



Original article

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## Quantitative determination of the saponin content and GC-MS study of the medicinal plant *Cassytha filiformis* (linn.) leaves

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### ABSTRACT

**Objective:** To determine the phytochemicals, total saponin content and types of saponin present in leaf extracts of *Cassytha filiformis*.

**Methods:** The leaves were extracted with *n*-hexane and methanol. The methanol extract was fractionated. The total saponin content of the butanol fraction was determined by colorimetry via a UV spectrophotometer and ginsenoside was used as the standard. Measurements were carried out at 550 nm. The butanol fraction was subjected to gas chromatography-mass spectrometer analysis.

**Results:** All screened phytochemicals were absent in the *n*-hexane extract while saponins, steroids, tannins and glycosides were present in the methanol extract. Flavonoids and alkaloids were absent. The total saponin content of the methanol extract is 73.47 µg ginsenoside Rb1 equivalent/g extract. The chromatography-mass spectrometer analysis gave eicosanoic acid, methyl ester as the most abundant compound and the steroidal saponin, cholestan-7-one and cyclic 1,2-ethanedienyl acetal as the most abundant saponin in the butanol fraction.

**Conclusions:** The leaves of *Cassytha filiformis* are rich in steroidal saponins.

## 1. Introduction

Plants have coexisted with humans from the beginning of life and people of every culture have different plants which they resort to for their primary healthcare. Those plants that possess therapeutic values are known as medicinal plants. Researches into medicinal plants have shown that medicinal plants contain secondary metabolites which possess a variety of structural arrangement and properties. The practice of the use of herbs for healthcare delivery is a phenomenon that is handed down from one generation to another. This practice is believed to be a sum of knowledge, skills, and practices which are based on the beliefs, theories and experiences of the indigenous people. Apart from the use of medicinal plants for therapeutic purposes, some of them possess phytoconstituents which can aid in the maintenance and improvement of health and also have the ability to prevent sicknesses and diseases[1]. The knowledge of bioactive compounds present in medicinal plants has led to more research work resulting into the discovery of novel drug candidates which are effective against diverse ailments. The phytoconstituents have been shown to be non phytotoxic, more systemic and easily biodegradable[2,3].

*Cassytha filiformis* L. (*C. filiformis*) is a member of the Lauraceae

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family. It is a parasitic plant on a variety of plants such as *Mangifera indica*, *Azadirachta indica*, etc. It is also found on herbaceous weeds, small bushes and low trees[4]. The stem is threadlike while the leaves are minute and spirally arranged like scales on the stem. This plant is widespread in America, Africa and Asia. The medicinal properties of this plant have been reported in some regions or countries such as Taiwan, Brazil, Japan and Benin[5]. Locally, it is used for the treatment of urinary tract infection, gonorrhea, malaria, dysentery and fever. Researchers have shown that *C. filiformis* L. possesses both biological and pharmacological activities[6-10]. Chemical constituents such as alkaloids and flavonoids have been isolated from the plant species found in Japan, Brazil and Taiwan[5]. The aim of this research work was to determine the total saponin content and types of saponin present in the methanol leaf extract.

## 2. Materials and methods

### 2.1. Chemicals and equipments

All chemicals used were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Genseys 10S v1. 200 217H311008 spectrophotometer was used for the UV absorbance measurement.

### 2.2. Plant material

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.

## 2.2. Extraction

The pulverized plant leaves were extracted with *n*-hexane and methanol respectively. These extracts were concentrated by distilling off the solvents to yield the crude extracts.

## 2.3. Phytochemical screening

The crude extracts and the extracts obtained from fractionation of the crude methanolic extracts were screened for the presence/absence of phytochemicals such as steroids, flavonoids, tannins, saponins, alkaloids, terpenoids and glycosides using the method described by Harborne[11].

## 2.4. Determination of the total saponin content

A total of 20 g of the extract was placed in a conical flask and 100 mL of 20% aqueous ethanol was added to it. The mixture was placed in a water bath and heated at a temperature of 55 °C for 4 h with continuous stirring. The mixture was filtered and re-extracted with 200 mL of 20% ethanol. The filtrates were combined and the volume was reduced to 40 mL using a water bath at a temperature of 90 °C. The concentrated filtrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added to it. This was shaken vigorously and allowed to separate into two layers. This extraction was carried out three times and the aqueous layer was recovered. The aqueous layer was extracted three times with 60 mL of *n*-butanol. The *n*-butanol extract was washed three times with 10 mL of 5% NaCl. The washed *n*-butanol extract was heated in a water bath to evaporate the *n*-butanol. The *n*-butanol extract was dried in the oven at a temperature of 50 °C to a constant weight to give the saponins. Vanillin-acetic acid (0.2 mL, 5% w/v) and 0.8 mL of perchloric acid were added to 50 µL of the *n*-butanol extract and placed in an oven at a temperature of 70 °C for 15 min. The mixture was cooled on an ice bath for 1 min, and then 5.0 mL of glacial acetic acid was added to it. The mixture was then scanned on a UV/Vis spectrophotometer at a wavelength of 550 nm. The butanol extract was also subjected to gas chromatography-mass spectrometer (GC-MS) analysis[12].

## 2.5. Preparation of standard

Ginsenoside Rb1 (2.0 g) was dissolved in 10 mL of ethanol and serial dilutions of 0.2, 0.6, 1.0, 1.4, 1.8, 2.2 and 2.6 were prepared in separate test tubes. These concentrations were evaporated to dryness

and 5% w/v of 2.0 mL vanillin-acetic acid and 0.8 mL of perchloric acid were added to each test tube. The mixture in each test tube was heated at 70 °C for 15 min, and then cooled on an ice bath for 20 s. Then 5 mL of glacial acetic acid was added to each test tube. The absorbance of each dilution was measured at a wavelength of 550 nm using a UV spectrophotometer. The absorbance data was used to plot a graph of absorbance against concentration.

## 2.6. GC-MS analysis

Gas chromatography-mass spectrometric (GC-MS) analysis was performed using Agilent 7890A/5975C GC-MSD instrument and split (50:1) injection system. The GC was fitted with an Agilent 19091S-433HP-5MS capillary column (30.00 m × 0.25 mm inner diameter, 0.25 µm phase thickness). The GC oven was programmed from 100 °C held for 4 min to final temperature of 300 °C at the rate of 4 °C/min and held isothermally at final temperature of 240 °C for 10 min. Helium at a constant flow rate of 1.5 mL/min was used as carrier gas and running time of 49 min. A total of 1 µL aliquot of sample was injected automatically. The samples were analyzed in the full scan mode. The electron ionization energy of 70 eV, source temperature of 250 °C and solvent delay of 5 min were employed. These compounds were identified based on their mass spectrum, molecular weight, and fragment ions obtained from the mass spectrum. These parameters were matched with those of reference compounds obtained from National Institute of Standards and Technology 2011 database which were incorporated into the computer system of the equipment.

## 3. Results

The leaf extracts of *C. filiformis* were screened for presence or absence of certain phytochemicals. It was observed that saponins, steroids and tannins were present in the methanolic extract while alkaloids, glycosides, terpenoids and flavonoids were absent. The *n*-hexane extract did not show presence of any of the screened phytochemicals as shown in Table 1. The quantitative determination of saponins gave 73.47 µg ginsenoside Rb1 equivalent/mg extract as the total saponin content. The GC-MS analysis of the butanol fraction containing the saponins revealed presence of eight compounds that could contribute to the medicinal property of the plant leaves. The retention time, peak area, molecular weight and molecular formula of the identified compounds are presented in Table 2.

**Table 1**  
Phytochemical screening of crude extracts of leaves *C. filiformis*.

Extracts	Saponins	Steroids	Tannins	Terpenoids	Flavonoids	Cardiac glycosides	Alkaloids
<i>n</i> -Hexane	-	-	-	-	-	-	-
Methanol	++	++	+	-	-	-	-

-: Absent; +: Moderately present; ++: Highly present.

**Table 2**

GC-MS analysis of the butanol fraction.

Peak number	Retention time	Peak area	Molecular mass	Molecular formula	Identified compound
1	7.120	16710	354	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	9,12-Octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester
2	10.085	28241	428	C <sub>29</sub> H <sub>48</sub> O <sub>2</sub>	5-Stigmastan-3, 6-dione
3	15.145	23671	502	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	Didodecyl phthalate
4	16.071	296320	326	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	Eicosanoic acid, methyl ester
5	20.106	26983	506	C <sub>36</sub> H <sub>74</sub>	Hexatriacontane
6	21.707	91291	400	C <sub>28</sub> H <sub>48</sub> O	Campesterol
7	26.855	107119	430	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	Cholestan-7-one, cyclic 1,2-ethanediyl acetal
8	30.940	75598	548	C <sub>31</sub> H <sub>48</sub> O <sub>8</sub>	Cholan-24-oic acid, 3,7,12-tris (acetyloxy)-, methyl ester, (3α, 5β, 7α, 12α)-

#### 4. Discussion

The importance of the knowledge of phytochemicals present in plants cannot be over emphasized. This knowledge can enhance the discovery of therapeutic agents and evaluation of their medicinal properties and also serve as an information bank of great value to researchers who are interested in synthesizing any of the identified therapeutic agents. It could also reveal the actual significance of the folkloric remedies. Plants have been shown to possess diverse chemical components that have therapeutic agents. Therefore, the determination of the phytochemicals present in plant extracts is an important step in the search for therapeutic agents. In this research work, evaluation of the phytochemicals present in both *n*-hexane and methanol extracts showed that the screened phytochemicals were all absent in the *n*-hexane extract while saponins, tannins and steroids were present; glycosides, alkaloids, flavonoids and terpenoids were absent in the methanolic extract. This is in contrast to that of Adonu *et al.*[13] and Mythili *et al.*[14] who showed that the leaves of *C. filiformis* do not contain steroids and saponins but has flavonoids. Tannins have been implicated as possessing antidiarrheal, antihemorrhoidal and hemostatic[15], antiviral, antibacterial, wound healing and antitumor properties. Tannins have also been shown to have ability to inhibit HIV replication, protect against microbiological degradation of dietary proteins in semen[16,17], and serve as an antiparasitic[18]. Saponins are antinutrients that are considered useful in human diets for the control of cholesterol[19]. Some plant steroids are biologically active compounds known as phytoanticipins or phytoprotectants that defend the plant system[20]. Thus, the identified steroids in *C. filiformis* leaves could possess ecological significance. In the quantitative determination of the saponin content of the methanol extract of *C. filiformis* ginsenoside Rb1 was used as the standard. A curve of  $y = 0.0205x - 0.0862$  with  $R^2 = 0.991$  was obtained and used to derive the concentration of saponin. GC-MS is an important tool in qualitative determination of certain compounds in complex mixtures such as plant extracts. The GC-MS analysis of the butanol fraction obtained from the methanolic extract revealed presence of eight compounds that could contribute to the medicinal properties of the plant leaves. The retention time, peak area and molecular weight of the unknown compounds were compared with those of National Institute of Standards and Technology (2011) stored in the computer data base library. Those that matched were used as a means for identifying the unknown compounds. Three esters, one hydrocarbon and four steroidal saponins were identified. The first compound to emerge was identified as 9,12-octadecadienoic acid (Z, Z)-2,3-dihydroxypropyl ester with retention time of 7.120 min while the last one to emerge was cholan-24-oic acid, 3,7,12-tris (acetyloxy)-, methyl ester, (3 $\alpha$ , 5 $\beta$ , 7 $\alpha$ , 12 $\alpha$ )- with retention time 30.940 min. The predominant compound was eicosanoic acid, methyl ester (44.50%) while the least was 9,12-octadecadienoic acid (Z, Z)-2,3-dihydroxypropyl ester (2.51%). Some of the identified compounds have been shown to possess biological properties. For example, 9,12-octadecadienoic acid (Z, Z)-2,3-dihydroxypropyl ester has antipyretic, anticonvulsant, antiseptic and analgesic properties[20], while didodecylphthalate has antimicrobial and antifouling activities[21].

The leaves of *C. filiformis* are rich in steroidal saponins. Moreover, this work is preliminary and qualitative. Therefore more research work is needed to isolate, quantify and determine the safety profile of the biologically active compounds.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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