GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Phytochemical profiles of Krishna Tulsi (*Ocimum tenuiflorum*) and Rose Periwinkle (*Catharanthus roseus*)

Velusamy Arulvendhan * and Periyakali Saravana Bhavan

Crustacean Biology Laboratory, Department of Zoology, School of Life sciences, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India.

GSC Biological and Pharmaceutical Sciences, 2024, 26(02), 079–092

Publication history: Received on 29 December 2023; revised on 11 February 2024; accepted on 14 February 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.26.2.0055

Abstract

This investigation explored the phytochemical profiles of two medicinal herbs, *Ocimum tenuiflorum* and *Catharanthus roseus*. Their leaves were powdered and subjected to ethanolic (polar solvent) withdrawal. The presence of secondary phytochemicals, such as alkaloids, terpenoids, flavonoids, tannins, phenols, saponins, glycosides, quinones and steroids were detected in these two medicinal herbs. The GC-MS revealed presence of nine secondary phytochemical metabolites in *O. tenuiflorum*, of which four have bioactive properties (Phenol, 2-methoxy-4-(2-propenyl)-, acetate; N-Hexadecanoicacid; 2-Piperidinone, n-[4-bromo-n-butyl]-; Dotriacontane). The presence of seven secondary metabolic compounds were detected in *C. roseus*, which four possessed bioactive principles (3-Methylmannoside; Squalene; Pentatriacontane; 2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene). Thus, these herbs have detectable levels of secondary phytochemicals and their metabolite components. Among these two herbs *C. roseus* possessed more numbers of detectable secondary phytochemicals.

Keywords: *Ocimum tenuiflorum*; *Catharanthus roseus;* Ethanolic extract; Phytochemicals; Secondary metabolites

1. Introduction

Medicinal plants are traditionally used for the treatment of various diseases in India and all over the world since the beginning of civilization. In fact, natural products are a source of synthetic and traditional herbal medicine. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Medicinal plants have been playing a vital role in the health and healing of man since dawn of human civilization. There is a cure for every disease or disorder in nature and traditionally the plants are being used to treat them.

"Thulasi" in Tamil, "Tulasi" in Sanscreet, "Tulsi" in Hindi and "Holy Basil" in English is the most sacred herb for Hindus in India. *Ocimum* (Lamiaceae), is an erect, softly hairy, aromatic herb, commonly cultivated in temple premises and households as a sacred plant [1]. Worldwide there are 63 species of *Ocimum* reported. In India, there are two types of *Ocimum* are in cultivation: one is sritulasi, ramatulasi, lakshmitulasi (*Ocimum sanctum*) with green leaves, and another one, *O. tenuiflorum*, with purple leaves known as shyamatulasi, krishnatulasi, karuntulasi. Plant is native of India and has a wide distribution, covering the entire Indian sub-continent ascending up to 1,800 m in the Himalayas and as far as the Andaman Nicobars islands**.** It is broadly distributed in Asia, and is also found in Australia, West Africa and some of the Arab countries [2]**.** *O. tenuiflorum* plant is an erect, much branched sub-shrub, 30–60 cm tall with purple sub quadrangular branches. Leaves are simple, opposite, elliptic oblong, obtuse or acute, with entire or sub serrate or dentate margins, pubescent on both sides, minutely gland dotted, with slender, hairy petioles flowers are purplish borne in elongate racemes in close whorls. The seeds are oval, flattened, shining having reddish-yellow colour.

* Corresponding author: Velusamy Arulvendhan

For centuries, the dried leaves of Tulasi have been mixed with stored grains to repel insects. Tulasi, has its important role in the traditional Ayurvedic and Unani system of medicine for its range of therapeutic activities [3]**.** It has been used for thousands of years in Ayurveda for its diverse healing properties water mixed with the petals is given to the dying to raise their departing souls to heaven. The whole plant of *O. tenuiflorum* has medicinal value; mostly leaves and sometimes the stem, flower, root and seeds are known to possess therapeutic potential [4,5,6]**.** Tulasi extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. Traditionally, *O. tenuiflorum* is taken in many forms, as herbal tea, dried powder or fresh leaf. They have been used for the treatment of various diseases like bronchitis, malaria, diarrhoea, dysentery, skin disease, arthritis, eye diseases, insect bites. The plant has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions [7,6]. Its oil possesses a pleasant odor characteristic of the plant, with an appreciable note of clove. The plant has several chemotypes, morphologically indistinguishable plants differing in their chemical elements. Ethanolic rich chemotypes dominate in *O. tenuiflorum* [8,9]. Large variability in volatile constituents from leaves inflorescence oil of *O. tenuiflorum* has been reported [10].

Catharanthus roseus is a potential medicinal plant of family Apocynaceae, it has many pharmacological actions such as antimicrobial, antioxidant, anthelmintic, antifeedant, antisterility, antidiarrheal, antidiabetic effect etc. [11]. The flower petals, seeds and other parts of *C. roseus* exhibit antioxidant properties. It has multiple applications in foods, cosmetics and pharmaceutical industries. It could synthesize more than 70 types of chemical constituents such as indole type of alkaloids, ajmalicine, serpentine and reserpine. Due to presence of those alkaloids in *C. roseus* has antihypertensive and antispasmodic properties. One of the important types of alkaloids is the vinblastine produced from *C. roseus* due to its antitumor activity and wide pharmaceutical use [12]. The compounds mentioned above have a wide range of applications mostly in the treatment of lymphocytic cancer, Wilkins's cancer, neuroblastoma and reticulum cell tumour, Hodgkin's disease besides lymphosarcoma, choriocarcinoma [13]. It contains significant amounts of volatile and phenolic compounds including caffeoylquinic acids and flavanol glycosides which are known to antioxidant activity. It has an important role in the body defines system that is acts as an antioxidant against reactive oxygen species (ROS), which are harmful by forming such products through normal cell aerobic respiration. Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, aging process and perhaps dementia [14]. The phenolic compounds have redox properties that act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Besides antioxidant activity, its phenolic compound exhibits antiallergic, anti-inflammatory, antimicrobial, anti-thrombotic, cardio protective and vasodilatory effects [15]. The main objective of the present study was to characterize the primary and secondary phytochemicals of one part, the leaves of two medicinal herbal plants, *O. tenuiflorum* and *C. roseus*.

2. Material and methods

2.1. Collection of plant material and preparation of *O. tenuiflorum* and *C. roseus* leaf extract

A medicinal plant identified as *Ocimum tenuiflorum* and *Catharanthus roseus* Figure 1. and Figure 2. was gathered in 2019 from March and April. The gathering took place in the Tamil Nadu village of Uthangarai, which is located in the Krishnagiri region of the Indian state. Plant specimens were transferred with the reference number BSI/SRC/5/23/2019/Tech/72/73 in order to undergo rigorous taxonomic categorization. The genuineness of the plant has been confirmed by the Botanical Survey of India (BSI), which is situated in Coimbatore, Tamil Nadu, India. The leaves samples of *O. tenuiflorum and C. roseus* were taken for this investigation**.**

2.2. Scientific classification

- Kingdom: Plantae
- Class : Angiosperm
- Order : Lamiales
- Family : Lamiaceae
- Genus : *Ocimum*
- Species : *tenuiflorum*

Figure 1 *Ocimum tenuiflorum* leaves

2.3. Scientific classification

- Kingdom: Plantae
- Class : Magnoliopsida
- Order : Gentianales
- Family : Apocynaceae
- Genus : *Catharanthus*
- Species : *roseus*

Figure 2 *Catharanthus roseus* leaves

2.4. Solvent extraction of *O. tenuiflorum* and *C. roseus*

The collected sample was cleaned well with freshwater to remove all the extraneous matter such as, sand particles and necrotic parts and brought to the laboratory in plastic bags. The sample was then thoroughly washed with freshwater and blotted (disembowelled). The two medicinal herbal plants leaves were separated, spread out and dried at room temperature for 2 weeks. After shade drying, the dehydrated plant parts stayed ground well using a mechanical blender into fine powder and transferred into airtight container with proper labelling for further use. The powdered sample (50 g) was packed in Whatman No.1 filter paper and Soxhlet extraction was done with 250 ml (1:5 w/v) of ethanol individually for 6-9 h (30 to 36 cycle) until a clear colourless solution was obtained. The extract was filtered by using double layer muslin cloth, concentrated at 40-50 °C using rotary vacuum evaporator (ROTAVAP) attached with ultra-cryostat and dried at 40 °C under hot air oven. The dark, gummy solid obtained was used for further investigation. Solute thus extracted was collected in a centrifuge tube and used for further studies.

2.5. Extract recovery percentage

The extracts of *O. tenuiflorum* and *C. roseus* recovered after Soxhlet was calculated [16]. Recovery % = Extract weight / Plant sample weight (g) × 100

2.6. Qualitative analysis of Preliminary phytochemicals

The solvent extract was subjected to primary phytochemical analysis such as presence of Alkaloids, Terpenoids, Flavonoids, Tannins, Phenols, Saponins, Glycosides, Quinones and Steroids by adopting the standard qualitative procedures [17].

2.7. Gas chromatography-mass spectrum (GC-MS) analysis of ethanolic extract of *O. tenuiflorum* and *C. roseus*

A Perkin-Elmer GC Clarus 500 system and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a BR-5MS, fused silica capillary column (30mm x 0.25mm 1D x 0.25 µMdf, made up of 5% Diphenyl / 95% Dimethyl poly siloxane) were utilized to perform the GC-MS analysis of these extracts. An electron ionization device with an ionizing energy of 70 eV has been used for GC-MS detection. At an injection volume of 2 µL and a split ratio of 10:1, helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1mL min-1. The injector temperature was 280 °C, and the ion source temperature was 250 °C. The oven was configured to start at 110 °C (isothermal for 2 minutes), ascend by10°C min-1 to 200 °C, then by 5 °C min-1 to 280 °C, then subsequently, finish with a 12-minute isothermal at 280 °C. Mass spectra were recorded at 70 eV with pieces ranging from 50 to 500 amu and a scan interval of 0.5 seconds. The GC played approximately 40-50 minutes in total. Mass spectra and chromatograms were processed using software called Turbomass, and the average peak area of each component was compared to the total areas to calculate its relative percent amount.

2.8. Identification of phytoconstituents

The National Institute of Standards and Technology (NIST) database, which contains more than 71,000 patterns, was utilized to interpret the mass spectrum of the GC-MS. The known components mass spectrum that was maintained in the NIST library was compared to the mass spectrum of the unknown component. By correlating the relevant peak areas to the TIC areas from the GC-MS, quantitative insights were made. The test materials labels, molecular masses, times of retention, and peak area fractions were identified. In matching each extract's mass spectrum to the data in the molecular weights, chemical identities, and structures of the bioactive components in the NIST and Wiley libraries have been determined for each extract [18].

3. Results and Discussion

3.1. The extraction yields

The successive solvent extract yield was shown in the Figure 3. The compounds of *O. tenuiflorum* present in ethanolic extract were according to polar nature. The leaf powder yielded 1.5% respectively. *O. tenuiflorum* extraction was mostly done with polar solvents. More yields were depending upon the solvent type which dissolves more of a particular compound. Similarly, the leaf powders of *C. roseus* yield were shown in the Figure 4. In *C. roseus* also the compounds present in leaf powder were according to polar nature. The leaf powder yielded 2.0% respectively. *C. roseus* extraction was mostly done with polar solvents. More yields were depending upon the solvent type which dissolves more of a particular compound.

Figure 3 Ethanol extract of *O. tenuiflorum*

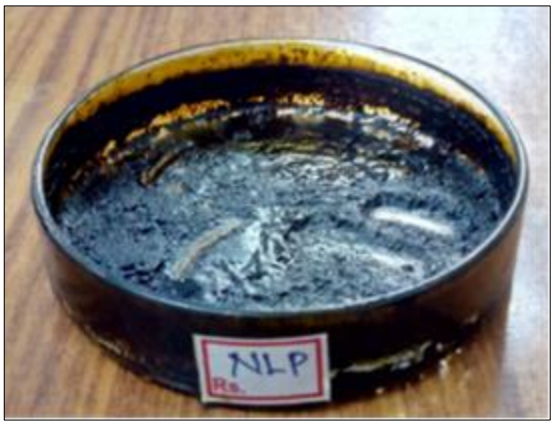


Figure 4 Ethanol extract of *C. roseus*

Hence, the ethanolic extraction of *C. roseus* contains more yields following by other solvents Figure 5.

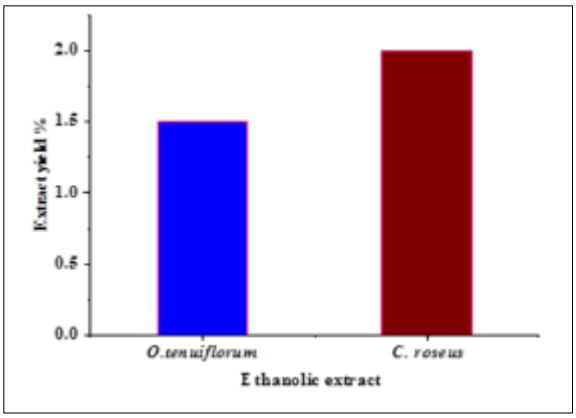


Figure 5 Extraction yields

3.2. Preliminary phytochemicals

The primary phytochemicals present in *O. tenuiflorum* and *C. roseus* presented in Table 1. depicts the quality primary photochemical compounds present in the ethanolic extract of leaf powder of *O. tenuiflorum.* There were five compounds, of which alkaloids, luxuriantly present and Terpenoids, Flavonoids and Phenols Moderate present and Glycosides poorly present.

Table 1 Secondary phytochemicals present in ethanolic extract of *O. tenuiflorum* and *C. roseus* leaves

Phytochemicals	**Ethanolic extracts of leaves (crude)**	
	O. tenuiflorum	***C. roseus***
Alkaloids	+++	+++
Terpenoids	++	++
Flavonoids	++	+++
Tannins	-	-
Phenols	++	+++
Saponins	-	+
Glycosides	+	-
Quinones	-	++
Steroids	-	+

+, Poor presence; **++**, Moderate presence; **+++**, Luxuriant presence; --, Absence

The other compound such as Tannins, Saponins, Quinones and Steroids were not present and the quality primary photochemical compounds present in the ethanolic extract of leaf powder Table 2 along with Figure 6. The *C. roseus* there were six compounds, of which alkaloids, flavonoids and phenols luxuriantly present. The other compound such as terpenoids and quinines were moderately present, saponins and Steroids was poorly present, and tannins and Glycosides were not present. The overall presence was almost similar in number of compounds among the two plant parts. Therefore, overall, five compounds in the *O. tenuiflorum* leaf and Seven compounds in the *C. roseus* were present. The overall presence was almost similar in number of compounds among the two plant parts. However, leaf of *C. roseus* showed three luxuriantly presence, whereas the *O. tenuiflorum* showed one luxuriantly present.

Table 2 Standard Methods for Phytochemical Analysis

Phytochemicals	Test name	Methodology	Observations
Alkaloid	Mayer's Test	To 1 mL Plant extract few drops of Mayer's reagent (Potassium Mercuric Iodide) were added.	Formation of a yellow- colored precipitate.
Flavonoids	Alkaline Reagent Test	1-2 mL of Plant extract was treated with 2-3 ml of dilute NaOH, followed by addition of 3-4 mL dilute HCl.	Formation of intense yellow color, which becomes colorless on addition of dilute HCl.
Glycosides	Salkowski Test	To 1 mL of Plant extract, 2 mL of chloroform was added, followed by the 2 mL of concentrated H_2SO_4 acid.	Formation of reddish brown colored steroidal ring.
Phenols	Ferric Chloride Test	To 2 mL of Plant extract, 3-4 drops of ferric chloride solution were added.	Formation of dark green color.
Saponins	Foam Test	1-2 mL Plant extract, was mixed with 5 mL distilled water in a test tube and shaken vigoursly.	The formation of stable foam.
Tannins	Braemer's Test	10% alcoholic ferric chloride was added to 2-3 mL of ethanolic plant extract.	Formation of dark blue or greenish grey color of the solution.
Terpenoids		1 mL of Plant extract was dissolved in 2 mL of methanol and then evaporated to dryness followed by the addition of 3 mL of Conc. H_2SO_4.	Formation of reddish-brown color.
Quinones		1 ml of extract was taken in test tube and added 2 ml of conc. HCl.	Formation of yellow or green color.
Steroids		Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture	A red color produced in the lower chloroform layer indicated the presence of steroids. The development of a greenish coloration indicated the presence of steroids

Alkaloids are reported to be biologically and therapeutically active (morphine, atropine and quinine) and have numerous medical applications in the present study [19]. alkaloids were present in both *O. tenuiflorum* and *C. roseus.* Terpenoids are reported to be useful in prevention and therapy of several diseases including cancer. Terpenoids are also known to possess antimicrobial, antifungal, anti-parasitic, anti-viral, anti-allergenic, anti-spasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties [20]. In this study, terpenoids are present in the leaf of *O. tenuiflorum*, and leaf of *C. roseus*. Flavonoids are reported to possess antioxidant, free radical scavenger, anti-leukemic, vasodilator and antibacterial properties and are reported to be useful for improving blood circulation in brain of Alzheimeric patients [21]. In the present study, flavonoids were present in both leaves of *O. tenuiflorum* and *C. roseus.* Flavonoids have anti-inflammatory, analgesic, antipyretic and antimicrobial effects. Flavonoids increased step down latency and acetyl cholinesterase inhibition and hence can be used in the treatment of cognitive disorders. Flavonoids have memory enhancing, hepatoprotective, antifertility, antiulcer, antidiabetic, antiarthritic, anticataract, antithyroid, anti-helminthic, anticataract, anti-amnesic and nootropic activity. Since *O. tenuiflorum* has been widely employed in traditional medicines, its phytoconstituents can be used in variety of disorders afflicting mankind [22]. Tannins are used in medicine as mild antiseptics in treatment of diarrhoea and to check small haemorrhages. In the present study, tannins were present only in the leaves of *O. tenuiflorum* and *C. roseus.* Phenols are structural and allelopathic components which are associated with diverse functions including activation of enzymes, nutrient uptake, protein synthesis and photosynthesis. In the present study, polyphenols were present in both *O. tenuiflorum* and *C. roseus.* Saponins have a

wide range of medicinal. Saponins have a wide range of medicinal properties including hypo-cholesterolemic, anticarcinogenic, anti-inflammatory, anti-microbial and antioxidant. In the present study, saponins were present in the leaves of *O. tenuiflorum* and in the leaf of *C. roseus.*

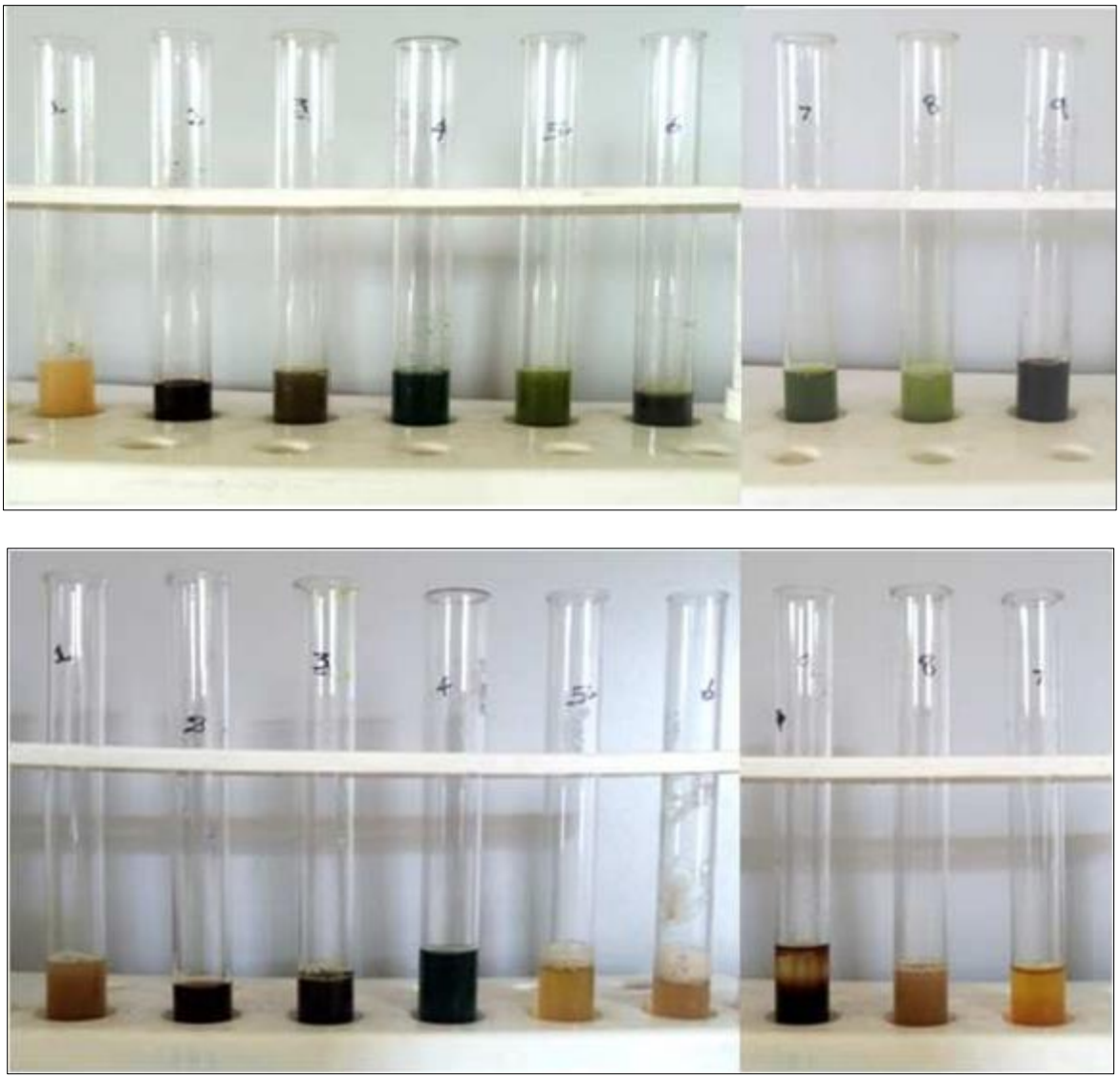


Figure 6 Different phytochemical tests peformed

The Glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Cardiac glycosides and catecchloamine are agents of choice in treatment of congestive cardiac failure [23]. In the present study, Glycosides were present in the leaf of *O. tenuiflorum* and in the leaves of *C. roseus.* Quinones are compounds very much used in pharmacopoeia in the treatment of malaria and more recently of tumours [24]. They are having good source of anti- inflammatory, antibacterial and immunomodulating potentials. In the present study, quinones were present only in the leaf of *C. roseus*.

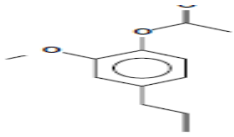
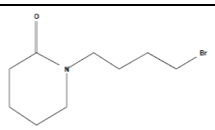
The presence of alkaloids, terpenoids, flavonoids, Phenols, and Glycosides were reported in *O. tenuiflorum* [25]. The presence of alkaloids, flavonoids, phenols, saponins, glycosides, tannins, quinones, anthraquinones, catechin, steroids, sugar, amino acid, xanthoprotein and fixed oils are reported in *C. roseus* [26]. Since, *O. tenuiflorum* is edible, its parts can directly be used for various ailments. But, all parts of *C. roseus* is poisonous, its primary phytochemicals can be isolated, separated and purified, after proper animal and human tests, they can be taken. Moreover, vincristin and vinblastin are used on human as anti-cancerous drug [27]. However, in traditional medicine, the periwinkle has been used for relieving muscle pain, depression of the central nervous system, also used for applying to wasp stings and to heal wounds. Its application ranges widely from the prevention of diabetes to treatment of stomach ache [28] as it has rich nutrients [29].

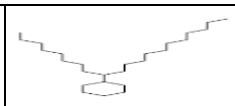
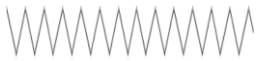
3.3. Gas chromatography-mass spectrum (GC-MS) analysis phytoconstituents

GC-MS analyses of ethanol extract of leaf powder of *O. tenuiflorum* showed presence of nine different secondary active principal compounds: Phenol, 2-methoxy-4-(2-propenyl)-, acetate; N-Hexadecanoic acid; 2-Piperidinone, n-[4-bromo-n-butyl]-; 2(3h)-Furanone, 3-(15-hexadecynylidene) dihydro-4-hydroxy-5-methyl-, Eicosane, 9-cyclohexyltlp; Dotriacontane; Sulfurous acid, pentadecyl 2-propyl ester; Sulfurous acid, 2-propyl tridecyl ester; and Heptacosane, 1-chloro (Table 3; Fig. 7) Of which, four compounds have biological properties: Phenol, 2-methoxy-4-(2-propenyl)-, acetate; N-Hexadecanoic acid; 2-Piperidinone, n-[4-bromo-n-butyl]-; and Dotriacontane.

In the present investigation for the four bioactive compounds secondary phytochemicals metabolic activities such as Phenol, 2-methoxy-4-(2-propenyl)-, acetate ($C_{12}H_{14}O_3$): is one of the synonyms of eugenol acetate. Eugenol acetate has been reported to exhibit significant antifungal, antibacterial, antiviral, and antioxidant properties [30]. N-Hexadecanoic acid ($C_{16}H_{32}O_2$): Hexadecanoic acid possesses some biological activity such as antioxidants, hypocholesterolemic, nematicide, and pesticide [31]. n-Hexadecanoic acid as the common compound in the leaves of *O. tenuiflorum* hexadecanoic acid, ethyl ester act as antifungal, antitumour, anti-bacterial. Hexadecanoic acid, found in the leaves of *O. tenuiflorum* plant extract act as hemolytic, pesticide, flavour, antioxidant show anti-inflammatory activities. 2-Piperidinone, n-[4-bromo-n-butyl]- ($C_9H_{16}BrNO$): 2-Piperidinone, N- [4- bromo-n-butyl]- is a bio-active component identified in the *O. tenuiflorum* plant extract by GC–MS analysis possess antimicrobial activity [32] it is an Alkaloid obtained from GC-MS analysis of ethanol extract of the *O. tenuiflorum*. Dotriacontane ($C_{32}H_{66}$): Dotriacontane and Pentatriacontane have antimicrobial, antioxidant, antispasmodic, antibacterial, and antiviral [33] GC-MS analysis of ethanol extract of the *O. tenuiflorum*.

Table 3 GC-MS profiles of secondary phytochemical metabolites in *O. tenuiflorum* leaf extract

Ethanol Solvents										
Ocimum tenuiflorum										
S. No.	**Peak RT**	**Name of the compounds**	**P**	**MW**	**MF**	**Chemical structure**	**Area%**	**SI**	**RSI**	**Biological properties**
1	16.75	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	27.95	206	$C_{12}H_{14}O_3$		33.15	630	855	Flavoring agent used in the manufacture of vanillin, Anti-Infective Agents, Anti-oxidant
2	21.12	N-Hexadecanoic acid	36.89	256	$C_{16}H_{32}O_2$		3.84	689	876	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor potent mosquito larvicide
3	22.46	2-Piperidinone, n-[4-bromo-n-butyl]-	39.25	233	$C_9H_{16}BrNO$		1.85	694	888	Antimicrobial Activity
4	23.93	2(3h)-Furanone, 3-(15-hex adecynylidene) dihydro-4-hydroxy-5-methyl-,	42.21	334	$C_{21}H_{34}O_3$		1.75	479	868	--

5	25.10	Eicosane, 9-cyclohexyltlp	44.56	364	$C_{26}H_{52}$		3.75	638	822	--
6	26.18	Dotriacontane	46.73	450	$C_{32}H_{66}$		5.91	740	939	Antimicrobial, antioxidant, Antispasmodic
7	27.25	Sulfurous acid, pentadecyl 2-propyl ester	48.84	334	$C_{18}H_{38}O_3S$		15.89	884	939	--
8	28.69	Sulfurous acid, 2-propyl tridecyl ester	50.28	320	$C_{17}H_{36}O_3S$		2.65	766	907	--
9	29.41	Heptacosane, 1-chloro	51.76	414	$C_{27}H_{55}Cl$		8.16	818	940	--

RT, Retention time; P, Probability; MF, Molecular formula; MW, Molecular weight; CS, Chemical structure; SI, Similar index; RSI, Reverse similar index

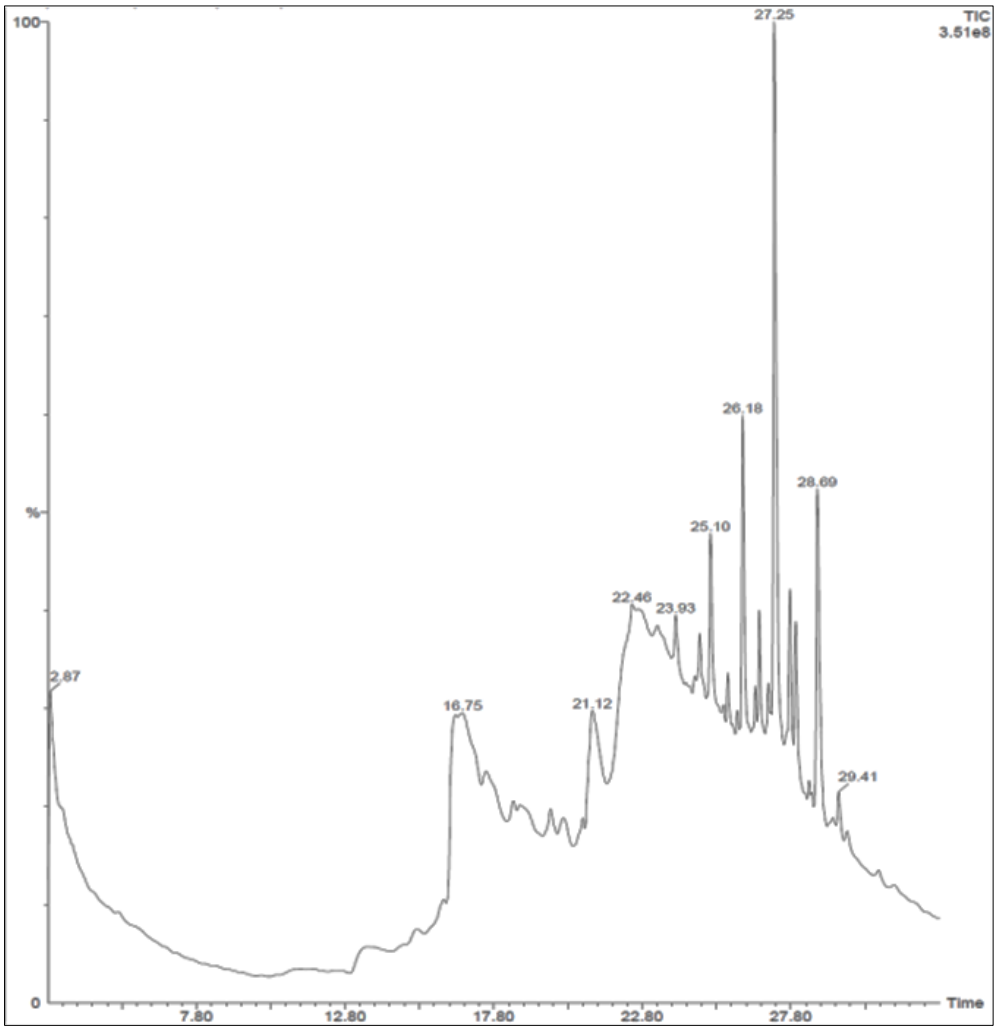
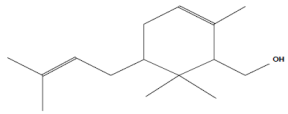


Figure 7 GC-MS chromatogram of *O. tenuiflorum* leaf extract

Table 4 GC-MS profiles of secondary phytochemical metabolites in *C. roseus* leaf extract

Ethanol Solvents										
Catharanthus roseus										
Sl. No	**Peak RT**	**Name of the compounds**	**P**	**MW**	**MF**	**Chemical Structure**	**Area%**	**SI**	**RSI**	**Biological properties by literature only**
1	21.84	3-Methylmannoside	36.60	194	C7H14O6		23.04	638	755	Innate immunity activities
2	24.39	Tetradecanoic acid, 5,9,13-trimethyl-, methyl ester	39.14	284	C18H36O2		22.17	471	770	--
3	25.09	Squalene	43.03	410	C30H50		2.11	834	931	Antioxidant, Chemopreventive, Antitumor and Hypocholesterolemic activities
4	25.46	Pentatriacontane	46.39	492	C35H72		8.10	721	867	Antioxidant activity, Antiinflammatory activities Antibacterial, Antiviral
5	26.63	Hexatriacontane	48.56	506	C36H74		11.06	863	949	--
6	27.17	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	52.21	222	C15H26O		1.96	325	835	Antimicrobial, Anti-inflammatory activities
7	28.49	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta,4.alpha,5.alpha.)-	53.27	468	C32H52O2		3.48	684	853	--

RT, Retention time; P, Probability; MF, Molecular formula; MW, Molecular weight; CS, Chemical structure; SI, Similar index; RSI, Reverse similar index

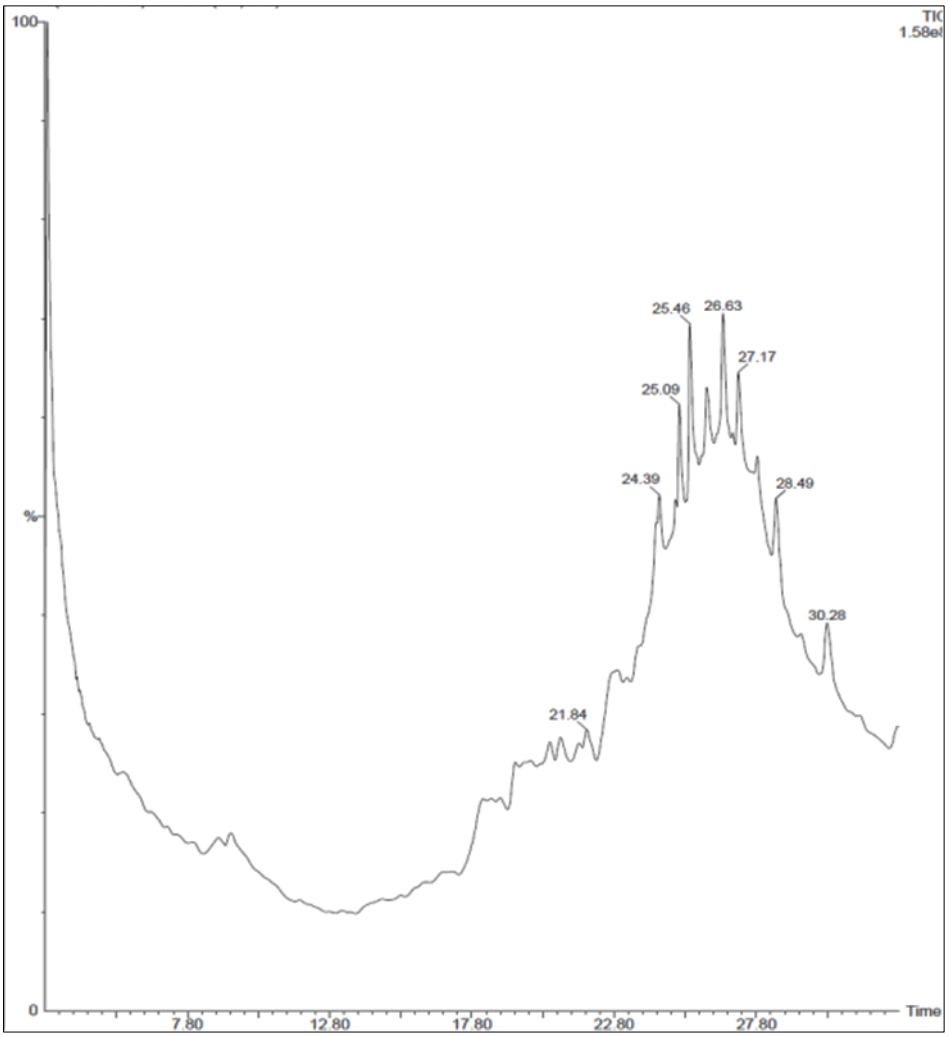


Figure 8 GC-MS chromatogram of *C. roseus* leaf extract

The ethanolic extract of leaf powder of *C. roseus* revealed presence of seven different secondary metabolites: 3-Methylmannoside; Tetradecanoic acid, 5,9,13-trimethyl-, methyl ester; Squalene; Pentatriacontane; Hexatriacontane; 2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene; 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta,4.alpha,5.alpha.)- (Table 4; Fig. 8) of these, four compounds having bioactive properties. They are, 3-Methylmannoside; Squalene; Pentatriacontane; and 2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexeneIn the present investigation for the four bioactive compounds secondary phytochemicals metabolic activities such as 3-methylmannoside ($C_7H_{14}O_6$): The *C. roseus* extract included primarily 3-methyl mannoside, according to the results of the GC-MS analysis. 3-methyl mannoside, formerly referred to as aglycone, is a glycoside of a plant-based flavonoid with a variety of biological properties, such as anti-inflammatory, anti-diabetic, anti-bacterial, antifungal, antiviral, and antioxidant properties [34]. Natural flavonoids usually appear in the form of O- or C-glycosides. O-glycoside, which is methylated at the first position, was the glycoside identified in the extract [35] Many research investigations demonstrate the effectiveness of 3-methyl mannoside in preventing bacterial growth via the lectin conjugate binding pathway. Additionally, it is utilized in prescribing, specifically focusing on antigen-presenting cells via mannose receptors [36]. Squalene ($C_{30}H_{50}$): Squalene biological activities linked to its emollient, skin-hydrating, antioxidant, and anticancer qualities were incorporated in the *C. roseus* extract. Its usage in cosmetic dermatology is widely recognized [37]. Pentatriacontane ($C_{35}H_{72}$): Pentatriacontane is an extremely hydrophobic, fully neutral, and insoluble molecule in water. Pentatriacontane is a *C. roseus* substance that is present. It is a naturally occurring substance that can be found in *C. roseus*, various plant essential oils, and parsley [38]. 2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene ($C_{15}H_{26}O$): ($C_{15}H_{26}O$) Sesquiterpene is a naturally occurring 15-carbon terpene that is formed in plant cells through pyrophosphate dephosphorylation. Its anti-inflammatory and antioxidant properties demonstrate the compound's significant therapeutic potential. Numerous pharmacological properties, including antibacterial, anticancer, and antioxidant effects, as well as functions in several organs and systems have been reported for this compound [39]. Based on GCMS spectra investigation, the medicinal plants demonstrate a foundation

of several secondary metabolites [40]. Used a methanolic *C. roseus* extract to determine most of the compounds were present. The extracts phytochemical investigation indicated the presence of alkaloids, phenols, flavonoids, terpenoids, and glycosides of these, all of the plant extract contains phenols, flavonoids, and alkaloids reports of similar observations have also reported [41].

4. Conclusion

The present study suggests that *O. tenuiflorum* and *C. roseus* have significant amounts of secondary phytochemicals and their metabolite components. Among these two herbs, *C. roseus* has more number of phytochemicals. Further, it is suggested that since these two herbs have many bioactive principle compounds, they can be taken to pharmaceutical industry for various preparations.

Compliance with ethical standards

Acknowledgments

The authors gratefully acknowledge Dr. C. Murugan, Scientist 'E', Southern Regional Centre, Botanical Survey of India (BSI), Coimbatore, India, for authentication of the Medicinal Plants. Sophisticated Instrumentation Facility (SIF), School of Advanced Sciences, Chemistry Division, VIT University, Vellore, Tamil Nadu, India, is acknowledged for providing GC-MS outsourcing service.

Disclosure of conflict of interest

The authors declare no conflict of interests.

References

[1] Yadav SR, Sardesai MM. Flora of Kolhapur district. Rajhuns Printing Press. 2002, 382.

[2] Pistrick K, Kruse J, Adler K. Obdiplostemonie in der *Gattung Allium L.* Flora. 2001, 196 (3):204-14.

[3] Anonymous Wealth of India, Vol 7. Publication and Information Directorate, CSIR, New Delhi. 1991, 79-89.

[4] Prakash PA, Gupta N. Therapeutic uses of *Ocimum sanctum Linn* (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian journal of physiology and pharmacology. 2005, 49(2):125.

[5] Rahman S, Islam R, Kamruzzaman M, Alam K, Jamal AH. Phytochemical and Pharmacological profile of *Ocimum sanctum L.* Am J Drug Discov Dev. 2011, 1-5.

[6] Pingale SS, Firke NP, Markandetya AG. Therapeutic activities of *Ocimum tenuiflorum* accounted in last decade: a review. J Pharm Res. 2012, 5(4): 2215-20.

[7] Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum Linn*. A reservoir plant for therapeutic applications: An overview. Pharmacognosy reviews. 2010, 4(7):95.

[8] Chadha KL, editor. Advances in horticulture. 11. Medicinal and aromatic plants. Malhotra Publishing House, 1995.

[9] Kothari SK, Bhattacharya AK, Singh CP, Singh K, Syamasundar KV, Ramesh S, Kumar S. A new methyl eugenol-rich and short duration strain of *Ocimum tenuiflorum*. 2001, 22(4a):385-8.

[10] Machado MI, de Vasconcelos Silva MG, Matos FJ, Craveiro AA, Alencar JW. Volatile constituents from leaves and inflorescence oil of *Ocimum tenuiflorum L.* f. (*syn. O. sanctum L.*) grown in Northeastern Brazil. Journal of Essential Oil Research. 1999, 11(3):324-6.

[11] Mardani-Nejad S, Khavari-Nejad RA, Saadatmand S, Najafi F, Azar PA. Potent antioxidant properties of rolB-transformed *Catharanthus roseus* (L.) G. Don. Iranian Journal of Pharmaceutical Research: IJPR. 2016, 15(2):537.

[12] Rischer H, Orešič M, Seppänen-Laakso T, Katajamaa M, Lammertyn F, Ardiles-Diaz W, Van Montagu MC, Inzé D, Oksman-Caldentey KM, Goossens A. Gene-to-metabolite networks for terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* cells. Proceedings of the National Academy of Sciences. 2006, 103(14):5614-9.

[13] Aslam J, Khan SH, Siddiqui ZH, Fatima Z, Maqsood M, Bhat MA, Nasim SA, Ilah A, Ahmad IZ, Khan SA, Mujib A. *Catharanthus roseus* (L.) G. Don. An important drug: it's applications and production. Pharmacie Globale (IJCP). 2010, 4(12):1-6.

[14] Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Riceevans C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Archives of biochemistry and biophysics. 1995, 322(2):339-46.

[15] Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. Journal of ethnopharmacology. 2004, 92(2-3):291-5.

[16] Revathi P, Parimelazhagan T, Manian S. Quantification of phenolic compounds, in vitro antioxidant analysis and screening of chemical compounds using GC-MS in *Acalypha alnifolia* Klein ex willd.: A leafy vegetable. Int J Pharma Biosci. 2013a. 2013, 4:973-86.

[17] Trease GE, Evans WC. Pharmacology, 11th ed., Bailliere Tindall Ltd., London. 1978, 60-75.

[18] Obaidullah AJ, Alanazi MM, Alsaif NA, Mahdi WA, Fantoukh OI, Tareq AM, Sami SA, Alqahtani AM, Emran TB. Deeper insights on *cnesmone javanica blume* leaves extract: chemical profiles, biological attributes, network pharmacology and molecular docking. Plants. 2021, 10(4):728.

[19] Shulka YM, Dhruve JJ, Patel NJ, Bhatnagar R, Talati JG, Kathiria KB, Plant secondary metabolites. New India Publishing Agency, New Delhi, India. 2009, 1-306.

[20] Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. Breast cancer research and treatment. 2009, 115: 223-39.

[21] Sharma DK. Pharmacological properties of flavonoids including flavonolignans–Integration of petrocrops with drug development from plants.2006.

[22] Ekta S, Sheel S, Jaya D, Swapnil S. Diversified potentials of *Ocimum sanctum Linn* (tulsi): an exhaustive survey. Journal of Natural Product and Plant Resources. 2012, 2(1):39-48.

[23] Tripathi KD. Cardiac glycosides and drugs for CCF, Essentials of medical pharmacology.2004.

[24] Bagnis CI, Deray G, Baumelou A, Le Quintrec M, Vanherweghem JL. Herbs and the kidney. American journal of kidney diseases. 2004, 44(1):1-1.

[25] Paul JJ, Devi SS. Asian Journal of Biochemical and Pharmaceutical Research.2013.

[26] Kabesh K, Senthilkumar P, Ragunathan R, Kumar RR. Phytochemical analysis of *Catharanthus roseus* plant extract and its antimicrobial activity. Int. J. Pure App. Biosci. 2015, 3(2):162-72.

[27] Das A, Sarkar S, Bhattacharyya S, Gantait S. Biotechnological advancements in *Catharanthus roseus* (L.) G. Don. Applied microbiology and biotechnology. 2020, 104:4811-35.

[28] Gajalakshmi S, Vijayalakshmi S, Devi RV. Pharmacological activities of *Catharanthus roseus*: a perspective review. International Journal of Pharma and Bio Sciences. 2013, 4(2):431-9.

[29] Radali D, Alka G. Periwinkle (*Catharanthus roseus*) Leaves and Lemongrass (*Cympoogon citratus*): An Analysis of Their Nutritional Composition, Anti-Nutritional Factors and Antioxidant Content. Int. J. Curr. Microbiol. App. Sci. 2018, 7(6):2130-5.

[30] Musthafa KS, Voravuthikunchai SP. Anti-virulence potential of eugenyl acetate against pathogenic bacteria of medical importance. Antonie Van Leeuwenhoek. 2015, 107:703-10.

[31] Beulah GG, Soris PT, Mohan VR. GC-MS determination of bioactive compounds of *Dendrophthoe falcata* (LF) Ettingsh: An epiphytic plant. Int. J. Health Sci. Res. 2018, 8:261-9.

[32] Ganesh M, Mohankumar M. Extraction and identification of bioactive components in *Sida cordata* (Burm. f.) using gas chromatography–mass spectrometry. Journal of food science and technology. 2017, 54:3082-91.

[33] Devi MS, Muthiah K, Rajeswaran PS, Srinivasan VM. GC-MS, FT-IR analysis and antibacterial study of bioactive compounds of chundaivattralchooranam-A siddha poly herbal formulation. Int J PharmTech Res. 2015, 8(10):204-9.

[34] Rajendran V, Krishnegowda A, Nachiappan V. Antihyperlipidemic activity of *Cassia auriculata* flower extract in oleic acid induced hyperlipidemia in Saccharomyces cerevisiae. Journal of food science and technology. 2017, 54:2965-72.

[35] Peng H, Liu Y, Ramakrishna S. Recent development of centrifugal electrospinning. Journal of Applied Polymer Science. 2017, 10, 134(10).

[36] Socrates SH, Mohan SC. Phytochemical analysis of flower extracts of different *Cassia* species by using gas chromatography-mass spectrometry. Int J Biol Chem. 2019, 13:1-1.

[37] Huang ZR, Lin YK, Fang JY. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 2009, 14(1):540-54.

[38] Nithya TG, Jayanthi J, Raghunathan MG. Phytochemical, antibacterial and GC MS analysis of a floating fern Salvinia molesta DS Mitchell (1972). Int. J. PharmTech Res. 2015, 8:85-90.

[39] Khan R, Sultana S. Farnesol attenuates 1, 2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats. Chemico-Biological Interactions. 2011, 192(3):193-200.

[40] Adekunle OA, Adefalu LL, Oladipo FO, Adisa RS, Fatoye AD. Constraints to youths' involvement in agricultural production in Kwara State, Nigeria. Journal of agricultural extension. 2009, 13(1).

[41] Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. Journal of food science and technology. 2015, 52:1212-7.