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PHYTOCHEMICAL SCREENING, GC-MS, LC-MS ASSISTED METABOLITE PROFILING, AND ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF NATURAL PRODUCTS FROM MEDICINAL PLANT *VITEX NEGUNDO*

Aziz Alans[1*], Amat Al-Qader F. Al-Anesi[2], Ali Alrabie[3], Mazahar Farooqui[3] and Deepak D. Kayande[1]

[1]S.B.E.S. College of Science, Aurangabad, Maharashtra, India-431004.
[2]Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431001, India.
[3]Department of Chemistry, Maulana Azad College of Arts, Science and Commerce, Aurangabad, India.

***Corresponding Author: Aziz Alans**

S.B.E.S. College of Science, Aurangabad, Maharashtra, India-431004.

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ABSTRACT

This study aimed to assess the biological activities, including antioxidant and antibacterial characteristics, of a methanol extract of *Vitex negundo* leaves, as well as their phytochemical composition, using chromatographic techniques. The results of the GC-MS and LC-MS analyses showed that the methanol extract was stable. The extract was also tested for its antioxidant and antibac erial activities in vitro. With 90 peaks in the LC-MS study, the methanolic extract contained 96 distinct phytochemicals; in contrast, the GC-MS analysis revealed 32 compounds in the extract. The methanolic extract showed potent antibacterial action against *A. aeroginosa*, *S. aureus*, and *S. pyogenes*, with a minimum inhibitory concentration (MIC) of 125 µg/mL. When tested with DPPH, a strong antioxidant response was seen. This is the first study that we are aware of that details the chemical makeup of *Vitex negundo* as well as its antioxidant and antibacterial capabilities. Extract from *Vitex negundo* may have potential as an antibacterial and natural antioxidant in food preservation and human health, according to the current study's findings.

KEYWORDS: *Vitex negundo*, LC-MS, GC-MS analysis, Antioxidant activity, Antibacterial activity.

INTRODUCTION

Phytomedicine is a significant modality of traditional medicine, practiced globally since ancient times as a treatment strategy for addressing particular pathological disorders.[1] Phytomedicine has been a culturally endorsed method for alleviating many ailments due to its cost-effectiveness and accessibility.[2] This entails the utilization of herbal substances that may comprise entire plants or its parts, which are recognized to encompass specific elements that may influence alterations in the human body. Consequently, diverse plants have been utilized for multiple functions in the lives of humans and animals, specifically as food for nutritional advantages and as medications for disease treatment.[3] Plants produce several chemical compounds known as phytochemicals. Phytochemicals are non-nutritive bioactive compounds with disease-preventive characteristics. They are fundamentally categorized into six primary groups based on their chemical structures and properties: carbohydrates, lipids, phenolics, terpenoids, alkaloids, and other nitrogenous substances. These categories are further subdivided during biogenesis to yield alkaloids, saponins, glycosides, lignans, flavonoids, tannins, triterpenes, coumarins, carotenoids, and others, which function alone or synergistically to produce beneficial or detrimental effects on the body.[4] These phytochemicals are recognized for their many roles and have been demonstrated to exhibit a multitude of biological activities, including antibacterial, antifungal, antiviral, antioxidant, anticancer, hepatoprotective, and antitumor properties[5], among others. Contemporary pharmacological research use ethnobotany to identify phytochemicals in plants that can mitigate severe adverse effects linked to manufactured medications.

The term Vitex originates from the Latin 'vieo,' which signifies to tie or bind, reflecting the flexible character of its stems and twigs.[6] The Sanskrit term 'Nirgundi' for V. negundo signifies 'something which safeguards the body from ailments'.[7] In 1753, Linnaeus erected the genus Vitex and classified it within the family Verbenaceae.[8] V. negundo is a widely utilised medicinal plant across various medical systems, including Ayurveda, Unani, Siddha, Folklore, Chinese, Tibetan, Homoeopathy, and Allopathy.[9] The majority of traditional medications

utilised in healthcare are derived from plants. In this context, *Vitex negundo*, generally referred to as the five-leaved chaste tree, is a large, aromatic, deciduous shrub characterised by quadrangular branches and numerous ethnomedicinal applications. Leaves are 3-5 foliolate, dark green on the upper surface and pale green with a tomentose texture on the underside.[10] Petite blooms exhibit a bluish-purple hue. It is typically located in open waste lands and deciduous woods adjacent to damp environments. It is indigenous to India, Sri Lanka, Pakistan, Afghanistan, Thailand, Malaysia, the Philippines, Myanmar, China, and Japan. It is planted as a commercial crop in regions of Asia, Europe, North America, and the West Indies. Every part of the plant, including roots, bark, leaves, flowers, seeds, and essential oil, possess therapeutic potential. They have been frequently utilised as components or entirety in pharmaceuticals to address various disorders. The leaves and roots of the plant are considered febrifuge and tonic. Heated leaves are utilised in the treatment of rheumatoid arthritis.[11] This study aimed at investigating the antibacterial activity and antioxidant potential and phytochemical analysis of the methanol extract of *Vitex negundo* (using LC-MS and GC-MS analysis).

Figure 1: *Vitex negundo* leaves.

MATERIAL AND METHODS

Collection of plant material

The leaves of the *Vitex negundo* plant were bought from a vendor in Aurangabad. At Dr. Babaseab Ambedkar Marathwada University in Aurangabad, Dr. Narayan Pandura oversaw the verification process. Following a thorough washing, the leaves were left to dry naturally at room temperature for over 20 days. After that, they were ground into a powder and stored in an airtight container until they were ready to be used.

Preparation of extract

By employing the Soxhlet apparatus for a period of six hours, fifty milligrams of powdered dried *Vitex negundo* leaves were extracted with five hundred milliliters of methanol that was 95%. After the extraction process was complete, the solvent was removed using a rotary vacuum evaporator, and the free solvent extract was stored in an amber bottle in the refrigerator thereafter.

Preliminary phytochemicals screening of plant extract

An extensive phytochemical investigation was conducted on extract following established protocols.[12] The extract was examined for the presence of carbohydrates, glycosides, proteins and amino acids, alkaloids. Phenols, saponins, flavonoids, fat and oils.

Gas Chromatography-Mass Spectrometry (GC-MS)

The analyses of phytoconstituents were performed using an instrument Thermo Scientific TSQ-800 GS-MS which was connected with silica capillary column TG-5-MS (dimensions-30 m × 0.25 mm, film thickness 0.25 µm). The run time for GC take place 22 minutes. Helium gas with a flow rate of 1 ml/min was used as a carrier. The oven temperature was initiated at 60°C for 2 min and then programmed to increase up to 280°C at a step of 5°C/min and later held isothermally for 10 min. The temperature of the injector port, ion source, and detector were set at 250°C, 260°C and 280°C respectively. The mass-spectrometric detector was operated in the form of electron impact ionization mode at a fragment of 70eV with a scanning mass range of 50-700 (m/z). The database of the National Institute of Standards & Technology (NIST) Library was used to identify the names, molecular weight and structures of the components.[13]

Liquid Chromatography–Mass Spectrometry (LC-MS)

At SAIF, IIT, Bombay, we used an LC-ESI-Q-TOF MS system (Agilent Technologies 6550 i-funnel) to identify active phytoconstituents in the sample's methanol extract. The two-solvent elution approach includes a gradient system of 0.1 % Formic acid in water (A) and 90 %

Acetonitrile + 10 % Water + 0.1 % Formic acid (B) at a flow rate of 0.3 ml/min and injection volume 5 µl. The gradient system started with 95 % of A and 5 % of B, reaching 0 % of A : 100 % of B at 25 minutes and then back to initial composition 95 % of A and 5 % of B in 1 minute and which was maintained at the same composition for 5 minutes. The MS analysis was carried out in dual positive (+ve) and negative (-ve) modes of ESI were used to ionize the compounds which were channelized using i-funnel Q-TOF. The parameters set in Q-TOF MS source were as follows: capillary voltage 3500 V, mass range 120-1000 m/z, gas flow 13 L/min, gas temperature 250°C, sheath gas temperature 250°C, sheath gas flow 11, nebulizing gas pressure 35 (psig), skimmer 65 V, fragmentor 175 V, RF peak 750 V. The Mass Hunter software was run for profiling, characterizing, identifying and quantifying the compounds present in the extract via high definition MS and MS/MS. The MS spectra received of the analyzed samples were explored against the Metlin database to find the presumptive compounds present in the sample.[14]

Antibacterial activity

The broth-dilution method was used to measure the MIC (Minimum Inhibition Concentration) of a methanol extract of *Vitex negundo* leaves. The following gram-positive bacteria were acquired from the Microcare Laboratory in Surat: Staphylococcus aureus (MTCC96) and Streptococcus pyogenes (MTCC442), as well as Escherichia coli (MTCC443) and Pseudomonas aeruginosa (MTCC1688). For the test microbe's overnight incubation at 37 °C, the control tube is subcultured with a medium that promotes its development. The medication and extract were mixed to create a stock solution with a concentration of 2000 µg/L. The extract was made in a serial dilution format for both primary and secondary evaluation and analysis. The main evaluation utilized quantities of the extract of 1000 µg/mL, 500 µg/mL, and 250 µg/mL. Second, we tested the extract against microbes using the same methods we used to find it active in the first round of testing. For the secondary evaluation, the active extract from the initial evaluation was diluted to obtain concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. Ensuring the reproducibility of the extract concentration is done by recording the Minimal Inhibition Concentration of the control microbe. The minimum inhibitory concentration (MIC) is defined as the concentration at which a 99 % inhibition zone is displayed.[15, 16]

Antioxidant property

The 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) was used to assess the antioxidant activity of the sample. With some minor adjustments, the spectrophotometric method (Sreejayan and Rao, 1996)[17] was used to find the DPPH scavenging activity. In order to get concentrations ranging from 10 to 50 µg/ml, 0.05 ml of extract dissolved in DMSO was mixed with 1.0 ml of ethanol to make it a 1.0 ml volume. Then, 200 µM of DPPH, obtained in 95% ethanol, was added. For the control group, we used a mixture of ethanol and DMSO at a ratio of 1:1. Triplicate aliquots were used for all studies. A spectrophotometer (Shimadzu UV-1800) was used to measure the decrease in absorbance of the test compounds at 515 nm following 20 minutes of dark incubation. The percentage of inhibition was then determined using the specified formula:

Calculate scavenging as:

$$\% \, Scavenging = \frac{A - B}{(A)} \, x100$$

Here, A represents the control absorbance, which is the measurement of the DPPH solution without extract, and B represents the sample absorbance, which is the measurement of the DPPH solution with extract. We employed ascorbic acid as our standard medication.

RESULT AND DISCUSSION

Preliminary phytochemicals screening of plant extract

Table 1 displays the findings of the different phytochemical screening tests that were conducted during the investigation. The phytochemical investigation of the methanolic extract revealed that carbohydrate, alkaloids, proteins and amino acids, saponins, glycosides, fat and oils, and flavonoids were present in the extract. Many people are interested in flavonoids because of the positive effects they may have on health. Many flavonoids have shown biological and pharmacological characteristics in recent years, including antibacterial, anti-inflammatory, antioxidant, and anti-tumor actions linked to their ability to scavenge free radicals. Some studies have shown that flavonoids can help lower blood sugar levels and even prevent diabetes. Flavonoids lower the chance of developing some malignancies and contain antioxidant properties that protect cells from oxidative damage.[18-20]

Table 1: Results of Phytochemical Screening Tests of Extract.

S.NO	Phytochemicals	Phytochemical tests	Methanol Extract
1	**Alkaloids**	a) Mayer test	+++
		b) Wanger test	++
2	**Carbohydrates**	a) Molish test	++
		b) Fehling test	++
3	**Proteins&Amino acids**	a) Biuret test	-
		b) Ninhydrin test	-
4	**Flavonoids**	a) Shinoda test	+++
		b) Lead acetate test	+++
5	**Glycosides**	a) Legal Test	++
		b) Killer Killan test	+++
6	**Phenols &Tannins**	Ferric chloride test	+++
7	**Terpenoids**	Salkowski test	++
8	**Saponins**	Foam test	++
9	**Fat &oils**	Spot test	++

The expectorant and cardiotonic effects of saponins are due to their glycosidic composition. The hypoglycemic and anti-diabetic properties of saponins have also been reported, making them valuable in the therapy of diabetes mellitus. It is well-known that tannins help alleviate inflammation and ulcers, and they also exhibit extraordinary anti-cancer and cancer prevention properties.[21] The results indicate that *Vitex negundo* shows promise as a source of pharmaceutically significant phytochemicals.

Fourier-transform infrared (FTIR)

Methanol extract of *plant* leaves exhibited a characteristic band at 2938.84 cm^{-1} indicating the presence of alkane (C-H stretch). The band at 3317.08 cm^{-1} might be due to dimer O-H of carboxylic acid RCO-OH, dimer O-H of carboxylic acid C=C-CO-OH, N-H stretch (H-bond) of amines R_2NH, ArO-H (H-bonded) of phenols. The bands at 2938.84 cm^{-1} and 2852.72 cm^{-1} were due to C-H stretch of alkane ($–CH_3$, $-CH_2-$). The band at 2114.96 cm^{-1} referred to C≡C stretch of alkynes, N=C in R-N=C=S, Si-H of silane. The band at 1639.63 cm^{-1} referred to C=C stretch of 3-ring alkenes, N-H out of plane of amides $RCONH_2$, C=O stretch of amides RCONHR' 6-ring, NH2 in plane bend of amines RNH_2. The band at 1608.54 cm^{-1} referred to C=C stretch of 5-ring alkenes, N-H out of plane of amides $RCONH_2$, NH2 in plane bend of amines RNH_2, C-O stretch of carboxylic acid RCO-O-. The band at 1515 cm^{-1} assigned to N-O of aromatic nitro compounds, N=O nitroso, N-O asymmetric nitro compounds. The bands at 1446.04 cm^{-1} and 1407.51 cm^{-1} assigned to S=O of sulfate ester, ArC-C stretch in ring. The band at 1363.75 cm^{-1} was due to S=O of sulfonyl chloride, S=O of sulfate ester, C-H rock of alkane.

The band at 1306.61 cm^{-1} referred to C-F stretch of alkyl halides, C-O stretch of carboxylic acid RCOOH, C-O stretch of ester. The band at 1269.01 cm^{-1} referred to C-F stretch of alkyl halides, C-N stretch of amines, P=O of phosphoramide, N-O of aromatic amine oxide, C-O stretch of carboxylic acid RCOOH, C-O stretch of ester, C-H wag of alkyl halide ($-CH_2-X$). The band at 1166.75 cm^{-1} assigned to C-F stretch of alkyl halide, C-N stretch of amines (RNH_2, R_2NH), C=S of thiocarbonyl, S=O of sulfonyl chloride, P-H bending of phosphine, P=O of phosphine oxides, P=O of phosphate, C-O stretch of carboxylic acid, C-O stretch of ester RCOOR'. The band at 1014.20 cm^{-1} was due to C-F stretch of alkyl halide, P-H bending of phosphine, P-OR of ester, Si-OR, C-O stretch of carboxylic acid RCO-OH, C-H stretch of ester RCOOR'.

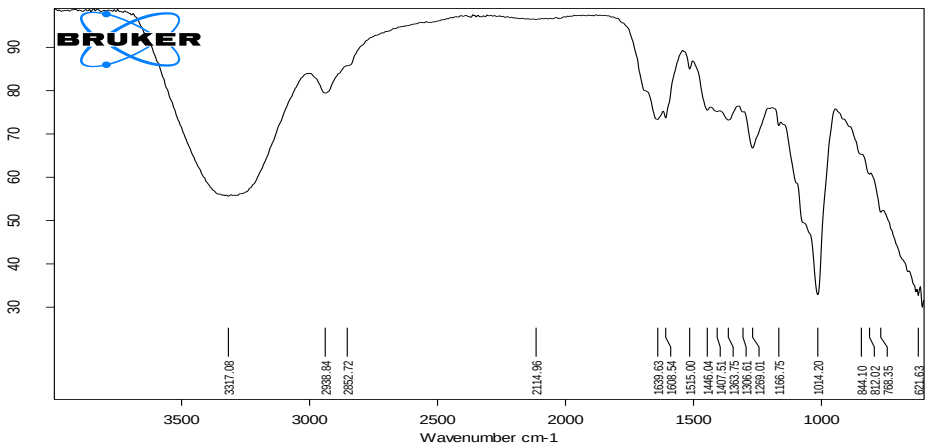

Figure 2: Fourier-transform infrared (FTIR) spectrum of methanol extract.

Gas Chromatography-Mass Spectrometry (GC-MS)

A new medicine can be derived from a medicinal plant. Medicinal plants are an indirect source for many of today's pharmaceuticals. In the battle against sickness, they have provided numerous components. Developing, updating, and controlling the quality of herbal formulations rely heavily on the examination and extraction of plant material. Protecting humans and other animals from naturally occurring toxins is another important goal of medicinal plant research. Therefore, the purpose of this work was to use gas chromatography and mass spectrometry to identify the bioactive components in methanolic extract of *Vitex negundo* leaves. There are thirty two bioactive phytochemical compounds in the methanolic extract of *Vitex negundo* leaves; Table 2 and Figure 3 indicate the active principles along with their retention time (RT), molecular formula, and concentration (peak area %).

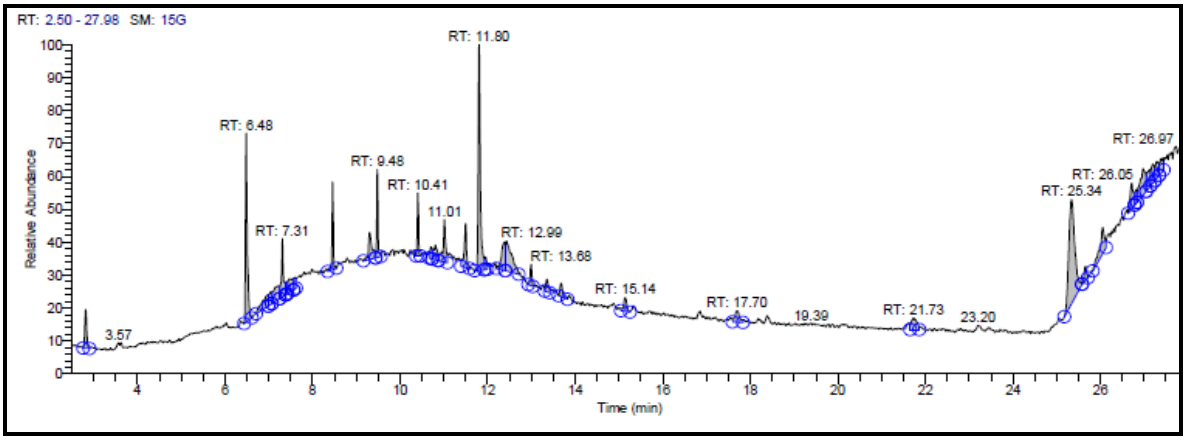

Figure 3: GC-MS chromatogram of methanolic extract of *Vitex negundo* leaves.

Table 2: GC-MS spectral analysis of methanolic extract of Vitex negundo leaves

S.NO	RT min	Name	Molecular Formula	Peak area %
1	2.82	2,2-Dimethoxybutane	$C_6H_{14}O_2$	1.96
		2-Methoxy-3-methyl-butyric acid, methyl ester	$C_7H_{14}O_3$	
		2-[2-[2-[2-(2-Butoxyethoxy) ethoxy]ethoxy]ethoxy]ethyl acetate	$C_{16}H_{32}O_7$	
2	7.48	Tetra-N-butylammonium chloride	$C_{16}H_{36}ClN$	8.66
		Tetrabutylammonium dichlorobromate	$C_{16}H_{36}BrCl_2N$	
3	6.97	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	2.03
		Desulphosinigrin	$C_{10}H_{17}NO_6S$	
		Gentamicin a	$C_{18}H_{36}N_4O_{10}$	
4	7.04	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	1.02
5	7.15	l-Gala-lido-octose	$C_8H_{16}O_8$	2.24
6	7.31	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	3.04
7	7.47	D-Streptamine, O-2-amino-2-deoxy-à-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-Cmethyl-3-(methylamino)-á-Larabinopyranosyl-(1-6))-2-deoxy	$C_{19}H_{38}N_4O_{10}$	1.69
8	7.59	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	$C_9H_{11}NO_4$	0.66
9	8.46	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	2.84
10	9.30	Diethyl Phthalate	$C_{12}H_{14}O_4$	2.51
		Phthalic acid, ethyl isoporpyl ester	$C_{13}H_{16}O_4$	
11	9.48	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	2.91
12	10.41	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	1.97
13	10.71	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyloxime)	$C_{23}H_{36}N_2O_4$	0.62
14	10.81	Z-10-Methyl-11-tetradecen-1-ol propionate	$C_{18}H_{34}O_2$	1.45
15	11.01	Di-sec-butyl phthalate	$C_{16}H_{22}O_4$	2.94
16	11.50	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6Si_7$	2.70
17	11.80	Phthalic acid, butyl 4-octyl ester	$C_{20}H_{30}O_4$	12.20
18	11.95	Pyrazole[4,5-b] imidazole, 1-formyl-3-ethyl-6-á-d-ribofuranosyl-	$C_{12}H_{16}N_4O_5$	0.63

19	12.38	N-Benzoyl-N-phenylhydroxylamine	$C_{13}H_{11}NO_2$	2.69
20	12.43	1-Benzoyloxy-2-formyloxybenzene	$C_{14}H_{10}O_4$	4.83
21	12.99	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	0.90
22	13.36	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans	$C_{28}H_{44}O_4$	0.81
23	13.68	Pregan-20-one, 2-hydroxy-5,6-epoxy-15 methyl-	$C_{22}H_{34}O_3$	0.98
24	15.14	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	1.12
25	17.70	Milbemycin b,13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)-	$C_{33}H_{46}ClNO_7$	1.41
26	21.73	Tetrahydroaraucarolone	$C_{20}H_{34}O_4$	1.62
27	25.34	Spiro[4.5]decan-7-one, 1,8-dimethy-l8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	16.21
28	25.65	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	0.92
29	26.05	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	3.14
30	26.71	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	2.28
31	26.81	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	$C_{27}H_{44}O_3$	0.65
32	26.97	Androstan-3,11,17-trione, 9-chloro-	$C_{19}H_{25}ClO_3$	3.78

Among the identified phytocompounds, Desulphosinigrin have been reported to have antibacterial, antiasthmatic, and anticancer properties of DES. It is also known as an inhibitor of Cyclin-dependent kinases and urease enzymes.[22] Gentamicin a have been reported as antibiotic used to treat several types of bacterial infections[23]; [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (antibacterial activity[24]); Spiro[4.5]decan-7-one, 1,8-dimethy-l8,9-epoxy-4-isopropyl- (anti-inflammatory activity[25]); 1-Monolinoleoylglycerol trimethylsilyl ether (antimicrobial, antioxidant, antiinflammatory, antiarthritic, antiasthma, diuretic[26]); Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- (Anti-microbial and anti-tumor.[27])

Liquid Chromatography–Mass Spectrometry (LC-MS)

The chemical composition of the active extract was determined using high resolution liquid chromatography mass spectrometry (HR-LC-MS). This technique was used to separate and identify phytoconstituents based on retention duration, database difference (library), experimental mass spectrometry, MS/MS fragments, metabolite class, and suggested compounds. Ninety phytoconstituents were identified in both modes (positive and negative ionization mode) table 3 & 4, figures 4& 5. The important phytoconstituents confirmed by (HR)-LCMS Analysis were Peganine (quinazolines), Riddelline (pyrrolizidine alkaloid), Apigenin 7-glucoside (flavonoid), Quercetin 3-(3-p-coumaroylglucoside) (flavonoid-3-o-glycosides), 3,5-Dihydroxyphenyl 1- O-(6-O-galloyl-beta-D-glucopyranoside) (phenolic glycosides), Tryptophyl-Phenylalanine (dipeptides), Diosmetin 7-O-beta-D-glucuronopyranoside (flavonoid-7-o-glycosides), Gardenin B (flavonoid), Glucosyloxyanthraquinone (monosaccharide), 2'-Hydroxy-3,4',5',7,8-pentamethoxyflavone (flavonoid), Atractylone (sesquiterpenoid), Chryso-obtusin (anthraquinone derivative), Myxalamid C (fatty amide), O-Methylsomniferine (morphinane alkaloid), Ganoderic acid F (triterpenoid), Physagulin C (withanolides), Ganosporelactone A (withanolides and derivatives), Sanguisorbic acid dilactone (hydrolyzable tannin), Hibiscitrin (flavonoid-3-o-glycosides), Amentoflavone (biflavonoid), Estriol 3-sulfate 16- glucuronide (steroidal glycosides), Kaempferol 3-xylosylglucoside (flavonoid-3-o-glycosides), Macrocarpal E (sesquiterpenoid). There are other plants that contain such compounds, and they have a variety of pharmacological effects. Among them, Apigenin 7-glucoside flavonoids have been reported to have antioxidant and anti-inflammatory activity[28], Diosmetin 7-O-beta-D-glucuronopyranoside shows antioxidant activity[29], Atractylone compound found in *Atractylodes lancea* (Thunb.) DC plant has shown anti-inflammatory and anti-hepatotoxic effects[30], ganoderic acid F has exhibited antitumor and antimetastatic activities[31], Isochamaejasmin used in traditional Chinese medicine (TCM) to treat tumors, tuberculosis, and psoriasis.[32]

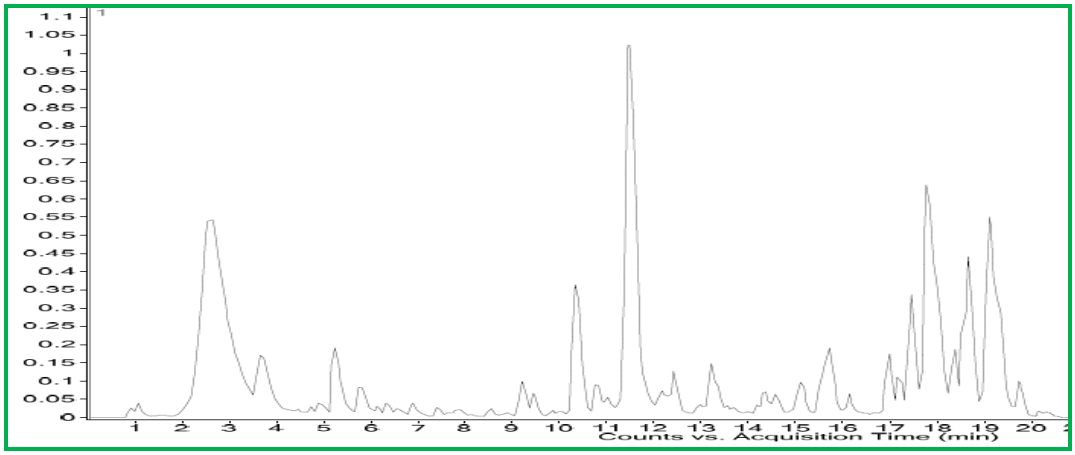

Figure 4: HR-LCMS chromatogram of extract, positive ionization mode.

Table 3: List of phytochemicals identified from methanolic extract of leaves of *Vitex negundo* using HR-LC-MS, positive ionization mode.

S.NO	NAME OF COMPOUND	MOLECULAR FORMULA	RT min	NOLE. MASS	m/z
1	Peganine	$C_{11}H_{12}N_2O$	2.112	188.09	189.1
2	cis-3-Hexenyl 2- aminobenzoate	$C_{13}H_{17}NO_2$	3.457	219.12	220.13
3	Primidone	$C_{12}H_{14}N_2O_2$	3.672	218.10	219.11
4	Riddelline	$C_{18}H_{23}NO_6$	4.529	349.15	372.14
5	(E)-10-Hydroxy-2- decene-4,6-diynoic acid	$C_{10}H_{10}O_3$	4.921	178.06	179.07
6	6-C-Galactosylluteolin	$C_{21}H_{20}O_{11}$	5.187	448.10	449.1055
7	Khellol glucoside	$C_{19}H_{20}O_{10}$	5.262	408.11	431.09
8	3-(4-Hydroxy-3-methoxyphenyl)-1,2- propanediol 2-O- (galloyl-glucoside)	$C_{23}H_{28}O_{13}$	5.477	512.15	535.14
9	Apigenin 7-glucoside	$C_{21}H_{20}O_{10}$	5.81	432.10	433.11
10	Hexyl 2-furoate	$C_{11}H_{16}O_3$	6.15	196.11	197.12
11	Cynometrine	$C_{16}H_{19}N_3O_2$	6.343	285.15	308.14
12	Quercetin 3-(3-p-coumaroylglucoside)	$C_{30}H_{26}O_{14}$	6.606	610.13	611.14
13	3,5-Dihydroxyphenyl 1- O-(6-O-galloyl-beta-D-glucopyranoside)	$C_{19}H_{20}O_{12}$	6.85	440.10	463.09
14	Tryptophyl- Phenylalanine	$C_{20}H_{21}N_3O_3$	7.09	351.16	352.16
15	trans-Lachnophyllol	$C_{10}H_{12}O$	7.41	148.09	149.09
16	Diosmetin 7-O-beta-D-glucuronopyranoside	$C_{22}H_{20}O_{12}$	7.74	476.09	477.09
17	9Z-Octadecen-12- ynoic acid	$C_{18}H_{30}O_2$	8.57	278.23	301.22
18	p-Coumaroyl quinic acid	$C_{16}H_{18}O_8$	9.22	338.10	361.10
19	3-tert-Butyl-5- methylcatechol	$C_{11}H_{16}O_2$	9.44	180.11	181.12
20	Myricetin 3,7,3',4'- tetramethyl ether	$C_{19}H_{18}O_8$	10.27	374.10	375.12
21	1-Peroxyferolide	$C_{17}H_{22}O_7$	10.77	338.14	361.13
22	Gardenin B	$C_{19}H_{18}O_7$	10.97	358.10	359.11
	Mycinamicin VII	$C_{29}H_{47}NO_7$	11.25	521.33	522.34
24	Glucosyloxyanthraquinone	$C_{20}H_{18}O_8$	11.381	386.10	387.11
25	2'-Hydroxy-3,4',5',7,8-pentamethoxyflavone	$C_{20}H_{20}O_8$	11.382	388.12	389.12
26	Mitoxantrone	$C_{22}H_{28}N_4O_6$	11.756	444.20	445.21
27	7-beta-D-Glucopyranosyloxybutylidenephthalide	$C_{18}H_{22}O_8$	11.918	366.13	389.12
28	6- Oxabicyclo[3.1.0]hexa ne-2-undecanoic acid methyl ester	$C_{17}H_{30}O_3$	12.275	282.22	305.21

29	Atractylone	$C_{15}H_{20}O$	12.277	216.15	217.16
30	Chryso-obtusin	$C_{19}H_{18}O_7$	12.37	358.10	359.11
31	17beta-Hydroxy-2-methylandrost-1,4- dien-3-one	$C_{20}H_{28}O_2$	13.098	300.21	301.22
32	Kanamycin	$C_{18}H_{36}N_4O_{11}$	13.222	484.24	507.22
33	3b,17a,21-Trihydroxypregnenone	$C_{21}H_{32}O_4$	13.229	348.23	349.24
64	Myxalamid C	$C_{24}H_{37}NO_3$	13.548	387.27	388.28
35	(-)-Abietadiene	$C_{20}H_{32}$	14.338	272.25	273.26
36	Gentamicin C2	$C_{20}H_{41}N_5O_7$	14.509	463.29	464.30
37	9E-Heptadecenoic acid	$C_{17}H_{32}O_2$	15.088	268.24	291.23
38	8,11-Heptadecadienal	$C_{17}H_{30}O$	15.618	250.23	273.22
39	23- Acetoxysoladulcidine	$C_{29}H_{47}NO_4$	15.937	473.35	496.34
40	6,10,14-Trimethyl- 5,9,13-pentadecatrien- 2-one	$C_{18}H_{30}O$	16.317	262.23	263.24
41	Irinotecan	$C_{33}H_{38}N_4O_6$	17.005	586.28	609.27
42	O-Methylsomniferine	$C_{37}H_{38}N_2O_7$	17.601	620.26	621.27
43	y-Morphine	$C_{34}H_{36}N_2O_6$	17.632	568.27	591.26
44	Hematoporphyrin	$C_{34}H_{38}N_4O_6$	17.634	598.28	621.27
45	Ganoderic acid F	$C_{32}H_{42}O_9$	17.778	570.28	593.27
46	Demethyl-desacetyl-rifamycin S	$C_{34}H_{41}NO_{11}$	17.797	638.27	639.28
47	Physagulin C	$C_{30}H_{38}O_9$	18.27	542.25	565.24
	Pyropheophorbide a	$C_{33}H_{34}N_4O_3$	18.317	534.26	535.27
49	Ganosporelactone A	$C_{30}H_{40}O_7$	18.608	512.28	535.27
50	Aralionine A	$C_{34}H_{38}N_4O_5$	18.922	582.29	605.27
51	Rifamycin W-hemiacetal	$C_{35}H_{43}NO_{11}$	18.99	652.29	653.29
52	Euphornin	$C_{33}H_{44}O_9$	19.052	584.30	607.29
53	C-13(2)- Carboxypyropheophorbide a	$C_{34}H_{34}N_4O_5$	19.679	578.25	579.26
54	Antimycin A1	$C_{28}H_{40}N_2O_9$	19.741	548.28	549.28

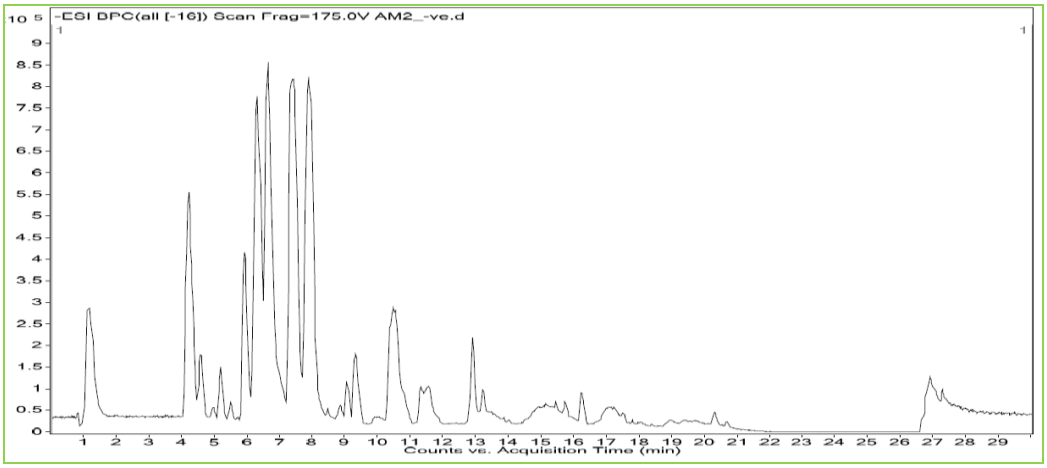

Figure 5: HR-LCMS chromatogram of extract, negative ionization mode.

Table 4: List of phytochemicals identified from methanolic extract of leaves of *Vitex negundo* using HR-LC-MS, negative ionization mode.

S. NO	NAME OF COMPOUND	MOLECULAR FORMULA	RT	NOLE. MASS	m/z
1	4-Mercapto-5-methyl- 3(2H)-thiophenone	$C_5H_6OS_2$	1.416	145.99	190.98
2	(E)-S-1-Propenyl thiosulfate	$C_3H_6O_3S_2$	2.806	153.98	152.97
3	Imazosulfuron	$C_{14}H_{13}ClN_6O_5S$	3.29	412.04	411.03
4	UDP-4-dehydro-6- deoxy-D-glucose	$C_{15}H_{22}N_2O_{16}P_2$	3.695	548.04	547.04
5	Halosulfuron-methyl	$C_{13}H_{15}ClN_6O_7S$	4.493	434.04	493.05
6	Sanguisorbic acid dilactone	$C_{21}H_{10}O_{13}$	4.548	470.01	529.03
7	Hibiscitrin	$C_{21}H_{20}O_{14}$	4.97	496.08	495.07
8	dTDP / Thymidine 5'- diphosphate	$C_{10}H_{16}N_2O_{11}P_2$	5.218	402.02	447.02

9	Fomesafen	$C_{15}H_{10}ClF_3N_2O_6S$	5.402	437.99	482.99
10	4,4',6,6'-Tetranitro- 2,2'-azoxytoluene	$C_{14}H_{10}N_6O_9$	5.506	406.05	465.062
11	6-(Dibromomethylene)- 17beta-hydroxyandrost-4-en-3- one	$C_{20}H_{26}Br_2O_2$	5.51	456.04	501.04
12	N-Adenylyl-L-phenylalanine	$C_{19}H_{23}N_6O_8P$	5.747	494.13	539.13
13	Quazepam	$C_{17}H_{11}ClF_4N_2S$	5.798	386.03	431.02
14	Amentoflavone	$C_{30}H_{18}O_{10}$	5.801	538.09	537.08
15	Cemandil (O-Formylcefamandole)	$C_{19}H_{18}N_6O_6S_2$	6.103	490.07	535.06
16	3,3'-Bisanigorufone	$C_{38}H_{22}O_4$	6.148	542.15	541.15
17	Trisjuglone	$C_{30}H_{12}O_9$	6.254	516.04	515.04
18	Estriol 3-sulfate 16- glucuronide	$C_{24}H_{32}O_{12}S$	6.477	544.17	543.16
19	Kaempferol 3- xylosylglucoside	$C_{26}H_{28}O_{15}$	6.483	580.14	579.13
20	Myricetin 3-(6- acetylgalactoside)	$C_{23}H_{22}O_{14}$	7.07	522.10	521.09
21	dTDP-2,6-dideoxy-D-glycero-hex-2-enos-4- ulose	$C_{16}H_{22}N_2O_{14}P_2$	7.23	528.05	587.07
22	4,4"-bis(N-feruloyl)serotonin	$C_{40}H_{38}N_4O_8$	7.241	702.28	701.27
23	Taccalonolide	$C_{36}H_{46}O_{14}$	7.744	702.28	701.27
24	Isomethiozin	$C_{12}H_{20}N_4OS$	8.754	268.14	327.15
25	Methotrimeprazine	$C_{19}H_{24}N_2OS$	9.088	328.16	327.15
26	19- Hydroxydeacetylnomilinic acid 17-beta-D-glucopyranoside	$C_{32}H_{46}O_{16}$	9.23	686.29	685.28
27	Eucommin A	$C_{27}H_{34}O_{12}$	10.835	550.20	595.20
28	Nummularine F	$C_{23}H_{32}N_4O_4$	11.494	428.25	487.26
29	Dulciol A	$C_{28}H_{32}O_6$	11.498	464.22	523.24
30	Leucyl-Tyrosine	$C_{15}H_{22}N_2O_4$	13.036	294.16	293.15
31	Isochamaejasmin	$C_{30}H_{22}O_{10}$	13.229	542.11	541.11
32	Macrocarpal E	$C_{28}H_{40}O_6$	14.125	472.28	471.27
33	Prupaside	$C_{27}H_{36}O_{12}$	15.976	552.22	597.22
34	Nisoldipine	$C_{20}H_{24}N_2O_6$	16.329	388.16	433.16
35	Surinamensin	$C_{22}H_{28}O_6$	16.562	388.19	387.18
36	Methionyl-Alanine	$C_8H_{16}N_2O_3S$	17.253	220.09	265.09

Biological activity

With chronic disorders rising to the top of the global health care burden, it is crucial to approach traditional herbal medicinal plants with a scientifically sound perspective. A unique and balanced approach that covers as many targets as feasible at the same time with as many active principles is preferred.[33, 34] Not only that, but the Indian subcontinent is famous for its varied flora and fauna, and many people there still use age-old medical herbs for a wide range of ailments. Before this culture disappears along with biodiversity, it is crucial to record this distinct information about the ethnic group.[35. 36] The therapeutic potential of many medicinal plants that have not been well studied in the literature has to be reestablished and validated through coordinated study.

Antibacterial activity

The antibacterial activities of the tested methanolic extract from the plant material in terms of the minimum inhibitory concentrations (MIC) is presented in Tables 5 & Figure 7. The extract displayed remarkable antibacterial potential against all tested bacteria. The extract showed high antibacterial activity against *S. aureus* exceeds the reference antibiotics Ampicillin.

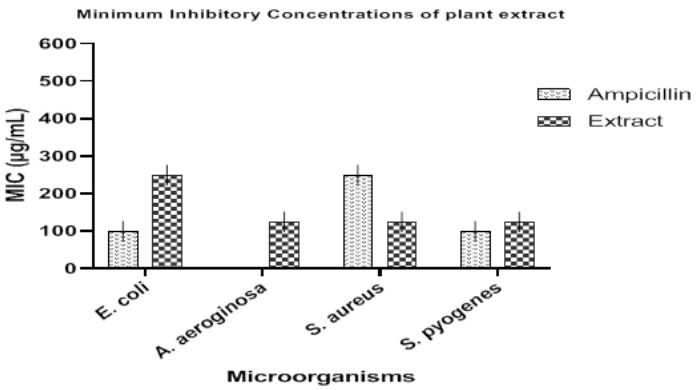

Figure 6: Minimum inhibitory concentration (MIC) (µg/mL) of extract.

Table 5: Minimum inhibitory concentration (MIC) (µg/mL) of extract.

Microorganisms	Ampicillin	Extract
E.coli	100	250
A.aeroginosa	-	125
S. aureus	250	125
S. pyogenes	100	125

Antioxidant activity

Vitex negundo leaf methanolic extract was evaluated for its antioxidant activity by comparing its DPPH free radical scavenging capacity to that of ascorbic acid. Figure 7 and Table 6 show the findings of comparing the plant extract's and the standard's DPPH radical scavenging capacity as half maximum inhibitory concentration (IC_{50}) values. Lower IC_{50} values indicate more effective DPPH radical scavenging. Based on the findings, the *Vitex negundo* methanolic extract exhibited notable DPPH activity, with an IC_{50} value of 3.03 µg mL^{-1}, in contrast to the 2.63 µg mL^{-1} found in the standard ascorbic acid.

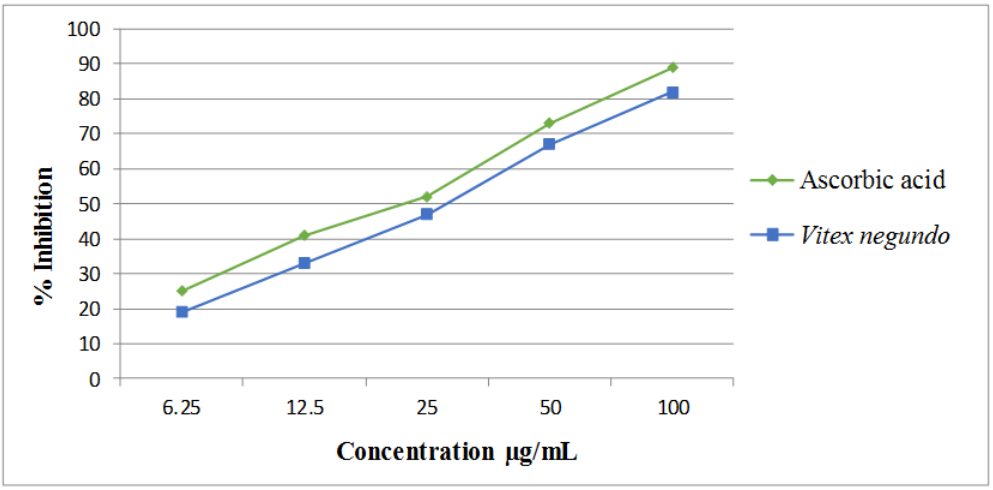

Figure 7: Percentage inhibitions of DPPH free radical activity of methanol extract of *Vitex negundo* leaves compared with standard antioxidant ascorbic acid.

Table 6: Percentage DPPH free radical scavenging activity of *Vitex negundo* leaves methanolic extract.

Concentration (µg/mL)	Ascorbic acid	*Vitex negundo*
6.25	25	19
12.5	41	33
25	52	47
50	73	67
100	89	82
IC_{50} (µg/mL)	2.63	3.03

CONCLUSION

The results of the current research showed that the leaves of Vitex negundo contain a wide variety of phytochemicals, several of which may be responsible for the putative qualities of the extract. In addition, the extract of the plant possesses actions that are both antioxidant and antibacterial.

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REFERENCES

1. Chaachouay N, Zidane L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs Drug Candidates., 2024; 3: 184-207. https://doi.org/10.3390/ddc3010011.
2. Liheluka E, Gibore NS, Lusingu JPA, et al. Community perceptions on the effectiveness of herbal medicines and factors associated with their use in managing diarrhoea among under-five children in North-eastern Tanzania. Trop Med Health., 2023; 51(48). https://doi.org/10.1186/s41182-023-00537-5.
3. Nadine AOR, Ian CNR, Sannon JRR, et al. Folk Medicine in the Philippines: A Phenomenological Study of Health-Seeking Individuals. International Journal of Medical Students, 2021; 9(1).

4. Christian S and Bharat M. A Review: On Phytochemical Analysis and Antioxidant activities with Medicinal uses of Combretaceae Family. International Journal of Scientific Research in Science and Technology, 2021; 8(2): 287-294. https://doi.org/10.32628/IJSRST218244.
5. Shokon P, Anousheh ZK, Hamid RB, et al. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants., 2020; 9(12): 1309. https://doi: 10.3390/antiox9121309.
6. Shep SU, Rangini T. Review on NIRGUNDI (Vitex Negundo Linn.) Plant. International Journal of Research Publication and Reviews, 2023; 4(12): 1021-1026.
7. Shendage AS, Bhanwase MS, Borade KD, Awhad JA, Kadam KB. Phytochemical and Physiological Evaluation of Vitex Negundo. IJNRD, 2023; 8(12): d660-d666.
8. ABHIJEET VP. Duranta Repens Linn. (Verbenaceae): A Comprehensive Review of Pharmacognostic, Ethnomedicinal, Pharmacological, and Phytochemical Aspects. Asian J Pharm Clin Res., 2018; 11(11): 91-96.
9. Nurkhalida K, Nurul SMA, Ivana NAR, Muhammed AHMA, et al. Traditional Medicinal Uses, Phytochemistry, Biological Properties, and Health Applications of Vitex sp. Plants (Basel)., 2022; 11(15): 1944. doi: 10.3390/plants11151944.
10. AJILA RSS, SANTHAN NN, PRASOBH GR, SURABHI GS, JIJI MMU. An Overview on Vitex Negundo. International Journal of Humanities Social Science and Management., 2022; 1(1): 50-55.
11. Choudhary M, Kumar V, Malhotra H, Singh S. Medicinal plants with potential anti-arthritic activity. J Intercult Ethnopharmacol., 2015; 4(2): 147-79. doi: 10.5455/jice.20150313021918.
12. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). Maedica (Bucur), 2019; 14(4): 350-356. doi: 10.26574/maedica.2019.14.4.350.
13. Ali A, Arwa A, Inas A, Vidya P, Mazahar F. Phytochemical screening, GC-MS analysis, Molecular docking study and evaluation of antioxidant, antimicrobial activity of Sapindus emarginatus seed kernel. Research Journal of Pharmacy and Technology, 2022; 15(5): 2117-2121. 10.52711/0974-360X.2022.00351.
14. Ali A, Nabeel AA, Mohammed A, Arwa A, et al. 2022. An integrative GC–MS and LC–MS metabolomics platform determination of the metabolite profile of Bombax ceiba L. root, and in silico & in vitro evaluation of its antibacterial & antidiabetic activities. Natural Product Research. https://doi.org/10.1080/14786419.2022.2149519.
15. Ola B, Samreen F, Ali A, Mazahar F. Supercritical fluid extraction of Cichorium intybus (L) and it's characterization. Journal of Chemical and Pharmaceutical Sciences, 2016; 9(4): 2936-2944.
16. Samreen F, Ola B, Mazahar F, Pathan MA. Phytochemical, and physicochemical properties of Hibiscus rosa sinensis leaves extract: A comparison between conventional and Microwave assisted extraction. European Journal of biomedical and pharmaceutical sciences, 2018; 5(7): 551-559.
17. Sreejayan N, Rao MN. Free radical scavenging activity by curcuminoids. Arzneimittelforschung., 1996; 46: 169-71.
18. Parker L, Rimbach G, Virgili F. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus maritima) bark pycnogenol. Free Rad Biol Med., 1999; 27: 704–724.
19. Ahmad M, Aktar MS, Malik T, et al. Hypoglycemic action of the flavonoids fraction of Cuminum seeds. Phytotherap Res., 2000; 14(2): 103–106.
20. Willis JA, Scott RS, Brown LJ, et al. Islet cell antibodies and antibodies against glutamic acid decarboxylase in newly diagnosed adult-onset diabetes mellitus. Diabetes Res Clin Pract., 1996; 33: 89–97.
21. Ruch RJ, Cheng SJ, Klaunig JE: Prevention of cytotoxicity and inhibition of Intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogens, 1989; 10: 1003-1008.
22. Morteza S, Mehdi SK, Sattar G, Mahmoud V, Farhad MK, Parham T. α-glucosidase inhibitory, antioxidant activity, and GC/MS analysis of Descurainia sophia methanolic extract: In vitro, in vivo, and in silico studies. Arabian Journal of Chemistry, 2022; 15(9): 104055. https://doi.org/10.1016/j.arabjc.2022.104055.
23. Liu Y, Chang H, Li Z, et al. Biodegradation of gentamicin by bacterial consortia AMQD4 in synthetic medium and raw gentamicin sewage. Sci Rep., 2017; 7: 11004. https://doi.org/10.1038/s41598-017-11529-x.
24. Rajani S, Alok M and Amita V. GC-MS Analysis of Phytocomponents in, Pet Ether Fraction of Wrightia tinctoria Seed. Phcog J., 2015; 7(4): 249-253.
25. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (Zingiber officinale) roscoe and bioactive chemical analysis using Gas chromatography mass spectrum. Orient J Chem., 2016; 32(2): 817-837.
26. Parthipan B, Suky MGT, V.R. Mohan. GC-MS Analysis of Phytocomponents in Pleiospermium alatum (Wall. ex Wight & Arn.) Swingle, (Rutaceae). Journal of Pharmacognosy and Phytochemistry., 2015; 4(1): 216-222.
27. MOHANAD JK, GHAIDAA JM and IMAD HH. In vitro Antibacterial, Antifungal and Phytochemical Analysis of Methanolic Extract of Fruit Cassia fistula. Oriental Journal of Chemistry, 2016; 32(3): 1329-1346.
28. Wei Wg, Ru-Feng Y, Zhen J, Li-Min H, Rong S, Dan D, You-Zhi T. Efficiency comparison of apigenin-7-O-glucoside and trolox in antioxidative stress and anti-inflammatory properties. Journal of

Pharmacy and Pharmacology, 2020; 72(11): 1645-1656.
29. Huahua W, Xiaoyu Z, Yangyang L, Yunyun Z, Yingyu W, Yunru P, Yongfang D. Diosmetin-7-O-β-D-glucopyranoside suppresses endothelial–mesenchymal transformation through endoplasmic reticulum stress in cardiac fibrosis. Clinical and Experimental Pharmacology and Physiology, 2023; 50(10): 789-805.
30. Jun X, Fu P, Lei Y, Cheng P. Pharmacological effects of medicinal components of Atractylodes lancea (Thunb.) DC. Chin Med., 2018; 13: 59. doi:10.1186/s13020-018-0216-7.
31. Supanimit T, Sasinun S, Chadarat A, Natthakarn, Noppamas R and Chaichan S. Pharmacokinetics of Ganoderic Acids A and Fafter Oral Administration of Ling Zhi Preparation inHealthy Male Volunteers. Evidence-Based Complementary and Alternative Medicine, 2012; 2012, 780892: 1-7. doi:10.1155/2012/780892.
32. Shou-De Z, Lei S, Wei L, Hong-Lin L, Wei-Dong Z. Isochamaejasmin induces apoptosis in leukemia cells through inhibiting Bcl-2 family proteins. Chinese Journal of Natural Medicines, 2015; 13(9): 660-666.
33. Fischer CL, Drake DRDawson DVBlanchette DR, B rogden KA, Wertz PW. Antibacterial Activity of Sphingoid Bases and Fatty Acids against Gram-Positive and Gram-Negative Bacteria. Antimicrob Agents Chemother., 2012; 56. https://doi.org/10.1128/aac.05151-11
34. Ali A, Nabeel AA, Mohammed A, et al. 2022. Martynia annua safety and efficacy: heavy metal profile, in silico and in vitro approaches on antibacterial and antidiabetic activities. Natural Product Research. https://doi.org/10.1080/14786419.2022.2097227
35. Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP . Fatty Acids and Derivatives as Antimicrobial Agents. Antimicrob Agents Chemother., 1972; 2(1). https://doi.org/10.1128/aac.2.1.23.
36. Ali A, Ola B, Mazahar F. Gas chromatography-mass spectrometry analysis, Inductively coupled plasma mass spectrometry investigation, and antimicrobial screening of Caesalpinia bonducella (L.) roxb seed kernel. Asian Journal of Pharmaceutical and Clinical Research, 2019; 12(4): 315-319. 10.22159/ajpcr.2019.v12i4.32067.