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SHORT COMMUNICATION

Comprehensive GC-MS Investigation of Phytoconstituents and Evaluation of Antioxidant Activities of *Salvia leucantha* Cav. Leaf Extract

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Abstract

Background: *Salvia* is the largest genera in the Lamiaceae family, and is renowned for its therapeutic properties. The genus has numerous phytocomponents that have significant medicinal use. **Aim:** The current investigation is the initial effort to uncover the antioxidant efficacy and phytochemicals present through Gas Chromatography-Mass spectrometry of *Salvia leucantha* leaves. **Methods:** The fresh leaves of *S. leucantha* were collected from the Western Ghats of Nilgiris, Tamil Nadu. The collected leaves were desiccated under shade, made into fine powder and extracted with methanol using a Soxhlet apparatus. Gas chromatography (CH-GCMSMS-02) and mass spectrometry were used to determine phytoconstituents in methanolic leaf extract. Assays like DPPH radical scavenging, nitric oxide scavenging, hydroxyl radical scavenging, reducing power, and total antioxidant activity were used to establish the *in vitro* antioxidant impact. **Results**: Among the 415 bioactive compounds identified by GC-MS, this study zeroed in on 30 compounds—among them, 9,12,15-octadecatrienoic acid, methyl ester, hexadecanoic acid, methyl ester, 3'-fluoro-a-pyrrolidinopropiophenone, methyl nicotinate, 5,7-Dimethylpyrimido-[3,4-a] a -indole Squalene, 9,12-octadecadienoic acid (Z, Z)-, campesterol, hexestrol dimethyl ether, gamma-sitosterol, and others due to their high peak values and potential medicinal use *viz*., antimicrobial, anti-inflammatory, antiviral, antioxidant, and cardioprotective effects were seen in the majority of the components. The leaves showed potent antioxidant activity in all assays, with free radical scavenging effects that increased with increasing dosage. **Conclusion:** This study suggests that *S. leucantha* has potential lead compounds like squalene, stigmasterol, and campesterol, making the plant a promising source for potential pharmaceutical applications, which was supported by strong antioxidant activities.

Major Findings: *Salvia leucantha* leaves, there are 30 major bioactive compounds, including squalene, stigmasterol, campesterol attributes to antioxidant, anticancer and antimicrobial effects. Methanolic extract of leaves displayed a clear dose-dependent antioxidant effect indicating potentials pharmaceutical development.

Keywords: Antioxidant Activities, Bioactive Compounds, GC-MS Analysis, Methanolic Leaves Extracts, *Salvia leucantha*

1. Introduction

In recent years pharmaceutical industry has focused on novel drug molecules from plant sources. Since before the civilisation, plants have been an important source of traditional medicine and used to cure chronic as well as transmissible diseases. India is home to more than 8,000 species of medicinal plants, making it the world's biggest botanical garden[1]. These plants have a long history of usage in the traditional Indian system of medicine, such as *Ayurveda*, *Sidda*, and *Unani*.

Major contributors to stress-related cellular damage and diseases are attributed to Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS); antioxidants are an excellent resource for eliminating these pollutants. The intermediate dosage of ROS and RNS is often associated with signal transduction and cellular defence. The excess accumulation of reactive

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species results in the damage of biomolecules, which leads to several degenerative disorders[2].

The antioxidant components in medicinal plants determine their therapeutic qualities. Several medicinal plants are known to contain substantial amounts of powerful antioxidants, particularly polyphenols. In addition to quenching singlet and triplet oxygen molecules, these chemicals bind and neutralise free radicals and decompose toxic peroxides. Their presence significantly enhances the plant's ability to protect against oxidative stress, making them highly valuable in both medicinal and therapeutic applications[3].

The pharmaceutical properties of the plants confide in the phytochemicals (secondary metabolites) present in the crude extracts. These phytochemicals eradicate various disorders by their individual, active and synergic effects[4]. The focus on phytochemicals increased because of their wide spectrum of applications with nil or fewer side effects compared to modern medicine. The progress of drug discovery is based on the identification of phytoconstituents. GC-MS serves as a highly effective tool for chemotaxonomic studies, particularly in the analysis of medicinal plants that contain biologically active compounds[5].

Salvia leucantha Cav. is an ornamental Herbaceous perennial shrub from Lamiaceae. Mexican bush sage is another name for this plant. Although it is now native to many parts of the globe, *S. leucantha* was originally from Central America and Mexico. It is possible to find this plant in the Indian states of Tamil Nadu, Kerala, Karnataka, and Uttarakhand. The medicinal, cosmetic, fragrance, and food sectors all make heavy use of the many flavouring compounds found in the aromatic sage, which is one of the most abundant genera in the plant kingdom (with over a thousand species).

Salvia species are highly valued for their diverse aromatic properties and bioactive compounds, making them essential ingredients in numerous products across these industries. Traditional medicine practitioners have relied on this plant species for centuries to alleviate a wide range of ailments. In particular, it has a reputation for alleviating symptoms of the common cold, bronchitis, haemorrhage, TB (tuberculosis), and irregular menstruation. Its significance in promoting health and well-being throughout the years is demonstrated by its extensive usage in traditional medicine[6]. The research investigation analyzed the methanolic leaf extract using GC-MS to identify potential chemical components and antioxidant properties.

2. Materials and Methods

2.1 Collection of Plant Material

Salvia leucantha plant leaves are collected from Doddabetta Hills, Western Ghats of Nilgiris, Tamil Nadu and the plant was authentically identified by the South Regional Centre, Botanical Survey of India, Coimbatore, with the ID No.: BSI/SRC/5/23/2023/Tech -213 (Figure1).

2.2 Study Area

The Western Ghats of Nilgiris are well-known for their rich plant diversity, containing nearly 5,000 species of vascular plants that belong to about 2,200 genera. This area is especially important because around 1,700 species, or 34 per cent, are endemic in the region. It is situated at a height of 2240 m above sea level. Club Hill, Elk Hill, Doddabetta Hill, and Snowdon Hill are the four main hills in this area. Doddabetta is the highest mountain in the Nilgiris Mountains at 2,637 meters (8,652ft). The plants were collected in the geographical range of Latitude 11.418713° and Longitude 76.72915°.

2.3 Preparation of Plant Extract

Using a Soxhlet apparatus at 60°C, the powdered plant material (25 g) was extracted with 250 mL of methanol until the substance lost its colour. The plant material was subjected to extraction for 8 h using a Soxhlet apparatus. A dried solid crude extract was prepared by condensing, evaporating, and drying the resultant extract under decreased pressure. This was then stored for future examination.

2.4 Analysis of Gas Chromatography and Mass Spectrometry (GC-MS) for Bioactive Components

To conduct the GC-MS analysis, a gas chromatograph system (Agilent CH-GCMSMS-02) and a mass spectrophotometer were meticulously used. The molecules were efficiently segregated using helium as the carrier gas, which flowed at a rate of 1.0 mL/min. A comprehensive study was possible because the entire elution procedure took 1 h and 11 min. By comparing

the size of each peak to the overall area of all peaks, we were able to determine the relative percentage of each component in the sample. By operating the system and collecting data using the source-provided MS solution program, we were able to guarantee accurate and dependable results throughout the analysis.

2.5 Identification of Compounds

Components were identified by analysing their retention indices, and their mass spectra were calculated by consulting the NIST database. An excellent tool for determining the identity of unknown compounds, this database includes more than 62,000 patterns of recognised molecules. The unknown components' spectra in the *S. leucantha* fraction were compared to known components' standard mass spectra in the NIST library (NISTII) in this investigation. The chemical profile of *S. leucantha* and its possible uses were better understood because of this comparison, which enabled more precise compound identification.

2.6. *In Vitro* Free Radical Scavenging Activity

2.6.1 DPPH Radical Scavenging Assay

Researchers in the area of natural products often use the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assessment to find out how well a natural medicine can neutralise free radicals. Antioxidant chemicals' capacity to efficiently scavenge stable free radicals, a recognised contributor to oxidative stress and a host of diseases, is the focus of this particular experiment.

This research used the DPPH radical as an *in vitro* assay to determine the extracts' free radical scavenging abilities. The approach was grounded on the specified procedure with some small tweaks to make it more accurate and easier to replicate[7]. A combination was made by mixing 0.8 mM DPPH solution with 1.0 mL of a *S. leucantha* methanolic extract, with doses varying from 200 to 1000 µg/mL. Next, the extract and DPPH radical were thoroughly combined by vigorously mixing the combination. Then, to avoid light-induced degradation of the DPPH radical, it was left to stand at room temperature for 30 min in dark conditions.

The solution's Optical Density (OD) was measured at 517 nm after the incubation time, with ascorbic acid acting as the standard antioxidant and a blank control. This measured tolerates one to determine the extent to which the extracts' antioxidant chemicals have scavenged the DPPH radicals. We used the following equation to get the DPPH radical inhibitory percentage.

$$\% \text{ inhibition} = [(\text{Control- Test})/\text{Control}] \times 100$$

2.6.2 Nitric Oxide Radical Scavenging Assay

In Griess reaction, sodium nitroprusside combines with oxygen and produces nitric oxide, meanwhile reducing the nitrite ions. This reaction is important for understanding how nitric oxide works in the body. In this experiment, we followed the steps to ensure our results were accurate[8].

In order to make the reaction mixture, 3.0 mL of phosphate-buffered saline (pH 7.4) with 10 mM sodium nitroprusside was mixed. Afterwards, *S. leucantha* extract in methanol was added at several quantities ranging from 200 to 1000 µg/mL. We chose this range so that we could see how varying the extract concentration affected the production of nitrite ions. Put the mixture in an incubator set at 25°C for half an hour after mixing. This period was necessary for the reaction to occur, which facilitated the conversion of sodium nitroprusside to nitric oxide and its subsequent interaction with nitrite ions.

Then, 5 mL of Griess reagent was added to the combination. Phosphoric acid (H_3PO_4) makes up 2% of the Griess reagent, which also includes 1% sulphanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). An essential step in creating the coloured chemical, this reagent contrasted the absorbance of the finished product at 546 nm with that of a blank sample. We may learn about the efficacy of the *S. leucantha* extract in inhibiting nitrite ion production from the absorbance values. Then, we used the following equation to determine the percentage of nitrite ions created.

$$\% \text{ inhibition} = [(\text{Control- Test})/\text{Control}] \times 100$$

2.6.3 Hydroxyl Radical Scavenging Assay

The approach was used to assess the extracts' ability to scavenge hydroxyl radicals[9]. It is crucial to determine the efficacy of natural extracts in neutralising hydroxyl radicals since these molecules are extremely reactive and can harm cells.

We mixed 1.0 mL of *S. leucantha* methanolic extract at doses ranging from 200 to 1000 µg/mL to create the reaction mixture. This will allow us to gauge the extract's efficacy in combating radicals. To the mixture, add 1.0 mL of an iron-EDTA solution (containing 0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1.0 mL of dimethyl sulfoxide (0.85% in 0.1 M phosphate buffer, pH 7.4), and 0.5 mL of 0.22% ascorbic acid, a representative antioxidant.

The tubes were sealed firmly after mixing to avoid contamination. They were then heated in a water bath at 80-90°C for 15 min. One way to make hydroxyl radicals is by heating the mixture. We introduced 1.0 mL of trichloroacetic acid (17.5%), which was ice-cold, to halt the process. The next step was to add 3.0 mL of Nash reagent to the solution. A one-litre solution of the Nash reagent was prepared by combining 3.0 mL of glacial acetic acid, 2.0 mL of acetylacetone, and 75.0 g of ammonium acetate with distilled water. This chemical is used for making colours.

To let the colour develop, let the liquid remain at room temperature for 15 min after adding the Nash reagent. To ensure the accuracy of our results, we assessed the resulting yellow colour at 412 nm with a blank sample. To evaluate *S. leucantha* extract's efficacy, ascorbic acid was utilised as a reference. Lastly, use the following equation to get the proportion of hydroxyl radicals scavenged.

"% inhibition = [(Control- Test)/Control] × 100."

2.6.4 Determination of Total Antioxidant Activity

Phosphomolybdenum analysis was used to determine the total antioxidant activity of the extracts[10]. The capacity of various compounds to decrease molybdenum ions, resulting in a coloured product that can be detected, is a typical metric for evaluating their antioxidant properties.

A reaction mixture was prepared by mixing 1.0 mL of *S. leucantha* methanolic extract at various concentrations, ranging from 200 to 1000 µg/mL. We can learn more about the effect of the extract's antioxidant activity at various concentrations from this spectrum. A standard reagent solution containing 0.6 M sulphuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate was combined with 1.0 mL of extracts of varying concentrations. For the reaction to proceed as planned, these components are required.

To prevent contamination, firmly shut the tubes after mixing. After that, the tubes were kept in a thermal incubator set at 95°C for half an hour. During this heating interval, the extract's antioxidants can combine with molybdenum ions to produce a molecule of stable colour. Upon completion of incubation, the tubes were allowed to come down to room temperature. Using a spectrophotometer and a reagent blank as a control, they measured the absorbance of the solution at 695 nm after it had cooled. The level of antioxidant activity may be determined by this measurement.

Per gramme of extract, the total antioxidant activity was measured in mg of Ascorbic Acid Equivalent (AAE). We may compare the antioxidant capacity of the *S. leucantha* extract to that of ascorbic acid, a well-known antioxidant, by utilising it as a reference. The following equation was used to compute the inhibitory percentage for total antioxidant activity.

"% inhibition = [(Control- Test)/Control] × 100."

2.6.5 Reducing Power Assay

This method is useful for measuring how well different substances can act as antioxidants by checking their ability to reduce iron ions, which shows how effective they are at neutralising harmful free radicals[11].

To prepare the reaction mixture, 1.0 mL of the extracts with varying concentrations, ranging from 200 to 1000 µg/mL, is combined. This range allows us to observe the effect of different extract concentrations on their reducing power. Next, 2.5 mL of oxidising agent potassium ferricyanide (1% concentration) and 2.5 mL of pH buffer sodium phosphate (0.2 M) were added to maintain a constant pH level throughout the process.

The mixture was then incubated at 50°C for 30 min after mixing. For the reaction to take place and the extracts to demonstrate their reducing potential, this window of opportunity is crucial. When the incubation period was up, 2.5 mL of 10% Trichloroacetic Acid (TCA) was added to halt the process. This aids in the removal of proteins and halts the process, leading to more discernible outcomes. Then, to separate the components, the mixture was centrifuged at 3000 rpm for 10 min. The top layer, which includes the antioxidant chemicals that are soluble, may be collected using this method. The next step was to combine 2.5 mL of the top layer with 2.5 mL of deionised water and 0.5 mL of

0.1% ferric chloride. In order to measure absorbance, we need a coloured complex, which ferric chloride produces.

Lastly, we compared the absorbance of the combination to a blank sample at 700 nm. A higher absorbance indicates that the extracts have a stronger ability to decrease iron ions and free radicals, suggesting that they are powerful antioxidants.

3. Results

3.1 Gas Chromatography-Mass Spectroscopy Profiling of Methanol Leaves Extract of *Salvia leucantha*

The GC-MS analysis of *Salvia leucantha* leaves extracted with methanol revealed 415 bioactive chemicals, each with its own unique phytochemical activity. The chemical constituents present in the leaves and their potential medicinal and health benefits are highlighted in this study. The chromatogram provides a visual depiction of the chemicals that were found and their retention durations. The compounds found, 30 were chosen for further analysis and are included in Table 1. Important details about each molecule are listed in this table, including their Retention Time (RT), name, molecular formula, area, and component structure.

Thymol is the only phenolic compound identified among the bioactive constituents, while the terpenoids include campesterol, squalene, stigmasterol, oleic acid, tetradecanoic acid, hexadecanoic acid methyl ester, n-hexadecenoic acid, 9,12-octadecadienoic acid (Z,Z)-methyl ester, and 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z)-Octocrylene. Additionally, amine derivatives such as Encainide, 1,3,5-Triazine-2,4(1H,3H)-dione, 6-(ethylamino)- (N-Ethylammelide), 6-Isoquinolinamine, Propylthiouracil, Hexestrol dimethyl ether, and Thiazole derivatives are present. The category of carboxylic acids and esters comprises 2-Furancarboxaldehyde, 5-methyl-, Thiophene, 2,2'-(1,1,2,2,3,3-hexafluoro-1,3-propanediyl) bis-, Dimethyl fumarate, Methyl nicotinate, Sarin, 3(2H)-Thiophenone, dihydro-2-methyl-, and Thiophene-2-carboxylic acid, 3-pentyl ester. No prior research has reported the phytochemical composition of *S. leucantha*.



Figure 1. *Salvia leucantha*.

4. Discussion

4.1 GC-MS and their Bioactive Compound Activities

The bioactive components identified from the methanolic leaf extract of *Salvia leucantha* were derived from various plants and synthetic sources exhibit significant pharmacological properties. For instance, 3'-fluoro-α-pyrrolidinopropiophenone is a crucial component of neurological and psychiatric medications, playing a role in neurotransmission and mental health treatments[12]. Cyclopentatone, a bioactive compound, has demonstrated antibacterial efficacy, making it a potential candidate for treating bacterial infections[13]. Encainide is a well-established antiarrhythmic drug used in managing irregular heartbeats[14].

A naturally occurring molecule in plants like *Cassia tora*, 2-furancarboxaldehyde, 5-methyl-has a diverse pharmacological activity which includes antibacterial, antioxidant, antiviral, anti-inflammatory, and anticancer effects[15]. The chemical thiophene, 2,2'-(1,1,2,2,3,3-hexafluoro-1,3-propanediyl) bis- has a number of useful medical properties, including antibacterial[16], analgesic, anti-inflammatory[17], antihypertensive[18], antiallergic[19], and anticancer effects[20].

Dimethyl fumarate, found in *Fumaria officinalis*, is an effective immunomodulator used in treating autoimmune diseases such as multiple sclerosis[21]. Methyl nicotinate, a derivative of *Nicotiana tabacum* (tobacco plant), is known for its ability to treat respiratory

Table 1. Phytochemical components identified in the methanol leaves extract of *Salvia leucantha* by GC-MS

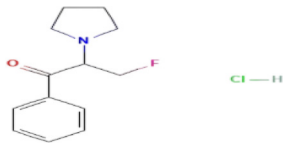
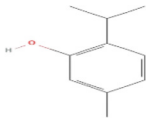
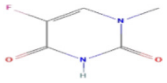
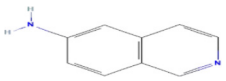
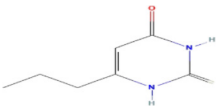
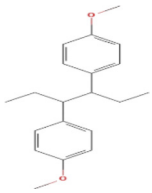
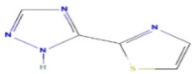
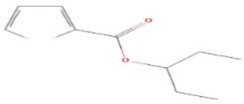
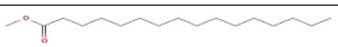
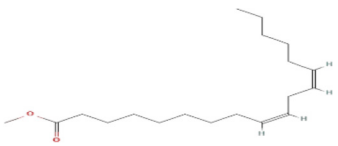
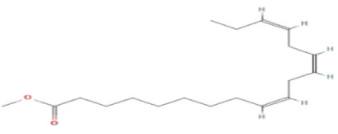
S. No.	RT (mins)	Name of the compound	Molecular formula	Component area	Structure of compounds
1	5.8067	3'-fluoro-a-Pyrrolidinopropiophenone	$C_{13}H_{16}FNO$	2511372.9	
2	6.1472	Cyclopentatone	C_5H_8O	1759826.2	
3	6.2266	Encainade	$C_{22}H_{28}N_2O_2$	12256.4	
4	6.5881	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	2030936.7	
5	6.9645	Thiophene,2,2'-(1,1,2,2,3,3-hexafluoro-1,3-propanediyl) bis-	$C_{11}H_6F_6S_2$	113407.6	
6	8.0613	Dimethyl fumarate	$C_6H_8O_4$	18832098.8	
7	11.3947	Methyl nicotinate	$C_7H_7NO_2$	2337390.8	
8	11.4275	Sarin	$C_4H_{10}FO_2P$	327361.5	
9	15.9306	1,3,5-Triazine-2,4(1H,3H)-dione, 6-(ethylamino)- or N-Ethylammelide	$C_5H_8N_4O_2$	2268751.7	
10	16.1739	Thymol	$C_{10}H_{14}O$	829976.2	
11	16.3751	3-Methyl-5-fluorouracil	$C_5H_5FN_2O_2$	502230.5	
12	16.8031	6-Isoquinolinamine	$C_9H_8N_2$	1765326.4	
13	19.8332	Propylthiouracil	$C_7H_{10}N_2OS$	10120548.5	
14	21.8936	Hexestrol dimethyl ether	$C_{20}H_{26}O_2$	578833.4	
15	22.6395	Thiazole, 2-(3-chloro-1H-1,2,4-triazol-1-yl)-	$C_5H_3ClN_4S$	133170.5	

Table 1. Continued...

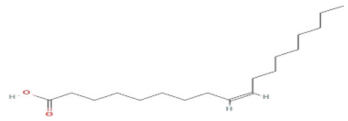
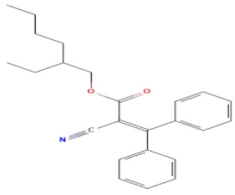
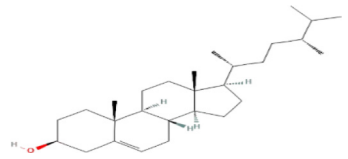
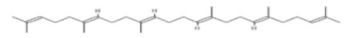
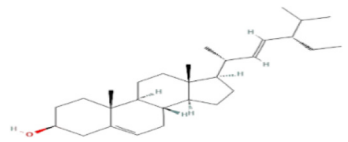
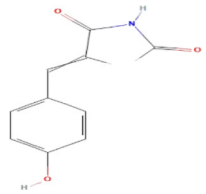
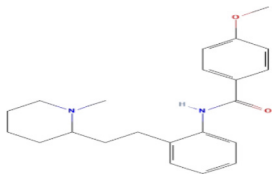
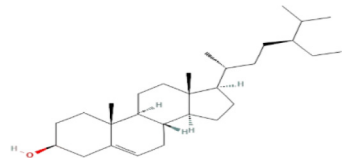
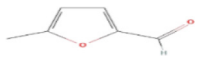
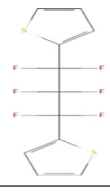
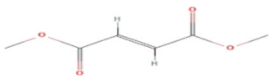
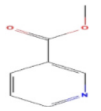
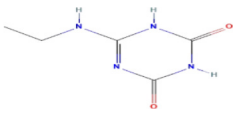
S. No.	RT (mins)	Name of the compound	Molecular formula	Component area	Structure of compounds
16	22.7434	3(2H)-Thiophenone, dihydro-2-methyl-	C_5H_8OS	73522717.9	
17	22.8124	Thiophene-2-carboxylic acid, 3-pentyl ester	$C_{10}H_{14}O_2S$	70587038.9	
18	24.2917	Tetradecanoic acid	$C_{14}H_{28}O_2$	11275731.3	
19	26.3298	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	158111360.8	
20	26.7947	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	52619320.7	
21	28.4231	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	205236230.6	
22	28.5147	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	296074869.1	
23	28.8957	Oleic Acid	$C_{18}H_{34}O_2$	10134786.8	
24	29.1325	Octadecanoic acid	$C_{18}H_{36}O_2$	13727013.0	
25	34.1512	Octocrylene	$C_{24}H_{27}NO_2$	18326844.5	
26	35.1]000	Campesterol	$C_{28}H_{48}O$	65928179.1	

Table 1. Continued...

S. No.	RT (mins)	Name of the compound	Molecular formula	Component area	Structure of compounds
27	35.7076	Squalene	$C_{30}H_{50}$	86388470.2	
28	36.0196	Stigmasterol	$C_{29}H_{48}O$	188902655.7	
29	37.5875	5-(4-Iodo-phenylamino)-thiazolidine-2,4-dione	$C_9H_7IN_2O_2S$	12327347.5	
30	37.6880	gamma-Sitosterol	$C_{29}H_{50}O$	78319419.3	

diseases, vascular disorders, and rheumatoid arthritis, while also exhibiting anti-tumour properties[22]. Sarin, though highly toxic, functions as a potent nerve agent affecting the central nervous system[23].

1,3,5-Triazine-2,4(1H,3H)-dione, 6-(ethylamino)- exhibits diverse therapeutic effects, including anticancer, antiviral, antimicrobial, anti-inflammatory, anti-amoebic, antibacterial, fungicidal, antimalarial, and antitubercular activities, making it a promising lead compound for pharmaceutical research[24]. Thymol, a major constituent of *Thymus vulgaris* (thyme), is widely recognised for its antifungal, anti-inflammatory, antiviral, antibacterial, antibiofilm, antileishmanial, and anticancer properties[25].

3-Methyl-5-fluorouracil is a chemotherapeutic agent utilised in the treatment of multiple cancers, such as breast, ovarian, colorectal, bladder, gastric, pancreatic, and liver cancer[26]. 6-Isoquinolinamine is a compound that may be found in several medicinal plants. It has a wide range of uses, including fighting against fungal infections, viruses, cancer, enzymes, spasms, and diabetes[27]. Propylthiouracil, a thyroid medication, is primarily used in the management of hyperthyroidism[28]. Hexestrol dimethyl ether demonstrates antitumor effects and is explored for its potential in cancer treatment[29].

Thiazole, 2-(3-chloro-1H-1,2,4-triazol-1-yl)- displays antibacterial[30], antiprotozoal[31], antitubercular[32], antifungal[33], and anthelmintic activities[34]. 3(2H)-Thiophenone, dihydro-2-methyl- functions as an antimicrobial[35], analgesic, anti-inflammatory[36], antihypertensive[20], and antitumor agent[37]. Thiophene-2-carboxylic acid, 3-pentyl ester, derived from plants like *Ipomoea batatas* (sweet potato), is studied for its potential in treating breast cancer[38].

Tetradecanoic acid, present in *Cocos nucifera* (coconut oil), is known for its cancer-preventive, antioxidant, nematicidal, lubricant, and hypocholesterolemic effects[39]. Hexadecanoic acid, methyl ester, found in *Azadirachta indica* (neem), healed inflammation, cancer, hepatitis, arthritic, and coronary issues[40], along with antioxidant, hypocholesterolemic, nematicidal, and pesticidal effects[41].

Green tea, or *Camellia sinensis*, contains the compound n-hexadecenoic acid, which has several beneficial effects, including reducing inflammation, improving insulin production, and lowering cholesterol and testosterone levels[42-44]. The 5-alpha-reductase inhibitor, antihistaminic, anti-coronary, insectifuge, antieczemic, and anti-acne agent. 9,12-octadecadienoic acid (Z,Z)-methyl ester is present in *Helianthus annuus* (sunflower) and has a number of other useful properties, an antiarthritic, antiandrogenic, hepatoprotective, anti-inflammatory, hypocholesterolemic, and nematicidal activities[45].

An anti-inflammatory, cancer-preventive, and heart disease-reducing compound, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z)- is prevalent in *Linum usitatissimum* (flaxseed)[46]. Anemiagenic, dermatitigenic, insectifuge, antibacterial, antiandrogenic, and cancer-preventive effects have been associated with oleic acid, which is present in olives (*Olea europaea*)[47].

Octadecanoic acid, naturally occurring in *Theobroma cacao* (cocoa butter), has anti-inflammatory, antiviral, and antioxidant properties and is effective in treating skin lesions, hypocholesterolemia, and androgenic disorders[48]. Octocrylene, a common sunscreen ingredient, is used in cosmetic formulations to protect against UV radiation[49].

Campesterol, a phytosterol found in *Brassica oleracea* (cabbage), exhibits anti-carcinogenic properties[50]. Squalene, derived from *Amaranthus caudatus* (amaranth) and *Olea europaea* (olive), functions as an antioxidant, antimicrobial, anticancer agent, and has gastroprotective, hepatoprotective, and sunscreen properties[51-53].

The compound stigmasterol, which is present in soybeans (Glycine max), is used to treat cancers of the liver[54], gallbladder[55], skin[56], stomach[57], breast[58,60], endometrial adenocarcinoma[58], prostate[59], and cervix[60]. The activity against hyperglycemia, tumours[61], arthritis[62], inflammation[63], and microbes[64] is well-known for 5-(4-Iodo-phenylamino)-thiazolidine-2,4-dione.

Gamma-sitosterol, present in *Arachis hypogaea* (peanut) and *Curcuma longa* (turmeric), exhibits antimicrobial, anticancer, hepatoprotective, anti-arthritic, and antiasthmatic effects, making it a promising compound in the pharmaceutical industry[65].

4.2 Antioxidant Activities

A set of tests was run on the methanolic *Salvia leucantha* leaf extract to determine its antioxidant capabilities. These included reducing power, total antioxidant capacity, DPPH, hydroxyl radical, and nitric oxide radical scavenging activities. The tests were carried out with concentrations varying between 200 and 1000 µg/mL. The results showed that the antioxidant potential was high and increased with increasing dosage in all the tests that were conducted.

4.2.1 DPPH Radical Scavenging Activity

Use of DPPH, a stable free radical with a nitrogen centre, allows researchers to examine the radical scavenging capabilities of various chemicals and

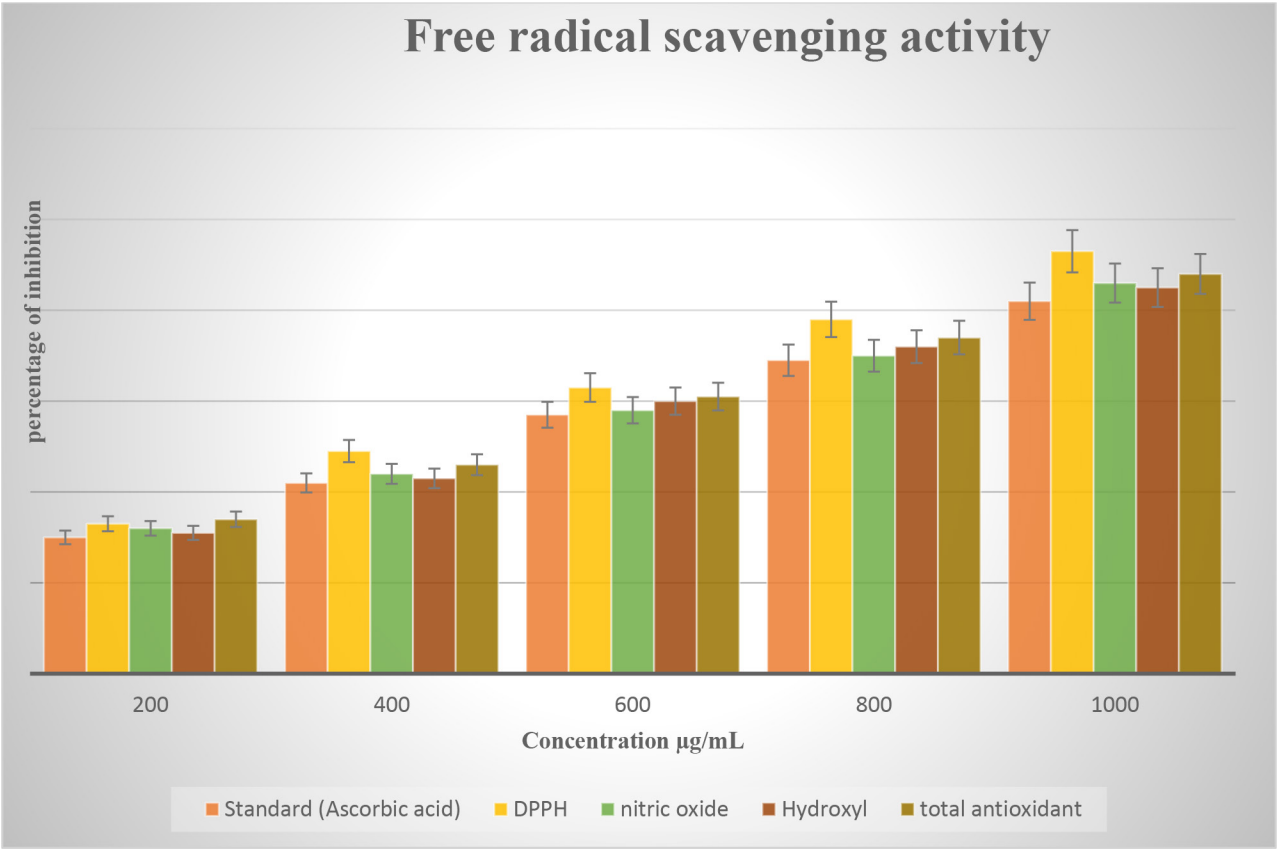


Figure 2. Free radical scavenging activity of methanolic extract of *Salvia leucantha* leaves.

biological extracts[66]. The stable DPPH radical may be transformed into 2-Diphenylpicrylhydrazine, a yellow molecule, by donating an electron to an antioxidant. The use of a UV spectrometer allows one to quantify this shift. Antioxidants and radical scavengers are molecules that help this process along.

Methanol leaf extract of *S. leucantha* showed various levels of DPPH radical scavenging activity according to the concentration range of 200-1000 µg/mL, with an IC_{50} value of 390 µg/mL. According to Figure 2, the methanol extract exhibited the highest DPPH radical scavenging activity at 1000 µg/mL, followed by 800 > 600 > 400 > 200 µg/mL. The standard and the methanol extract both inhibited DPPH radicals in a dose-dependent manner. Particularly noteworthy is the fact that the methanolic leaf extract showed more effective free radical scavenging capabilities than standard ascorbic acid, in terms of antioxidant activity.

4.2.2 Nitric Oxide Radical Scavenging Activity

A diffusible free radical, nitric oxide, acts as an effector molecule in several biological systems and exerts strong pleiotropic regulation across numerous physiological systems[67]. The *S. leucantha* methanol extract has a nitric oxide radical scavenging activity that ranges from 200 to 1000 µg/mL. The production of nitric oxide was significantly decreased by the various concentrations of *S. leucantha*. The radical scavenging activity of the methanol extract was greatest, as shown in Figure 2, with values ranging from 1000 to 200 µg/mL. The nitric oxide radical inhibition was best at 1000 µg/mL concentration when compared to other concentrations, with the IC_{50} value of 707 µg/mL. An increase in nitric oxide scavenging activity compared to conventional ascorbic acid was one indication of the methanolic extract's promising role in reducing oxidative stress.

4.2.3 Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are very damaging to nearby biomolecules because they are the most reactive oxygen-centred species. Using ascorbic acid-iron EDTA to generate OH radicals allowed for the observation of the scavenging action of hydroxyl radicals[68]. Among several doses tested, the methanolic leaf extract of *S. leucantha* exhibited the greatest ability to scavenge hydroxyl radicals at 1000 µg/mL, with an IC_{50} value of 713 µg/mL. The extracts' scavenging activity was displayed in Figure 2 in a decreasing order of 1000 > 800 > 600 > 400 > 200 µg/mL. *S. leucantha* scavenged hydroxyl radicals in a concentration-dependent manner when added to the reaction mixture at various concentrations. We can attribute the methanolic extract's free radical scavenging ability to the presence of phenolic compounds. The methanolic leaf extract showed a substantial capacity to neutralise damaging radicals and protect biological systems from oxidative damage, with a hydroxyl radical scavenging capability that was much higher than that of conventional ascorbic acid.

4.2.4 Total Antioxidant Activity

Using spectrophotometry, the phosphomolybdenum approach was used to assess the overall antioxidant activity of the methanol extracts. This methodology is based on the reduction of Mo (VI) to Mo (V), with maximal absorbance at 695 nm. The overall antioxidant activity was best at 1000 µg/mL for the *S. leucantha* methanol extract when compared to other doses. The IC_{50} value was 343 µg/mL, and the total antioxidant activity dropped as indicated in Figure 2, with 1000 > 800 > 600 > 400 > 200 µg/mL being the highest and lowest values. A substantial electron-donating capacity was exhibited by the methanolic leaf extract, which showed a reducing power that was much larger than that of ordinary ascorbic acid. Because of this, it may have the ability to protect biological systems from free radical damage and combat oxidative stress as a powerful natural antioxidant.

4.2.5 Reducing Power Assay

One important feature of phenolic antioxidant qualities is their ability to donate electrons, and one popular way to measure this is by Fe (III) reduction[69]. Reductones, which are antioxidants, are a good indicator of a compound's reducing power since they donate a hydrogen atom to interrupt the free radical chain[70]. The reductive capacities of *S. leucantha* methanol extract are illustrated in Figure 3. The extracts' reducing power, like their antioxidant activity, rose with concentration. When compared to other concentrations, the methanol extract at 1000 µg/mL had the greatest reducing power. The IC_{50} value was 253 µg/mL, and the decreasing power ability was as follows: 1000 > 800 > 600 > 400 > 200 µg/mL. The ferrous form of the Fe3+/ferricyanide complex was reduced by the antioxidant principles found in *S. leucantha* extracts, demonstrating their ability to

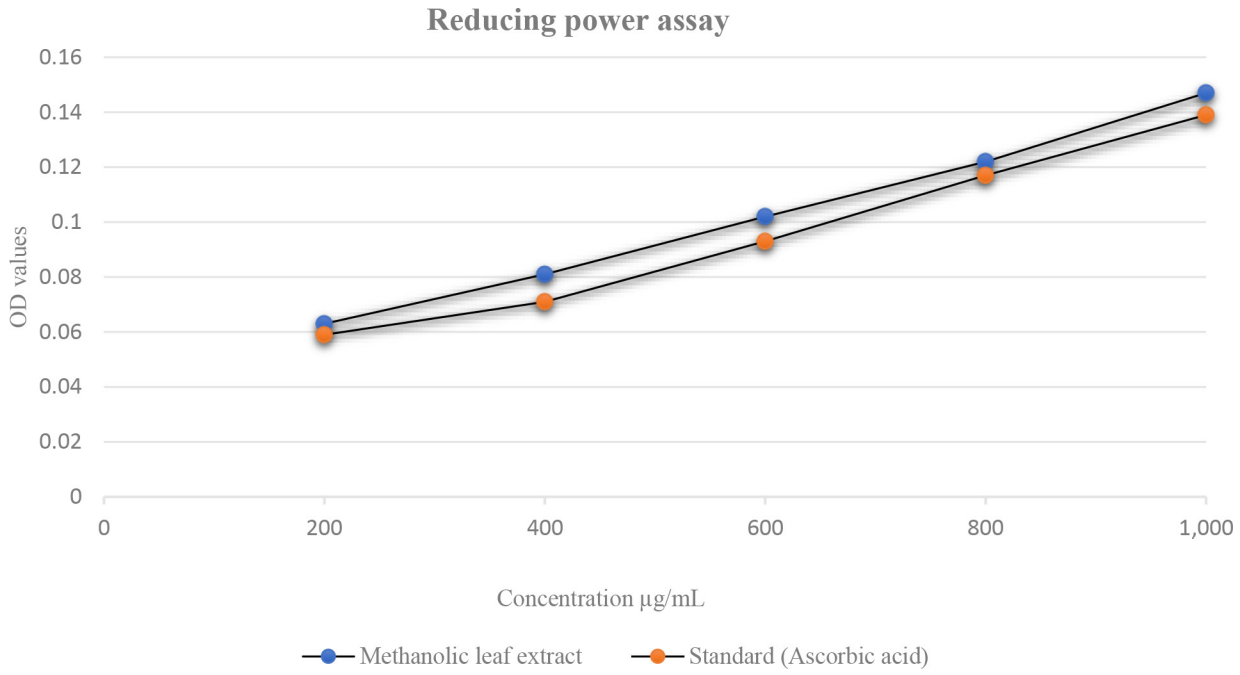


Figure 3. Reducing the power activity of the methanolic extract of *S. leucantha* leaves.

decrease. As an example of its superior electron-donating capability, the methanolic leaf extract showed a reducing power that was much higher than that of conventional ascorbic acid. Its ability to help reduce oxidative stress and protect biological systems from free radical damage highlights its potential as a powerful natural antioxidant.

5. Conclusion

This study shows that *Salvia leucantha* contains many important natural compounds with strong potential for medicinal uses. The GC-MS analysis identified 30 important phytochemical constituents, of bioactive compounds analyzed in this study demonstrate diverse pharmacological effects, including antimicrobial, anticancer, anti-inflammatory, neuroprotective, and immunomodulatory properties. Apart from thymol and 9, octadecenoic acid, all other compounds were new to the genus *Salvia*. The occurrence of these components in both natural and synthetic forms underscores their significance in advancing modern medical treatments. Their interaction with multiple biological pathways highlights their potential as key candidates for drug development.

Ongoing pharmacological research on these compounds emphasizes the necessity for further investigation to determine their precise mechanisms of action, enhance therapeutic efficacy, and reduce adverse effects. The integration of nanotechnology and advanced drug delivery methods may improve their effectiveness.

The methanolic leaf extract of *Salvia leucantha* exhibited strong antioxidant properties across multiple radical scavenging assays, confirming its effectiveness as a natural antioxidant. The extract showed a concentration-dependent ability to neutralize DPPH, nitric oxide, and hydroxyl radicals, outperforming standard ascorbic acid. In addition, its improvement of oxidative stress was demonstrated by its enhanced electron-donor capacity in the total antioxidant activity and reducing power assays. The plant's high antioxidant activity might be attributed to its phenol, terpene, carboxylic, and amine groups.

The natural chemicals found in *Salvia leucantha* have the potential to be a powerful new tool in the fight against cancer, infections, and inflammation. A hopeful candidate for future therapeutic uses, this plant shows great promise for further study and medication development.

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8. Author Contribution

Suresh Kumar- collection of plant samples, analysis and writing the original manuscript. Rama Prabha- plans, edits, supervises and drafts the manuscript. All authors have read and agreed to the published version of the manuscript.

9. References

1. Ahmedull M, Nayar MP. Red data book for Indian plants. Bot Surv India. 1997; 4: 346- 350
2. Xu JG, Hu QP, and Liu Y. Antioxidant and DNA-protective activities of chlorogenic acid isomers. J Agric Food Chem. 2012; 60(46):11625-11630. https://doi.org/10.1021/jf303771s PMid:23134416
3. Anderson KJ, Teuber SS, Gobeille G, Cremin P, Waterhouse AL, Steinberg FM. Walnut polyphenolics inhibit *in vitro* human plasma and LDL oxidation: Biochemical and molecular action of nutrients. J Nutr. 2001; 131(11):2837-2842. https://doi.org/10.1093/jn/131.11.2837 PMid:11694605
4. Patel DK. Plant as a source of medicine. Med Aromat Plants. 2015; S(3). https://doi.org/10.4172/2167-0412.S3-e001
5. Héthelyi E, Tétényi P, Dabi E, Dános B. The role of mass spectrometry in medicinal plant research. Biomed Environ Mass Spectrom. 1987; 14(11):627-632. https://doi.org/10.1002/bms.1200141110 PMid:2962668
6. Jash SK, Gorai D, Roy R. Salvia genus and triterpenoids. Int J Pharm Sci Res. 2016; 7(12):4710-4732.
7. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem. 1992; 40(6):945-948. https://doi.org/10.1021/jf00018a005
8. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem. 1982; 126(1):131-138. https://doi.org/10.1016/0003-2697(82)90118-X PMid:7181105
9. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating systems. Biochemistry. 1981; 20(21):6006-6012. https://doi.org/10.1021/bi00524a013 PMid:6272833
10. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269(2):337-341. https://doi.org/10.1006/abio.1999.4019 PMid:10222007
11. Oyaizu M, Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986; 44:307-315. http://dx.doi.org/10.5264/eiyogakuzashi.44.307
12. Springer D, Fritschi G, Maurer H. Metabolism of the new designer drug α-pyrrolidinoproppiophenone (PPP) and the toxicological detection of PPP and 4/'-methyl-α-pyrrolidinopropiophenone (MPPP) studied in rat urine using gas chromatography-mass spectroscopy. J Chromatogr B. 2003; 796(2):253-266. https://doi.org/10.1016/j.jchromb.2003.07.008 PMid:14581066
13. Nandhini RS, Nithya RN, Vidhya K. GC-MS analysis of phytochemical compounds in different extracts of *Curculigo orchiode*. Research J Pharm Tech. 2021; 14(8):4355. https://doi.org/10.52711/0974-360X.2021.00756
14. Antonaccio MJ, Gomoll AW, Byrne JE. Encainide. Cardiovasc Drugs Ther. 1989; 3(5):691-710. https://doi.org/10.1007/BF01857621 PMid:2518680
15. Tailulu A, Li M, Ye B, Al-Qudaimi R, Cao F, Liu W, *et al*. Antimicrobial and anticancer activities of Hainan dry noni fruit alcoholic extracts and their novel compounds identification using UPLC-Q-Exactive Obitrap- MS/MS. J Pharm Biomed Anal. 2022; 220:114989. https://doi.org/10.1016/j.jpba.2022.114989 PMid:35998428
16. Russel RK, Press JB, Rampulla RA, McNally JJ, Falotico R, Keiser JA, *et al*. Thiophene systems. 9. Thienopyrimidinedione derivatives as potential antihypertensive agents. J Med Chem. 1998; 31(9):1786-1793. https://doi.org/10.1021/jm00117a019 PMid:2842504
17. Chen HJ, Wang WL, Wang GF, Shi LP, Gu M, Ren YD, *et al*. Rational design and synthesis of 2,2-bisheterocycle tandem derivatives as non-nucleoside hepatitis B virus inhibitors. ChemMedChem. 2008; 3(8):1316-1321. https://doi.org/10.1002/cmdc.200800136 PMid:18663717
18. Mongevega A, Aldama I, Robbani MM, Fernandez E. The synthesis of 11H-1,2,4-triazolo[4,3-b] pyridazino[4,5-b] indoles,11H-tetrazolo[4,5-b] pyridazino[4,5-b] indoles and 1,2,4-triazolo[3,4-f]-1,2,4-triazino[4,5-a] indoles. J Heterocycl Chem. 1980; 17(1):77-80. https://doi.org/10.1002/jhet.5570170116
19. Burnett DA, Caplen MA, Davis Jr HR, Burrier RE, J W Clader JW. 2-Azetidinones as inhibitors of cholesterol absorption. J Med Chem. 1994; 37(12):1733-1736. https://doi.org/10.1021/jm00038a001 PMid:8021912
20. Russell RK, Press JB, Rampulla RA, McNally JJ, Falotico R, Keiser JA, *et al*. Thiophene systems. 9. thienopyrimidinedione derivatives as potential antihypertensive agents. J Med Chem. 1988; 31:1786-1793. https://doi.org/10.1021/jm00117a019 PMid:2842504

21. Vandermeeren M, Janssens S, Wouters H, Borghmans I, Borgers M, Beyaert R, *et al*. Dimethyl fumarate is an inhibitor of cytokine-induced nuclear translocation of NF-kappa B1, but not Rel A in normal human dermal fibroblast cells. J Investig Dermatol. 2001; 116(1):124-130. https://doi.org/10.1046/j.1523-1747.2001.00211.x PMid:11168807
22. Hussein HM. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and Fourier-Transform infrared spectroscopy. Res J Pharm Biol Chem Sci. 2016; 7(4):2529-2555.
23. PubChem. Compound Summary for CID 7871. Sarin. https://pubchem.ncbi.nlm.nih.gov/compound/sarin
24. Singh S, Mandal MK, Masih A, Saha A, Ghosh SK, Bhat HR, *et al*. 1,3,5-Triazine: A versatile pharmacophore with diverse biological activities. Arch Pharm (Weinheim). 2021; 354(6):e2000363. https://doi.org/10.1002/ardp.202000363 PMid:33760298
25. Kowalczyk A, Przychodna M, Sopata S, Bodalska A, Fecka I. Thymol and thyme essential oil-new insights into selected therapeutic applications. Molecules. 2020; 25(18):4125. https://doi.org/10.3390/molecules25184125 PMid:32917001 PMCid: PMC7571078
26. Bethesda (MD). LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Fluorouracil. [Updated 2018 Feb 2]. https://www.ncbi.nlm.nih.gov/books/NBK548781/
27. Sharma D, Sharma N, Manchanda N, Prasad SK, Sharma PC, Thakur VK, *et al*. Bioactivity and *in silico* studies of isoquinoline and related alkaloids as promising antiviral agents: An insight. Biomolecules. 2023; 13(1):17. https://doi.org/10.3390/biom13010017 PMid:36671402 PMCid:PMC9856122
28. Okamura K, Ikenoue H, Shiroozu A, Sato K, Yoshinari M, Fujishima M. Reevaluation of the effects of methyl mercaptoimidazole and propylthiouracil in patients with Graves' hyperthyroidism. J Clin Endocrinol Metab. 1987; 65(4):719-723. https://doi.org/10.1210/jcem-65-4-719 PMid:3654917
29. Morton IK, Hall JM. Concise dictionary of pharmacological agents: Properties and synonyms. Springer Science and Business Media; 2012.
30. Oh CH, Cho HW, Baek D, Cho JH. Synthesis and antibacterial activity of 1β-methyl-2-(5-substituted thiazole pyrrolidin-3-ylthio) carbapenem derivatives. Eur J Med Chem. 2002; 37(9):743-754. https://doi.org/10.1016/S0223-5234(02)01397-1 PMid:12350291
31. Tapia RA, Prieto Y, Pautet F. Synthesis and antiprotozoal evaluation of benzothiazolopyrroloquinoxalinones, analogues of kuanoniamine A. Bioorg Med Chem. 2003; 11(16):3407-3412. https://doi.org/10.1016/S0968-0896(03)00311-0 PMid:12878135
32. Kumar GVS, Rajendraprasad Y, Mallikarjuna BP, Chandrashekar SM, Kistayya C. Synthesis of some novel 2-substituted-5-[isopropylthiazole] clubbed 1,2,4-triazole and 1,3,4-oxadiazoles as potential antimicrobial and antitubercular agents. Eur J Med Chem. 2010; 45(5):2063–2074. https://doi.org/10.1016/j.ejmech.2010.01.045 PMid:20149496
33. Samadhiya P, Sharma R, Srivastava SK, Srivastava SD. Synthesis of 2-oxoazetidine derivatives of 2-aminothiazole and their biological activity. J Serb Chem Soc. 2012; 77(5):599-605. https://doi.org/10.2298/JSC110616002S
34. Srivastava SK, Yadav R, Srivastava SD. Synthesis and biological activity of 4-oxothiazolidines and their 5-arylidenes. Indian J Chem. 2004; 43B(2):399-405. https://doi.org/10.1002/chin.200422150
35. Tehranchian S, Akbarzadeh T, Fazeli MR, Jamalifar H, Shafiee A. Synthesis and antibacterial activity of 1-[1,2,4-triazol-3-yl] and 1-[1,3,4-thiadiazol-2-yl]-3-methylthio-6,7-dihydro-benzo[c]thiophen-4(5H) ones. Bioorg Med Chem Lett. 2005; 15(4):1023-1025. https://doi.org/10.1016/j.bmcl.2004.12.039 PMid:15686905
36. Pillai AD, Rathod PD, Xavier FP, Pad H, Sudarsanam V, Vasu KK. Tetra substituted thiophenes as anti-inflammatory agents: Exploitation of analogue-based drug design. Bioorg Med Chem. 2005; 13(24):6685-6692. https://doi.org/10.1016/j.bmc.2005.07.044 PMid:16125391
37. Chen Z, Ku TC, Seley KL. Thiophene-expanded guanosine analogues of gemcitabine. Bioorg Med Chem Lett. 2015; 25:4274-4276. https://doi.org/10.1016/j.bmcl.2015.07.086 PMid:26316465 PMCid: PMC4579529
38. Burnett AD, Caplen AM, Davis RH, Clader J. 2-Azetidinones as inhibitors of cholesterol absorption. J Med Chem. 1994; 37(12):1733-1736. https://doi.org/10.1021/jm00038a001 PMid:8021912
39. Prabakaran R, Joseph B, Pradeep PN. Phyto medicinal compounds from *Urginea indica* Kunth: A synthetic drugs potential alternative. Br J Pharm Res. 2016; 11(5):1-9. https://doi.org/10.9734/BJPR/2016/25216
40. Surender S, Vinod N, Sweety J, Gupta YK. Evaluation of anti-inflammatory activity of plant lipids containing -linolenic acid. Indian J Exp Biol. 2008; 46(6):453-456.
41. Sheela D, Uthayakumari F. GC-MS analysis of bioactive constituents from coastal sand dune taxon –*Sesuvium portulacastrum* (L.) Biosci Discov. 2013; 4(1):47-53.
42. Shettima AY, Karumi Y, Sodipo OA, Usman H, Tijjani MA. Gas Chromatography–Mass Spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of *Guiera senegalensis* J.F. Gmel. J App Pharm Sci. 2013; 3(3):146-150.
43. Astudillo AM, Meana C, Guijas C, Pereira L, Lebrero P, Balboa MA, *et al*. Occurrence and biological activity of palmitoleic acid isomers in phagocyte cells. J Lipid Res. 2018; 59(2):237-249. https://doi. org/10.1194/jlr.m079145 PMid:29167413 PMCid: PMC5794419

44. Saravanakumar K, Raj A, Umaiyambigai D. GC-MS and FT-IR profiling of leaves methanol extract from the *Pleiospermium alatum* (Wall. ex Wt. and Arn) Swingle Rutaceae family. J Phytopharm. 2016; 5(5):201-204. https://doi.org/10.31254/phyto.2016.5506
45. Nishanthini A, Mohan VR, Jeeva S. Phytochemical, FT-IR, and GC-MS analysis of stem and leaf of *Tiliacora acuminata* (Lan.) Hook F and Thomas (Menispermaceae). Int J Pharm Sci Res. 2014; 5(9):3977-3986.
46. Jananie RK, Priya V, Vijayalakshmi, K. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. N Y Sci J. 2011; 4:16-20.
47. Abubacker MN, Devi PK. *In-vitro* antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Wild. (Acanthaceae) inforescence. Asian Pac J Trop Med. 2014; 7:190-193. https://doi. org/10.1016/s1995-7645(14)60230-3 PMid:25312119
48. Prabakaran R, Joseph B, Pradeep PN. Phyto medicinal compounds from *Urginea indica* Kunth: A synthetic drugs potential alternative. J Pharm Res Int. 2016; 11(5):1-9. https://doi.org/10.9734/bjpr/2016/25216
49. Berardesca E, Zuberbier T, Viera MS, Marinovich M. Review of the safety of octocrylene used as an ultraviolet filter in cosmetics. J Eur Acad Dermatol Venereol. 2019; 33(7):25-33. https://doi.org/10.1111/jdv.15945 PMid:31588614
50. Choi JM, Lee EO, Lee HJ, Kim KH, Ahn KS, Shim BS, *et al*. Identification of campesterol from *Chrysanthemum coronarium* L. and its antiangiogenic activities. Phytother Res. 2007; 21(10):954-959. https://doi.org/10.1002/ptr.2189 PMid:17604370PMid:17604370
51. Sunitha S, Nagaraj M, Varalakshmi P. Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats. Fitoterapia. 2001; 72(5):516-523. https://doi.org/10.1016/S0367-326X(01)00259-3 PMid:11429246
52. Ukiva M, Akihisa T, Tokuda H, Suzuki H, Mukainaka T, Ichiishi E, *et al*. Antitumor promoting effects and cytotoxic activity against human cancer lines of triterpene diols and triols from edible chrysanthemum flowers. Cancer Lett. 2002; 177(1):7-12.
53. Katerere DR, Grev AI, Nash RJ, Waigh RD. Antimicrobial activity of pentacyclic triterpenes isolated from African combretaceae. Phytochemistry. 2003; 63(1):81-88. https://doi.org/10.1016/S0031-9422(02)00726-4 PMid:12657301
54. Kim YS, Li XF, Kang KH, Ryu B, Kim SK. Stigmasterol isolated from marine microalgae *Navicula incerta* induces apoptosis in human hepatoma HepG2 cells. BMB Rep. 2014; 47(8):433-438. https://doi.org/10.5483/BMBRep.2014.47.8.153 PMid:24286323 PMCid: PMC4206714
55. Pandey P, Bajpai P, Siddiqui MH, Sayyed U, Tiwari R, Shekh R, *et al*. Elucidation of the chemo preventive role of stigmasterol against Jab1 in gall bladder carcinoma. Endocr Metab Immune Disord Drug Targets. 2019; 19(6):826-837. https://doi.org/10.2174/1871530319666190206124120 PMid:30727937
56. Ali H, Dixit S, Ali D, Alqahtani SM, Alkahtani S, Alarifi S. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma. Drug Des Devel Ther. 2015; 9:2793-2800. https://doi.org/10.2147/DDDT.S83514 PMid:26060396 PMCid: PMC4454197
57. Li K, Yuan D, Yan R, Meng L, Zhang Y, Zhu K. Stigmasterol exhibits potent antitumor effects in human gastric cancer cells mediated via inhibition of cell migration, cell cycle arrest, mitochondrial mediated apoptosis and inhibition of JAK/STAT signalling pathway. J Buon. 2018; 23(5):1420-1425.
58. Newill H, Loske R, Wagner J, Johannes C, Lorenz RL, Lehmann L. Oxidation products of stigmasterol interfere with the action of the female sex hormone 17 beta-estradiol in cultured human breast and endometrium cell lines. Mol Nutr Food Res. 2007; 51(7):888-898. https://doi.org/10.1002/mnfr.200700025 PMid:17579897
59. Scholtysek C, Krukiewicz AA, Alonso JL, Sharma KP, Sharma PC, Goldmann WH. Characterising components of the Saw Palmetto Berry Extract (SPBE) on prostate cancer cell growth and traction. Biochem Biophys Res Commun. 2009; 379(3):795-798. https://doi.org/10.1016/j.bbrc.2008.11.114 PMid:19059205
60. Ayaz M, Sadiq A, Wadood A, Junaid M, Ullah F, Khan NZ. Cytotoxicity and molecular docking studies on phytosterols isolated from *Polygonum hydropiper* L. Steroids. 2019; 141:30-35. https://doi.org/10.1016/j.steroids.2018.11.005 PMid:30444979
61. Chadha N, Bahia MS, Kaur M, Silakari O. Thiazolidine-2, 4-Dione derivatives: Programmed chemical weapons for key protein targets of various pathological conditions. Bioorg Med Chem. 2015; 23(13):2953-2974. https://doi.org/10.1016/j.bmc.2015.03.071 PMid:25890697
62. Da Rocha Jr LF, Rêgo MJ, Cavalcanti MB, Pereira MC, Pitta MG, de Oliveira PS. Synthesis of a novel thiazolidinedione and evaluation of its modulatory effect on IFN- γ, IL-6, IL-17A, and IL-22 production in PBMCs from rheumatoid arthritis patients. BioMed Res Int. 2013. https://doi.org/10.1155/2013/926060 PMid:24078927 PMCid: PMC3773918
63. Youssef AM, White MS, Villanueva EB, El-Ashmawy IM, Klegeris A. Synthesis and biological evaluation of novel Pyrazolyl-2, 4-Thiazolidinedionesas anti-inflammatory and neuroprotective agents. Bioorg Med Chem. 2010; 18(5):2019-2028. https://doi.org/10.1016/j.bmc.2010.01.021 PMid:20138770
64. Albrecht U, Gordes D, Schmidt E, Thurow K, Lalk M, Langer P. Synthesis and structure-activity relationships of 2-Alkylidenethiazolidine-4, 5-diones as antibiotic agents.

Bioorg Med Chem. 2005; 13(14):4402-4407. https://doi.org/10.1016/j.bmc.2005.04.046 PMid:15905094

65. Zeb MA, Khan SU, Rahman TU. Isolation and biological activity of β-sitosterol and stigmasterol from the roots of *Indigofera heterantha*. Pharm Pharmacol Int J. 2017; 5(5):204-207. https://doi.org/10.15406/ppij.2017.05.00139
66. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites. 2009; 60(4):405-412. https://doi.org/10.3989/gya.010109
67. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharmacol Exp Ther. 1993; 264(1):11-16. https://doi.org/10.1016/S0022-3565(25)10238-3 PMid:7678645
68. Sasikumar V, Kalaisezhiyen P. Evaluation of free radical scavenging activity of various leaf extracts from *Kedrostis foetidissima* (Jacq.) Cogn. Biochem Anal Biochem. 2014; 3(2). https://doi.org/10.4172/2161-1009.1000150
69. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Pharmacogn Mag. 2009; 5(18):122-126.
70. Meir S, Kanner J, Akiri B, Hadar SP. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. J Agric Food Chem. 1995; 43(7):1813-1819. https://doi.org/10.1021/jf00055a012