International Journal of Plant & Soil Science

Volume 35, Issue 19, Page 994-1007, 2023; Article no.IJPSS.105028
ISSN: 2320-7035

Bioassay of Phytochemicals Isolated from Chloroform Extracts of *Azadirachta indica* Leaves

Nitin Vitthal Gurav [a*], Rajendra Marotrao Gade [b++] and Rupeshkumar Jagannath Choudhari [a#]

[a] *Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India.*
[b] *Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola-444104, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i193636

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105028

Original Research Article

Received: 14/06/2023
Accepted: 19/08/2023
Published: 30/08/2023

ABSTRACT

The present study investigated the chemical constituents isolated from *Azadirachta indica* chloroform extract. The extraction of leaves was done using Soxhlet extraction apparatus. To isolate and identify the antibacterial fraction from *Azadirachta indica* chloroform extract, TLC-bioautography was carried out. Phenol 3,5-bis (1,1-dimethyl ethyl), Phthalic acid bis (7-methyloclyl), Dido decyl phthalates, Oxalic acid, allyl hexadecyl ester, and 2-Piperidinone, N-(4-bromo-n-butyl) were the five primary antibacterial chemicals identified by the GC-MS study. The findings of the FTIR study revealed the presence of functional groups C-H str, C=O str, and C=C str as well as alcohol and the carboxylate ion. While the ^{13}C NMR data demonstrated the existence of carbonyl, aromatic carbon, quaternary carbon, olefinic carbon, and methyl group, and the ^{1}H NMR results

---

++ *Associate Dean of Instruction;*
# *PhD Research Scholar;*
**Corresponding author: E-mail: 2016guravnitin@gmail.com;*

revealed the presence of aliphatic OH, methyl, aromatic OH, and olefinic proton. The phytoconstituents detected using GC-MS analysis showed a wide range of pharmacological properties, including antioxidant, antibacterial, antifungal, anti-inflammatory, and antimalarial effects. Thus, *Azadirachta indica* plant has a high concentration of medicinal phytoconstituents that can be used to make antimicrobial medications to fight against plant pathogenic bacteria.

Keywords: Azadirachta indica; phytoconstituents; soxhlet extraction; TLC-bioautography; GC-MS; FTIR; NMR.

1. INTRODUCTION

Secondary metabolites are the broad category of organic chemicals that are isolated by the plants, many of which don't seem to directly contribute to growth and development [1]. These substances are a very diverse set of organic materials produced by a wide range of organisms, including plants, fungi, bacteria, algae, and mammals. It has been estimated that, between 14 and 28 percent of higher plant species are used for medicinal purposes, and from that 74% of plant species are utilised pharmacologically, based on research into the ethnomedical uses of the plants [2]. Many therapeutic benefits are demonstrated by the diverse array of phytochemicals that medicinal plants produce, including phenolic acids, flavonoids, tannins, and other substances [3]. Terpenes, phenolics, and chemicals with nitrogen are the three primary categories of secondary metabolites. In this, phenolics include phenolic acids, coumarins, flavonoids, tannins, and lignin, while nitrogen-containing chemicals include alkaloids and glycosylates. Terpenes include plant volatiles, cardiac glycosides, carotenoids, and sterols.

Many efforts have been made to find novel antimicrobial chemicals from a variety of sources, including microorganisms, animals, and plants. Plants defend themselves against microbial infection and degeneration because of the discovery of novel, powerful antibacterial chemicals from plants [4]. As a result of the increased public knowledge of the dangerous side effects of synthetic chemicals, the employment of biocontrol agents is becoming increasingly important. Researchers have started to focus on using plants and microbes as a biocontrol agent as they play a crucial role in controlling different diseases of crops.

The plant *Azadirachta indica*, also known as neem, belongs to the Meliaceae family and has already risen to the top of the list of potential biocontrol agents. Many pharmacological activities, including antibacterial, antifungal, antiulcer, antifeedant, repellant, pesticide, inhibitor, and sterilant, have been linked to the neem leaf, bark, fruit, stem, and flower. The presence of bioactive chemicals makes it commercially viable and enables it to be employed against a variety of plant diseases historically. Due to the plant's medicinal and bioactive properties, researchers from around the world are interested in studying its bioactive components [5]. It has been claimed that bioactive substances derived from natural sources can be used to diagnosis a variety of diseases [6].

Alkaloids, tannin, flavonoids, phenolic compounds and dicarboxylic acid type chemicals are among the bioactive substances mostly found in *Azadirachta indica* [7]. These groups of substances exhibit a variety of pharmacological properties, including antimicrobial and antimalarial activity [8], antioxidant activity [9], anti-inflammatory activity [10], antifouling activity [11], anti-hypersensitive activity [12], cancer [13].

Thus, the aim of this paper is to report on the isolation and partial characterization of bioactive antimicrobial compounds from chloroform extract of *Azadirachta indica* using GCMS, FT-IR and NMR (^{1}H and ^{13}C).

2. MATERIALS AND METHODS

Preparation of plant extract: The fresh leaves of *Azadirachta indica* were collected and thoroughly washed under running tap water to get rid of dirt and other contaminants. The leaves were dried individually under shade with occasional shifting for around 3 to 4 weeks. Then the leaves were ground into a powder using a grinder, then stored in an airtight container for later use. The Soxhlet's method procedure given by Gupta et al. [14], was carried out for extraction of leaves. Following extraction, the supernatant was collected in the flask individually by filtering it using Whatman No. 1 filter paper and allowed to evaporate at room temperature. Air dried extracts

were weighed separately and kept in small tubes at 5 °C in the refrigerator.

Thin layer chromatography: To determine the chemical composition of *Azadirachta indica* leaf chloroform extract, thin layer chromatography was used. The TLC plates were made by combining 25 g of silica gel-G (Hi media) with 50 ml of distilled water, and then using a spreader to evenly spread the resulting slurry across the plates with a thickness of 0.25 mm. The plates were heated in an oven at 110˚C for one hour after being allowed to dry at room temperature. With the aid of capillary tubes, a 10 µl sample of the *Azadirachta indica* chloroform extract was put on TLC plates at identical distances after being diluted in DMSO. The TLC plate was retained and allowed to run until it reached the 3/4 position in the hexane: ethyl acetate (1:1) solvent system. The produced chromatogram on the TLC plates was examined with visible and ultraviolet light after being allowed to air dry. The distance travelled by the solvent front and the solute front was used to calculate the bands' Rf values (Relative front).

Bioautography: According to Ahmed and Beg 2001, the bioautography approach was used to isolate the bioactive chemicals. On TLC plates, the zone of inhibition was visible as a transparent spot on a red background. The position of the compound exhibiting antibacterial activity was confirmed using bioautography technique on the TLC plate. Later the antibacterial fraction was scraped out from the silica gel and was thoroughly dissolved in chloroform. It was then centrifuged for 10 minutes at 10,000 rpm. For complete solvent evaporation, the supernatant was evaporated at 60°C for 50 min using a vacuum concentrator.

FT-IR, GC-MS, ^{1}H and ^{13}C NMR analysis: Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Fourier Transform Infrared (FT-IR) spectroscopy (FT-IR) techniques were used to conduct additional study on the purified chemical compound. Nuclear Magnetic Resonance (NMR) was done using JNM-ECZ600R spectrometer for which 1 mg crude sample was dissolved in methanol-d4 (deuterated methanol) solvent. Gas Chromatography-Mass Spectrometry (GC-MS) was done using AccuTOF-GCv mass spectrometer for which 1 mg crude sample was dissolved in methanol solvent. Fourier Transform Infrared (FT-IR) spectroscopy (FT-IR) analysis was carried out in FTIR interferometer using methanol solvent. For this analysis, separate 70 mg active compound was stored in small, sterile glass vials. The samples were sent to the Sophisticated Analytical Facility, IIT, Powai, Bombay, for chemical analysis using FT-IR, GC-MS, and ^{1}H and ^{13}C NMR. Tables and Figures showed the interpreted data.

3. RESULTS

Extraction yield of *Azadirachta indica*: Extraction yield of *Azadirachta indica* leaves in different solvents are presented in Table 1.

The results revealed that distilled water exhibited (9.08%) maximum extraction from *Azadirachta indica* leaves whereas minimum extraction yield was observed in petroleum ether (2.9%).

Thin Layer Chromatography (TLC): Thin layer chromatography was used for separation of different chemical constituents present in chloroform extract of *Azadirachta indica.*

TLC-Bioautography: To assess the antibacterial activity of isolated compounds against the tested bacterium, bioautography technique was utilized on TLC plates run in hexane: ethyl acetate (1:1). The TLC plate displayed a transparent zone of inhibition against a red background around the band that contained the active ingredient responsible for the antibacterial activity after being sprayed with 2, 3,5-tri phenyl tetrazolium chloride. One compound from *Azadirachta indica* extract demonstrated well-resolved suppression of *Xanthomonas axonopodis* pv. *citri* at Rf- 0.74 while emitting a pink color when illuminated by UV light.

GCMS analysis of *Azadirachta indica*: Chloroform leaf extract from *Azadirachta indica* revealed the existence of five major components together with their retention times, peak areas, and molecular weights. Phenol 3, 5-bis (1,1-dimethyl ethyl) at retention time 9.88, mol. wt. 206, and peak area 659085.64 was the first compound. Phthalic acid bis (7-methyl octyl) ester was second compound at retention time 15.58, mol. wt. 418, and peak area 1188911.27. The third compound was Dodecyl phthalates with retention time 20.17, mol. wt. 502 and peak area 3732124.99. The fourth and fifth compound are Oxalic acid, allyl hexadecyl ester, and 2-Piperidinone, N-(4-bromo-n-butyl) at retention times of 27.24 and 31.34, mol. wt. 354 and 233, their peak areas were 1528807.42 and

2565012.55, respectively, (Table 2 and Figs. 1,2,3,4 and 5).

FTIR analysis of *Azadirachta indica*: The following peaks and functional groups were identified using FT-IR analysis. Peak 3427 indicates the existence of a hydroxyl methyl group, while peak 2952 and peak 1734 indicate C-H stretching and carboxylic acid-like C=O stretching, respectively. C-H bending was found at 1089.46 and 801.61 peak, and C=C stretching was seen at 1465.19. (Table 3 and Fig. 6).

^{1}H NMR analysis of *Azadirachta indica*: Different signals for various proton types were discovered by ^{1}H NMR analysis. The initial signal indicated the existence of an aromatic proton between 6.95 and 7.78. The existence of olefinic proton was indicated by the second signal, 6.67 to 6.81. At 4.51 to 4.72 signal, phenolic hydroxyl group presence was evident. While the 4.21 to 3.31 signal indicated the presence of a hydroxyl group, or a methylene group connected to an electronegative atom. At 3.62 to 3.70 signal, the ester group was present, and at 2.62 signal, the ketone group. And the final signal (0.9-1.48) indicated the existence of a methyl group (Table 4 and Fig. 7).

^{13}C NMR analysis of *Azadirachta indica*: Carbonyl group was present at signals 169.50, 67.1, and 67.3 according to ^{13}C NMR study. Quaternary carbons were observed at signals 129.7 to 132.4 while aromatic carbons were present at signals 116.7 to 127.57. Likely at 27.85 to 23.79 and 14.52 signals, methyl groups were present respectively, (Table 4a and Fig. 8).

Table 1. Effect of different solvents on per cent extraction yield from dry weight of leaves

Plant	Solvent	Yield in %
Azadirachta indica	Petroleum ether	2.9
	Chloroform	6.7
	Dichloromethane	4.64
	Distilled water	9.08

Table 2. Identification of compounds from chloroform extract of *Azadirachta* indica leaves by GC-MS

Sr. no.	Name of compound	Formula	MW	Retention time	Peak area
1	Phenol 3,5-bis (1,1-dimethyl ethyl)	$C_{14}H_{22}O$	206	9.88	659085.64
2	Phthalic acid bis (7-methyloclyl) ester	$C_{25}H_{42}O_4$	418	15.58	1188911.27
3	Didodecyl phthalates	$C_{32}H_{54}O_4$	502	20.17	3732124.99
4	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354	27.24	1528807.42
5	2-Piperidinone, N-(4-bromo-n-butyl)	$C_9H_{16}BrN_0$	233	31.34	2565012.55

Table 3. Identification of functional group from chloroform extract *Azadirachta* indica leaves by FTIR analysis

Sr.no.	Peak	Functional group	Average range
1	3427	R-CH2OH, R_2-CHOH R_3-C-OH	3400-3600
2	2952	C-H str. Hydrocarbons aliphatic aromatic	2850-3000
3	1734	C=O str. Carbonyl group	1650-1800
4	1465.19	C=C str. Aromatic compounds	1450-1600
5	1089.46 and 801.61	C-H bending hydrocarbons, aliphatic aromatic	650-1000

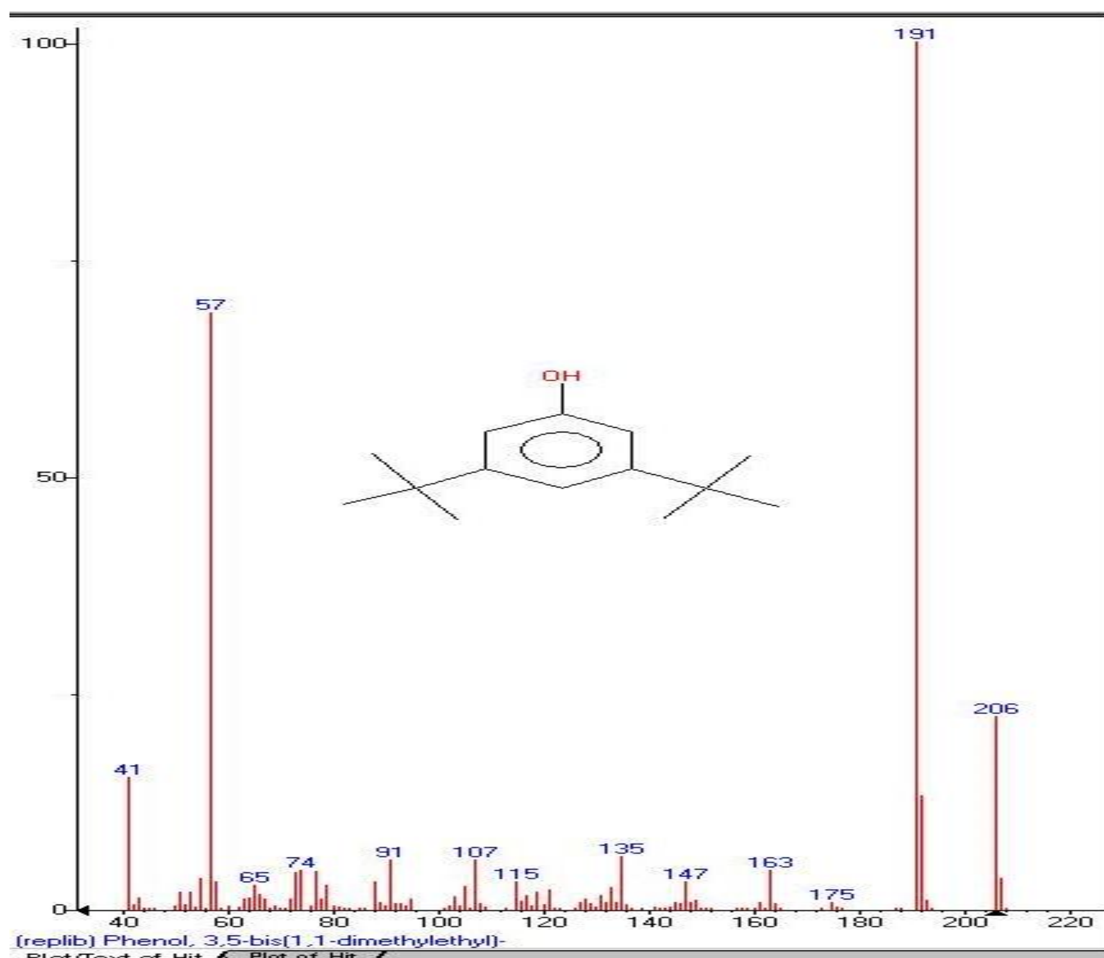

Fig. 1. Mass spectra of compound Phenol 3, 5, bis (1,1-dimethyl ethyl) from chloroform extract of *Azadirachta indica* leaves

Table 4. Identification of types of protons from *Azadirachta indica* leaves by Proton NMR analysis (deuterated methanol)

Sr. no.	(δ) Chemical shift	Type of proton
1	6.95-7.78	Aromatic proton
2	6.67-6.81	Olefinic proton
3	4.51-4.72	Phenolic OH
4	4.21-4.31	CH_2 or CH attached to electronegative atom
5	3.62-3.70	CH_2 or CH or Ar-O=CH_3 or R-O-C-CH_3 (ester)
6	2.62	Ar-CH_3 or O=C-CH_3 (ketone)
7	0.9-1.48	R-CH_3

Table 4a. Identification of types of carbon from *Azadirachta indica* leaves by ^{13}C NMR analysis (deuterated methanol)

Sr. no	(δ) Chemical shift	Type of carbon
1	169.50	C=O
2	129.7-132.4	Quaternary carbon aromatic
3	116.7-127.57	Aromatic carbon, Olefinic carbon
4	31.9-49.67	CH or CH_2 attached to electronegative atom, O-CH_3
5	27.85 and 23.79	R-CH or R-CH_2
6	14.52	R-CH_3

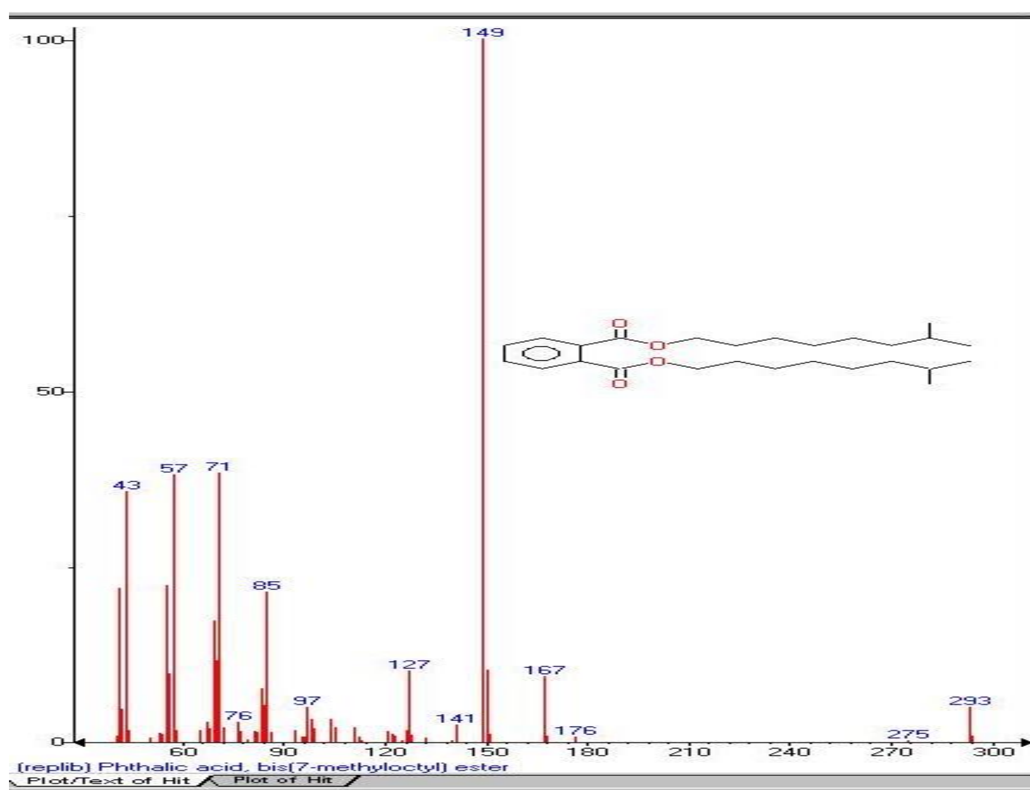

Fig. 2. Mass spectra of compound Phthalic acid bis (7-methyloctyl) ester from chloroform extract of *Azadirachta indica* leaves

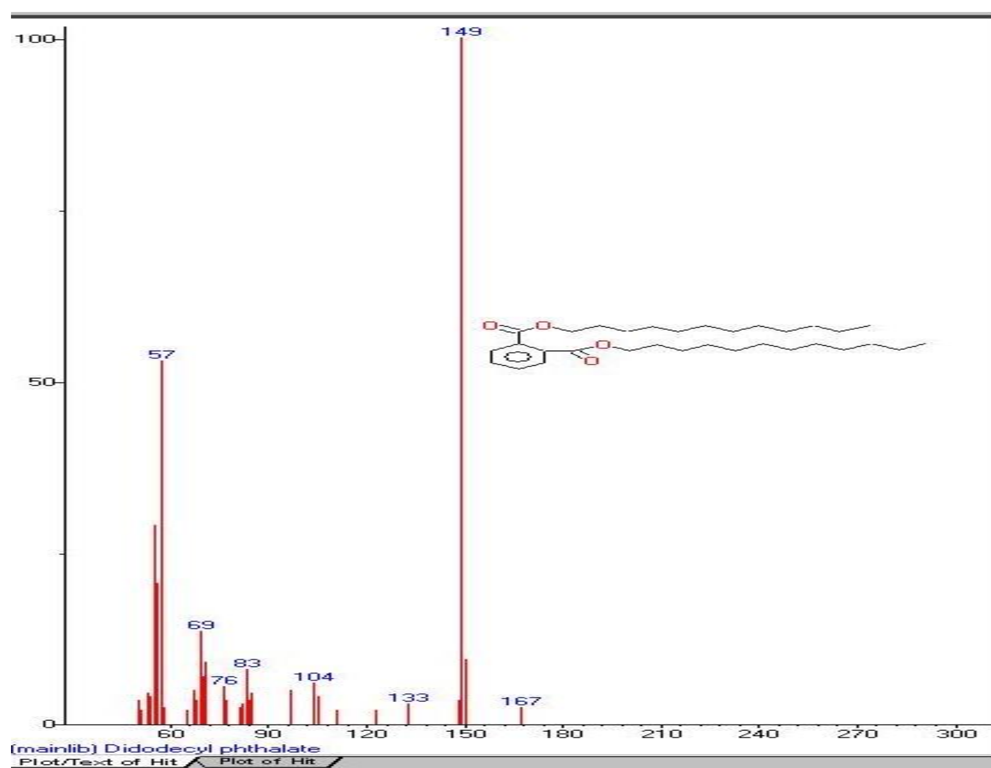

Fig. 3. Mass spectra of compound Didodecyl phthalate from chloroform extract of *Azadirachta indica* leaves

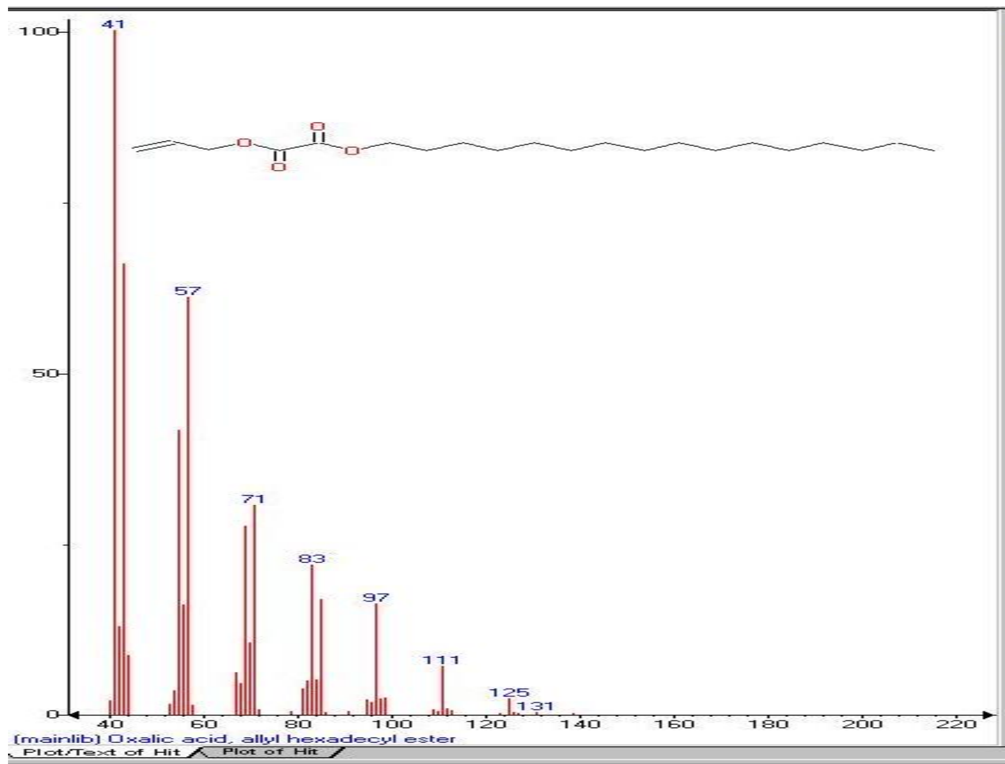

Fig. 4. Mass spectra of compound Oxalic acid, allyl hexadecyl ester from chloroform extract of *Azadirachta indica* leaves

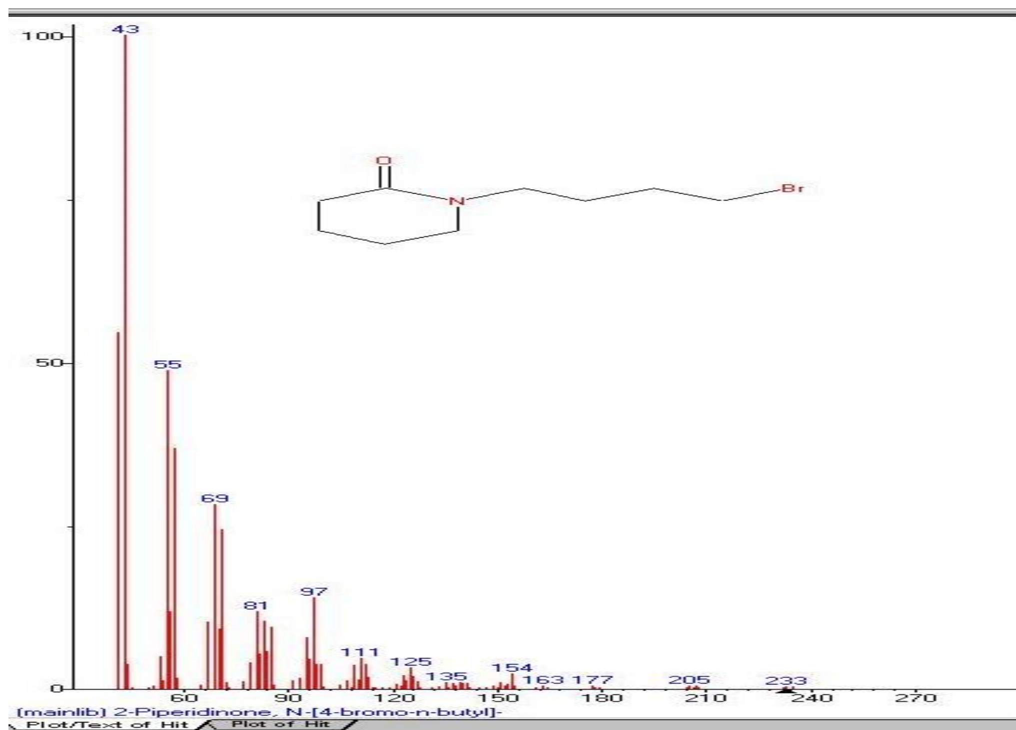

Fig. 5. Mass spectra of compound 2-piperidinone, N- (4-bromo-n-butyl) from chloroform extract of *Azadirachta indica* leaves

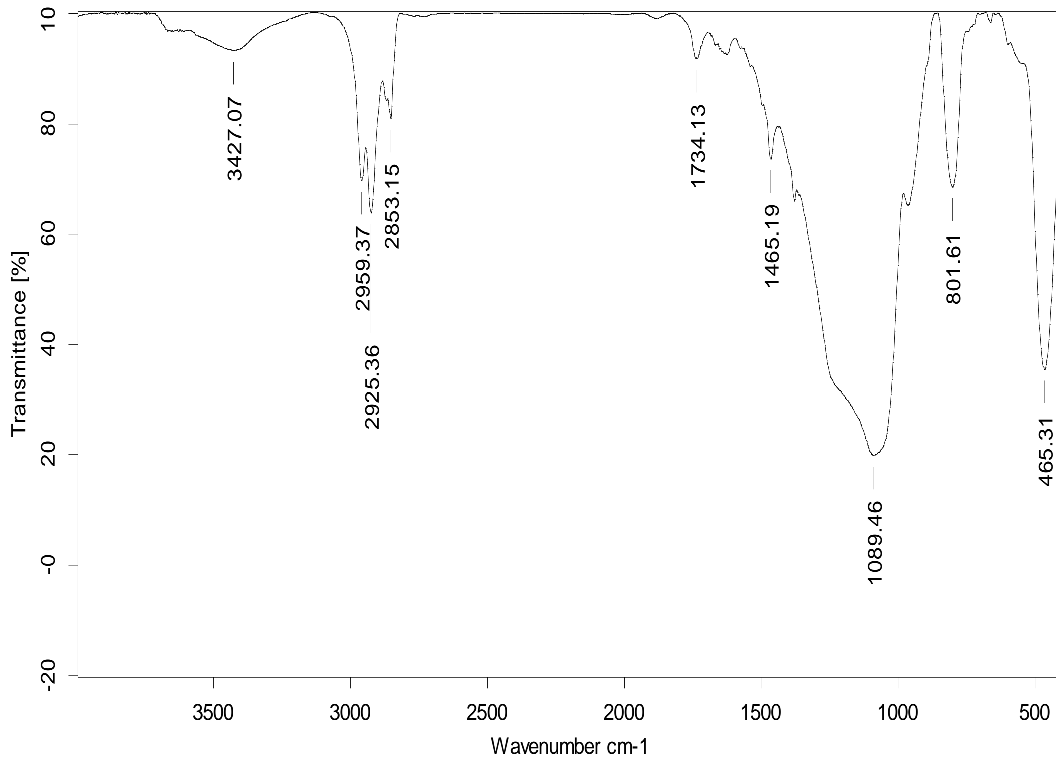

Fig. 6. FTIR spectra of compound isolated from chloroform extract of *Azadirachta indica* leaves

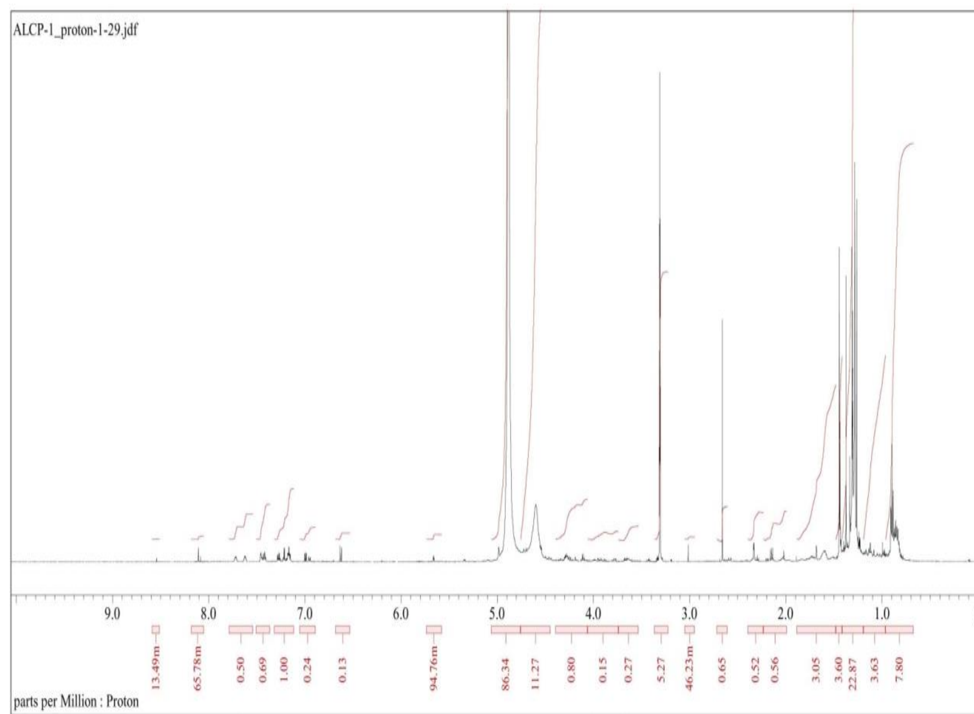

Fig. 7. ¹H NMR spectra of compound isolated from *Azadirachta indica* leaves (deuterated methanol)

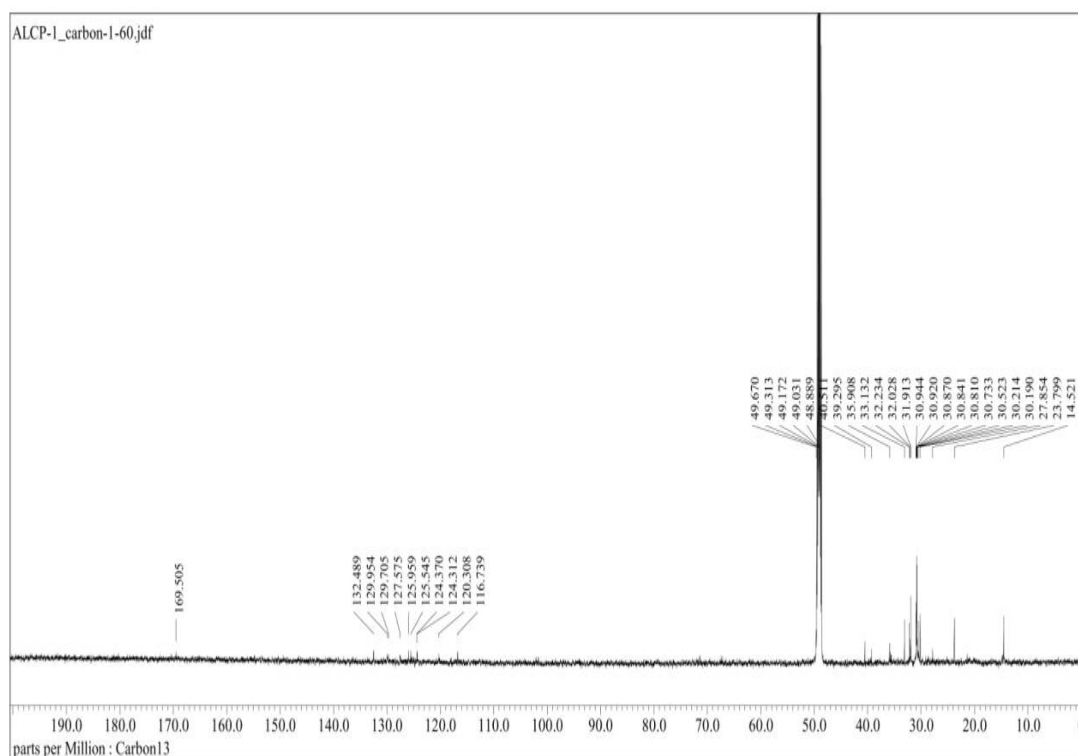

Fig. 8. ^{13}C NMR spectra of compound isolated from *Azadirachta indica* leaves (deuterated methanol)

4. DISCUSSION

Extraction: Table 1 provides the extraction yields of the various solvents employed in this study. The polarity and capacity of a solvent to extract additional chemical compounds from the *Azadirachta indica* plant determines how extractable it is. *Azadirachta indica* was recognised to yield more bioactive compounds when extracted with distilled water compared with other solvents. The findings were like those of Raja Pandiyan et al. [15], who found that water extract had the maximum extraction yield (4.5g) due to the presence of highly polar alkaloids, flavones, and sugars. Babu et al. [16] also previously recorded the highest extraction yield (0.6882g) from *Azadirachta indica* leaves in water extract.

Thin layer chromatography: Several antibacterial fractions or secondary metabolites responsible for antibacterial activity were separated using thin layer chromatography. The findings of the above investigation, revealed the retention factors (Rf) of ethanol extracts of *Azadirachta indica* in various solvent systems, are consistent with those of Mondali et al. (2014). In hexane: ethyl acetate (1:1) solvent system, the ethanol extracts generated nine fractions with Rf 0.09, 0.10, 0.19, 0.22, 0.38, 0.48, 0.58, 0.66, and 0.91. In the current study, the same solvent system hexane: ethyl acetate (1:1) produced the maximum band separation using chloroform extract. As a result, the TLC results show that chloroform extracts contain a variety of chemical components.

Bioautography: The distinct antibacterial fraction of *Azadirachta indicia* chloroform extract eluted on TLC plates in a hexane: ethyl acetate (1:1) solvent system was identified using the bioautography method. The 2,3,5-triphenyl tetrazolium chloride was sprayed onto TLC plates, which displayed a whitish or translucent zone of inhibition against a pink or red background at Rf-0.74. Kruzselyi et al. [17] used High Performance Liquid Chromatography (HPLC) and electrospray ionization mass spectrometry to identify the active chemicals present in *Azadirachta indica* oil and designated them as linoleic and oleic acid. Using TLC-bioautography and spectroscopic analysis, Shubham et al. [18] also reported the existence of an active chemical tetra nor-triterpenoid limonoid with an Rf- 0.56 and a retention period of 3.8 minutes in *Azadirachta indica* leaves.

GCMS analysis: The several bioactive components found in *Azadirachta indica* chloroform extract are identified using Gas Chromatography and Mass Spectrometry (GC-MS) technique. Five main chemicals that are responsible for *Azadirachta indica* therapeutic potential were discovered using GC-MS research. Table 2 lists the recognised compounds along with their retention time, molecular weight, molecular formula, and peak area. These components fall under the categories of alkaloid, phenolic, dicarboxylic, and plasticizer chemicals.

Phenol, 3,5, bis-(1,1-dimethyl ethyl), Dodecyl phthalate, 2-piperidinone, N-(4-bromo-n-butyl), Oxalic acid, allyl hexadecyl ester, and Phthalic acid, bis (7-methyloctyl) ester were the most prevalent of the above-mentioned described compounds. Except the first compound Phenol,3,5, bis-(1,1-dimethyl ethyl), all the remaining four compounds were found to be new compounds from *Azadirachta indica* chloroform extracts.

The first compound Phenol, 3,5, bis-(1,1-dimethyl ethyl), was discovered in the leaves of *Indoneesiella echiodes* [8], *Azadirachta indica* [19], *Hibiscus micranthus* [20], *Nerium oleander* [21], and *Ninbapatradi chooram* [10]. This phytochemical demonstrated a variety of activities, including antimicrobial [8,19,22,23,24,10] (Wagay and Rothe 2016), antioxidant [9,25,24]. Lawal et al. [22] and Rukhsana et al. [26] and Govindappa et al. [27], antimalarial [8], anti-inflammatory [10], analgesic, anesthetic, antiseptic, antiviral, cancer preventive and fungicidal [24].

Dodecyl phthalate, the second compound, was recognised for its plasticizing properties [28]. These phthalates are present in cosmetics, detergents, lubricating oils, alternatives for polychlorinated biphenyls, carriers in pesticide formulations, solvents, and building materials like flooring, sheeting, and films [29]. This phytocompound was identified in a variety of plant extracts, including *Mukia maderaspatana* [12], *Sarcostemma secamone* [11], *Blighia sapida* [30], *Viola odorata* [31] flower and *Trigonella foenum* [28]. Additionally, a variety of activities, including antimicrobial and antifouling [11,28,32,33], anti-hypersensitive, vasodialator, diuretic, and angiotensin ATZ receptor antagonist, were demonstrated by this compound [12].

The third compound, 2-piperidinone, N-(4-bromo-n-butyl), was reported to be found in a variety of plants, including the leaves extract of *Microcosmus exaspeatus* [34], *Asparagus racemosus* [35], sesame seed [36], the leaves, fruit, and latex of *Croton bonplandianum* [37], and in *Aspergillus tamarii* and *Penicillium islandium* [38]. This compound showed antibacterial, anti-inflammatory [37,34], and antioxidant properties [34].

The presence of fourth antibacterial compound Oxalic acid, allyl hexadecyl ester was found by various researchers in other plant species like in *Laurencia brandenii* [39], *Aloe vera* plants [40], Nigerian rice [41] and in *Pongamia pinnata* (Anuradha and Krishnamoorthy 2012). This compound produced a variety of activities such as antimicrobial [42], acaricide, irritant, pesticidal, renotoxic and varroacidial [43].

The final compound, Phthalic acid, bis (7-methyloctyl) ester was similarly known for its plasticizing properties [44,45]. Similarly, different plant taxa including *Tabebuia argentea* [46], *Aporosa lindleyana* [45], *Calotropis gigantea* [47], *Purpura persica* [44], and *Centratherum punctatum* [48] were shown to contain this compound. According to Ramakrishnan and Venkataraman [45], the antibacterial compound exhibited antibacterial and antifouling properties as well as tumour-fighting properties against mice sarcoma 180 cell lines [13].

FTIR analysis: *Azadirachta indica* chloroform extract was further analysed using Fourier Transform Infrared Spectroscopy to detect the various functional groups that were present. Table 3 presents the findings of the discovered functional groups by FTIR analysis together with their peaks and average range. The results of the FTIR analysis discussed above were consistent with those of Shaikh et al. [49], who found that O-H str was present at peak 3456.55, C=O str was present at peak 1653.05, and alkene and alkyl halide group were present at peak 675.11. Moreover, the existence of C=O str and C=C str was noted at maxima in 1730 and 1452, respectively [50]. At peaks 873.75 and 721.38, 2922.26 and 2852.72, 1463.97 and 1741.72, C-H bend, C-H str, C=C, and C=O were all detected. [51].

NMR analysis: To determine the types of protons and carbon contained in various compounds from the *Azadirachta indica* plant, the Nuclear Magnetic Resonance technique was

used. The first type of NMR is ^{1}H, which identifies the proton type, and the second type is ^{13}C, which identifies the carbon type contained in the corresponding plant extracts. Tables 4 and 4a show the chemical shifts, types of protons, and carbon atoms. Similar findings were made in the study of ^{1}H NMR analysis by Kumar et al. [52,53], who indicated the existence of an aromatic proton at peak 7.2–6.8, a CH_2 group at peak 3.1–3.8, a CH_3 group at peak 2.2–2.9, and a methyl group at peak 1.28. Furthermore, the methyl groups at 0.77–0.78 and 1.14–1.33 [54] and aromatic protons at 6.19-7.2, 7.54, 6.40 and 6.88 peaks were given by Sambadam et al. [55,56-61].

The above mentioned FTIR and NMR (^{1}H and ^{13}C) data revealed various functional groups, proton types, and carbons, which as a result are responsible for producing various pharmacological actions as demonstrated by the bioactive compounds from the *Azadirachta indica* plant that were found. These chemical constituents and the groups they were placed in the study play a significant part in having various activities against plant pathogens, which will be more crucial in the future for controlling the most serious horticultural and agricultural diseases.

5. CONCLUSION

The above study concluded that highest percent extraction yield of *Azadirachta indica* crude leaf extracts was obtained from distilled water (9.08%) while lowest was obtained from petroleum ether (2.09 %). Thin Layer Chromatography- bioautography results revealed the antibacterial fraction from the *A. indica* chloroform extracts at Rf- 0.74 against the *Xanthomonas axonopodis* pv. *citri* bacterium emitting a pink color when illuminated by UV light. Thus, based on TLC- bioautography results the antibacterial fraction obtained was subjected for GC-MS, FTIR and NMR analysis. From GCMS analysis, partial or most probable structure of compounds isolated from *Azadirachta indica* leaves were Phthalic acid, bis (7-methyloclyl ester), Di dodecyl phthalates, Oxalic acid, allyl hexadecyl ester, 2-piperidinone, N(4-bromo-n-butyl) and Phenol, 3,5-bis(1,1-dimethylethyl). The functional groups identified using FTIR analysis were hydroxyl methyl, C-H stretching, carboxylic acid-like C=O stretching, C-H bending and C=C stretching respectively. The ^{1}H NMR reports showed the presence of aromatic, olefinic, phenolic, hydroxyl, methylene, ester, ketone, and methyl proton groups. And the ^{13}C NMR results showed presence of carbonyl, quaternary, aromatic and methyl carbon groups present respectively. Hence it is concluded that above phytoconstituents found using GC-MS analysis showed a wide range of pharmacological properties, including antioxidant, antibacterial, antifungal, anti-inflammatory, and antimalarial effects. Thus, concluding that *Azadirachta indica* contains a high concentration of bioactive constituents that can be used to control plant pathogenic bacteria's.

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented in the conference: 6th International Conference on Strategies and Challenges in Agricultural and Life Science for Food Security and Sustainable Environment (SCALFE-2023) on April 28-30, 2023 in Himachal Pradesh University, Summer Hill, Shimla, HP, India. Web Link of the proceeding: https://www.shobhituniversity.ac.in/pdf/Souvenir-Abstract%20Book-Shimla-HPU-SCALFE-2023.pdf

ACKNOWLEDGEMENT

We are thankful to Mujahid sir, Department of Chemistry, Shri Shivaji College of Arts, Commerce and Science, Akola for their valuable help in interpreting the spectroscopic data. Also, we are thankful to Vasantrao Naik College of Agricultural Biotechnology, Yavatmal for providing necessary facilities for conducting this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hartmann T. Alkaloids. In herbivores; their interaction with secondary plant metabolites, The chemical participants, 2nd ed, Rosenthal GA and Berenbaum MR eds Academic press, San Diego. 1991;1:33-85.
2. Ncube RNS, Afolayan AJ and Okoh A. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afri J Biotech. 2008;7(12):1797-1806.

3. El-najjar N, Saliba N, Talhouk S and Gali-muhtasib H. *Onopordum cynarocephalum* induces apoptosis and protects against 1, 2-dimethylhydrazine induced colon cancer. Oncology Reports. 2007;17: 1517-1523
4. Cowan MM. Plant products as antimicrobial agents. Clin Microbial Rev. 1999;12:564- 582.
5. Luo XD, Ma YB, Wu SH, Wu DG. Two novels azadirachtin derivatives from *Azadirachta indica*. J Nat Prod. 1999;62(7):1022–1024.
6. Hamburger H, Hostettmann K. The link between phytochemistry and medicine. Phytochemistry. 1991;30:3864-3874.
7. Hill RA. Terpenoids in Thomson RH, (ed). Chemistry of Natural Products, Blackie Academic and Professional. London. 1985;106-134
8. Elaiyaraja A, Chandramohan G. Comparative phytochemical profile of *Indoneesiella echio*ides (L) Nees leaves using GC-MS. J of Pharmaco and Phytochem. 2016;5:158-171.
9. Ajayi GO, Olagunju JA, Ademuyiwa O and Martins OC. Gas chromatography- mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. J Med Plan Res. 2011;5(9):1756-176.
10. Chandrasekar T, Mudiganti RKR, Vijaya KR, Prabhu K, Nandha KS, Divya D. GC-MS analysis, antimicrobial, antioxidant activity of an Ayurvedic medicine, Ninbapatradi Chooram. J Chem and Pharma Res. 2015;7(8):124-136.
11. Kumari TK, Muthukumarasamy, Mohan VR. GC-MS analysis of ethanol extract of *Sarcostemma secamone* (l) bennet (Asclepiadaceae) Sci Res Rep. 2012; 2(3):187-191.
12. Mallikadevi T, Paulsamy S, Jamuna S and Karthika K Analysis for phytochemicals and bioinformatics approach for the evaluation of therapeutic properties of whole plant methanolic extract of *Mukia maderaspatana* (l.)– A traditional medicinal plant in western districts of Tamilnadu, INDIA. Asian J Pharm Clin Res. 2012;5(4):163-168.
13. Lee SM, Ha CS, Cho WJ. Antitumor and antiangiogenic activities of phthalic acid derivative polymers with medium molecular-weight. Mol Crys and Liquid Crys Sci and Tech. 2000;354:287-301.
14. Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS and Singh A. Phytochemical screening and antimicrobial assessment of leaves of *Adhatoda vasica*, *Azadirachta indica* and *Datura stramonium*. U K J Pharma Biosci. 2013; 1(1):42-47.
15. Rajapandiyan K, Shanthi S, Murugan AM, Muthu GA, Singh AJR. *Azadirachta indica* - cow urine extract, a novel controlling agent towards Clinically significant Multi Drug Resistant Pathogens. J App Pharm Sci. 2011;1(10):107-113.
16. Babu SK, Naik VKM, Latha J, Ramanjaneyulu K. Extraction, isolation and phytochemical investigation of natural products by using chromatographic (TLC) method. Int J Pharm Pharma Res. 2016;7(10:380-393.
17. Kruzselyi D, Nagy R, Ott PG, Móricz AM. Rapid, bioassay-guided process for the detection and identification of antibacterial neem oil compounds. J Chrom Sci. 2016;54(7):1084–1089.
18. Shubham, Bhardwaj U, Sharma N, Mathur A. Evaluation of potent hydro-alcoholic extract of leaves of *Azadirachta indica* for isolation and identification of anti-helminthic compound. Int J Med Res Health Sci. 2016;5(5):88-95.
19. Sandanasamy JN, Hamid A, Tajuddin, Nizam S, Hamid NA. Chemical characterization and biological study of *Azadirachta indica* extracts. Euro J of Acad Essa. 2014;1:9-16.
20. Kumar KA, Shetty SR, Narsu ML. GC-MS analysis of n-hexane extracts of *Hibiscus micranthus* Linn. Asian J of Chem. 2011;23(2):561-565.
21. Dey P, Chaudhuri TK. Comparative phytochemical profiling and effects of *Nerium oleander* extracts on the activities of murine peritoneal macrophages. Arch Biol Sci. 2016;68(3):515-531.
22. Lawal RA, Odesanmi OS, Ozaslan MD, Ebuehi OAT, Karagoz ID, Kilic IH, Uyar C, Badmus IA. Gas chromatography-mass spectrometry and cytotoxicity of *Securidaca long* epedunculata (polygalaceae) root bark extract. Fountain Journal of Natural and Applied Sciences. 2016;5(1): 19 – 24.
23. Arora M, Mahajan AM and Sembi JK. Essential oils analysis of pseudobulbs of *Crepidium acuminatum* (D.DON) SZLACH by GC-MS. Asian Pac J Health Sci. 2017;4(3):198-204.

24. Rai DK, Sharma V, Pal K, Gupta RK. Comparative phytochemical analysis of *Cuscuta reflexa* Roxb. Parasite grown on north India by GC-MS. Trop Plant Res. 2016;3(2):428–433.
25. Victoria DT, Samrot AV. Identification of antioxidant activity of bark of Aegle Marmelos. Der Pharma Chemical. 2016;8(18):359-363.
26. Rukhsana K, Varghese V, Akhilesh VP, Jisha KEK, Baskaran KP, Bindu PU, Sebastian CD. GC-MS determination of chemical components in the bioactive secretion of Anoplodesmus saussurii (Humbert, 1865). Int J Pharma Sci and Res. 2015;6(4):650-653.
27. Govindappa M, Hanabusa R, Sadananda TS, Chandrappa CP, Umashankar T. Identification of bioactive metabolites by GC-MS from an endophytic fungus, *Alternaria alternata* from Tabebuia argentea and their in vitro cytotoxicity activity. Int J Biolog Pharmac Res. 2014;5(6):527-534
28. Priya V, Jananie RK, Vijayalakshmi K. GC/MS determination of bioactive components of Trigonella foenum grecum. J Chem Pharm Res. 2011;3(5):35-40.
29. George C, Prest H. Determination of phthalate esters by positive chemical ionization MS with retention-time locked GC. NORTH AMERICA. 2002;20(2).
30. Ojo OA, Ajiboye BO, Imiere OD, Adeyonu O, Olayide I, Fadaka A. Antioxidative properties of blighia sapida K.D. koenig stem bark extract and inhibitory effects on carbohydrate hydrolyzing enzymes associated with non-insulin dependent diabetes mellitus. Pharmacogn J. 2018;10(2):376-383.
31. Jasim SF, Baqer NN and Alraheem E. Detection of phytochemical constituent in flowers of viola odorata by gas chromatography-mass spectrometry. Asian J Pharm Clin Res. 2018;11(5:):262-269.
32. Chandel E, Kumar B. Antimicrobial activity and phytochemical analysis of *Cynodon dactylon*: A review. World J Pharm and Pharmaceu Sci. 2015;4(11):515- 530.
33. Kalaiarasan A, Kumar P, Ahmed JS. Biochemical Investigation of *Bulbophyllum kaitense* RECHIB. Root By GC-MS. Eastern ghats of India. Nature and Science. 2012;10(2):29-31.
34. Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M and Chamundeswari KP. GC-MS determination of the bioactive components of Microcosmus exasperates. J Curr Chem Pharm Sci. 2012;2(4):271-276.
35. Selvam PR, Srinivasam V, Gunasekaran S, Palani S. Phytochemical and GCMS analysis of ethanolic extracts of *Asparagus racemosus*. J Curr Chem Pharm Sci. 2014;2(4):271-276.
36. Olaleye OO, Kukwa RE, Eke MO, Aondo TO. Extraction, physicochemical and phytochemical characterization of oil from sesame seed. Asian Food Sci Jou. 2018;1(4):1-12.
37. Vennila V, Udayakumar R. GC-MS analysis of leaf, fruits, and latex of *Croton bonplandianum* Baill. Int J of Biochem Res & Rev. 2015;5(3):187-197.
38. Hady HA, Abdel-Wareth MTA, El-Wakil EA, Helmy EA. Identification and evaluation of antimicrobial and cytotoxic activities of *Penicillium islandicum* and *Aspergillus tamarii* ethyl acetate extracts. World J of Pharm and Pharmaceu Sci. 2016;5(9):2021-2039.
39. Manilal A, Sujith S, Sabarathnam B, George SK, Selvin J, Shakir C, Aaron P, Lipton. Biological activity of the red alga *Laurencia brandenii*. Acta Bot Croat. 2011;70(1):81–90.
40. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World J Agri Sci. 2009;5:572-576.
41. Adekoyeni OO, Adegoke AF, Sogunle KA. Volatile aromatic components of two varieties of parboiled Nigerian rice. Life Journal of Science. 2018;20(1).
42. Sathya S, Lakshmi S and Nakkeeran S Combined effect of biopriming and polymer coating on chemical constituents of root exudation in chilli (*Capsicum annuum* L.) cv. K 2 seedlings J of Appl and Nat Sci. 8(4):2141-2154.
43. Zayed MZ, Samling B. Phytochemical constituents of the leaves of *Leucaena eucocephala* from Malaysia. Int J Pharm Pharma Sci. 2016;8:174-179.
44. Santhi V, Sivakumar V, Jayalakshmi S, Thilaga RD, Mukilarasi Isolating M. Bioactive compound from marine prosobranch *Purpura persica* from Tuticorin Coast. Int J Env Protection and Policy. 2016;4(3):64-76.
45. Ramakrishnan S, Venkataraman R. Screening of antioxidant activity, total

phenolics and gas chromatography-mass spectrophotometer (GC-MS) study of ethanolic extract of *Aporosa lindleyana* Baill. Afri J Biochem Res. 2011;5(14):360-364.

46. Melappa G, Shree SCB, Basava C, Prakash B. *In vitro* antimitotic, antiproliferative and GC-MS studies on the methanolic extract of endophytic fungi, penicillium species of *Tabebuia argentea* bur & k. Sch Farmacia. 2017;65(2):301-309.
47. Singh M, Javed K. Comparative study of chemical composition of *Calotropis gigantea* flower, leaf, and fruit essential oil. Euro Chem Bull. 2015;4:477-480.
48. Sivasubramanian R, Brindha P. In-vitro cytotoxic, antioxidant and GC-MS studies on Centratherum punctatum. Int J Pharm Pharm Sci. 2013;5:364-367.
49. Shaikh TN, Chaudhari S. Characterization of green synthesized silver nanoparticles using *Azadirachta indica* (Neem) leaf extract. Int J Adv Res in Sci and Eng. 2017;6(9):1127-1138.
50. Resmi CR, Sreejamol P, Pillai P. Green synthesis of silver nanoparticles using *Azadirachta indica* leaves extract and evaluation of antibacterial activities. Int J. Adv Bio Res. 2014;4(3):300-303.
51. Banerjee K, Thiagarajan N, Thiagarajan P. *Azadirachta indica* A. juss based emollient cream for potential dermatological applications. Ind J Pharm Sci. 2016;78(3):320-325.
52. Kumar VS, Navaratnam V, Rajasekaran A, Nair N, Soundaraj D, Matharasi P, Narasimhan S, Subramaniam. Isolation and characterization of glucosamine from *Azadirachta indica* leaves: An evaluation of immunostimulant activity in mice. Asian Pacific J of Trop Biomed. 2012;1561-1567.
53. Kumar DR, Sharma V, Pal K, Kumar RG. Comparative phytochemical analysis of *Cuscuta reflexa* roxb. parasite grown on north India by GC-MS. Trop Plant Res. 2012;3(2):428–433.
54. Patil V, Dhunjibhoy K and Dasgupta D. Novel antioption bacterium activity of embelin and chebulagic acid on screening of Indian medicinal plants. Int. Res. J. Pharm. 2017;8 (5):45-52.
55. Sambadam B, Thiyagarajan D, Ayyaswamy A, Raman P Extraction and isolation of f flavonoid quercetin from the leaves of Trigonella foenum-graecum and their antioxidant activity. Int J Pharm Pharm Sci. 8(6):120-124.
56. Govindappa CM, Chandrappa CP, Sadananda TS. *In vitro* antidiabetic activity of three fractions of methanol extracts of loranthus micranthus, identification of phytoconstituents by GC-MS and possible mechanism identified by GEMDOCK method. Asian J of Biomed and Pharmaceu Sci. 2014;4(34):34-41.
57. Zhaoa JT, Davisb LC, Verpoortec R. Elicitor signal transduction leading to production of plant secondary metabolitesǁ. Biotech Adv. 2005;23:283–333.
58. Stamp, Nancy. Out of the quagmire of plant defense hypotheses. The Quar Rev of Bio. 2003;78(1):23 55.
59. Oyewale AO, Audu PA, Amupitan JO. A survey of the chemical constituents and biological activities of some medicinal plants. Chem Class J. 2004;162-165.
60. Rajender B, Saikumar A, Venkatesham A. Comparative antibacterial activities of combined crude leaf extracts of *Eucalyptus globules*, *Azadirachta indica* and *Ocimum scantum*. J Pharm Res. 2015;4(4):164-166.
61. Mondall NK, Mojumdar A, Chatterje SK, Banarjee JK, Gupt AS. Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species in vitro culture medium. J Appl Sci Environ Manage. 2009;13(1):49–53.

© 2023 Gurav et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105028