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ABOUT COVER

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ORIGINAL ARTICLE

Clinical and Translational Research

Chemical profiling of bioactive compounds in the methanolic extract of wild leaf and callus of *Vitex negundo* using gas chromatography-mass spectrometry

Gunjan Garg, Alok Bharadwaj, Shweta Chaudhary, Veena Gupta

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Abstract

BACKGROUND

The investigation of plant-based therapeutic agents in medicinal plants has revealed their presence in the extracts and provides the vision to formulate novel techniques for drug therapy. *Vitex negundo* (*V. negundo*), a perennial herb belonging to the *Varbanaceae* family, is extensively used in conventional medication.

AIM

To determine the existence of therapeutic components in leaf and callus extracts from wild *V. negundo* plants using gas chromatography-mass spectrometry (GC-MS).

METHODS

In this study, we conducted GC-MS on wild plant leaf extracts and correlated the presence of constituents with those in callus extracts. Various growth regulators such as 6-benzylaminopurine (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthylacetic acid (NAA), and di-phenylurea (DPU) were added to plant leaves and *in-vitro* callus and grown on MS medium.

RESULTS

The results clearly indicated that the addition of BAP (2.0 mg/L), 2,4-D (0.2 mg/mL), DPU (2.0 mg/L) and 2,4-D (0.2 mg/mL) in MS medium resulted in rapid callus development. The plant profile of *Vitex* extracts by GC-MS analysis

showed that 24, 10, and 14 bioactive constituents were detected in the methanolic extract of leaf, green callus and the methanolic extract of white loose callus, respectively.

CONCLUSION

Octadecadienoic acid, hexadecanoic acid and methyl ester were the major constituents in the leaf and callus methanolic extract. Octadecadienoic acid was the most common constituent in all samples. The maximum concentration of octadecadienoic acid in leaves, green callus and white loose callus was 21.93%, 47.79% and 40.38%, respectively. These findings demonstrate that the concentration of octadecadienoic acid doubles *in-vitro* compared to *in-vivo*. In addition to octadecadienoic acid; butyric acid, benzene, 1-methoxy-4-(1-propenyl), dospan, tridecanedialdehyde, methylcyclohexenylbutanol, chlorpyrifos, n-secondary terpene diester, anflunine and other important active compounds were also detected. All these components were only available in callus formed *in-vitro*. This study showed that the callus contained additional botanical characteristics compared with wild plants. Due to the presence of numerous bioactive compounds, the medical use of *Vitex* for various diseases has been accepted and the plant is considered an important source of therapeutics for research and development.

Key Words: Leaf extracts; Callus extracts; Methanolic extract; Octadecadienoic acid; Hexadecanoic acid; Methyl ester; Gas chromatography-mass spectrometry analysis

Core Tip: Phytopharmacological analysis of medicinal plants and their extracts can show the presence of important elements and gives insight into new methods for drug therapy. *Vitex negundo* (*L.*) is a perennial herb belonging to the *Varbanaceae* family, an aromatic small tree widely used in conventional medicine. In the present study, we report the gas chromatography-mass spectrometry analysis of leaf extracts from wild plants and correlate the existence of these components with those present in callus extracts. The medicinal application of *Vitex negundo* for various diseases has been recognized due to the identification of various bioactive compounds, and the plant is recognized as an important botanical remedy in medical research and development.

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INTRODUCTION

India has always been considered a treasure trove rich in medicinal plants and the application of medicinal plants in various forms has been considered a way of life[1]. Eighty percent of the world's population relies on herbal medicine as primary healthcare. These medicinal plants are an important source of secondary metabolites (SMs), which are small organic molecules that are important for the longevity of plants, but they do not play a role in the growth and development of plants. On the basis of their structures and biosynthesis, these SMs are divided into the following three categories: (1) Phenolic compounds; (2) Terpenoids; and (3) Alkaloids[2]. These SMs are beneficial to human health and effective in preventing diseases. The use of *in-vitro* propagation techniques facilitates the rapid propagation of rare and commercially important plants. The massive expansion of medicinal plants through the use of plant biotechnology has the potential to meet the pharmaceutical industry's need for raw materials for herbal preparations. Numerous medicinal plants have been extracted from the natural flora for commercial drug production[3]. The collection of phytochemicals from plant tissues has been studied for over 30 years, and the information obtained contributes to the use of cell cultures to produce the desired phytochemicals. In the last few years, the development of plant tissue culture has increased, and the corporate utility of this technique as a measure of producing precious phytochemicals has received feedback from the scientific community. Cell suspension culture has been found to be the best method for the study of biosynthesis, and callus tissue is the most abundant cell mass obtained during culture production. Cell cultures are useful tools for studying and producing important SMs[4]. Chemical evaluation of plant SMs includes qualitative, quantitative, and biochemical tests. Qualitative and quantitative drug testing can be used to identify the important bioactive components in plant samples.

Vitex negundo (*V. negundo*), is a small perennial, woody, aromatic and flowering ornamental medicinal herb, commonly known as chaste tree, belonging to the *Verbenaceae* family. This plant is deciduous in nature and has a medium height of 4-5 m. It has 3 or 5 palmate-sized flowers, with deep-violet purple aromatic flowers and rounded black-ripe fruit. Plants of *Vitex* species have different ethno-botanical and pharmacological applications. *V. negundo* contains many bioactive phytochemicals such as glycosides, phenolic compounds, flavonoids, terpenes and phytosteroids. Terpenoids are thought to be one of the most abundant metabolites in the *Vitex* plant. Chemically, they contain five carbon isoprene units,

forming monoterpenes (five carbon atoms), hemiterpenes (C5), sesquiterpenes (fifteen carbon atoms), diterpenes (twenty carbon atoms), and triterpenes (thirty carbon atoms). The leaves of *Vitex* are used to reduce breast tenderness, regulate hormones related to fertility, menstrual cycle, menstrual pain and amenorrhea symptoms. Pharmacologically, this species showed anti-oxidative, antimicrobial, anti-inflammatory and anti-tumor properties. It reduces the level of serum prolactin in hyper-prolactinemia and mastodynia. The leaves of *V. negundo* are aromatic, bitter, pungent, and have astringent, analgesic, anti-inflammatory, antipyretic, and anthelmintic properties. Literature has revealed that leaves and flowers of *Vitex* are the main sources of SMs. Leaves are a good source of alkaloids, vitamin C, carotene, glycol-nonanol, benzoic acid, β-sitosterol, flavonoids (such as luteolin-7-glycosides, ricin, iridoid glycosides, C-glycosides), terpenes oil (such as caryophyllene epoxide, δ-guaiene, and ethyl-hexadecenoate), while flowers contain oil such as α-selinene, (E)-nerolidol, carryophyllene epoxide, and germacren-4-ol. To date, there is no comprehensive record available on the phytochemical study of wild leaf and callus culture (*in-vitro*) samples obtained from this plant. Thus, to determine the therapeutic importance of *V. negundo*, a detailed comparative study of phytochemical constituents is essential. Hence, the aim of this study was to analyze and identify the phyto-constituents of wild leaves and *in-vitro* cultured leaf calluses and to spectroscopically identify bioactive compounds in crude extracts prepared in methanol by gas chromatography-mass spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Collection of plant samples

The fresh young leaves of *V. negundo* (*L.*) were collected from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The leaves were carefully rinsed with distilled water to eliminate dust particles and dried at room temperature for 15 d until the dry weight stabilized. The dried herb was placed in liquid nitrogen, ground to a fine powder, and stored it in an airtight container until use.

Callus preparation and culture

To prepare the callus, fresh leaves were collected from plants and washed thoroughly with water to remove dust, then the surfaces were disinfected with 0.1% $HgCl_2$. Leaf explants were excised aseptically and cultured on MS medium containing 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP), and diphenylurea (DPU). The highest and fastest response for callogenesis with a green and friable callus was observed in the MS medium with the addition of BAP (2.0 mg/L) and 2,4-D (0.2 mg/mL) (*i.e.,* M2 media); and DPU (2.0 mg/L) with 2,4-D (0.2 mg/L) (*i.e.,* M4 media). The peculiarity of the combination is the difference between the formation of green brittle and white loose calluses, respectively (Table 1). The callus of *V. negundo* (*L.*) was produced from the primary callus of leaf explants by sub-culturing on MS media (M2 and M4) enriched with standard growth regulators, *i.e.,* M2-media: BAP (2.0 mg/L) with 2,4-D (0.2 mg/mL); and M4-media: DPU (2.0 mg/L) with 2,4-D (0.2 mg/L).

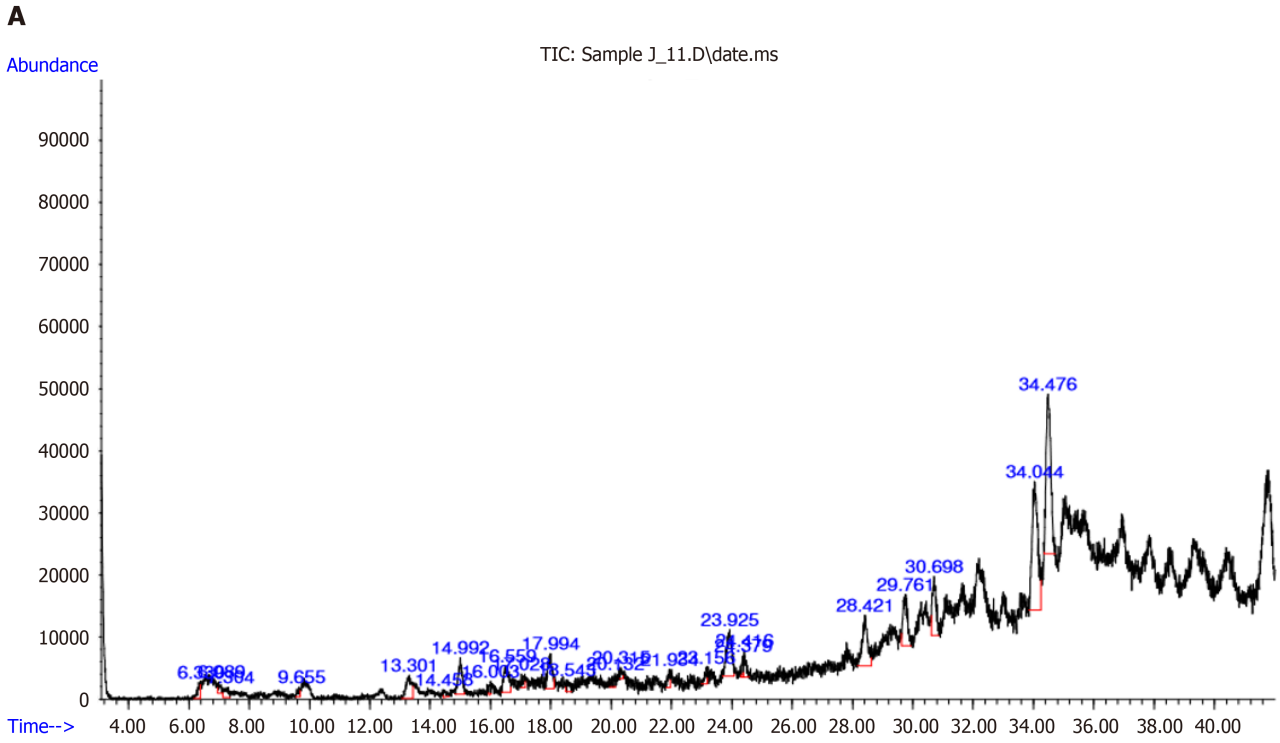
Preparation of plant extracts and GC-MS analysis of bioactive compounds present in the samples

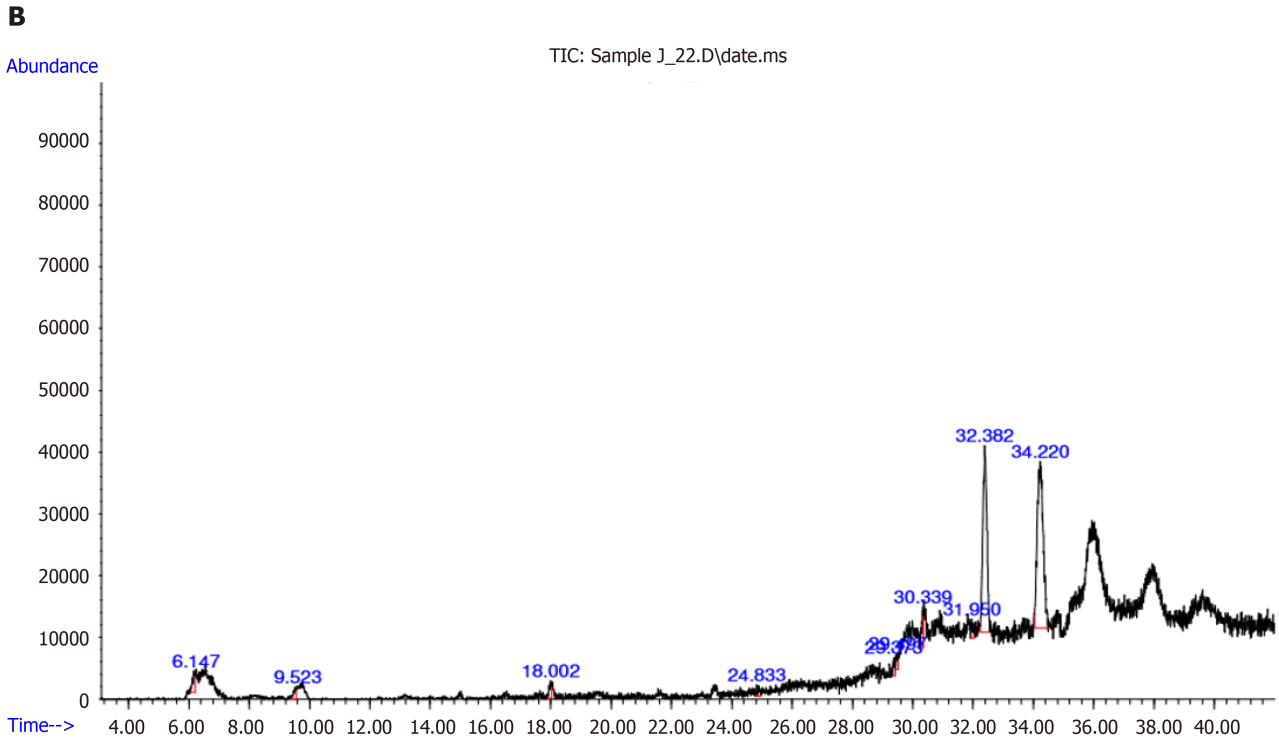
Preparation of the plant extracts was conducted by following the modified method of Anwar *et al*[5]. The fresh young leaves of *V. negundo* (*L.*) were collected from the herbal garden, NBPGR, New Delhi, and calluses were produced by subculture from leaf explants of *V. negundo* in M2 and M4 medium, which were then selected for further quantitative analysis by GC-MS. For leaf sample (LS) preparation, the leaves were carefully rinsed in tap water to eliminate dust particles, then rinsed with sterilized distilled water, dried at room temperature for 24 h, and ground to a fine powder in a mechanical grinder. For callus sample preparation, we selected 6-8-wk-old green friable callus (CS1) and white loose callus (CS2) from the M2 and M4 medium, respectively. The medium particles were removed by washing with double distilled water and then drying in an oven at 62 ± 5 °C for 28 h. Dried samples were ground to a fine powder and stored in sterilized, airtight polythene bags until use. For methanol extraction, 2 g of the powdered plant samples (LS, CS1, and CS2) were weighed and soaked in 10 mL of GC-MS grade methanol for 14 h (or incubated overnight) in a flask. The solution was thoroughly mixed with a shaker. After mixing, the solution was filtered through a Whatman No. 41 filter paper and the filtrate collected. This solution was used for GC-MS analysis.

GC analysis was performed using an Agilent gas chromatograph equipped with a split/split-less injector (230 °C) and mass spectrometer detection (230 °C). The gas used was helium (1 mL/min), and HP-5MS 5% Phenyl Methyl Silox (325 °C: 30 m × 250 μm × 0.25 μm) was employed as the capillary column. The sample (2 μL) was injected through a split-less system with the following program: 170 °C for 1 min, 250 °C for 2 min at 8 °C/min, and finally 3 °C/min at 310 °C for 2 min. The mass selective detector was operated at 70 eV in the 70-600 amu range. The final running time was 39 min, and the data were analyzed using total ion count for identification and quantification of compounds. Each extract spectrum reflects the retention time in the column, and the peaks detected correspond to the relative abundance (%) of bioactive compounds found in specific regions. By comparing the mass spectra of the detected components with the mass spectra of known components available in the National Institute of Standards and Technology library, the bioactive phytochemical compounds of *V. negundo* were identified[6]. Compound concentrations were determined from the GC peak area of the total ion current.

RESULTS

The GC-MS chromatogram spectra were achieved in all three extracts of *V. negundo i.e.*, wild *in-vivo* leaves, green-compact and white-loose *in-vitro* callus. The results showed that bioactive compounds were present in both *in-vivo* and *in-vitro* sample extracts (Figure 1). Using GC-MS analysis we detected twenty-four, ten, and fourteen bioactive compounds in the methanolic extract of wild-leaves, green, and white loose callus, respectively (Tables 2-4). The results showed that octadecadienoic acid, hexadecanoic acid and methyl esters were the main components in the methanol extract of leaves and callus. Among these, octadecadienoic acid was the most common compound in all samples[7,8]. The maximum concentration of octadecadienoic acid in leaves, green callus and white loose callus was 21.93%, 47.79% and 40.38%, respectively. Our results confirmed that the concentration of octadecadienoic acid doubled *in-vitro* compared to *in-vivo*. In earlier research, the results of GC-MS analysis revealed similar compounds to those obtained in the present study[9].





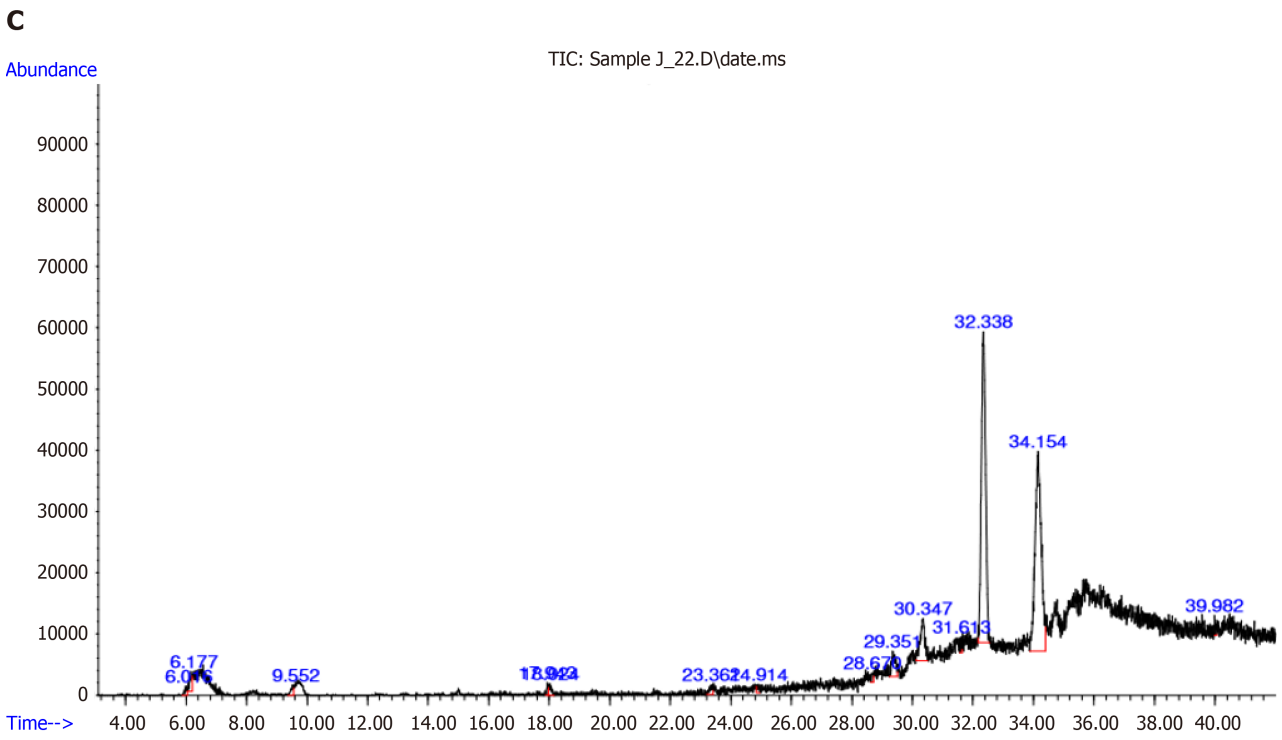


Figure 1 Gas chromatography-mass spectrometry chromatogram of the methanol extract of wild leaves, methanol extract of green callus, and methanol extract of white loose callus of *Vitex negundo* (*L.*). A: Methanol extract of wild leaves; B: Methanol extract of green callus; C: Methanol extract of white loose callus.

DISCUSSION

In *V. negundo* plants, octadecadienoic acid is present along with plant glycosides[10]. Plant glycosides are a diverse group of natural compounds found in various parts of plants, including leaves, stems, roots, and seeds. They consist of a sugar molecule (glycone) attached to a non-sugar, biologically active compound (aglycone or genin), which has both medicinal and toxic properties[11]. Along with the plants' glycosides, octadecadienoic acid develops bitterness in leaves, which frightens herbivores as the leaves are toxic or unpleasant when ingested[12]. Some important glycosides, *e.g.*, anthocyanin glycosides, along with octadecadienoic acid are responsible for the pigmentation (red, blue, and purple) in leaves, which act as stressors in plant defense against temperature stress[13].

Other bioactive constituents such as 1-oxo-dimethyl-methylene-hexahydrocyclopentanol pyran (7.91%), veridiflorol (6.79%), pyrrolo-carbazole (CAS) (6.79%), and dimethyl-phenyl (6.79% peak area) were identified in the methanolic leaf extract. It was observed that other bioactive compounds such as dursban, butyric acid, benzene, 1-methoxy-4-(1-propenyl), nor-ses-terterpene-diester, tri-decanedial, chlorpyrifos, methyl-cyclohexenyl-butanol, and anhalonine were present in good amount in *in-vitro* developed callus (both green-compact and white-loose) along with the major compounds (octadecanoic acid, hexadecanoic acid, and methyl ester) (Tables 3 and 4). The results obtained in the present study were analogous with the data obtained by Kaliyannagounder *et al*[9]. In medicinal plants, octadecadienoic acid is an important polyunsaturated fatty acid. It is also known as linoleic acid and plays several important roles in plant growth and development. Octadecadienoic acid triggers the production of defensive compounds in plants and plays a major role in plant defense mechanisms. It is considered a core-structural integral element of phospholipids that make up the lipid bi-layer of plant cell membranes. It maintains the integrity of the plant cell and controls the movement of molecules in and out of the cell along with other fatty acids. Finally, it adjusts the fluidity and stability of membranes under different environmental stresses, such as temperature extremes, drought, and pathogen attacks. Furthermore, it acts as a precursor of jasmonic acid, which is a signaling molecule involved in the plant's response to herbivores, pathogens, and other stressors in plant defense mechanisms. These findings are similar to those obtained by Ahuja *et al* [14].

Hexadecanoic acid and methyl esters have antioxidant, cholesterol-lowering, antiandrogenic, hemolytic, and alpha-reductase inhibitory properties. Hexadecanoic acid (palmitic acid) is a common saturated fatty acid, while hexadecanoic acid methyl ester (methyl palmitate) is a chemically modified derivative of palmitic acid. On the basis of data available in the literature, plant biomass with good amounts of methyl palmitate (a chemically modified derivative of hexadecanoic acid) is used in biodiesel production. The present study found that *in-vitro* grown callus (white-loose) is a good medium for the synthesis of methyl esters, and we can consider this approach in the near future as a source of renewable fuel for our future energy demands, if appropriate research is carried out[15].

In the present study, two major compounds were found *i.e.*, a sesquiterpenoid compound "viridiflorol" and anhalonine (naturally occurring alkaloid) in the *in-vitro*-derived white loose callus extract of *V. negundo*[16,17]. Viridiflorol showed anti-inflammatory, antioxidant, and anti-mycobacterium tuberculosis activity. Anhalonine is considered an important

Table 1 Effect of combinations of Plant Genetic Resources in mass spectrometry medium on callus induction in *Vitex negundo*

MS + hormone (mg/L)	Medium name	Source of explants	Number of explants inoculated	Callus response (%)	Callus genesis	Callus initiation (time/d)	Color	Texture
MS + no hormone	M1	Leaf	13	-	-	-	-	-
MS + BAP (2.0) + 2,4D (0.2)	M2	Leaf	13	15 d	+++	100%	Green	Friable
MS + BAP (2.0) + NAA (0.2)	M3	Leaf	13	15 d	++	70%	Greenish white	Friable
MS + DPU (2.0) + 2,4D (0.2)	M4	Leaf	13	15 d	+++	90%	White-yellowish	Loose
MS + DPU (2.0) + NAA (0.2)	M5	Leaf	13	15 d	+	40%	White-yellowish	Loose

MS: Mass spectrometry; BAP: 6-benzylaminopurine; NAA: α-naphthylacetic acid; DPU: Di-phenylurea.

Table 2 Bioactive components detected in the wild leaves methanol extract of *Vitex negundo* (*L.*)

Peak	Retention time	Area (%)	Name of components
1	6.330	1.12	Dimethylphosphine-D1
2	6.989	0.72	2-[2-hydroxyethyl]-9-[beta-d-ribofuranosyl] hypoxanthine
3	7.304	1.31	7-tetradecenyl-1-(N-acetyl)amine
4	9.655	0.56	1-propanol, 3-mercapto-sulfanyl-propanol
5	13.301	3.62	3-methylpyridazine pyridazine
6	14.458	0.55	2-octyldodecan-1-ol
7	14.992	3.96	3-hydroxyphenylacetylene 3-ethynylphenol
8	16.003	0.59	Z-citral 2,6-Octadienal, 3,7-dimethyl-, (Z)- (CAS)
9	16.559	03.80	1H-indenol
10	17.028	0.82	2-fluorobenzyl alcohol, benzene-methanol
11	17.994	4.28	Benzene, 1-methoxy-4-enylanisole
12	18.543	0.91	N-hexadecylpyridinium bromide
13	20.132	0.90	9-chloro-8-oxatetracyclotridecan
14	20.315	0.58	5-chlorovaleric acid, octyl ester
15	21.934	1.09	Dimethyl-2-phenyl-2-propenyl phosphite
16	23.156	0.92	4-chromanol-benzopyran-4
17	23.925	6.79	D-viridiflorol
18	24.379	0.75	8,9-epoxy-6,6-dimethyl-3,4-undecadien
19	24.416	1.51	Drimenol
20	28.421	7.91	1-oxo-5,5-dimethyl-6-methylene-hexahydrocyclopentano pyran
21	29.761	6.79	Pyrrolo-carbazole (CAS)
22	30.698	6.74	Dimethyl (phenyl) silane
23	34.044	21.93	9,12-octadecadienoic acid
24	34.476	21.84	2-hexadecen-1-OL, TETRAM

naturally occurring alkaloid. A very small amount of this compound was obtained from *Lophophora williamsii* (a rare species of cactus). It may serve as a chemical defense mechanism in some plants, and may be toxic to herbivores and pathogens. Hence, by producing anhalonine, plants may discourage herbivores and reduce the risk of damage from grazing animals. It seems that anhalonine also showed allelopathy. Such compounds are released by the plants into the soil through their root system and inhibit the growth of nearby competing plants and provide a competitive advantage

Table 3 Bioactive components detected in the green callus methanol extract of *Vitex negundo* (*L.*)

Peak	Retention time	Area (%)	Name of components
1	6.147	2.60	N-butyric-D7 acid
2	9.523	1.50	Benzyloxy-6-tetrabutyldimethyldimethylsilyl-oxy-methyl
3	18.002	1.76	Benzene, 1-methoxy-4 (CAS) anethole-p propenyl isole
4	24.833	1.23	Tridecanedial
5	29.373	1.50	Nor sesterterpene diene ester
6	29.497	1.38	But-2-enyliden-6-tert-butyl-7-methyl-tetrahydrocy
7	30.339	3.77	Hexadecanoic acid, methyl ester
8	31.950	1.63	Tricyclo-undecan-10-imine
9	32.382	36.82	Dursban
10	34.220	47.79	10,13-octadecadienoic acid, methyl ester

Table 4 Bioactive components detected in the white loose callus of methanol extract of *Vitex negundo* (*L.*)

Peak	Retention time	Area (%)	Name of components
1	6.016	0.58	Acetoxy-beta-caryophyllene
2	6.177	1.43	Anhalonine
3	9.552	0.84	1,3,5-cycloheptatriene-cyclohep-triene-cycloheptatrien
4	17.943	0.46	2,3-dichloropyridine 2, 3-dichloropyridine
5	18.024	0.83	1-methoxy-4-propenyl benzene
6	23.361	0.80	6,7-dimethyl-triazolo-triazine
7	24.914	0.51	Benzene, 2-methylthio-ethenyl
8	28.670	0.63	Spiro-decan-7-one, 1,8-dimethy epoxy-4-isopropyl
9	29.351	3.08	Methyl-cyclohex-1-enyl
10	30.347	6.31	14-pentadecynoic acid, methyl ester (CAS)
11	31.613	0.55	2,5-furandione, di hydro-dodecenylsuccinic anhydrid
12	32.338	42.98	Chlorpyrifos
13	34.154	40.38	Octadecadienoic acid, methyl ester
14	39.982	0.61	8-alpha,12-epoxy-hexanorlabdane

for the producing plant. It was noted in the present work that plants of *Vitex* grown in the *in-vivo* field condition usually inhibited the growth of weeds in their nearby area. This may be due to the presence of anhalonine, which was not detected in the methanolic extract of the wild leaf sample. The compound was present in very good amounts (peak area 1.43%) in white loose callus. Anhalonin, used as a psychotropic drug, can change the function of the nervous system and results in alterations of perception, mood, cognition, and behavior in our traditional folk medicinal/herbal system. It also showed antimicrobial properties, which protect the plants from microbial infections and diseases by acting as natural antimicrobial agent[18]. The exact role of anhalonine in plants has not been extensively studied. Detailed research on the functions of anhalonine in different plant species is continuing, and further studies are required to clarify its ecological and physiological significance in the plant system.

Similar outcomes were obtained in a previous study conducted by Lad *et al*[19]. The methanolic extract of *V. negundo* showed the presence of phytol compounds, which have other biological effects such as hypocholesterolemic cancer prevention, insecticidal, hepatoprotective, inhibition of 5-alpha reductase, anti-inflammatory, anti-rash, nematocidal, antihistamine, anti-acne, and anti-inflammatory antibiotic properties. The same bioactive SM of phytol has been previously reported to have various medicinal properties in some aquatic plant species such as *Hydrilla verticillata*, *Gracilaria*, and *Carissa carandas*[20,21]. Octadecanoic acid is a well-known example of a saturated fatty acid and possesses antihypertensive properties along with the ability to decrease low density lipoprotein cholesterol and increase high density lipoprotein cholesterol levels[22].

In the present study, octadecanoic acid was present in both leaf and callus extracts of *V. negundo*. The results of the present study were in accordance with reports on *Cleistanthus collinus*, *Goniothalamus umbrosus*, *Kigelia pinnata*, and *Melissa*

officinalis which contained n-hexadecanoic and octadecadienoic acids[23,24]. The study revealed the presence of various bioactive compounds present in all the methanolic extracts of *in-vivo* and *in-vitro* plant samples[25]. However, it has become clear from the present study that the callus contained additional phytochemicals in comparison to wild-type plants that display significant medicinal properties. Further studies such as bio-prospecting are necessary to support the biological properties and the importance of these inventive bio-molecules.

CONCLUSION

Screening of the methanolic extracts of *Vitex negundo* revealed the presence of twenty-four, ten, and fourteen bioactive compounds in the wild-leaves, green, and white loose callus, respectively. The results confirmed that octadecadienoic acid, hexadecanoic acid, and methyl ester were found to be the key constituents in the methanolic extract of leaves and callus[26]. Octadecadienoic acid was the predominant potential bioactive compound identified in all samples. Results of the present research confirmed that the concentration of octadecadienoic acid in *in-vitro* conditions was twice that in *in-vivo* conditions. Hence, in the case of *Vitex*, we can use micro-propagated plants as a potent source of phyto-compounds for commercialization without destroying the wild plant population[27,28]. A recent literature review showed that plant biomass with good amounts of methyl palmitate (a chemically modified derivative of hexadecanoic acid) is used in biodiesel production[29,30]. The findings of the present study showed that *in-vitro* grown callus is a good medium for the synthesis of methyl esters. Hence, we can consider this approach in the near future as a source renewable fuel for our future energy demands. Active sesquiterpenoid compounds such as viridiflorol and anhalonine (naturally occurring alkaloid) were found in the *in-vitro* derived white loose callus extract of *Vitex negundo*. Viridiflorol showed anti-inflammatory, antioxidant, and anti-mycobacterium tuberculosis activity, while anhalonine may be used as a psychotropic drug in animals and is also responsible for showing allelopathy in plants. This study showed that the callus contained additional botanical characteristics in comparison to wild plants. Due to the presence of numerous bioactive compounds, the medical use of *Vitex* callus for various diseases has been accepted and the plant is considered an important source of therapeutics for research and development.

ARTICLE HIGHLIGHTS

Research background

Gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic leaf extract of *Vitex negundo* has been previously demonstrated in a few studies. The present study has significance as it investigated the plant-based therapeutic agents in the medicinal plant *Vitex negundo*, determined their presence in extracts and provides the vision to formulate novel techniques for drug therapy.

Research motivation

This study identified dospan, butyric acid, benzene, 1-methoxy-4-(1-propenyl), n-sec-terpene-diester, tris-decandial, chlorpyrifos, methyl-cyclohexenyl-butanol, anhalonine, and other important active compounds. These substances are only found in calluses produced *in-vitro*. The results demonstrated that callus has more botanical properties than wild plants. The medicinal application of *Vitex negundo* for various diseases has been recognized due to the identification of various bioactive compounds, and the plant is recognized as an important botanical remedy in medical research and development.

Research objectives

The exact role of anhalonine in animals and plants has not been well-studied.

Research methods

In the present study, we report a GC-MS investigation of leaf extracts from wild plants and correlate the existence of components with those present in callus extracts. Various concentrations of growth regulators such 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid, 6-benzylaminopurine (BAP), and di-phenylurea (DPU) were added to *in-vitro* callus and plant leaves and grown on MS medium. The maximum and rapid response for callogenesis with a green and friable callus was observed in the MS medium with BAP (2.0 mg/L) and 2,4-D (0.2 mg/mL) and DPU (2.0 mg/L) with 2,4-D (0.2 mg/L). The plant profile of *Vitex negundo* extracts underwent GC-MS analysis, from which 24, 10, and 14 bioactive compounds were detected from leaf, green callus, and white loose callus methanolic extracts, respectively.

Research results

Screening of the methanolic extracts of *Vitex negundo* revealed the presence of twenty-four, ten, and fourteen bioactive compounds in the wild-leaves, green, and white loose callus, respectively. Our research data confirmed that octadecadienoic acid, hexadecanoic acid, and methyl ester were the key constituents in the methanolic extract of leaves and callus. Octadecadienoic acid was the predominant potential bioactive compound identified in all samples. The results of our research confirmed that the concentration of octadecadienoic acid in *in-vitro* conditions was twice that in *in-vivo* conditions. Hence, in the case of *Vitex*, we can use micro-propagated plants as a source of potent phyto-compounds for

commercialization without destroying the wild plant population.

Research conclusions

A recent literature review showed that plant biomass with good amounts of methyl palmitate (a chemically modified derivative of hexadecanoic acid) is used in biodiesel production. In the present study, we found that *in vitro* grown callus is a good medium for the synthesis of methyl esters. Hence, we can consider this approach in the near future as a source of renewable fuel for our future energy demands. We also found the active sesquiterpenoid compound viridiflorol and anhalonine (naturally occurring alkaloid) in the *in vitro*-derived white loose callus extract of *Vitex negundo*. Viridiflorol showed anti-inflammatory, antioxidant, and anti-mycobacterium tuberculosis activity, while anhalonine may be used as a psychotropic drug in animals and is responsible for showing allelopathy in plants.

Research perspectives

Detailed research and further studies on the functions of anhalonine in different plant species are required to clarify the physiological significance of anhalonine in plants. Findings from our research show that *Vitex negundo* is a phyto-pharmaceutically important plant.

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FOOTNOTES

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