ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 8, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL EVALUATION, GC-MS ANALYSIS OF PHYTOACTIVE COMPOUNDS, AND ANTIBACTERIAL ACTIVITY STUDIES FROM *LINUM USITATISSIMUM*

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Received: 14 May 2019, Revised and Accepted: 21 June 2019

ABSTRACT

Objective: The flaxseed or linseed (*Linum usitatissimum*) is a minor oil seed belonging to the family Linaceae and genus Linum, which has been used as a precious nutritional food grain and folklore medicine in human diets for thousands of years and more. Recently, it has been used as a source of nutraceuticals and identified as a healthy component whose benefits on health are generally attributed to the high concentration of linolenic acids (omega 3) and lignans as well as significant quantities of dietary fiber including soluble and insoluble fibers.

Methods: The present study was carried out to assess the various phytochemical compositions, Gas chromatogram and Mass spectrometry (GC-MS) analysis, and antibacterial potential of different extracts of *L. usitatissimum*.

Results: The crude extracts of *L. usitatissimum* revealed the presence of several biologically active phytochemicals with the highest quantity of steroids, terpenoids, flavonoids, tannins, cardiac glycosides, protein, and amino acids. The antibacterial potency was explored against pathogenic bacteria, and the highest inhibitory activity of ethanol and methanol extracts was obtained against *Staphylococcus aureus*. The GC-MS analysis provides different peaks determining the presence of 10 phytochemical compounds with different therapeutic activities. The phytoactive principles were described with their molecular formula, retention time, molecular weight, and peak area (%).

Conclusion: Our results confer the utility of this plant extract in developing novel broad-spectrum therapeutic agent.

Keywords: Phytochemical, Antimicrobial, GC-MS, *Linum usitatissimum* (flaxseeds).

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i8.34126

INTRODUCTION

Medicinal plants generally play a unanimous role in maintaining the healthy state among human individual. As the population increases day by day, the rate of infections also gets increased. The best remedial source for curing these sorts of human ailments relies only in medicinal plants. Many of the health organizations are now in a state of utilizing these medicinal plants for the purpose of ethnomedicine.

The search for novel high-quality but cheap source of protein, energy, fat and antioxidant property has been attaining popularity in developing countries for meeting the challenges of hunger, starvation on one side and prevention and control of non-communicable diseases through diet on the other side. The attention has been focused on cheap grains containing relatively high amounts of protein and good quality fat with antioxidant property that can help to enhance the quality of foods of a large segment of population. Flaxseed (*Linum usitatissimum*) is one of the grains gaining popularity in this aspect. Flaxseed has an extensive property in the treatment of various human ailments such as cough, bronchitis, gastrointestinal infections, diabetes mellitus, constipation, healing of wounds, mental disorders, and gingival disorders and also in curing eczema and psoriasis. They also play a prime role in reducing the risk of tumor growth. The present study is to analyze the phytoactive compounds and utilizing these compounds to make drugs for various diseases.

Flaxseed is grown in about 4–5 lakh hectare of land, with an average productivity of 395 kg/ha in India [1]. The important flaxseed-growing states of the country are Madhya Pradesh, Uttar Pradesh, Chhattisgarh, Bihar, Rajasthan, Orissa, and Karnataka [2]. Flaxseeds, also known as common flax, reach to the height of 1.2 m, where the stems are found to be slender. Their leaves were 20–40 mm long and 8 mm broad and remains green in color. The flowers are glowing blue with 15–22 mm in diameter, with a maximum of 5–6 petals. The fruits are found to be dry in nature with 5–9 mm in diameter containing shiny brown-shaded seeds of about 4–7 mm long.

Scientific classification

Kingdom Plantae – plants
Subkingdom Tracheobionta – vascular plants
Superdivision Spermatophyta – seed plants
Division Magnoliophyta – flowering plants
Class Magnoliopsida – dicotyledons
Subclass Rosidae
Order Linales
Family Linaceae – flax family
Genus Linum L. – flax P
Species *L. usitatissimum* L. – common flax.

METHODS

Collection of plant materials

Flaxseeds were collected from Aadhya Organic Ltd., Tiruchirappalli. Further, taxonomic identification was authenticated by the director of the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Trichy. The sample was completely cleansed with tap water and allowed to shade dry at room temperature. Then, they were coarsely powdered by means of mechanical grinder.

Preparation of the sample extract

Around 10 g of dried seed sample was gently soaked in 100 ml of various solvents such as methanol, ethanol, petroleum ether, chloroform, and aqueous, respectively. Then, they were subjected to the process of continuous agitation on a rotator shaker for about 3 consecutive days at 190–220 rpm. Then further, the plant extracts were filtered through Whatman filter paper no. 42 (125 mm) separately. The extracts were

then preserved in an airtight container under refrigeration for future use.

Phytochemical screening

Preliminary phytochemical screening of various solvents was performed in order to determine the presence or absence of the phytocomponents such as alkaloids, steroids, terpenoids, tannins, glycosides, proteins, and amino acids [3-5]; the following standard procedures were used.

Test for alkaloids

To 1 ml of the sample, 1 ml of Mayer's reagent was added. Cream-colored precipitate indicates the presence of alkaloids.

Test for steroids

To 0.5 g of the sample, 2 ml of acetic anhydride and 2 ml of concentrated sulfuric acid were added. The change of color from violet to blue or green indicates the presence of steroids.

Test for terpenoids

To 0.5 g of the sample, 2 ml of chloroform and 2 ml of concentrated sulfuric acid were added. A reddish brown interface indicates the presence of terpenoids.

Test for flavonoids

Few drops of ammonia solution were added to the sample, and further, concentrated hydrochloric acid was layered; the appearance of yellow color shows the presence of flavonoids.

Test for saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath; 10 ml of the filtrates was mixed with 5 ml of the distilled water shaken vigorously for froth formation.

Test for phenolic compounds

To 1 ml of extract, few milliliters of gelatin solution was added. White precipitate indicates the presence of phenolic compounds and tannins.

Test for tannins

To 1 ml of extract, few drops of lead acetate solution were added. Intense white solution indicates the presence of tannins.

Test for cardiac glycosides

To 2ml of the sample, 2 ml of glacial acetic acid, few drops of ferric chloride solution and about 1 ml of concentrated sulfuric acid were gently layered.Brown ring indicates the presence of deoxy sugar, violet ring may appear the brown ring, and sometimes, green ring may also appear just above the brown ring.

Test for proteins

To 1 ml of the extract, 1 ml of concentrated nitric acid was added, then it is boiled and cooled for few minutes, and about 20% of sodium hydroxide solution was added. Orange color indicates the presence of proteins.

Test for amino acids

To few milliliters of the sample, three drops of ninhydrin solution were added, and they were gently heated in a boiling water bath for 10 min. The appearance of purplish blue indicates the presence of amino acids.

Test for carbohydrates

To 1 ml of the extract, 2 ml of Molisch's reagent was added; reddish brown ring indicates the presence of carbohydrates.

Gas chromatographic–mass spectrophotometric (GC-MS) analysis

GC-MS analysis of ethanol extract of flaxseeds was performed using Perkin Elmer Clarus 500 GC-MS unit [6,7]. The analysis was carried out to detect the possible compounds present in the active fraction of column type used as PE-S (equivalent to DB-5) with a column length of 30 m. The flow rate maintained was 1 ml/min, with an initial column temperature of 50° and final temperature of 250°. The rate of temperature change in the column was maintained at 5°/min. 1-m volume of the sample was taken for injection. The identification of phytoactive compounds of ethanol extract was identified by comparing their retention indices and patterns of mass spectra with reference to the Wiley Registry of Mass Spectral Data, New York (Wiley 8), and National Institute of Standards and Technology (NIST 14 LIB).

Antibacterial activity

The crude extracts (methanol, ethanol, chloroform, petroleum ether, and aqueous) obtained from the seeds of *L. usitatissimum* were studied for its inhibitory action against *Staphylococcus aureus* by means of disc diffusion method [8]. The test organism was inoculated in a sterile conical flask containing 100 ml of nutrient broth. Mueller Hinton agar (HI media) was prepared, and about 20 ml of media was poured into sterile Petri plates. After solidification, the media in each plate were inoculated with the test organism from the seeded broth using sterile cotton swabs.

In disc diffusion method, sterile discs were taken and they were impregnated with varying concentration such 10 µl, 20 µl, 30 µl, and 40 µl of the seed extracts of various solvents, respectively, and they were layered carefully on the upper portion of the inoculated agar plates. Simultaneously, antibiotics such as streptomycin and ampicillin were used as a positive control. Then, the plates were incubated at 37°C for 24 h; after the period of incubation, the degree of sensitivity was measured by identifying the zone of inhibition around the sterile discs with sample loaded. The diameter of clear zone was calibrated by measuring scale and recorded, and this is possible by means of certain factors; composition of medium; and concentration of the sample, pH, and the period of incubation.

RESULTS

Preliminary phytochemical screening of flaxseeds

Phytochemical analysis is very useful in the evaluation of some phytoactive compounds of medicinal plants. The phytochemical screening carried on the *L. usitatissimum* (flaxseeds) revealed the presence of some active ingredients such as cardiac glycosides; protein and amino acids were present in all the extracts. Terpenoids were present in all extracts except aqueous. Saponins were present in all extracts except petroleum ether. Alkaloids, phenols, tannins, and flavonoids were present in methanol, ethanol, and aqueous extracts. Steroid was present in methanol, ethanol, and aqueous extracts. Carbohydrate was present in the petroleum ether extract (Table 1).

Identification of phytoactive compounds from ethanol extract of the flaxseeds by GC-MS

The results obtaining to GC-MS analysis lead to the identification of 10 compounds from the GC fractions of the ethanolic extract of flaxseeds (*L. usitatissimum)*. These compounds were distinguished through mass spectrometry attached with GC. The various components present in the flaxseeds that were detected by the GC-MS are shown in Table 2. The GC-MS spectrum confirmed the presence of various components with a different retention time, as illustrated in Fig. 1.

The pharmacological activities recorded are based on Dr. Dukes Phytochemical and Ethnobotanical Database by Dr. Jim Duke of the Agricultural Research Service/USDA. The GC-MS analysis of *L. usitatissimum* ethanolic seed sample extract revealed the presence of major phytochemicals. The available compounds and their biological activity [9-14] are presented in Table 3.

Antibacterial activity

The results of antibacterial activity were carried out on methanol, ethanol, chloroform, petroleum ether, and aqueous extracts from the flaxseeds (*L. usitatissimum)*. The maximum antagonistic effect was

Table 1: Phytochemical screening of flaxseed extract with various solvents

Photochemical	Methanol	Ethanol	Chloroform	Pet ether	Aqueous
Alkaloids	+	+	−	−	+
Steroids	++	+	+++	−	−
Terpenoids	+++	+++	+++	++	−
Flavonoids	++	+	−	−	++
Saponins	+	+	+	−	+
Phenols	+	++	−	−	+
Tannins	+++	++	−	−	+++
Cardiac glycosides	++	++	+	+	++
Proteins	+++	+++	++	++	+++
Amino acids	+++	+++	++	++	+++
Carbohydrates	−	−	−	+	−

+++: Strongly indicated, ++: Moderately indicated, +: Present, −: Absent

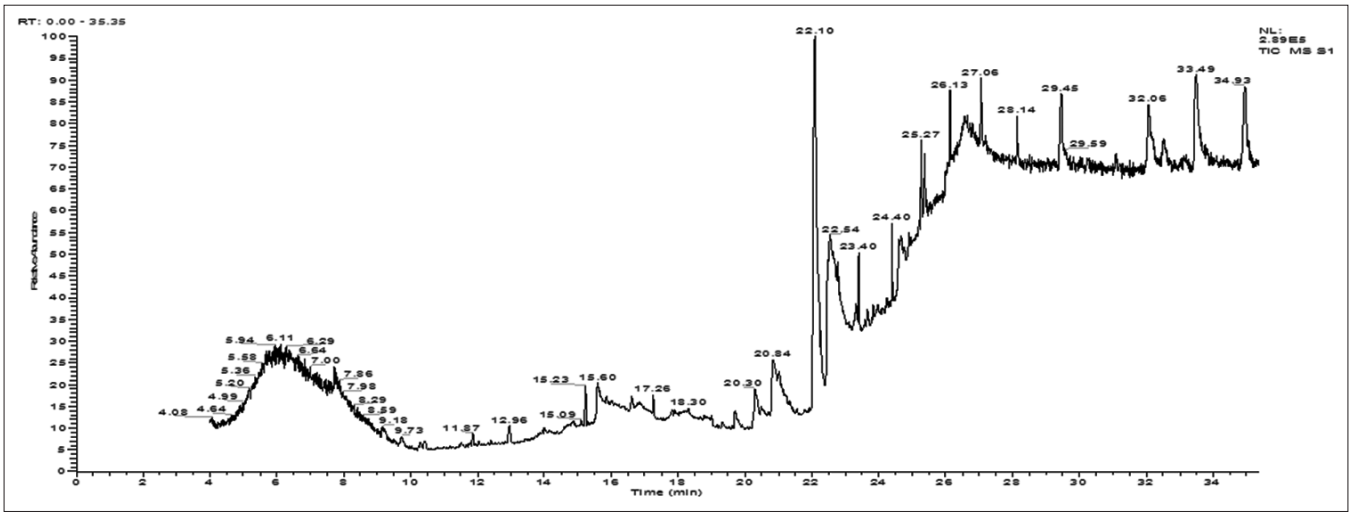
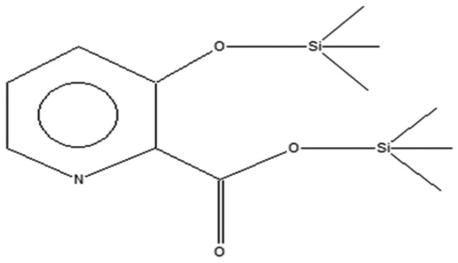
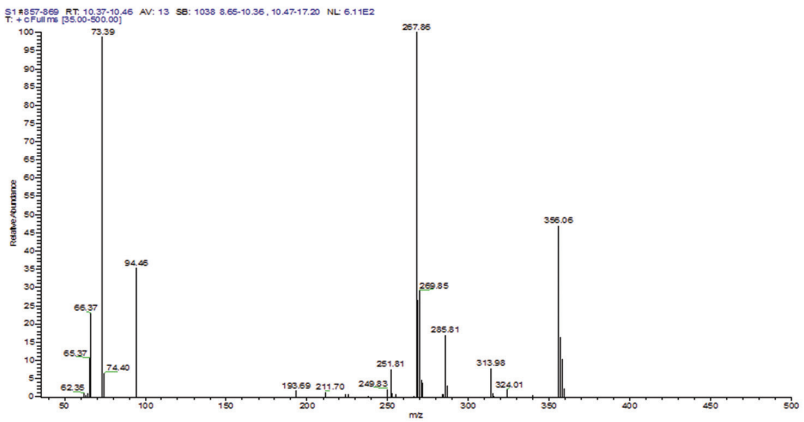


Fig. 1: Graphical representation of gas chromatogram and mass spectrometry (GC-MS)

Compounds for the respective peaks

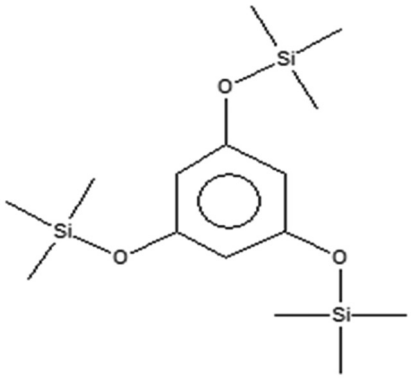
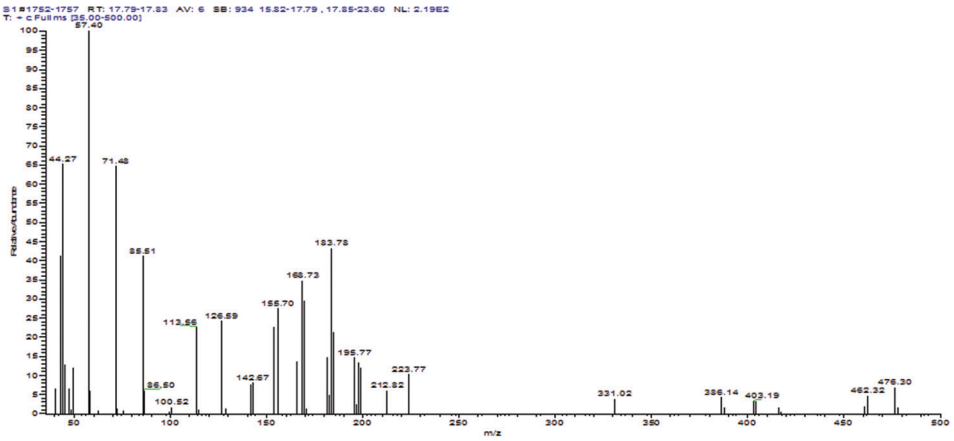
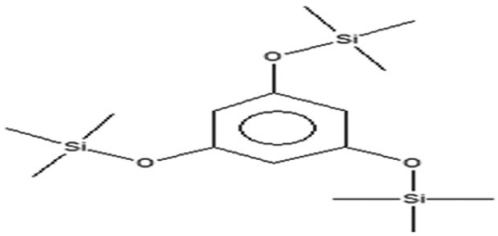
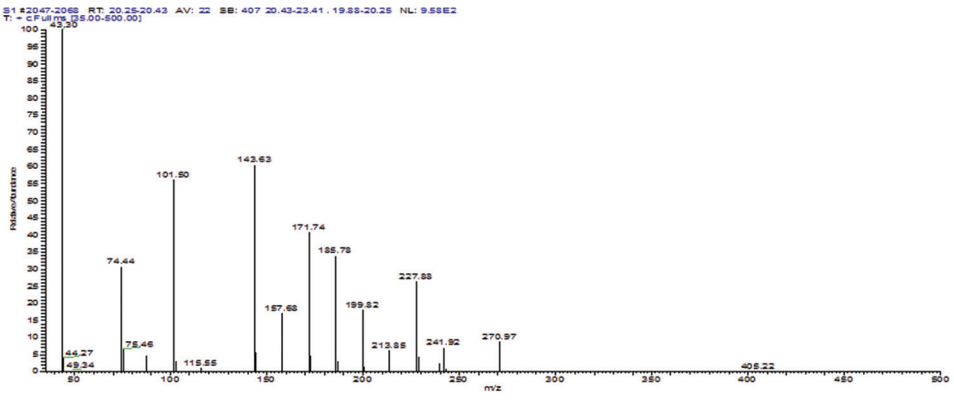
S. No.	Peak	Compound
1.	10.42	3-Hydroxy picolinic TMS 2 Formula C12H21NO3Si2, MW 283, CAS# 36972-81-3, Entry# 189928 Trimethylsilyl 3-[(trimethylsilyl)oxy]-2-pyridinecarboxylate #





(Contd...)

Compounds for the respective peaks *(Continued)*

S. No.	Peak	Compound
2.	11.87	1,3,5-Tris(trimethylsiloxy)benzene Formula C15H30O3Si3, MW 342, CAS# 10586-12-6, Entry# 30422 Silane, [1,3,5-benzenetriyltris(oxy)]tris[trimethyl-  
3.	12.96	1,3,5-Tris(trimethylsiloxy)benzene Formula C15H30O3Si3, MW 342, CAS# 10586-12-6, Entry# 204542 Silane, [1,3,5-benzenetriyltris(oxy)]tris[trimethyl-  

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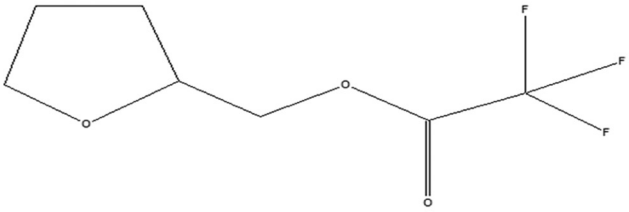
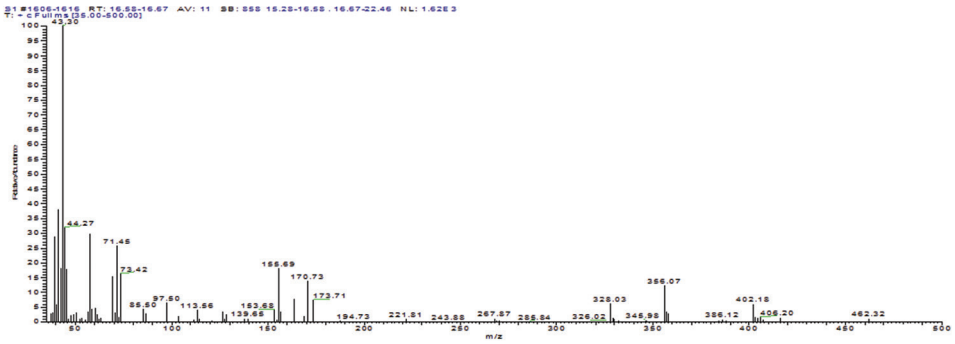
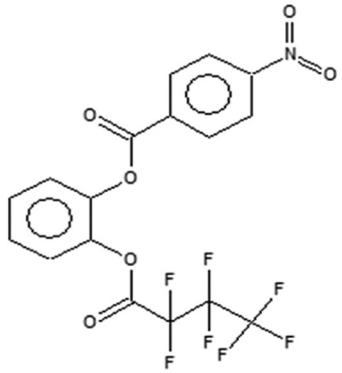
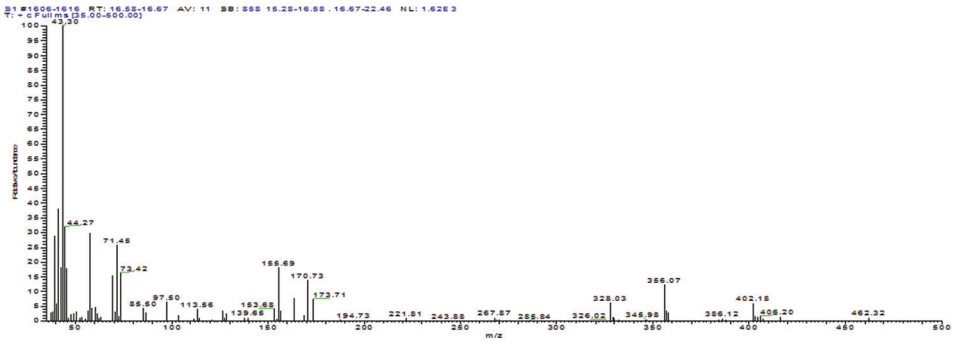
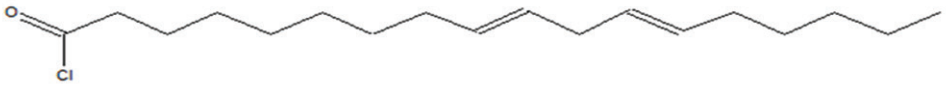
Compounds for the respective peaks *(Continued)*

S. No.	Peak	Compound
4.	16.61	Trifluoroacetic acid, 2-tetrahydrofurylmethyl ester Formula C7H9F3O3, MW 198, CAS# 71239-15-1, Entry# 35011 Tetrahydro-2-furanylmethyl trifluoroacetate #  
5.	19.73	1,2-Benzenediol, o-(2,2,3,3,4,4,4-heptafluorobutyryl)-o'-(4-nitrobenzoyl)- Formula C17H8F7NO6, MW 455, CAS# NA, Entry# 124137  
6.	20.10	9,12-Octadecadienoyl chloride, (Z,Z)- Formula C18H31ClO, MW 298, CAS# 7459-33-8, Entry# 4686 Linoleoyl chloride 

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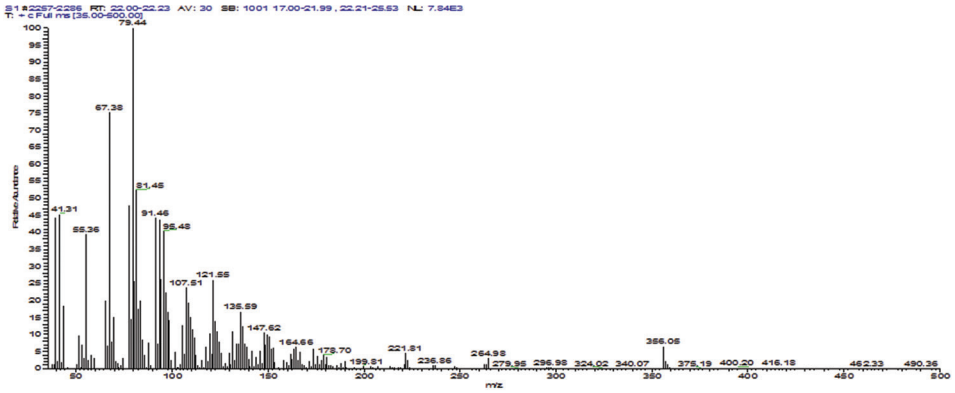
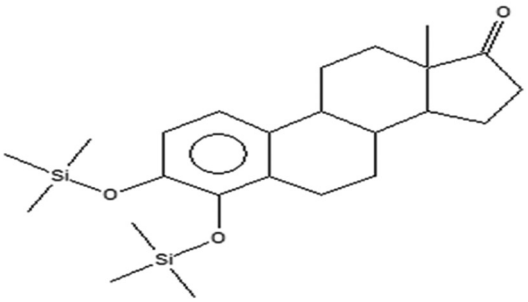
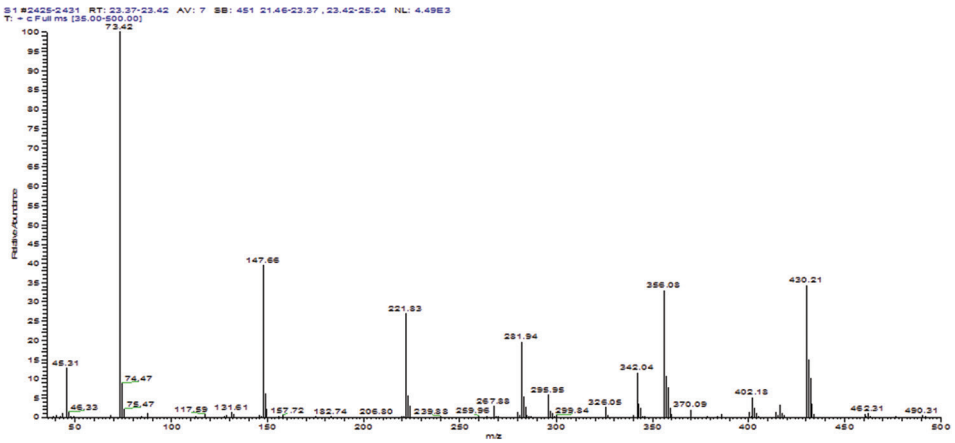
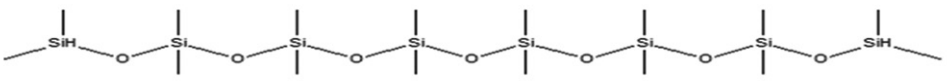
Compounds for the respective peaks *(Continued)*

S. No.	Peak	Compound
7.	23.40	 Estra-1,3,5(10)-trien-17-one, 3,4-bis[(trimethylsilyl)oxy]- Formula C24H38O3Si2, MW 430, CAS# 51497-39-3, Entry# 210593 3,4-Bis[(trimethylsilyl)oxy]estra-1,3,5(10)-trien-17-one #  
8.	24.40	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- Formula C16H50O7Si8, MW 578, CAS# 19095-24-0, Entry# 39429 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane # 

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Compounds for the respective peaks *(Continued)*

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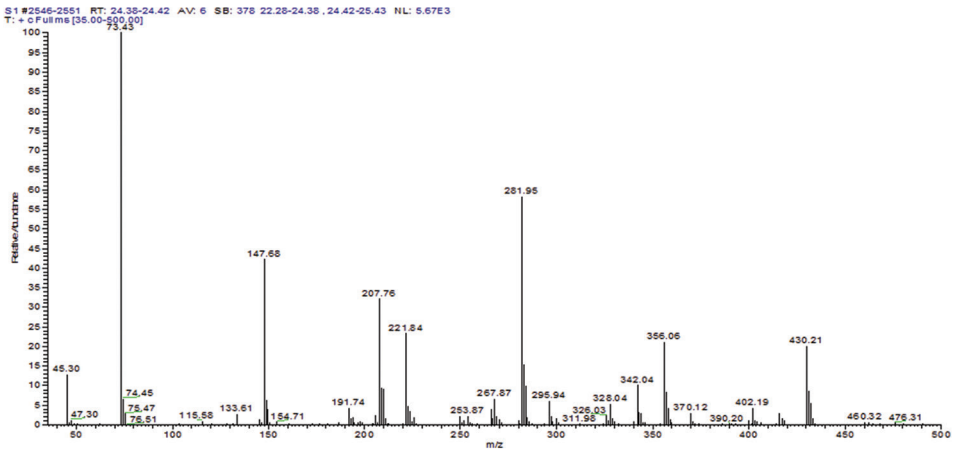
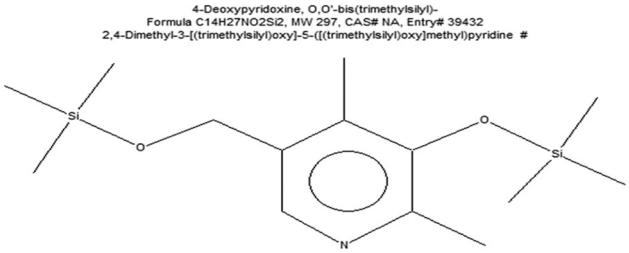
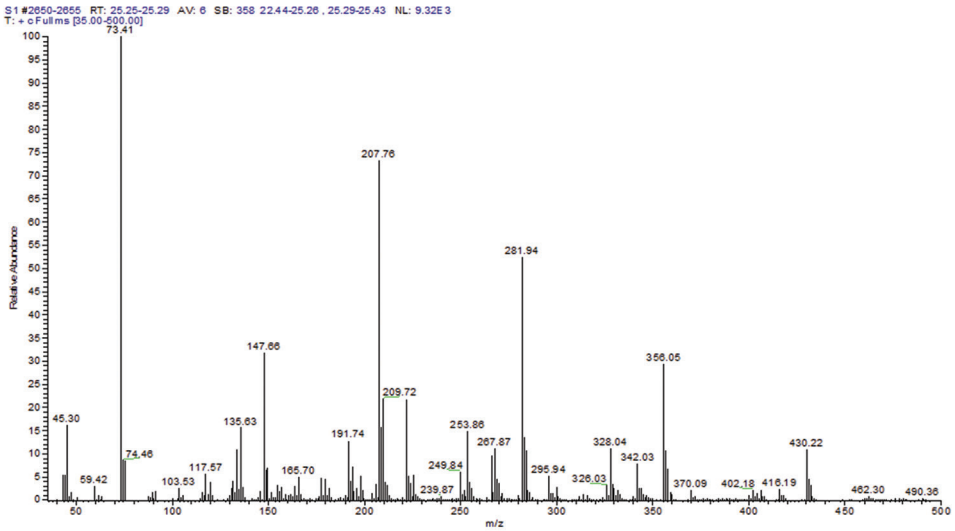
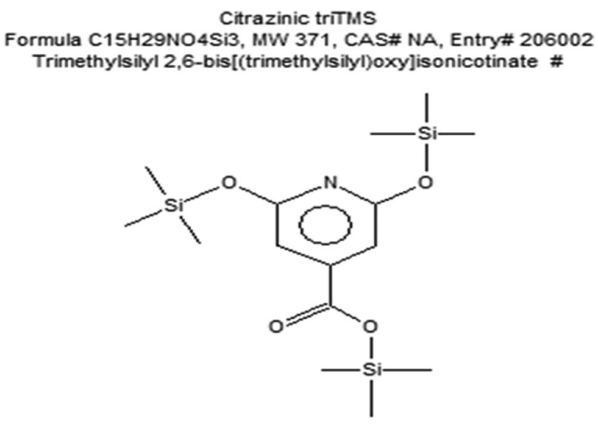
		
9.	25.27	 
10.	28.14	

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Compounds for the respective peaks *(Continued)*

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		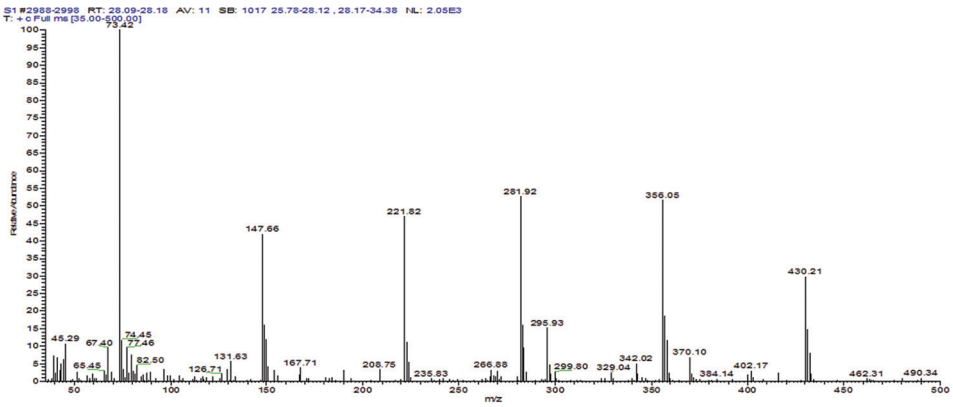

Table 2: Gas chromatogram and mass spectrometry analysis of *Linum Usitassimum*

S. No.	Peak value	Name of the compounds
1.	10.42	3-Hydroxy picolinic TMS 2
2.	11.87	1,3,5-Tris (trimethylsiloxybenzene)
3.	12.96	1,3,5-Tris (trimethylsiloxybenzene)
4.	16.61	Trifluoroacetic acid, 2-tetrahydrofurylmethyl ester
5.	19.73	1,2 Benzenediol O-(2,2,3,3,4,4,4 hepatafluoro×-(4 nitrobenzyl)-
6.	20.10	9,12-Octadecadienoyl chloride (Z, Z)-
7.	23.40	Estra-1,3,5 (10)-trien-17-one-3,4-bis trimethylsiyl (oxy)-
8.	24.40	Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,1 3,13,15,15 hexacamethyl
9.	25.27	4-Deoxypyridoxine 0-0×-bis (trimethylsilyl)
10.	28.14	Citrazinic tri-TMS

Table 3: Compound nature and biological activity of flaxseeds in ethanol extract

S.No.	Compound	Biological activity
1.	3-Hydroxy picolinic TMS 2	Antifungal activity
2.	1,3,5-Tris (trimethylsiloxybenzene)	Wound healing, anti-inflammatory activity
3.	1,3,5-Tris (trimethylsiloxybenzene)	Wound healing, anti-inflammatory activity
4.	Trifluoroacetic acid, 2-tetrahydrofurylmethyl ester	Antimicrobial and larvicidal activity
5.	1,2 Benzenediol O-(2,2,3,3,4,4,4 hepatafluoro×-(4 nitrobenzyl)-	No activity found
6.	9,12 Octadecadienoyl chloride (Z, Z)-	Antioxidant, hepatoprotective, nematicide
7.	Estra-1,3,5 (10)-trien-17-one-3,4-bis trimethylsiyl (oxy)-	anti-arrhythmic activity
8.	Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11, 13,13,15,15 hexacamethyl	Antimicrobial activity
9.	4-Deoxypyridoxine 0-0×-bis (trimethylsilyl)	No activity found
10.	Citrazinic tri-TMS	No activity found

Table 4: Antibacterial activity of *Linum usitatissimum*

Extracts	Concentration	*Staphylococcus aureus* (Zone of inhibition) mm in dm
Methanol	10 µl	2.0
	20 µl	2.5
	30 µl	3.0
	40 µl	3.5
Ethanol	10 µl	1.0
	20 µl	1.5
	30µl	2.0
	40 µl	2.5
Chloroform	10µl	1.0
	20 µl	2.0
	30 µl	2.5
	40µl	3.0
Petroleum ether	10 µl	0.7
	20µl	1.0
	30 µl	1.5
	40 µl	2.0

Values are mean±standard deviation of triplicates

found against methanol, ethanol, and chloroform extracts against *S. aureus*. The activity was carried out in different concentrations such as 10 µl, 20 µl, 30 µl, and 40 µl. The minimum activity was found in aqueous extract and petroleum ether extract. In most of the cases, the zone of inhibition gets an increase with increasing concentration of the extracts. The different extracts of *L. usitatissimum* showed potent antibacterial activity against *S. aureus* (Table 4).

DISCUSSION

Our data from the present work clearly confirm that the phytochemicals may be effective in combining or preventing disease due to their antioxidant effect [15]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. The identified phytochemical compounds may be the bioactive constituents responsible for the antimicrobial activity. The current study was initiated because of the increasing resistance to antibiotics including bacteria. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents. As a result, the antimicrobial activity of flaxseed extracts of *L. usitatissimum* was screened against *S. aureus*. In general, methanol, ethanol, and chloroform extracts of the flaxseed extracts appeared to be an effective source of active antimicrobial agents. A previous study reveals that the antimicrobial activity of flaxseed against *S. aureus* showed maximum inhibitory results [16]. GC-MS analysis helps to predict the formula and structure of 10 biomolecules.

Further investigation may lead to the isolation of bioactive compounds, and their structural elucidation and screening of pharmacological activity will be helpful for further drug development [17].

CONCLUSION

The present study revealed that the methanol, ethanol, chloroform, petroleum ether, and aqueous extracts of flaxseeds (*L. usitatissimum)* exhibited potent in various types of phytochemical constituents. Plant extract is the best source of phytochemicals, and it is the natural medicine for many illnesses. Among the variety of secondary metabolites in flaxseeds: tannins and terpenoids are predominant antioxidant compounds present which have anti-inflammatory and hyperglycemic activities [18]. The alkaloids are used as analgesics, stimulants, anesthetic, hallucinogens, and antibacterial agents [19]. The glycosides are reported to possess strong antibacterial activities [20,21]. In this study, ten chemical compounds have been identified from ethanol extract of flaxseeds by gas chromatogram mass spectrometry (GC-MS) analysis. The presence of phytoactive compounds proves the use of flaxseeds (*L. usitatissimum)* for various disorders by folklore practice. Therefore, it is recommended as a source of phytochemical significance. However, the more spectral analysis must be a compass to evaluate the mechanism of action with various activities. Further, research is needed to isolate and identify these phytoactive compounds which will pave the way for the progress of cost-effective drugs with hardly side effects.

ACKNOWLEDGMENT

The authors are grateful to Cauvery College for Women, Annamalai Nagar, Trichy - 620 018, for provided laboratory support to complete the work.

CONFLICT OF INTEREST

Authors we declared that have no conflict of interest.

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