions, based on their similarities in their spot(s) and Rf values were grouped together as follows:

F_{23} (F_1)
F_{43} (F_2)
F_{50} (F_3)
F_{61} and F_{81} (F_4)
F_{33}, F_{71} and F_{65} (F_5)
F_{27}, F_{58}, F_{75}, and F_{84} (F_6)

Phytochemical analysis was then carried out on the six fractions and the following results were obtained as presented in Table 4.

Table 4: Results of the phytochemical analysis of the TLC fractions

Phytoconstiteunts	Fractions					
	F_1	F_2	F_3	F_4	F_5	F_6
Alkaloids	-	-	-	-	+"	-
Steroids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	-	-	+	+	+	-
Phenolics	-	-	-	-	-	-

Fraction 5 which was observed to have flavonoids, saponins, and trace amount of alkaloids was then taken for GC-MS analysis.

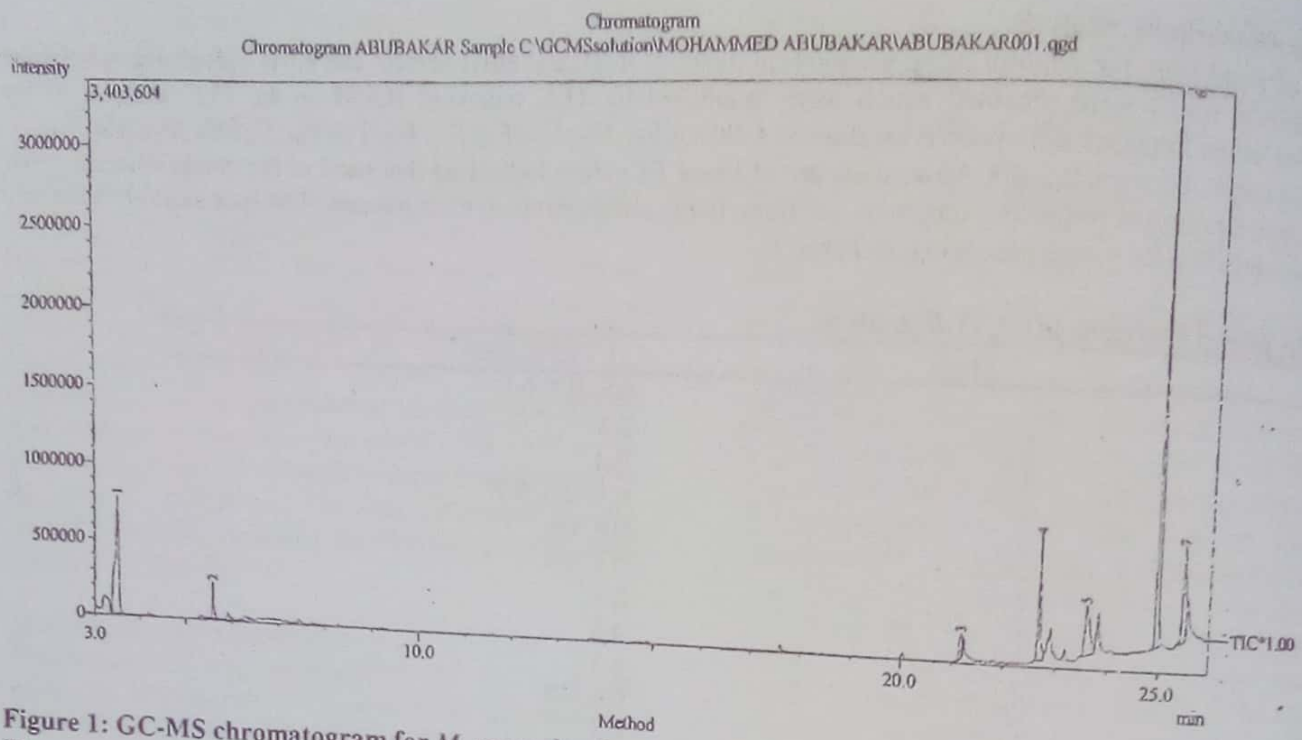

Figure 1: GC-MS chromatogram for *M. senegalensis subfraction fraction*

The compounds present in the methanol subfraction identified by GC-MS were shown in Figure 1. Peaks with peak area % as well as the suggested compounds are shown in Table 5.

Table 5: GC-MS for the methanol subfraction

Peaks	Compound name	Area %	Signals
1	Pyruvic aldehyde dimethyl acetal	20.07	117,102,87.
2	2methyl-1-cyclopentene-1-carbxylate	2.73	126,111,95.
3	Palmitic acid	2.85	
4	13-hexyloxacyclotridec-10-en-2-one	11.81	256,227,213,185
5	Oleic acid	10.50	280,166,151,137.
6	Methyl ricinoleate	38.05	264,137,123,97.
7	-	166	298,194.

CONCLUSION

Preliminary phytochemical investigation of *Maytenus senegalensis* revealed the presence of flavonoids, saponins and alkaloids, while the GC-Ms analysis identified compound such as pyruvic dimethyl acetal, 13-hexyloxacyclo-tridec-10-ene and methyl ricinoleate as the major constituents present. Their classes of compounds were earlier associated with antidiabetic, antimalarial and analgesic activities Further work is required to isolate and spectrally elucidate the active component identify and isolate the active components using NMR analysis.

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