

ORIGINAL ARTICLE

Solvent Specific Variations in Secondary Metabolites of *Mucuna nivea* (Roxb). DC. Revealed by GC-MS Analysis

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ABSTRACT

Mucuna nivea (Roxb.) DC. is a rare, wild, and underutilized leguminous climber plant from the family Fabaceae, it recognized for its significant medicinal and nutritional potential. Traditionally, it has been extensively used in ancient healthcare systems such as Ayurveda for the management of various ailments. The present study focuses on the qualitative phytochemical screening and Gas Chromatography Mass Spectrometry (GC-MS) analysis of leaves and seeds of *Mucuna nivea* using five different solvents. Preliminary phytochemical evaluation revealed the presence of important secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, steroids, and terpenoids. GC-MS profiling identified twenty bioactive compounds in the leaves and twenty-two in the seeds, with their occurrence varying depending on the solvent used. Previous studies have reported that *Mucuna nivea* exhibits a wide range of pharmacological properties, such as antioxidant, antifungal, antimicrobial, antimalarial, antidiabetic, anticancer, and hypocholesterolemic activities. The presence of these bioactive constituents highlights the plant's nutritional importance and provides scientific support for its traditional use by local healers and tribal communities, particularly in the treatment of various diseases using leaves and seeds.

Keywords: Analysis, Extractions, Medicinal, *Mucuna nivea*, Phytochemicals and Solvents.

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INTRODUCTION

The conventional healthcare system makes use of native plants as traditional remedies to treat many kinds of illnesses and disorders. Chemical elements extracted from natural sources have grown in popularity recently due to their many pharmacological advantages [1]. Researchers are interested in phytoconstituents produced from natural sources because of their possible applications in therapeutics [2]. Huge numbers of synthetic drugs have been studied recently, but traditional treatments gaining their preference. Moreover, plants are one of the important sources for essential secondary metabolites due to structural complexity, as chemical synthesis is not a feasible substitute [3]. However, due to their accessibility and possible advantages, alternative eco-friendly herbal medications are receiving a lot of attention [1, 4, 5]. Plant-derived phytoconstituents are classified into two types, e.g., the primary and secondary metabolites, based on their functions and characteristics. For plants to grow and develop, the fundamental metabolites; amino acids, proteins, lipids, carbohydrates, and other macromolecules are essential [5]. Whereas secondary metabolites are important components of plant defence mechanisms

against biotic and abiotic stressors, including alkaloids, flavonoids, phenolic compounds, essential oils, terpenoids, tannins, saponins, and cardiac glycosides [6]. Furthermore, these primary phytoconstituent act as a vital source of nutrition for human growth and development, and secondary metabolites playing an important role in human medicine, flavourings, and relaxing agents [3, 7, 8]. While the demand for food is rising daily because of ever-growing universal population. Wild plants are now essential as an origin of food and medicine to adequately satisfy the world's food demands [9]. In addition to being a rich source of nutrients, biotic and abiotic issues, wild plants are stronger than cultivated ones [5]. Furthermore, there is a long history of using these plants as food and nutraceuticals. Notably, their use in conjunction with farmed crop plants aids in meeting the world's needs for nutrient-rich food and medicine. *Mucuna nivea* (family Fabaceae) is an important tropical medicinal plant utilized in ayurveda medicine and other traditional medical systems across the globe because of its existence of diverse metabolites in leaves and seeds [1, 10, 11]. It is commonly known as kahj-khujli and cowitch (Figure 1) [12]. There are 100 species of climbing vines and shrubs in the genus *Mucuna*, most of which are found in tropical regions, particularly in tropical Africa, the Caribbean, and India [1, 13]. This plant has important uses, including antibacterial, inflammatory treatment, and flavouring agent for cakes, sweet breads, and candies [14, 15]. Because *Mucuna* seeds have a high content of L-DOPA, the pharmaceutical industry uses *Mucuna* extensively. [13]. It is also a very good source of nutrients [16]. This plant is also utilized in the treatment of neurodegenerative diseases like Parkinsonian disease [17]. Several metabolites from *M. nivea* have intriguing uses in medicine and are primarily linked to antibacterial properties as well as antioxidant properties in different parts of plants. Thus, the goal of this study is to identify the metabolites from the leaves and seeds of *Mucuna nivea*, a wild, underappreciated, tropically significant legume plant, and to determine whether these metabolites have any potential medical use.



Figure 1: Flowering twig (A), Mature pods (B), and (C) Harvested mature seeds of *M. nivea*.

MATERIAL AND METHODS

Plant Material Collection

The leaves and seeds of *Mucuna nivea* (Roxb.) DC. were obtained from the S. G. B. Amravati University campus (Figure 1). In accordance with the phonological calendar, a regular field protocol for collecting plant samples was followed.

Identification of plant material

Using standard flora, such as the Flora of Maharashtra and the local regional flora like Flora of Amravati District, the plant samples were identified [18, 19].

Sample preparation

Fresh leaves and dried pods of *Mucuna nivea* were harvested. It was taken care to extract the seeds from the dry pods without gathering any immature or infected seeds. The healthy, fresh seeds were cleaned and cut into small pieces then crushed in an electric grinder. Liquid nitrogen was utilized to compress freshly chopped leaves and further ground seeds. The prepared leaves and seed powder were refrigerated for later use in an airtight plastic container.

Extraction

Using Soxhlet extraction apparatus, 10 g of leaves and seed powder were placed within a thimble made up of filter paper. The leaves and seed powder were then extracted successively one at a time over the course of 24 hours using solvents such as petroleum ether, methanol, acetone, chloroform, and ethanol and in 180 ml. Every solvent's boiling point was maintained at the apparatus's temperature. Different solvents with distinct properties were used for the extractions, arranged in increasing order of polarity. To get a free and transparent extract, following extraction, the materials were passed through Whatman filter paper number 42. These extracts were concentrated to 10 ml by evaporating them. The resulting 10 ml extract was re-filtered before being kept in tiny, sterile, sealed vials in the refrigerator at 4 °C.

Preliminary phytochemical screening

Preliminary phytochemical tests were performed using the standard protocol for all extracts to confirm the presence of many chemical categories, including steroids, alkaloids, glycosides, phenols, tannins, saponins, and terpenoids, in plant parts extracted in solvents like water, petroleum ether, acetone, chloroform, ethanol, and methanol. [20, 21, 22]. For each chemical group, three replicative experiments were selected to confirm their existence in the solvent extract previously indicated. Mayer's reagents and Wagner reagents tests were used to identify alkaloids; ferric chloride and lead acetate tests were applied to confirm phenols; the Salkowski test was carried out to detect terpenoids and steroids; gelatine and lead acetate tests were used to screen tannins; alkaline and lead acetate tests were used to identify flavonoids; the killer-killani and sodium hydroxide tests were utilized to identify glycosides; and the froth and foam tests were applied to identify saponins. Plant samples that were extracted using various solvent are used for phytochemical assessment.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The plant extracts were analyzed at the Indian Institute of Technology Bombay, at Sophisticated Analytical Instrument Facility. GC-MS system based on Agilent Technologies 7880 is fitted with an HP-5 column (30 mm length, 0.25 mm I.D., and 0.32 μ film thickness) was utilized to analyze the aliquots (2 μ l). A 1 ml/min flow rate of helium gas was employed as the carrier. A temperature of 100 °C was selected for the injector. The oven's temperature was adjusted to range from 50 °C to 280 °C, with increments of 10 °C per minute to 200°C, 10°C per minute to 250°C, and a minute isothermal at 280°C. 50:1 split mode injection of the sample was used. The mass spectra (MS) of substances at 70 eV were obtained using the electron ionization and detector.

Identification of compounds

Based on the compounds' m/z values, retention times, and fragmentation patterns, they were identified. The MS value of known compounds stored in the National Institute of Standards and Technology library database were compared with the mass spectra of obtained result of plant samples via GC-MS to identify the unknown substance. The names, molecular weights, molecular formulas, and structures of the compounds were determined using the similarity percentage data. The relative amount of each compound was determined for quantitative analysis using the following formula [23].

RESULTS AND DISCUSSION

Phytochemical screening

Mucuna nivea leaf and seed extracts were subjected to qualitative phytochemical testing, which revealed a wide range of phytochemicals with varying properties depending on the solvent (Table 1 and 2). The phytoconstituents from each sample includes saponins, alkaloids, glycosides, tannins, flavonoids, phenols, steroids, and terpenoids, were identified by qualitative tests. However, in qualitative tests, the significant results were observed for flavonoids, phenols, tannins, saponins, and glycosides (Table 1). In addition to saponins, steroids, and terpenoids, other phytochemicals were also found in methanolic seed extracts as well as in ethanolic seeds extract are flavonoids, phenols, tannins, and glycosides. Similar research was done by Dhawan and Gupta [26], who compared the ability of several solvents to extract phytochemicals from *Datura metel* leaves. The plant's synthesized array of secondary metabolites is an important source for the food, nutraceuticals, and pharmaceutical industries [27, 28].

Similarly, in our study, all these phytochemicals (except phenols and glycosides) derived from leaves were recorded in aqueous extracts. Preliminary screening has revealed no alkaloids in the leaves extracted in acetone, chloroform, pet ether, or ethanol. On the other hand, acetone-extracted seed exhibits phenols, tannins, glycosides, terpenoids, and flavonoids (Table 2). Moreover, aqueous seeds extracted shows presence of tannins, saponins, flavonoids, phenols, and alkaloids. Petroleum ether and chloroform extracts of leaves did not contain the common secondary metabolites that were taken into consideration during the preliminary phytochemical screening; however, the presence of tannins, saponins, steroids, and terpenoids was found in the seeds. Out of all the investigated solvents, water, ethanol, and methanol solutions were shown to be the most effective for extracting secondary metabolites from leaves and seeds. Therefore, the preliminary phytochemical analysis could provide an idea about the potential application of *M. nivea* (leaves and seeds) in traditional and modern medicine.

Table1: Preliminary Phytochemical constituents in leaves of *Mucuna nivea*.

Phyto-Chemicals	Reagents (Tests)	Solvents					
		Pet. Ether	Chloroform	Acetone	Ethanol	Methanol	Water
Alkaloids	Mayers reagents	-	-	-	-	+	++
	Wagner reagent	-	-	-	-	+	++
Flavonoids	Alkaline reagent	-	+	+	+++	+++	++
	Lead acetate	-	+	+	++	+++	++
Phenols	Ferric chloride	+	+	+	++	+++	-
	Lead acetate	+	+	+	++	+++	++
Tannins	Gelatin	-	+	-	+++	+	++
	Lead acetate	-	+	-	+++	+	++
Saponins	Froth	+	+	-	++	-	+++
	Foam	+	+	-	++	-	+++
Glycosides	Sod. hydroxide	+	+	+	++	++	-
	Killer Killani	+	+	+	++	+++	-
Steroids	Salkowaski	-	-	+	+	++	+++
Terpenoids	Salkowaski	-	-	-	+	++	+++

(+++)= Highly present, (++) = moderately present, (+) = present, (-) =absent

Table 2: Preliminary Phytochemical constituents in seeds of *Mucuna nivea*.

Phyto-Chemicals	Reagents (Tests)	Solvents					
		Pet. Ether	Chloroform	Acetone	Ethanol	Methanol	Water
Alkaloids	Mayers reagents	-	-	-	+	+	++
	Wagnerreagent	-	-	-	+	+	++
Flavonoids	Alkaline reagent	-	-	+	++	++	++
	Lead acetate	-	-	+	++	+++	++
Phenols	Ferric chloride	-	-	+	+++	+++	++
	Lead acetate	-	-	+	+++	+++	++
Tannins	Gelatin	+	-	+	++	+++	++
	Lead acetate	+	-	+	++	+++	+++
Saponins	Froth	+	+	-	-	-	+++
	Foam	+	+	-	-	-	+++
Glycosides	Sod. hydroxide	-	-	+	+++	++	-
	Killer Killani	-	-	+	+++	++	-
Steroids	Salkowaski	+	+	+	-	-	-
Terpenoids	Salkowaski	+	+	+	-	-	-

(+++)= Highly present, (++) = moderately present, (+) = present, (-) =absent

GC-MS analysis

Utilizing the National Institute of Standards and Technology database, the mass spectrum derived from GC-MS analysis was examined. [23]. The NIST library's spectrum of known components was used to match the mass spectra of observed unknown substances. Tables 3 and 4 exhibit the bioactive compounds together with their chromatograms in Figures 2 and 3, as well as their biological activity, molecular formula, molecular weight (MW), retention time (RT), and relative percentage (%). From the results, it was found that the phytoconstituents of leaves (3,7,11,15 Tetramethyl 2 hexadecen 1-ol; Phytol; Oxirrane;heptadecyl; 1-Octacosanol; Stigmasterol; Azulene; Pentanoic acid,5-hydroxy-,2,4-di-t-butylphenyl ester; Dibutyl phthalate; 2-Cyclohexen-1-one,3,5-dimethyl; 1-Piperidineethanamine; 1,2Benzenedicarboxylic acid, mono(2-ethylhexyl) ester; Piperidine,1-methyl 5-Azauracil; 3.7.11.15-Tetramethyl-2-hexadecen-1-ol; 2,2,6,6 – Tetramethyl 4-piperidone; Naphthalene; Benzenepropanoic acid 3,5-bis (1,1-dimethylethyl) -4-hydroxy-,methyl ester;Tetracosamethyl cyclo-do-decasiloxane) were extracted in five different solvent systems (Figure 2 and Table 3).

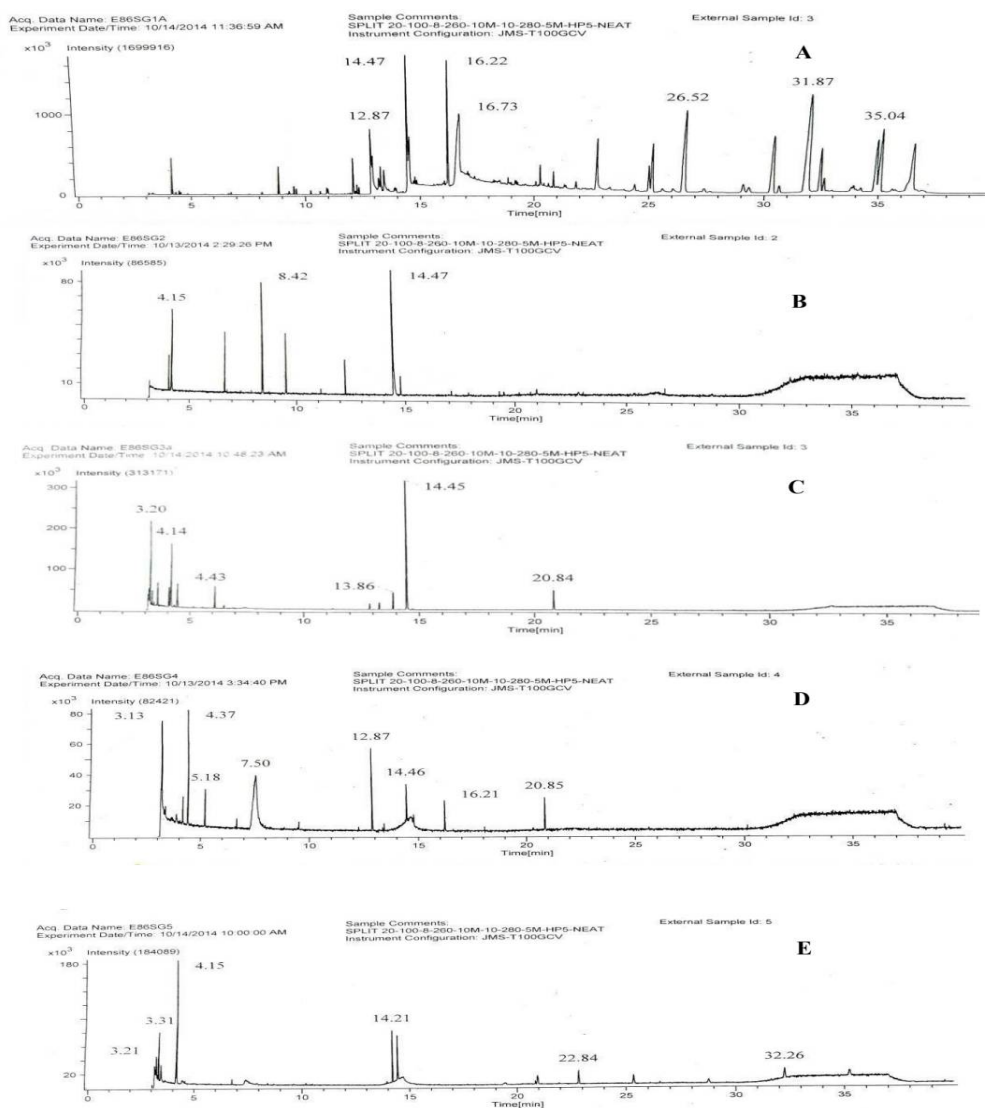


Figure 2: GC-MS results of leaves in Petroleum ether (A), chloroform (B), acetone (C), ethanol (D), and methanol (E) extracts

Leaves taken out of petroleum ether showed 5.19% of 3,7,11,15 Tetramethyl 2 hexadecen-1-ol and 10.23% phytol compounds have antimicrobial and antioxidant potential, as reported by Nisa et al. [29] from *Rumex dentatus*. Azulene (15.71%), Pentanoic acid, 5 hydroxy, 2, 4 di-t-butylphenyl ester (20.03%), and Dibutyl phthalate (29.83%) compounds were there in the chloroform extract of leaves of *Mucuna nivea* and showed antimicrobial, antibacterial, and antioxidant potential, as per an earlier investigation of black pepper extracted in chloroform [30]. Similarly, 2-Cyclohexen-1-one,3,5-dimethyl (26.95%), 1-Piperidineethanamine (5.75%), and 1,2Benzenedicarboxylic acid and mono(2-ethylhexyl) ester (5.50%) compounds were extracted in acetone from the leaves of *Mucuna nivea* with antioxidant, antimicrobial, antiviral, anticancer, and antidiabetic potential, as earlier reported in *Tephrosia bracteolata* leaf extract [31]. In the present investigation, secondary metabolites like Piperidine,1-methyl (12.63%), 5-Azaauracil (4.02%), 3,7,11,15-Tetramethyl 2 hexadecen-1-ol (8.73%), and phytol (3.21%) were identified in ethanolic extracts of the leaves of *M. nivea* and possessed potential antioxidant, antimicrobial effects according to previous literature. Naphthalene (29%) was identified in the methanol leaf extract of *Mucuna nivea* with antimicrobial qualities, as mentioned in *Ficus religiosa* by Aryal et al. [32]. Similarly, Tetra-cosam-ethyl cyclo-dodecasiloxane (3.95%) was identified in the methanol extract of the leaves of *M. nivea* as hepatoprotective, and anti-rheumatic activities were previously reported in the methanolic extract of *Amaranthus caudatus* [33] (Fig. 2 and Table 3). The GC-MS-analysed phytoconstituents in the seeds of *Mucuna nivea* are: Ethanone,1-(2-methylcyclopropyl); n-Hexadecanoic acid; 1-Dodecyne; 1-Hexacosene;

Azulene; Oxalic acid, allyl dodecyl ester; Oxalic acid, allyl tridecyl ester; Decanamide, N-(2-hydroxyethyl); Octanamide, N (2-hydroxyethyl); 2-Cyclohexen-1-one,3,5-dimethyl; Dibutyl phthalate; Decanamide, N-(2-hydroxyethyl); Propane,1,1,3-triethoxy; Propane,2-ethoxy; Pentadecanoic acid,2,6,10,14-tetramethyl-methyl ester;1,2 Benzene-dicarboxylic acid, mono (2-ethylhexyl) ester;2-Pentanone; 4-methoxy-4-methyl-Tridecanoic acid, methyl ester; Benzene-propanoic acid; 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester; and Octadecanoic acid, methy ester (Figure 3 and Table 4). The petroleum ether extract of seeds showed the presence of n-Hexadecanoic acid (39.38%), having antibacterial potential as earlier recorded in the GC-MS analysis of *Skimmia anquetilia* in the n-hexane extract of the root [34]. Likewise, Ethanone, 1-(2-methylcyclopropyl) (1.90%), and 1-Dodecyne (27.58%) compounds were obtained from petroleum ether extracts of seeds that were previously not screened for any potential biological activity. Similarly, Azuline (2.25%) was detected in the chloroform extract of the seeds of *Mucuna nivea*, which indicated potential antioxidant and antibacterial properties reported by Venkata Raman et al. [35].

2-Cyclohexen-1-one,3,5-dimethyl (16.96%) and Dibutyl phthalate (58.27%) compounds were obtained from acetone extracts of seeds of *M. nivea*, which was previously reported with antioxidant, antimicrobial, and antifungal activities [36]. Propane, 1, 1, 3-triethoxy (25.07%), Propane, 2-ethoxy (8.33%), Oxalic acid, allyl dodecyl ester (4.30%), Oxalic acid, allyl-tridecyl ester (4.48%), Pentadecanoic acid, 2, 6, 10, 14 tetramethyl-methyl ester (6.93%), and 1, 2-Benzene-dicarboxylic acid, mono (2 ethyl-hexyl) ester (2.78%) compounds were identified in ethanolic extract of seeds, performed an important role in therapeutic treatments as antimicrobial, antidiabetic, and anti-cancerous effects [37, 38]. The methanolic extract of seeds contains Benzenepropanoic acid, 3,5-bis (1,1-methylethyl)- 4 - hydroxy-methyl ester (2.94%), a compound with antioxidant and antifungal properties that had previously been identified in *Azadirachta indica* leaves extracted in hexane [38, 39]. Similarly, octadecanoic acid methyl ester (2.82%), which was obtained from the methanolic extract of *M. nivea* seeds, had antimicrobial and antibacterial activities, while tridecanoic acids methyl esters (3.51%) was reported to have antimicrobial and antioxidant potential [36]. Additionally, the methanolic extract of seeds showed presence of 2-Pentanone.4-methoxy-4-methyl- (75.65%) having antimicrobial properties [27] (Fig. 3 and Table 4).

Table 3: GC- MS Analysis of different solvent extract of *M. nivea* leaves with NIST library search.

S.N	RT	Phytochemicals	Rel. %	Class	MF	MW	Activity
Petroleum ether							
1	12.87	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5.19	Terpene alcohol	C ₂₀ H ₄₀ O	296	Antimicrobial, Antioxidant
2	16.21	Phytol	10.23	Diter pene	C ₂₀ H ₄₀ O	296	Antimicrobial, Antioxidant
3	30.33	Oxirrane,heptadecyl	4.63	Cyclic Ether	C ₁₉ H ₃₈ O	282	Antimicrobial, Adhesive
4	31.87	1-Octacosanol	7.69	Alcohol	C ₂₈ H ₅₈ O	410	Antiviral, Anti-inflammatory
5	35.03	Stigmasterol	5.17	Steroid	C ₂₉ H ₄₈ O	412	Antioxidant, Antiparkinsonism
Chloroform							
6	4.15	Azulene	15.71	Aromatic Hydro-carbon	C ₁₀ H ₈	128	Antioxidant, Antibacterial
7	8.42	Pentanoic acid,5 hydroxy ,2,4 di-t-butylphenyl ester	20.03	Carbo xylic acid	C ₁₉ H ₃₀ O ₃	306	Antibacterial,
8	14.47	Dibutyl phthalate	29.83	Aromatic Hydro-carbon	C ₁₆ H ₂₂ O ₄	278	Antimicrobial, Antifouling
Acetone							
9	3.19	2-Cyclohexen 1 one,3,5 dimethyl	26.95	Cyclic Alkene	• C ₈ H ₁₂ O	124	Antioxidant, Antimicrobial
10	6.09	1-Piperidineethanamine	5.75	Amine	C ₇ H ₁₆ N ₂	128	Antibacterial, Antiviral
11	20.83	1,2Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	5.50	Carboxylic acid	C ₁₆ H ₂₂ O ₄	278	Antioxidant, Anticancer, Antidiabetic
Ethanol							
12	4.37	Piperidine,1-methyl	12.63	Amine	• C ₆ H ₁₃ N	99	Antioxidant,

							Antimicrobial
13	5.17	5-Azaauracil	4.02	Purine Base	C ₃ H ₃ N ₃ O ₂	113	-
14	12.86	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	8.73	Terpene Alcohol	C ₂₀ H ₄₀ O ₁₁	296	Antimicrobial, Antioxidant
15	16.20	Phytol	3.21	Diterpene	C ₂₀ H ₄₀ O	296	Antimicrobial, Antioxidant
Methanol							
16	3.31	2,2,6,6-Tetramethyl-4-piperidone	9.76	Amine	• NO C ₉ H ₁₇	155	Antimicrobial, Anti-inflammatory
17	4.15	Naphthalene	29	Aromatic Hydrocarbon	C ₁₀ H ₈	128	Antimicrobial, Antifouling
18	14.21	Benzenepropanoic acid, 3,5 bis (1,1-dimethylethyl) 4 hydroxy, methyl ester	9.91	Fatty Acid	C ₁₈ H ₂₈ O ₃	292	Antioxidant, Antifungal
19	25.35	Tetracosamethyl-cyclododecasiloxane	3.95	Silicon Ether	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888	Hepato protective activity, Anti-rheumatic, Insecticides for mosquito control

RT= Retention Time, Rel=Relative Percent, MF = Molecular Formula, MW = Molecular Weight

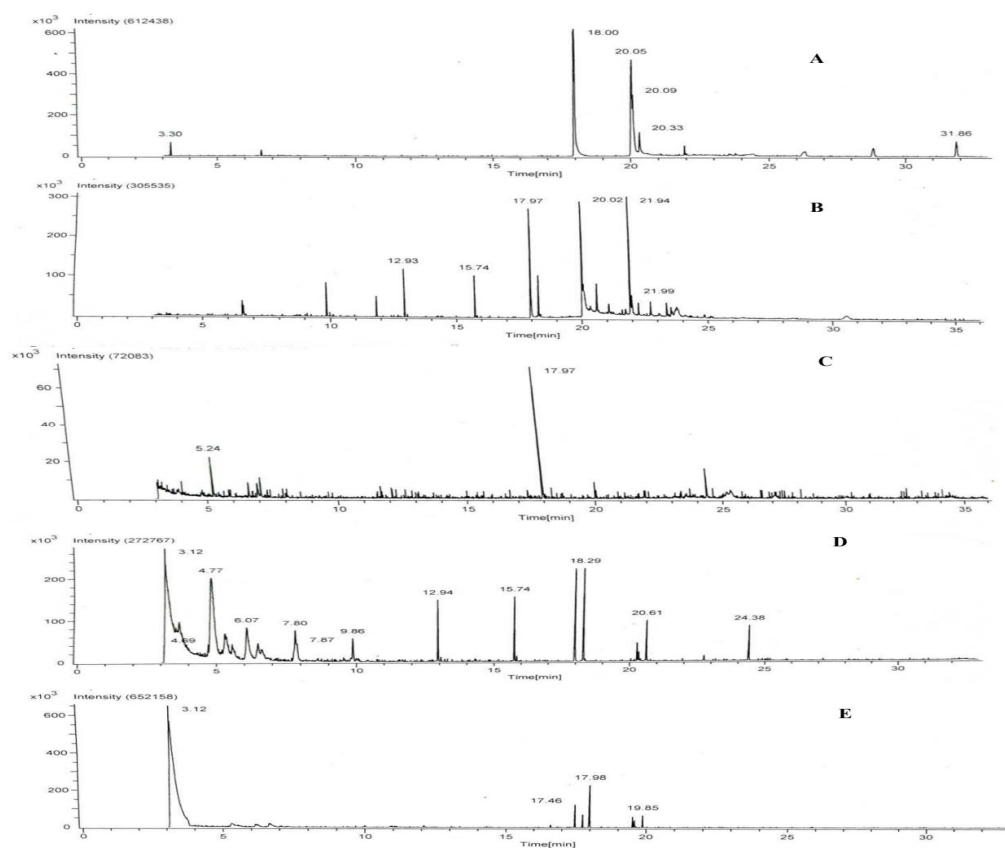


Figure 3: GC-MS results of Seed in Petroleum ether (A), chloroform (B), acetone (C), ethanol (D), and methanol (E) extracts

Table 4: GC- MS Analysis of different solvent extract of *M. nivea* seeds with NIST library search.

Sr.No.	R.T.	Phytochemicals	Rel%	Class	MF	MW	Activity
Petroleum ether							
1	3.30	Ethanone,1-(2-methylcyclopropyl)	1.90	Cyclic Ketone	C ₆ H ₁₀ O	98	-
2	18.00	n-Hexadecanoic acid	39.38	Fatty Acid	C ₁₆ H ₃₂ O ₂	256	Antifungal, Antioxidant, Antimicrobial,
3	20.05	1-Dodecyne	27.58	Alkyne	C ₁₂ H ₂₂	166	-
Chloroform							
1	6.56	1-Hexacosene	2.64	Alkene	C ₂₆ H ₅₂	364	
2	6.60	Azulene	2.25	Aromatic Hydro-carbon	C ₁₀ H ₈	128	Antioxidant, Antibacterial
3	12.93	Oxalic acid, allyl dodecyl ester	6.00	Fatty Acid	C ₁₇ H ₃₀ O ₄	298	-
4	15.74	Oxalic acids, allyltridecyl esters	5.68	Fatty Acid	C ₁₈ H ₃₂ O ₄	312	-
5	20.02	Decanamide,N (2 hydroxyethyl)	15.64	Amine	C ₁₂ H ₂₅ NO ₂	215	Anticonvulsant
6	21.94	Octanamide,N(2-hydroxyethyl)	18.64	Amine	C ₁₀ H ₂₁ NO ₂	187	
Acetone							
7	5.24	2-Cyclohexen-1-one,3,5-dimethyl	16.96	Cyclic Alkene	C ₈ H ₁₂ O	124	Antioxidant, Antimicrobial
8	17.97	Dibutyl phthalate	58.27	Aromatic Hyro-carbon	C ₁₆ H ₂₂ O ₄	278	Antifouling, Antimicrobial
9	20.01	Decanamide,N-(2-hydroxyethyl)	8.76	Amine	C ₁₂ H ₂₅ NO ₂	215	Anticonvulsant
Ethanol							
10	4.77	Propane,1,1,3-triethoxy	25.07	Ether	C ₉ H ₂₀ O ₃	176	-
11	6.07	Propane,2-ethoxy	8.33	Ether	C ₅ H ₁₂ O	88	-
12	12.94	Oxalic acid, allyl dodecyl ester	4.30	Fatty acid	C ₁₇ H ₃₀ O ₄	298	-
13	15.74	Oxalic acids, allyltridecyl esters	4.48	Fatty acid	C ₁₈ H ₃₂ O ₄	312	-
14	18.29	Pentadecanoic acid, 2, 6, 10, 14 tetramethyl methyl ester	6.93	Fatty acid	C ₂₀ H ₄₀ O ₂	312	Antibacterial
15	24.38	1,2 Benzene-dicarboxylic acid, mono(2-ethylhexyl) esters	2.78	Fatty acid	C ₁₆ H ₂₂ O ₄	278	Antioxidant, Anticancer, Antidiabetic
Methanol							
16	3.12	2-Pentanone.4-methoxy-4-methyl-	75.65	Ketone	C ₇ H ₁₄ O ₂	130	-
17	17.46	Tridecanoic acid, methyl ester	3.51	Fatty acid	C ₁₄ H ₂₈ O ₂	228	Antioxidant, Antimicrobial
18	17.74	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-,methyl ester	2.94	Fatty acid	C ₁₈ H ₂₈ O ₃	292	Antioxidant, Antifungal
19	19.85	Octadecanoic acid, methy ester	2.82	Fatty acid	C ₁₉ H ₃₈ O ₂	294	Antimicrobial Antibacterial

RT=Retention Time, Rel%=Relative Percent, MF=Molecular Formula, MW=Molecular Weig

CONCLUSION

The preliminary phytochemical tests and GC-MS-based phytochemical profiling revealed of the bioactive constituents in leaves and seeds of *M. nivea*. The secondary metabolites screened from leaf and seed extracts of *M. nivea* previously displayed a range of biological functions. The current study revealed that *M. nivea* is one of the key sources for nutraceuticals and a variety of bioactive phytoconstituents. However, it required further study for purification of compounds and their specific biological activity. It is also important to study a proper cultivation practice for domestication of these species.

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AUTHOR CONTRIBUTIONS

KCM and AK: Designed the experiments, analyzed the data, and finalized the manuscript, DBS and MRC: Analyzed data and prepared final draft of the manuscript, ST: Performed the experiments, collected, and analyzed the data, PG, AK, SM, and HBS: Analyzed data and prepared draft manuscript. All authors read and approved the final version of manuscript.

CONFLICT OF INTEREST

The authors declare no known competing interest.

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