Research Article

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Determination of the Total Phenolic, Flavonoid Contents; Antioxidant Activity and GC-MS Study of the leaves of the Medicinal Plant *Sarcocephalus latifolius*

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ABSTRACT

Sarcocephalus latifolius is a medicinal plant whose leaves are useful for the treatment of fever, pain, diarrhea, etc. The aim of the present study was to determine the phytochemical constituents and quantify the total phenolic and flavonoid contents of the plant leaves. The phytochemical screening of the methanol extract revealed presence of saponins, tannins, flavonoids, steroids and alkaloids while anthraquinone glycosides were absent. Only steroids were observed present in the n-hexane extract. The total phenolic content was 72.12 ± 0.09 mg Gallic acid equivalent/mg extract while that of total flavonoid content was 52.20 ± 0.13 mg quercetin equivalent/mg extract. GC-MS analysis of two of the fractions obtained from fractionation of the methanol extract revealed presence of sixteen compounds in the butanol and seven compounds in the ethyl acetate fractions. The most abundant compound in the ethyl acetate fraction was 2-hydroxy-5-methylbenzaldehyde (60.079%) and the least quinic acid (3.274%) while quinic acid (31.124%) was the major component in the butanol fraction and 4-H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl was the least (1.076%). The composition of both fractions is made up of phenolics, carbonyls, alkaloids, fatty acid esters, other esters and substituted aromatic hydrocarbons.

Keywords: *Sarcocephalus latifolius;* phenolics; flavonoids; Phytochemicals; GC-MS.

INTRODUCTION

Each country of the world is endowed with plant species that are useful for the healthcare of its people. The traditional health practitioners are able to identify those plant species that possess medicinal properties. The study of plants have shown that they possess limitless ability to synthesize both aromatic and non aromatic compounds of which less than 10% has been isolated[1]. Several studies have shown that these compounds can serve as effective combatants of diseases and sicknesses. Such compounds include flavonoids, steroids, tannins, polyphenolics, saponins, alkaloids, terpenoids, etc[2-4]. Several of these phytochemicals can stop nitrosation, the formation of DNA adducts or stimulate the activity of the protective enzymes such as Phase II enzyme gluthathione transferase, inhibit cyclooxygenase enzyme and lipid peroxidation, possess antiviral, antimicrobial activities, etc[5]. Several medicinal plants possess antioxidant properties[6]. These antioxidants from natural sources aid the antioxidant capacity of the plasma and also help in reducing the risk of contracting certain ailments such as cancer[7]. Synthetic antioxidants have been reported to have several side effects such as risk of liver damage and carcinogenesis in laboratory animals[8-11]. There is therefore, the need for a less toxic and more effective antioxidants and these qualities can be found in medicinal plants[12]. There is an increase in the use of plant based products in food, pharmaceutical and cosmetic industries in both developed and developing countries due to the non toxic, less side effects and availability at affordable prices[13]. It is therefore pertinent to find active components from plants that are needed by these industries. This requires a systematic study of medicinal plants that involves the identification of those medicinal plants that possess more of the desired components that are required by these industries. Furthermore, it is also important to determine the safety profile of the phytochemicals through quantitative estimation of a broader category of phytochemicals such as total phenolic and flavonoid contents, etc[14]. *Sarcocephalus latifolius* is an important medicinal plant that has diverse uses.

Examples of tradomedical uses include the treatment of Fever, pain, malaria, septic mouth, diarrhea, dysentery, epilepsy[15-18].

In Nigeria the plant is found in the Southeast, South-south and the Middle belt regions. The objective of this study is to evaluate the antioxidant activity, total phenolic and flavonoid contents and identify those phytochemicals present in the leaves of *Sarcocephalus latifolius* by GC-MS analysis.

MATERIALS AND METHODS

Sample collection and preparation

The plant was collected from a forest in Omu aran, Kwara State by a traditional health practitioner. It was taken to Landmark University laboratory for identification in the Department of Biological Sciences.

The leaves were stripped from the plant and air dried in the laboratory. The dried leaves were pulverized into fine powder and stored in air tight containers to avoid contact with moisture.

Extraction

The pulverized plant leaves were extracted with n-hexane and methanol respectively.

These extracts were concentrated by distilling off the solvents. They were later evaporated to dryness to yield the crude extracts.

Phytochemical screening

The crude extracts and the extracts obtained from fractionation of the crude methanolic extract were screened for the presence and absence of the phytochemicals steroids, flavonoids, tannins, saponins, alkaloids, terpenoids and glycosides using the method described by Harborne 1993[19].

Determination of total phenolic content

Folin-Ciocalteau method was used for the determination of the total phenolic content of the methanolic extract. 1 ml of the dilute solution of the extract was prepared.

A mixture of 2.5 ml of diluted Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%) was added to 1 ml of the diluted extract and incubated for 30 mins at 40°C. It was then transferred to a uv spectrophotometer and the absorbance measured at 760 nm.

Gallic acid was used as the standard different concentrations of Gallic acid were prepared and the absorbance measured at 760 nm.

This was used to plot a graph and the total phenolic content was calculated from it.

Determination of Total flavonoid content

10 ml of 30% (v/v) ethanol was mixed with 0.7 ml of 5% (w/w) sodium nitrite and a dilute solution of the extract.

It was stirred for 5 mins and 0.7 ml of 0% aluminum chloride (w/w) was added.

The mixture was stirred again, and then 5ml of 1 mol/l sodium hydroxide added.

The mixture was diluted with 5ml of 30% (v/v) of ethanol and left standing for 10 mins. This mixture was then placed in a UV spectrophotometer and the absorbance reading taken at a wavelength of 500 nm. Quercetin was used as the standard and different concentrations of it were prepared and the absorbance readings obtained at 500 nm. This was used to obtain a graph and the total flavonoid content determined from the graph.

Solvent-solvent extraction

The crude methanolic extract was partitioned between water and four organic solvents. The organic solvents used are n-hexane, chloroform, ethyl acetate and n-butanol. The extracts obtained from this fractionation were concentrated by distilling off the solvent using a rotary evaporator. The ethyl acetate and the butanol extracts were subjected to GC-MS analysis.

GC-MS analysis

Table 1: Procedure for GC-MS analysis of *Sarcocephalus latifolius*

Gas Chromatographic programme	
Equipment	Agilent 5975C inert MSD with triple axis detector
Column	Agilent 19091S-433HP-5MS (30 m x 250 µm x 0.25 µm 5% phenyl methylsiloxane)
Carrier gas	Helium (constant flow rate 1.5 ml/min)
Sample injected	1µl
Injection temperature	240°C
Oven temperature Transfer temperature	100°C 300°C
Total GC running time	49 min
Mode	Split
Split ratio	50:1
Run time	49 min
Mass spectrometric programme	
Inlet line temperature	200°C
Source temperature	250°C
Electron energy	70eV
Mass scan (m/z)	50-600 amu
Solvent delay	5.0 min
Library	NIST version year -2011

RESULTS

The information obtained from preliminary qualitative phytochemical screening of plant extracts is essential for drug discovery. The present study was carried out to determine the phytochemicals that are present and absent in the leaves of *Sarcocephalus latifolius*. It was noted that saponins, tannins, flavonoids, steroids and alkaloids are present while anthraquinone glycosides are absent in the methanolic extract. The n-hexane extract showed presence of only steroids while saponins, flavonoids, tannins, alkaloids and terpenoids were absent. In the fractionation of the crude methanolic extract the phytochemicals were distributed into the fractions as shown in Table 3. Saponins were identified in the n-hexane, chloroform and n-butanol fractions; tannins in ethyl acetate, n-butanol; flavonoids in $CHCl_3$, ethyl acetate and n-butanol while alkaloids were present in the $CHCl_3$ and ethyl acetate fractions. The total flavonoid content of the methanolic leaf extract of *Sarcocephalus latifolius* is 52.20 ± 0.13 mg quercetin equivalent/mg extract. This was obtained from the quercetin standard curve (0.012x -0.011, R^2 = 0.992) while the total phenolic content obtained is 72.12±0.09 mg Gallic acid equivalent/mg extract. This quantity was derived from the linear Gallic acid curve (y = 0.023x +0.292, R^2 = 0.946) (Table 4). The antioxidant activity of the plant leaves is 83.98 ± 0.05. The GC-MS analysis was carried out for the butanol and ethyl acetate fractions obtained from the fractionation of the methanolic extract. The

chromatograms are presented in figures 1 and 2. The GC-MS analysis revealed presence of sixteen compounds in the butanol fraction and seven compounds in the ethyl acetate fraction. The identified compounds with their molecular masses, retention times, peak areas and molecular formulae are shown in Tables 5 and 6.

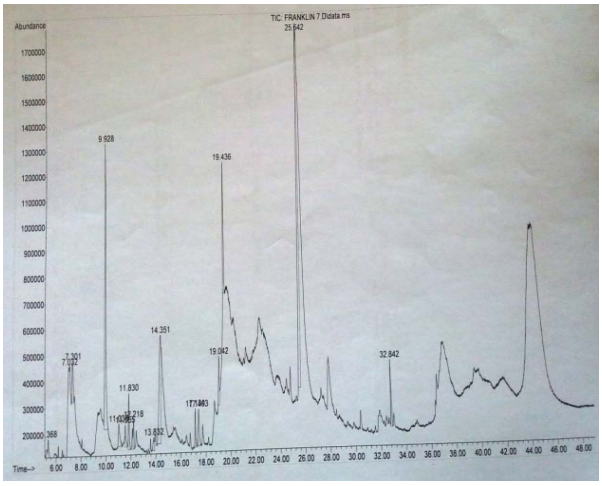

Figure 1: Chromatogram of the Butanol fraction

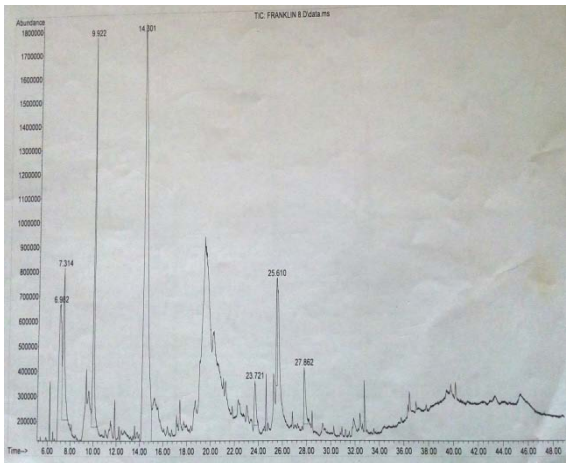

Figure 2: Chromatogram of the ethyl acetate fraction

Table 2: Phytochemical screening of crude extracts of *Sarcocephalus latifolius* leaves

Extracts	Saponins	steroids	tannins	terpenoids	flavonoids	Alkaloids	Anthraquinone glycosides
n-hexane	-	+	-		-	+	-
Methanol	+	+	+		+	+	-

Table 3: Phytochemical screening of methanolic fractions of *Sarcocephalus latifolius*

Fractions	saponins	steroids	tannins	terpenoids	flavonoids	Alkaloids	Anthraquinone glycosides
n-hexane	+	-	-		-	-	-
Chloroform	+	-	-		+	+	-
Ehyl acetate	-	+	+		+	+	-
n-butanol	+	+	+		+	-	-
Aqueous	+	-	-		+	-	-

Table 4: Total phenolic, flavonoid contents and antioxidant activity of the methanolic extract of *Sarcocephalus latifolius*

Total phenol content	Total flavonoid content (mg quercetin equivalent/mg extract)	Antioxidant activity
72.12±0.09	52.20±0.13	83.98±0.05

Table 5: GC-MS analysis of the butanol fraction of *Sarcocephalus latifolius*

Peak number	Retention time	Peak area	Molecular mass	Molecular formula	Identified compound
1	5.368	356384	144	$C_6H_8O_4$	4-H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl
2	7.032	3008145	110	$C_6H_6O_2$	Catechol
3	7.301	899783	120	C_8H_8O	Benzofuran, 2, 3-dihydro
4	9.928	6274473	150	$C_9H_{10}O_2$	2-methoxy-4-vinylphenol
5	11.036	674126	154	$C_8H_{10}O_3$	2, 4-dimethoxyphenol
6	11.555	419173	207	$C_{12}H_{17}NO_2$	3-pyridinecarboxylic acid, hexyl ester
7	11.830	914977	202	$C_{11}H_{20}O_3$	Decanoic acid, 3-hydroxy-, methyl ester
8	12.218	411242	281	$C_{19}H_{28}NO$	(M)-2-aminomethyl-1-(2´-hydroxy-4´, 6´-dimethylphenyl)-5, 6, 7,8-tetrahydronapthalene
9	13.832	370872	204	$C_{13}H_{16}O_2$	Menthanone (1-hydroxycyclohexyl) phenyl

10	14.351	4184072	136	$C_8H_8O_2$	Benzaldehyde, 2-hydroxy-4-methyl
11	17.134	559846	207	$C_{15}H_{13}N$	Butanamide, N-(2-methoxyphenyl)-3-oxo
12	17.403	843427	280	$C_{18}H_{32}O_2$	9, 12-octadecadienoic acid, methyl ester
13	19.042	866173	326	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
14	19.436	2058180	173	$C_9H_{19}NO_2$	N-propyl-2-hydroxy-1-oxohexahydro-1H-azepine
15	25.642	10307158	192	$C_7H_{12}O_6$	Quinic acid
16	32.842	968571	312	$C_{20}H_{40}O_2$	Hexadecanoic acid, butyl ester

Table 6: GC-MS analysis of the ethyl acetate fraction of *Sarcocephalus latifolius*

Peak No.	Retention time	Peak area	Molecular mass	Molecular formula	Name of identified phytoconstituent
1	6.982	3241897	110	$C_6H_6O_2$	Catechol
2	7.314	6559951	120	C_8H_8O	Benzofuran, 2, 3-dihydro
3	9.922	9976671	150	$C_9H_{10}O_2$	2-methoxy-4-vinylphenol
4	14.301	38649175	136	$C_8H_8O_2$	2-hydroxy-5-methylbenzaldehyde
5	23.721	1669835	207	-	unknown
6	25.610	2106084	192	$C_7H_{12}O_6$	Quinic acid
7	27.862	2126593	281	$C_{15}H_{11}N_3OS$	7-phenyl-5H-thiazolo [5, 4e] pyrrolo [1. 2. 4]-1, 4-diazepine-10 (9H) -one

DISCUSSION

Plants can be referred to as chemical store houses where man and other animals can resort to for their primary healthcare and alleviation of illness. The phytochemicals present in plants have been shown to exhibit biological and pharmacological properties such as antimicrobial, antiinflammatory, anticancer, antioxidant, etc[20]. In the present study saponins, tannins, flavonoids and alkaloids were identified present in the plant leaves. These phytochemicals could be responsible for the plant leaves biological activities. The presence of tannins confirms the ability of the plant to act as an antidiarrhoal and antihemorrhagic agent while the presence of alkaloids also confirms its use as a detoxifying and antihypertensive agent. Flavonoids are known to prevent oxidative damage in living cells[21-23]. Phenolics are also important antioxidants that could prevent the formation of carcinogens[24]. Saponins have been shown to possess antifungal, antiviral effects and cholesterol lowering ability and enhancement of mucosal drug absorption[25-27]. The identification of the phytochemicals separated by the GC and the interpretation of the MS spectra were carried out based on the database of the National Institute of Standards and Technology (NIST) 2011. The molecular weight of the unknown compound was obtained from the mass spectrum. The spectrum of the unknown compound was then compared with that obtained from NIST library which gave the name and structure of those compounds that match. In the butanol fraction the most abundant component was quinic acid (31.124%) while 2-hydroxy-5-methylbenzaldehyde (60.079%) was the most abundant in the ethyl acetate fraction. Most of the identified compounds belong to the class of phytochemicals known as alkaloids, Phenolics. Others are hydrocarbons, oxygenated hydrocarbons and nitrogenous compounds. The identified secondary metabolites could exert a wide range of biological activity on physiological systems.

CONCLUSION

Saponins, flavonoids, alkaloids and steroids are important phytochemicals present in the plant leaves. These compounds could be responsible for the biological activity of the leaves. Therefore, further research is on going to isolate, characterize and determine some biological parameters of the plant leaves.

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