ISSN 1022-8594
JUJS
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Vol. 41, No.1, pp.67-78

Phytochemical Screening of Crude Extracts and Identification of Nonpolar Compounds from Nonpolar Fractions of the Roots of *Spermacoce latifolia*

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Abstract

Phytochemical screening of the crude extracts from roots of *Spermacoce latifolia* confirmed the presence of different class of compounds like steroids, terpenoids, phenolic compounds, coumarins, quinones, flavonoids and alkaloids. Two nonpolar fractions of chloroform extract from roots of *Spermacoce latifolia* were investigated by GC-MS analysis to determine the chemical compounds present in the fractions. A total of 18 compounds were identified from these fractions by this experiment which belongs to the class of carbonyl compound, alcohol, phenol, ester, fatty acid, epoxide, amide, haloaliphatic and steroid.

Keywords: *Spermacoce latifolia*, phytochemical screening, GC-MS analysis, nonpolar fractions, identification.

Introduction

The genus *Spermacoce* which is belonging to the family Rubiaceae comprises 250-300 species widespread in tropical and subtropical America, Africa, Asia and Europe [1]. Some species in this genus play an important role in the treatment of various diseases as traditional medicine [2].The plant *Spermacoce latifolia* (Synonym:*Borrerialatifolia)* whichislocally known as GhuiojhilShakin different region all over Bangladesh belongs to the family of Rubiaceae and genus *Spermacoce*. It is an herb and an important member of medicinal plants. The plant is widely distributed in Sri Lanka, India, Bhutan, Malay Peninsula and tropical Africa. It occurs throughout Bangladesh [3]. This plant has attracted the attention of the chemists for its medicinal value.

Different parts of the plant are being used for the treatment of different diseases. The leaves of this plant are used as ophthalmic, inflammation of eye, blindness, carache, fever, spleen complaints and sore. Leaves are also used against hemorrhoids and conjunctivitis [4]. Leaf paste used by

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Tanchangya ethnic people in Bangladesh for treatment of boils [3]. It was reported that the root juice of this plant can be used to treat malaria [1]. Biological investigations such as antimicrobial, cytotoxic and antioxidant activities of methanolic and ethanolic extracts of the plant have been performed and the result showed moderate to strong activity [5-7]. Literature survey showed a few phytochemical studies have been done on this plant so far. In these studies iridoid glycosides, a diterpenoid and pentacyclictriterpene acids were isolated from this plant [8-9]. In recent time, isolation of four compounds: stigmasterol, 2,6-di-t-butyl-4-hydroxymethelene-cyclohexa-2,5-dienone, 3β-hydoxy-12-oleanen-28-oic acid & phthalic acid, and the identification of 35 nonpolar compounds from the aerial parts of *Spermacoce latifolia* have been reported by our research group [10-11].

As a continuation of our phytochemical study on the plant *Spermacoce latifolia*, we herein report the phytochemical screening of the crude extracts from the roots of the plant and identification of compounds from the nonpolar fractions of chloroform extract of roots by gas chromatography coupled to mass spectrometry (GC-MS) analysis.

Materials and Methods

Collection of the Plant Material

The roots of matured *Spermacocelatifo lia*plants were collected from Jahangirnagar University campus during May 2016. The plant was identified by Bangladesh National Herbarium at Dhaka and a voucher specimen (specimen no. 37755) was deposited at the herbarium.

Extraction and Fractionation

At first, roots were cut into small pieces, dried well under the shade and then powdered by using a grinder machine. The dried plant material (420 g) was extracted successively with chloroform and methanol. The plant material was soaked in chloroform at room temperature for 72 hrs. The extraction solvent was then removed by filtration and fresh distilled solvent was added to the plant material. The extraction process was repeated three times and the combined filtrate was evaporated and dried completely by using a rotary evaporator under reduced pressure to get greenish crude chloroform extract (2.96 g). The residual plant material was then soaked in

methanol at room temperature for 72 hrs. The extraction process was done by the same way as for chloroform and also repeated for three times to get the brownish crude methanol extract (7.49 g).

As a part of the isolation of pure compounds from the roots of *Spermacoce latifolia*, the crude chloroform extract (2.9 g) was subjected to column chromatography over silica gel and the column waseluted with pet. ether, pet. ether-ethyl acetate, ethyl acetate and ethyl acetate-methanol in gradient manner. A total of 184 collections with 15 mL each were collected separately and they were divided into 12 fractions according to their TLC behaviors. The fractions 3 (30 mg, sample **1**) and 5 (35 mg, sample **3**) were selected for the identification of nonpolar compounds using GC-MS analysis. A smaller part of fraction 3 was esterified by excess methanol in acidic medium and esterified fraction (8 mg, sample **2**) was also preserved for GC-MS analysis.

Test for Qualitative Estimation of Bioactive compounds

Phytochemical screening of the chloroform and methanol extracts of the roots of *Spermacoce latifolia* was done by the following qualitative tests [12-13].

Test for Steroids and Terpenoids

Liebermann-Burchard test

10 mg of the extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 mL of concentrated sulphuric acid. Blue colour in chloroform layer which changed to green shows the presence of steroids, whereas the appearance of pink colour in chloroform layer showed the presence of terpenoids.

Test for Flavonoids

Shindo’s test

10 mg of the extract was dissolved in methanol. Magnesium turnings were added into this followed by concentrated HCl. A pink colour showed the presence of flavonoids.

Test for Phenolic compound

10 mg of extract was dissolved in chloroform and few drops of 2.5% ferric chloride solution was added to that. The reddish brown colour indicated the presence of phenolic compound.

Test for Coumarins

10 mg of the extract was dissolved in methanol and alcoholic KOH was added. The appearance of yellow colour which decolourizes while adding concentrated HCl showed the presence of coumarin.

Test for Quinones

10 mg of the extract was dissolved in methanol and was treated with sulphuric acid. The development of colour indicated the presence of Quinone.

Tests for Saponins

A small quantity of extract was diluted with distilled water and shaken vigorously. Formation of foams indicated the positive test.

Tests for Alkaloids

For testing of alkaloids, Mayer's reagent was prepared initially. The reagent was prepared with the combination of mercuric chloride (1.36 g) and KI (5.0g) in water (100 mL). Then 10 mg of the extract was dissolved in concentrated HCl separately. A few drops of solution were poured into the center of watch glass. Mayer's reagent was added along the sides of the watch glass with the help of a glass rod. Formation of a gelatinous white precipitate at the junction of the two liquid indicated the presence of alkaloids.

GC-MS Analysis

The three nonpolar samples (2 mg/mL each) were diluted to 500 times with chloroform separately and analyzed by Electron Impact (EI) method on GC-2010 plus Shimadzu Gas Chromatograph, coupled to a GC-MS QP 2010 plus Shimadzu Mass Spectrometer. RTX-5MS fused silica capillary column (30 cm × 2.5 mm; 0.25 µm film thickness), coated with DB-5ms (J&W) was used. Column temperature was 40°C (hold 2 min) to 220°C (hold 5 min) at the rate of 10°C/min, maintained with carrier gas helium at

a constant pressure of 90 kPa (acquisition parameters full scan; scan range 40-550 amu). Samples were injected by splitting with the split ratio 10. Mass spectra were taken at 70 ev. The constituents of the samples were identified by calculation of their retention times under temperature-programmed conditions by comparison of their mass spectra with those of the internal reference mass spectral NIST-05 library.

Results and Discussion

The results of the qualitative analysis of $CHCl_3$ and MeOH extracts of roots of *Spermacoce latifolia* are presented in Table 1. The medicinal value of a plant lies in some chemical substances that have definite physiological action on human body. The most important of these bioactive constituents are alkaloids, steroids, terpenoids, flavonoids, coumarins, quinones and saponins. Results showed the presence of steroids, terpenoids, phenolic compounds, coumarins and quinones in the chloroform extract but the methanol extract of roots contain alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, coumarins and quinones. Both the extracts contain no saponins.

Table 1: The results of phytochemical screening of $CHCl_3$ and MeOH extracts of roots of *Spermacocelatifolia*

Tests for	**$CHCl_3$ extract**	**MeOH extract**
Steroids	+ ve	+ ve
Terpenoids	+ ve	+ ve
Flavonoids	- ve	+ ve
Phenolic compounds	+ ve	+ ve
Coumarins	+ ve	+ ve
Quinones	+ ve	+ ve
Alkaloids	- ve	+ ve
Saponines	- ve	- ve

The GC-MS analysis identified nine compounds in sample **1**, five compounds in sample **2** and four compounds in sample **3**. The mass spectrum of the unknown component was compared to the mass spectrum of the known component stored in the NIST (National Institute of Standard and Technology) library. The retention time, molecular weight and molecular formula of the components in the sample materials were

recorded separately and presented in Table 2 (for sample **1**), Table 3 (for sample **2**) and Table 4 (for sample **3**). TIC of sample **1**, **2** and **3** are presented in Figure 1, 3 and 5 respectively.

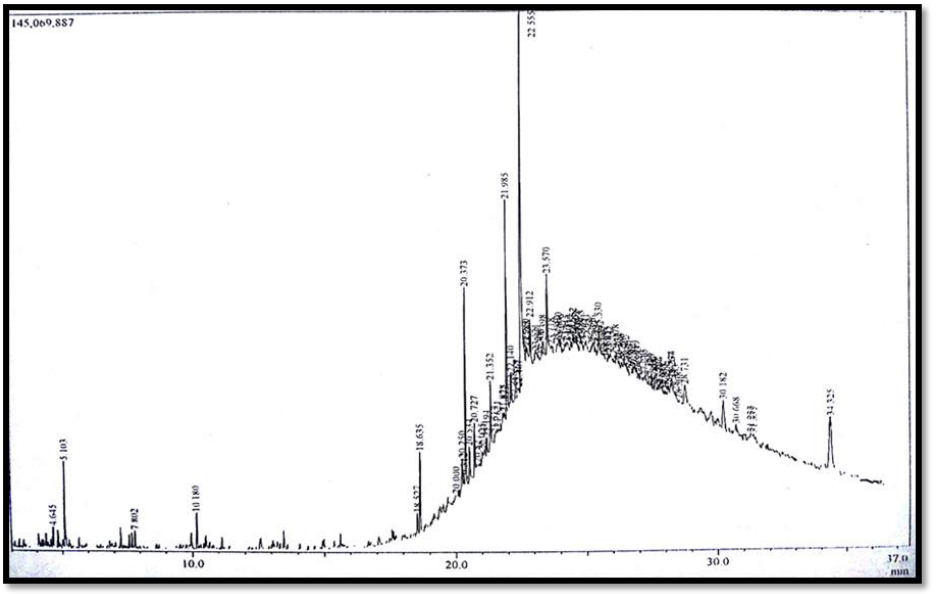


Figure 1: TIC of the GC-MS analysis of sample **1**

Table 2: Results of the GC-MS analysis of sample **1**

Sl no.	Retention time (min.)	Name& number of the compound	Molecular weight	Molecular formula	Class of compound
1	5.103	Hexachloroethane (1a)	234	C_2Cl_6	Halo alkane
2	10.180	2,4-Di-*tert*-butylphenol (1b)	206	$C_{14}H_{22}O$	Phenol
3	18.635	2-Pentacosanone (1c)	366	$C_{25}H_{50}O$	Ketone
4	20.373	6,10,14-Trimethyl-2-pentadecanone (1d)	268	$C_{18}H_{36}O$	Ketone
5	20.727	Bis (2-ethylhexyl) phthalate (1e)	390	$C_{24}H_{38}O_4$	Ester
6	21.352	Tetracontane-1,40-diol (1f)	594	$C_{40}H_{82}O_2$	Alcohol
7	22.555	(Z)-13-Docosenamide (1g)	337	$C_{22}H_{43}ON$	Amide
8	22.912	Hexadecyloxirane (1h)	268	$C_{18}H_{36}O$	Epoxide
9	23.570	2-Heptacosanone (1i)	394	$C_{27}H_{54}O$	Ketone

The sample **1** was a light reddish brown gummy substance. It was completely soluble in chloroform. Results of the GC-MS analysis of sample **1** showed that nine identified compounds are belonging in different classes like halo alkane, phenol, alcohol, ketone, ester, amide and epoxide. But minor fatty acids were not identified in this analysis. To identify fatty acid components, the sample **1**was esterified with methanol and the esterified sample (sample **2**) was subjected to GC-MS analysis.

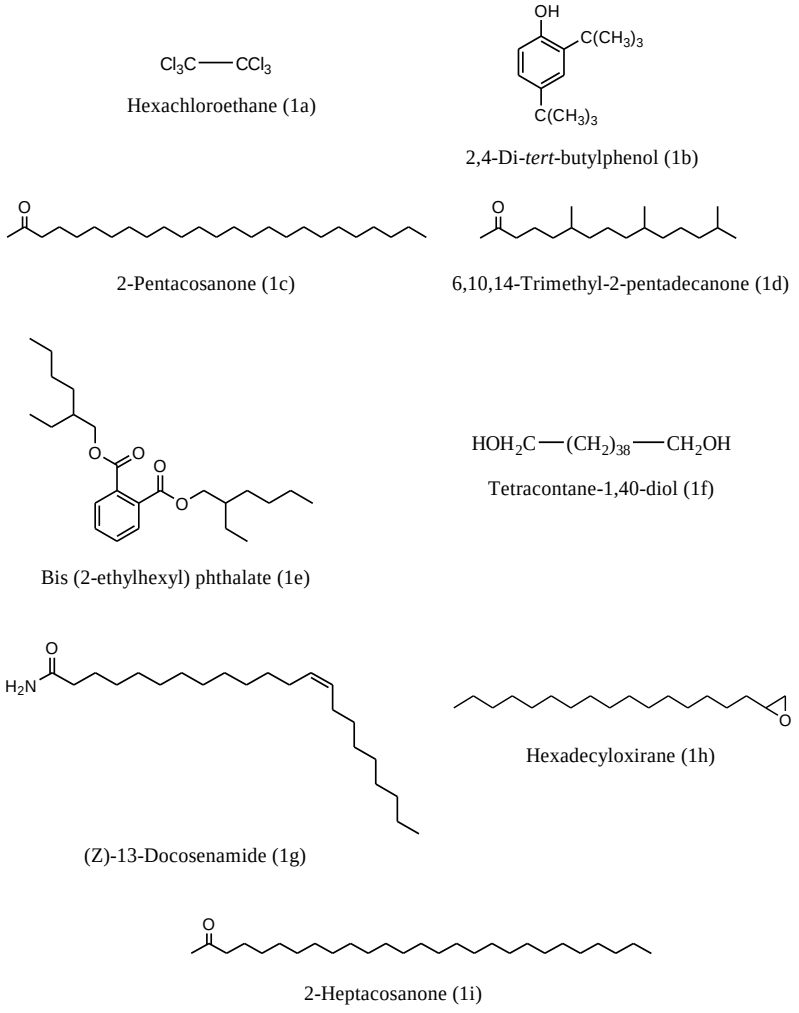


Figure 2: Structure of identified compounds from sample **1** by GC-MS analysis

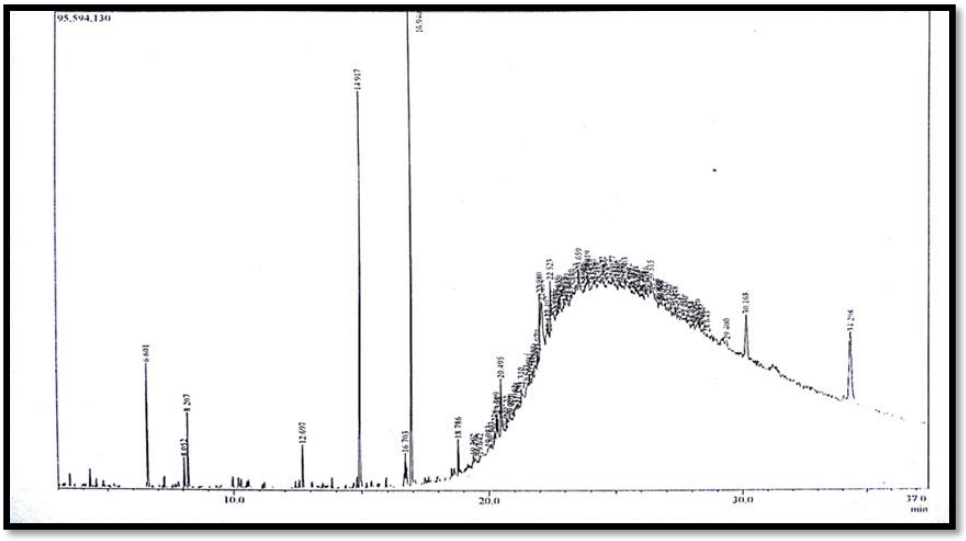
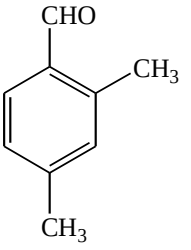


Figure 3: TIC of the GC-MS analysis of sample **2**

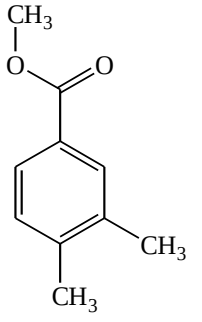
Table 3: Results of the GC-MS analysis of sample **2**

Sl no.	**Retention time (min.)**	**Name and number of the compound**	**Molecular weight**	**Molecular formula**	**Class of compound**
1	6.601	2,4-Dimethylbenzaldehyde (2a)	134	$C_9H_{10}O$	Aldehyde
2	8.207	Methyl 3,4-dimethylbenzoate (2b)	164	$C_{10}H_{12}O_2$	Ester
3	12.697	Methyltetradecanoate (2c)	242	$C_{15}H_{30}O_2$	Ester
4	14.917	Methyl 14-methylpentadecanoate(2d)	270	$C_{17}H_{34}O_2$	Ester
5	16.940	Methyl 16-methylheptadecanoate (2e)	298	$C_{19}H_{38}O_2$	Ester

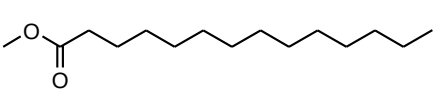
The sample **2** was a gummy substance and was soluble in n-hexane and chloroform. The GC-MS analysis of this esterified sample confirmed that sample **1** contains four fatty acids: 3,4-dimethylbenzoic acid,tetradecanoic acid,14-methylpentadecanoic acid and 16-methylheptadecanoic acidthose were not identified in the analysis of sample **1** due to their very minor amounts compared to others.



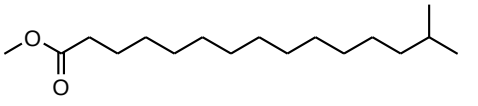
2,4-Dimethylbenzaldehyde (2a)



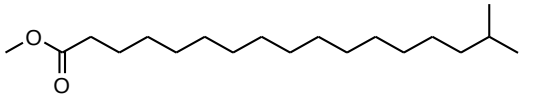
Methyl 3,4-dimethylbenzoate (2b)



Methyl tetradecanoate (2c)



Methy 14-methylpentadecanoate (2d)



Methyl 16-methylheptadecanoate (2e)

Figure 4: Structure of identified compounds from sample 2 by GC-MS analysis

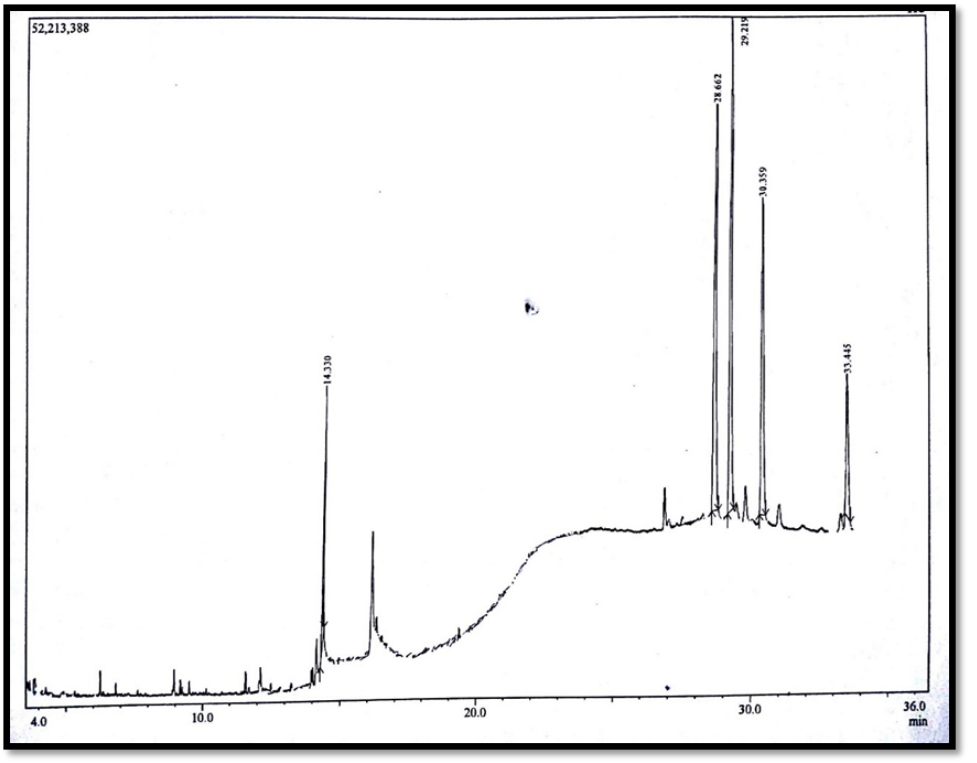


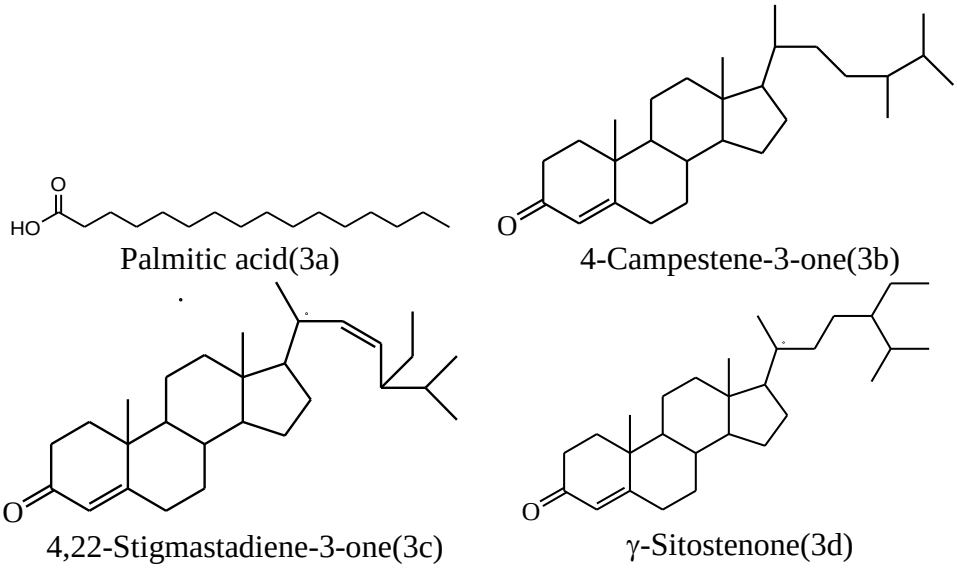
Figure 5: TIC of the GC-MS analysis of sample **3**

Table 4: Results of the GC-MS analysis of sample **3**

Sl no.	**Retention time (min.)**	**Name and number of the compound**	**Molecular weight**	**Molecular Formula**	**Class of compound**
1	14.330	Palmitic acid (3a)	256	$C_{16}H_{32}O_2$	Fatty acid
2	28.662	4-Campestene-3-one (3b)	398	$C_{28}H_{46}O$	Steroid
3	29.219	4,22-Stigmastadiene-3-one (3c)	410	$C_{29}H_{46}O$	Steroid
4	30.359	γ-Sitostenone (3d)	412	$C_{29}H_{48}O$	Steroid

The sample **3** was a brownish gummy solid. It was soluble in chloroform. Only four compounds were identified by GC-MS analysis in the case of

sample **3** in which one is fatty acid and other three are steroids. Phytochemical screening results of the chloroform extract of roots (Table 1) showed positive test for steroids which was supported by the GC-MS analysis of sample **3**.



Palmitic acid(3a) 4-Campestene-3-one(3b)

4,22-Stigmastadiene-3-one(3c) γ-Sitostenone(3d)

Figure 6: Structure of identified compounds from sample **3** by GC-MS analysis

Conclusion

As a part of our phytochemical study on the roots of *Spermacocelatifolia*, preliminary phytochemical screening of chloroform and methanol extracts and GC-MS analysis of two non-polar fractions of chloroform extract have been done. The results of these studieswill help to understand the types of compounds present in the plant in relation to its traditional medicinal activities and will encourage scientists for extensive work on the roots of this plant.

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