https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(4s) (December 2024); 15164-15177
Research Article

Phytochemical Investigation of *Erinocarpus nimmonii* Using FTIR and GC-MS Analysis

Rupali Aundhakar[1], D.K.Gaikwad[2*]

[1]Department of Botany Shivaji University, Kolhapur,Maharashtra,India-416004.
[*2]Raja Bhagwantrao Junior College, Aundh, Satara, Maharashtra, India.

***Corresponding Author**–Rupali Aundhakar
*Email: rupaliaundhakar@gmail.com

Abstract
Erinocarpus nimmonii, an endemic medicinal plant found in the Western Ghats of India, has been traditionally used for its therapeutic properties. The plant is rich in bioactive phytochemicals, including flavonoids, phenolics, and alkaloids, which contribute to its medicinal value. This study aims to identify and characterize the key bioactive compounds present in the leaf and seed extracts of *E. nimmonii* using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS). Ethanolic and cow urine extracts were analyzed to compare their phytochemical composition and bioactive potential. The FTIR analysis confirmed the presence of hydroxyl (-OH), carbonyl (C=O), and aromatic (C=C) functional groups, indicating the presence of polyphenols, flavonoids, and other bioactive compounds. GC- MS profiling revealed the presence of catechol, neophytadiene, tetradecane, squalene,and linoleic acid ethyl ester, which exhibit significant antioxidant, antimicrobial, andanti-inflammatory properties.The findings suggest that *E. nimmonii* possesses a rich phytochemical profile with potential pharmaceutical and nutraceutical applications. The bioactive compounds identified in this study highlight the plant's potential as a natural antioxidant and antimicrobial agent, paving the way for future research on its therapeutic applications.

Keywords: *Erinocarpus nimmonii*, GC-MS, FTIR, bioactive compounds, medicinal plant, phytochemical profiling, antioxidant, antimicrobial, therapeutic applications.

***Author of Correspondence: Email:** *rupaliaundhakar@gmail.com*

Accepted: 06-12-2024 *Received: 20-11-2024*

DOI: https://doi.org/10.53555/AJBR.v27i4S.7604

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1. Introduction

Medicinal plants have been used for centuries as natural sources of therapeutic compounds, contributing significantly to traditional and modern medicine. More than 60% of anticancer bioactive molecules are natural in origin. In India 45,000 medicinal plant species, are used by 3000 traditional users[1].These plants contain diverse bioactive phytochemicals, including alkaloids, flavonoids, terpenoids, and phenolics,[2,3]which exhibit various pharmacological properties suchas antimicrobial, antioxidant, and anti-inflammatory activities [4]. The increasing demand for plant-based medicineshas led to extensive research on phytochemical composition and their biological significance [5].Such bioactive compounds of plant origin are secondary metabolites can used in prevention of various diseases induced by oxidative stress, prevention of cardiovascular diseases, neurodegenerative diseases and cancers[6].

Erinocarpus nimmonii belongs to the family

Malvaceae**,** it is a monotypic genus, meaning it is the only species under the genus *Erinocarpus* [7]. It is a small to medium- sized evergreen tree with distinct palmate leaves and fibrous bark. *E. nimmonii* is an endemic plant of the Western Ghats**,** a globally recognized biodiversity hotspot in India. It is primarily found in Maharashtra, Goa, Karnataka, and Kerala**,** growing in moist deciduous and semi-evergreen forests at elevations from 600 to 1000 meters [8]. *Erinocarpus nimmonii* has long been used in folk medicine for various ailments. *Erinocarpus nimmonii* plant seeds are used as femine food, bark is used in rope making [9,10]. The pharmacognostic studies on *erinocarpus nimmonii* leaves shows anticancerous activities by using Allium cepa root tip assay method [11]. The *E.nommonii* leaf extract shows amylase inhibitory activity may be due presence of phytochemicals to reduce high blood sugar level[12]. *E.nimmonii* leaf extract shows anti inflammentory to treat inflammation and blood pressure and prevent cardio vascular diseases due to anti oxidant along with antihypertensive property [13]. Anti microbial theorapy, rich nutreal, mineraland anti oxidant supplement of Erinocarpus leaves protect from malnutrition[14].This study focuses on the phytochemical investigation of *Erinocarpus nimmonii*, by using FTIR and GC-MS analysis to identify the key bioactive compounds present in the leaf and seed extracts. Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR) are widely used analytical techniques that provide detailed insights into plant extract composition [15].

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

The plant specimens of *Erinocarpus nimmonii* were collected from Janai-Malai Hill, Satara, Maharashtra, a region known for its rich biodiversity and endemic flora. satara district is a district of Maharashtra state in western India and falls within the Deccan traps area. It lies between 17033’16” N and 74022’33”E. The collection was conducted during the peak flowering and fruiting seasons to ensure the highest yield of bioactive compounds. Proper documentation of geographic coordinates, altitude, and environmental conditions was maintained during the collection process.

Following collection, the plant samples, including leaves and seeds, were carefully cleaned with distilled water to remove any surface impurities. The specimens were authenticated by a qualified taxonomist and cross-verified with existing herbarium records. The leaves and seeds were then shade-dried at room temperature to preserve their phytochemical integrity. Once fully dried, they were finely ground using a mechanical grinder, sieved to obtain a uniform powder, and stored in airtight containers under controlled conditions for further extraction and analysis.

2.2 Extraction Techniques

Bioactive compounds from *Erinocarpus nimmonii* were extracted using ethanol and cow urine to compare their phytochemical extraction efficiency. Powdered leaves and seeds were mixed with ethanol or filtered cow urine followed by maceration and sonication at 20°C for 30 minutes. The mixtures were centrifuged (10,000 rpm, 10 min), filtered, and concentrated using a rotary evaporator, the semisolid left over have been collected. The sticky extract was then stored at 4°C in amber vials [34].

2.2.1 Storage Conditions to Maintain Bioactivity

To preserve the stability and bioactivity of the extracts, the concentrated samples were stored in amber-colored glass vials to protect them from light-induced degradation. The vials were tightly sealed and kept at 4°C to prevent oxidation and microbial contamination. These controlled storage conditions ensured the long-term stability of the bioactive compounds, maintaining their structural integrity for subsequent GC-MS and FTIR analysis.

2.3 Instrumental Analysis:

2.3.1 Fourier Transformed Infrared Spectroscopy

FTIR, or Fourier Transform Infrared Spectrophotometer, is the most efficient tool for determining the various kinds of functional groups and chemical bonds found in phytochemicals. A prominent characteristic of the chemical bond is the wave length of the light absorbed. A compound's chemical bonds can be ascertained by analyzing its infrared absorption spectra. Comparative analysis of spectra was conducted by using the Essential FTIR software, which provides a clear understanding of similarities in the spectra, as shown in graph 1and 2 indicate the spectra which were analyzed by the Alpha Bruker instrument. British Wiley library was used for FTIR data analysis. A 0.1gm/ml ethanolic and in cow urine extract of dried leaves and seed powder was sonicated at 20°C for 45 minutes and centrifuged at 10,000 rpm for 5 minutes. The supernatant was subjected to FTIR analysis using ALPHA Bruker, Germany.(CFC Shivaji University, Kolhapur).FTIR analysis was performed on the extract of leaves and seed sample in ethanol and cow urine. The Spectra were taken in the IR region of 4000-400 cm^{-1} for analysis of functional groups [16].

2.3.2 GC-MS/MS (Gas Chromatography-Mass Spectrometry) analysis:

GC-MS was used to identify bioactive compounds in ethanolic and cow urine extracts of *Erinocarpus nimmonii*. GC-MS/MS analysis was performed on Shimadzu, Japan TQ 8050 plus HS 20. Helium was used as the carrier gas. For analysis following conditions were maintained; Column oven temperature: 50.0°C, Injection temperature: 250.00°C, Injection mode: Split, Flow control mode with linear velocity, Pressure: 54.4 kPa, Total flow:54.6 ml/min, Column flow: 1.01 ml/min, Linear velocity :36.5 cm/sec, Purge flow :3.0 ml/min, Split Ratio:50.0. Initially, the oven temperature was set to 50°C and held for 2 minutes, followed by rate 5 for 180°C held for 2 minutes, followed by rate 5 for 250ºC held for 2 minutes and then rate 5 at 260°C held for 2 minutes. For GC ion temperature was maintained at 200°C, interference temperature at 270°C with a solvent cut

time of 3.00 min and detector gain mode is relative to tuning. The result detector gain is about 1.17 kV and a threshold was 0. For MS, the start time was 3.00 min and the end time was 50 min. The acq. Mode on Q3ScanandEvent Time was about 0.3sec with scan speed 1666. The Start m/z 45.00 and end m/z 500. Detected compounds were identified by comparing mass spectra with NIST, Wiley, and PubChem data bases.

3. Result:

3.1 FTIR Analysis of Leaf and Seed Extracts

Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify the functional groups present in the ethanolic extracts of *Erinocarpus nimmonii*. The FTIR spectra provide valuable insights into the phytochemical composition by detecting characteristic functional groups associated with bioactive compounds such as alcohols, alkanes, aromatics, carboxylic acids, halogen compound and alkylhalide in the ethanolic extract of *Erinocorpus nimmonii* leaves and seed (Table 1,2 and graph 1,2).

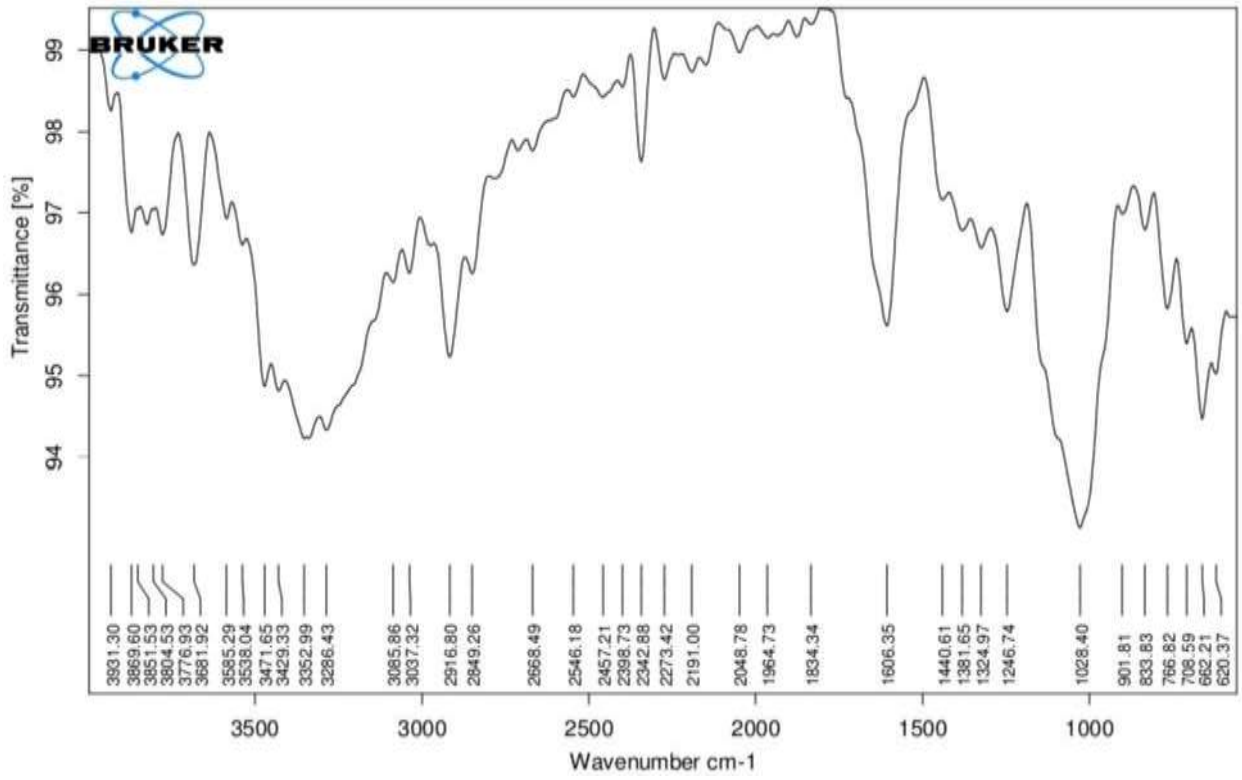
3.1.1 FTIR Analysis of Leaf Extract

The Table (1) and graph (1) demonstrates the presence of 36 functional groups recognized from the ethanolic extract of *Erinocorpus nimmoni* leaves. The strong instance peaks are identified at 3352.99, 3286.43,2916.80, 1606.35, 1028.40 cm-1 which are assigned the H- bonded and O- stretch, asymmetric stretching of –CH(CH2)vibration, C=O stretching and PO3 stretch with hydroxy compounds, alcohols, phenols, saturated aliphatic compounds- lipids, ketone compound and phosphate ion respectively. The peak at 2849.26 cm-1 attributes to symmetric stretching of –CH(CH2) vibration, lipids, fatty acids and protein[17]. Some other groups such as carboxylc acids, nitriles, terminal alkynes, ketone compound, aromatic compounds, phenol or tertiary alcohol, acids are absorbed at 3471.65,3429.33,2398.73,2457.21,2273.42,2191.00,1606.35,3085.86,2546.18,1440.61,1381.65,3352.99,2668.49,1028.40cm-1respectively.Thenumerous functional groups such as carboxylic acid, amines, amides, alkene, halogen compounds are present. [18].

Table1 :FTIR interpretation of compounds of ethanolic extract of *E.nimmoni* (Leaf)

Sr. no.	Wave no.cm- 1 (test sample)	Waveno. cm-1 (Reference article)	Functional Group Assignment	Expected phyto Compounds identified
1.	3869.60	> 3500	Non bounded O-H stretch	Hydroxy group
2.	3851.53	> 3500	Non bounded O-H stretch	Hydroxy group
3.	3804.53	> 3500	Non bounded O-H stretch	Hydroxy group
4.	3776.93	> 3500	Non bounded O-H stretch	Hydroxy group
5.	3681.92	> 3500	Non bounded O-H stretch	Hydroxy group
6.	3585.29	> 3500	Non bounded O-H stretch	Hydroxy group
7.	3538.04	> 3500	Non bounded O-H stretch	Hydroxy group
8.	3471.65	3500-2400, 3570-3200	O-H stretch carbolic group, Acidic, H-bonded	Carboxylic acid, Hydroxy compound
9.	3429.33	3500-2400, 3570-3200	O-H stretch carbolic group, Acidic, H-bonded	Carboxylic acid, Hydroxy compound
10.	3352.99	3570-3200	H-bonded, O-H stretch	Hydroxy compound, alcohols, phenols
11.	3286.43	3570-3200	H-bonded, O-H stretch	Hydroxy compound, alcohols, phenols
12.	3085.86	3100-3000	C-H stretch	Aromatic
13.	2916.80	2935-2915	A symmetric stretching of CH(CH2) vibration	Saturated aliphatic compound-lipids
14.	2849.26	2865-2845	Symmetric stretching of CH(CH2) vibration	Fatty acids, Lipids, Protein.
15.	2668.49	3300-2500	O-H stretch	Acid
16.	2546.18	3300-2500	O- H Stretch	Acid
17.	2457.21	3500-2400	O-H stretch, carboxylic group, Acidic	Carboxylic Acids
18.	2398.73	3500-2400	O-H stretch, carboxylic group, Acidic	Carboxylic Acids
19.	2273.42	2300-1900	Multiple bonding	Nitrile compounds
20.	2191.00	2260-2100	Carbon-carbon triple bond Multiple bonding	Nitrile compounds, Terminal alkynes
21.	2048.78	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
22.	1964.73	2100-1800	Carbonyl compound frequency	Transition metal carbonyls

23.	1834.34	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
24.	1606.35	1650-1600	C=O stretch	Ketone compound
25.	1440.61	1600-1400	C=C stretch	Aromatic
26.	1381.65	1410-1310	O-H bond, Alcoholic group	Phenol or tertiary Alcohol
27.	1324.97	1360-1210	C-H stretch	
28.	1246.74	1320-1210	C-O stretch, C-N stretch	Acid
29.	1028.40	1100-1000	Po3 stretch	Phosphate ion
30.	901.81	1000-675	=C-H bonding	Alkene
31.	833.83	1000-675	=C-H bonding	Alkene
32.	766.82	1000-675	=C-H bonding	Alkene
33.	708.59	1000-675 730-500	=C-H bonding C-Cl	Alkene, Halogen compound (chloro compound)
34.	662.21	620-490 730-500	C-I, C-Cl	Halogen compound
35.	620.37	620-490 730-500	C-I, C-Cl	Halogen compound (chloro compound, Iodo compound) Alkyl halides



Graph.1:FTIR spectrum analysis of ethanolic extract of leaf of *Erinocorpus nimmonii*

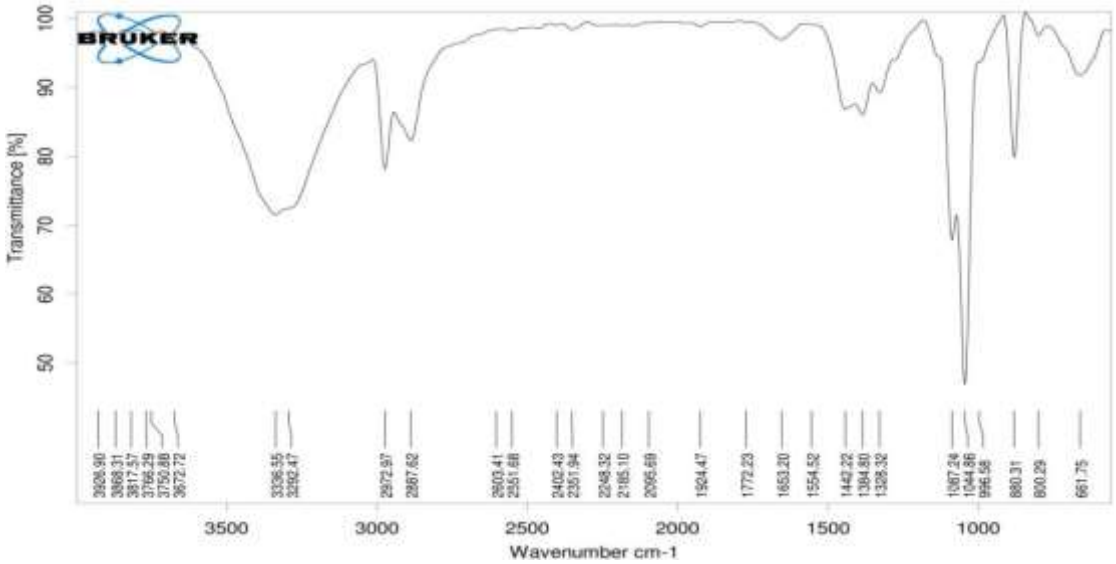
3.1.2 FTIR Analysis of Seed Extract

Table (2) and graph (2) demonstrate the ethanolic seed extract of *Erinocorpus nimmonii* shows various peak ratio of components. FTIR analysis confirms the presence of functional groups such as non bonded, O-H stretch carboxylic groups, acids, H- bonded, C-H stretch, C=N stretch, C=O stretch, C=C stretch, C-I, C-Cl, N-H bonding ≡ C-H bending. The major peak values at 3926.90, 3868.31, 3817.57, 3766.29, 3750.88, 3672.27 all are having higher peak value than 3500(> 3500) with hydroxy group. 3336.65, 3292.47 cm-1 have higher peak value and shows hydroxy compound with carboxylic acid and phenols. 2972.97, 2887.62 cm-1 has C-H stretch are aromatic and alkane. 1087.24, 1044.86 cm-1 which are most higher peak value in seed sample shows presenceofPO3stretch and phosphate ions. The peak value 2351.94,2248.32,2185.10cm-1 shows presence of nitrile compounds respectively. 2095.69 and 1924.47 cm-1 peak shows presence of metal carbonyl compounds. The peak value at 1772.23, 1653.20, 1554.52, 1442.22, 1384.80 and 1328.32 cm-1 shows presence of functional groups such as C = O stretch, C=C stretch, N-H bending, O-H bend with carbonyl alkene, amide, aromatic, phenols or tertiary alcohol respectively. At peak 880.31,800.29 and 661.75cm- 1

shows presence of halogen compound with = C- H bending, C-I, C-Cl.

Table 2:FTIR interpretation of compounds of ethanolic extract of seed of *E.nimmoni.*

Sr.no.	Wave no. cm-1 (Test sample)	Wave no. cm- 1 (reference article)	Functional group Assignment	Expected phyto compounds identified
1	3926.90	> 3500	H bonded, O-H stretch	Hydroxy group
2	3868.31	> 3500	H bonded, O-H stretch	Hydroxy group
3	3817.57	> 3500	H bonded, O-H stretch	Hydroxy group
4	3766.29	> 3500	H bonded, O-H stretch	Hydroxy group
5	3750.88	> 3500	H bonded, O-H stretch	Hydroxy group
6	3672.72	> 3500	H bonded, O-H stretch	Hydroxy group
7	3336.55	3570-3200	H bonded, O-H stretch	Hydroxy compound, alcohols, Phenols
8	3292.47	3500-2400 5700-3200	O-H stretch, carboxylic group, Acidic , H bonded, O-H stretch	Carboxylic acids, hydroxy compounds
9	2972.97	3100-3000	C-H stretch	Aromatic
10	2887.62	3000-2850	C-H stretch	Alkane
11	2603.41	3300-2500	O-H stretch	Acid
12	2551.68	3300-2500	O-H stretch	Acid
13	2402.43	3500-2400	O-H stretch	Carboxylic acid
14	2351.94	2248-2376	C=N stretch	Nitriles
15	2248.32	2248-2376	C=N stretch	Nitriles
16	2185.10	2260-2100 2300-1900	Carbon carbon triple bond, multiple bonding	Nitrile compounds, Terminal alkynes
17	2095.69	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
18	1924.47	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
19	1772.23	1820-1670	C=O stretch	Carbonyl
20	1653.20	1680-1620	C=C stretch	Alkene
21	1442.22	1600-1400	C=C stretch	Aromatic
22	1384.80	1410-1310	O-H bond, Alcoholic group	Phenol or tertiary alcohol
23	1328.32	1360-1210	C-H stretch	Amine
24	1087.24	1100-1000	PO3 stretch	Phosphate ions
25	1044.86	1100-1000	PO3 stretch	Phosphate ions
26	880.31	1000-675	=C-H bonding	Alkene
27	800.29	1000-675	=C-H bonding	Alkene
28	661.75	620-490 730-500	C-I, C-Cl	Halogen compound (chloro compound)



Graph.2:FTIR spectrum analysis of ethanolic extract of seed of *E.nimmonii*.

3.2 GC-MS Analysis: Identified Bioactive Components

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to identify the major bioactive compounds present in the ethanolic and cow urine extracts of *E. nimmonii*.. This study discovered that compounds from ethanolic and cow urine extract of leaves and seed of *Erinocarpus nimmonii* contains medicinally and nutritionally significant substances. Table 3-6 provides a summary of the bioactive compounds found in *Erinocarpus nimmonii* has been reported to exhibit antibacterial, anticancer, anti angiogenic and anthelmintic activities etc. The identified compounds have diverse chemical natures and functionalities, ranging from alkaloids, esters, phenols, and steroids to bile acids, macrolides, and sesquiterpenoids and shows diverse pharmacological activities, including antimalarial, antibacterial, antifungal, anti oxidant, antiviral, anticancer, anti-inflammatory etc. (Table 3-6). The technique enables precise identification of phytochemicals based on their mass spectra and molecular formulas, retention time, molecular weight, area and therapeutic potential of the plant.

3.2.1 GC-MS Profile of Ethanolic Leaf Extract

The GC-MS analysis of the ethanolic extract of *Erinocorpus nimmonii* leaves identified several bioactive phytocomponents, indicating its potential pharmaceutical and nutraceutical applications. Among the detected compounds, catechol, neophytadiene and tetradecane stand out due to their significant biological activities (**Table 3**). Decane ($C_{10}H_{22}$) and Dodecane ($C_{12}H_{26}$) shows antibacterial, antimicrobial and antifungal activities [22,23]. Catechol ($C_6H_6O_2$), a potent antioxidant, exhibited a retention time (R.T.) of 10.672 minutes with an area percentage of 8.43%. It is widely known for its radical scavenging properties, effectively neutralizing free radicals and protecting against oxidative stress- related disorders [24].Neophytadiene ($C_{20}H_{32}$), detected at an R.T. of 18.987 minutes with an area percentage of 11.29%, is a diterpene compound recognized for its strong anti-inflammatory and antimicrobial activities, making it a promising candidate for therapeutic applications against infections and inflammatory diseases [29].Tetradecane ($C_{14}H_{30}$), observed at an R.T. of 12.875 minutes with a notable area percentage of 13.77%, is a long-chain alkane known for its antibacterial potential, commonly found in medicinal plant extracts with antimicrobial properties [25].

Additionally, other phytoconstituents with significant biological roles were detected. Heptadecane ($C_{17}H_{36}$), with an area percentage of 13.50%, is another long-chain hydrocarbon known for its antimicrobial, anti-inflammatory activities[27]. Benzenepropanoic acid, 3,5-bis[1,1-dimethyl]-4-hydroxy-methyl-ester ($C_{18}H_{28}O_3$), constituting 11.35% of the extract, has potential pharmacological benefits, including antioxidant and anti-inflammatory effects. Dibutylphthalate($C_{16}H_{22}O_4$), detectedatan R.T.of 21.699 minutes, has been studied for its role in biological interactions, though its effects require further evaluation[31].

Notably,1-(+)Ascorbicacid2,6-dihexadecanoate($C_{38}H_{68}O_8$),identifiedatanR.T. of 21.943 minutes with an area of 5.41%, is an esterified form of ascorbic acid, known for its role in antioxidant defense mechanisms play important role in cancer, heart and other diseases[32]. The presence of phytol acetate ($C_{22}H_{42}O_6$), which is a diterpenoid acetate with antimicrobial, antileishmanial, antitrypanosomel and anti- inflammatory properties, further enhances the medicinal relevance of the extract[34].

The identification of these bioactive molecules through GC-MS profiling strongly supports the therapeutic potential of *E. nimmonii* leaf extract, suggesting its application in the formulation of novel pharmaceutical and nutraceutical products.

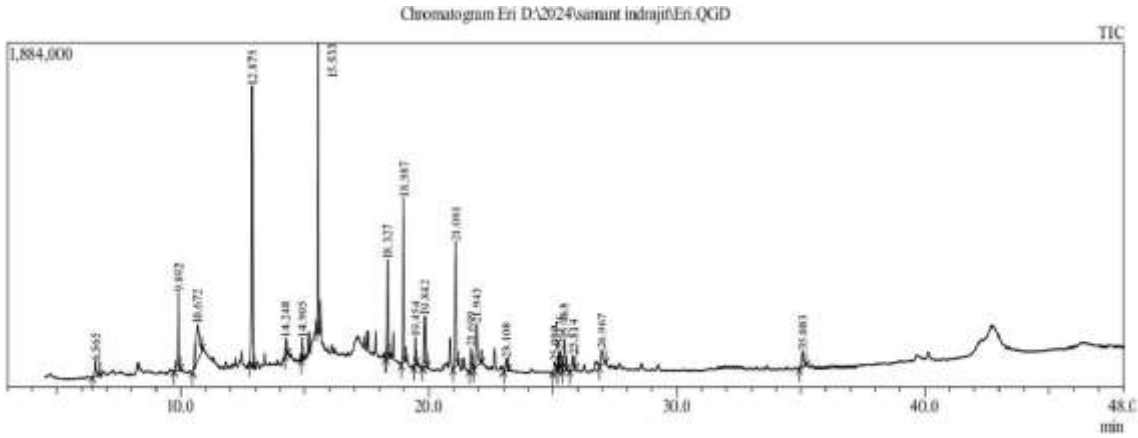


Figure3: GC-MS chromatogram of ethanolic leaf extract of *E.nimmonii*.

Table no.3:phytocomponent identified in the ethanolic extract of leaf of *E. nimmonii* by GC-MS/MS analysis.

Sr. No.	Compound Name	Molecular Formula	Molecular weight	R.T.	Properties
1.	Decane	$C_{10}H_{22}$	142	6.565	Antibacterial, Antimicrobial, Antifungal activity[22]
2.	Dodecane	$C_{12}H_{26}$	170	9.892	Antibacterial, Antifungal activity[23]
3.	Catechol	$C_6H_6O_2$	110	10.672	Antiseptic, Anti-oxidant activity[24]
4	Tetradecane	$C_{14}H_{30}$	198	12.875	Antibacterial activity [25]
5	2,6-Difluorobenzoic acid,tridec-2-ynyl ester	$C_{20}H_{26}F_2O_2$		14.279	Anti-oxidant, Pesticide, anti- inflammatory, Anti-androgenic, Hemolytic, antimicrobial, anticancer, 5-Alpha Reductase inhibitor, antipsychotic [26]
6	Nonadecane,9- methyl	$C_{20}H_{42}$	1282	14.905	Antibacterial activity
7	Heptadecane	$C_{17}H_{36}$	240.473	15.533	Antibacterial, anti-inflammatory, ,Antioxidant activity [27]
8	Eicosane	$C_{20}H_{42}$	282	18.372	Antifungal activity, found in person suffering from perkinsons disease[28]
9	Neophytadiene	$C_{20}H_{32}$	287.5	18.987	anti-inflammatory, antimicrobial, Antibacterial, anti fungal,Anti oxidant, anti cancer activity [29]
10	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	$C_{20}H_{40}O$	296	19.454	anti-inflammatory, antimicrobial activity[30]
11	Benzenepropanoic acid,3,5-bis[1,1- dimethyl]-4-hydroxy-methyl- ester	$C_{18}H_{28}O_3$	292	21.081	Antioxidant, antifungal activity
12	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	21.699	anti-inflammatory, antimicrobial activity [31]
13	1-(+) Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$		21.943	Antioxidant, play role in heart and diseases[32]
14	1-Octadecanol methyl ether	$C_{19}H_{40}O$	284	23.108	Antitumor, Antifungal and Antibacterial activity.
15	9,12,15-Octadecatrienoic- acid, methyl ester, (z,z,z)	$C_{19}H_{32}O_2$	292	22.463	Medicinally valuable bioactive compound for drug development against various diseases[33]
16	Phytol acetate	$C_{22}H_{42}O_6$	338	25.814	Anti inflammatory, anti leishmanial and antitrypanosomel activity[34]
17	Dichloroacetic acid, bis (2-ethylhexyl) ester	$C_{15}H_{42}C_{l2}O_2$	307.39	26.967	Antimicrobial activity
18	Hexanedioic acid, bis (2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.69	35.083	antitumor, Antifungal and Antibacterial activity.

The GC-MS profile of *E. nimmonii* leaf extract revealed a diverse range of bioactive phytocompounds with significant pharmacological potential. The presence of catechol, neophytadiene, and tetradecane suggests strong antioxidant, anti-inflammatory, and antimicrobial properties, respectively. Other identified compounds further contribute to the extract's medicinal value, supporting its potential application in drug formulation, skincare, and nutraceutical industries. Further pharmacological studies are warranted to explore the full therapeutic potential of these bioactive constituents.

3.2.2 GC-MS Profile of Ethanolic Seed Extract

The GC-MS analysis of *Erinocorpus nimmonii* seed extract identified several bioactive compounds, including styrene, linoleic acid ethyl ester, and squalene, which are known for their significant pharmacological properties (**Table** 4). These compounds contribute to the extract's potential applications in oncology and preventive medicine.

Styrene (C_8H_8), detected at a retention time (R.T.) of 5.887 minutes with an area percentage of 34.97%, is a naturally occurring aromatic hydrocarbon, used in rubber, latex and single used plastic[35]. It has been reported for its cytotoxic effects on cancer cells, indicating its potential role as an anticancer agent.

Linoleic acid ethyl ester ($C_{20}H_{36}O_2$),observed at an R.T. of 31.890 minutes with an area percentage of 28.66%, is a bioactive lipid known for its strong antioxidant, antifungal properties. It plays a crucial role in protecting cellular membranes from lipid peroxidation, thereby reducing oxidative stress-related damage [36]. Squalene ($C_{30}H_{50}$), identified at an R.T. of 49.993 minutes with an area percentage of 25.19%, is a well-established bioactive compound with anticancer and immune-modulatory properties. It has been extensively studied for its role in preventing tumor progression and enhancing immune responses, making it a promising candidate for cancer prevention, nurodegenarative diseases and therapy [37]. Hexadecenoic acid, ethylester($C_{18}H_{36}O_2$) at R.T. 34.086 min with an area 11.17% is well established anti oxidant with anti androgenic, hemolytic properties and also used in flavouring agent. It also used as pesticides and lubricant[30].

The presence of these bioactive molecules under scores the therapeutic significance of *E. nimmonii* seed extract, particularly in oncology, antioxidant defense, and immune regulation. These findings suggest that the extract could be explored further for potential applications in pharmaceutical and nutraceutical formulations.

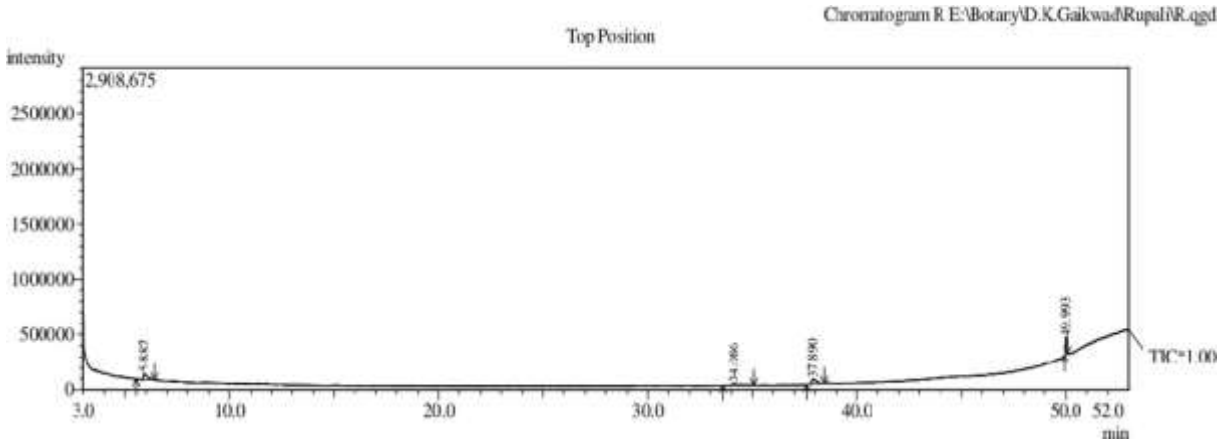


Figure 4: GC-MS chromatogram of ethanolic seed extract of *E.nimmonii*.

Table no. 4 :phytocomponents identified in the ethanolic extract of seed of *E. nimmonii* by GC-MS/MS.

Sr. no.	Compound name	Molecular formula	Mol.wt. Gm/mol.	R. time	Properties
1	Styrene	C_8H_8	104	5.887	Used in rubber, plastic[35]
2	Hexadecenoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	34.086	Antitumor, Antifungal and Antibacterial, antioxidant, antiandrogenic[30]
3	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	31.890	Antifungal, anti oxidant[36]
4	Squalene	$C_{30}H_{50}$	410	49.993	Antioxidant, cyto protective, cancer and cardiovascular diseases[37]

The GC-MS analysis of *E. nimmonii* seed extract confirms the presence of bioactive compounds with significant medicinal properties. Styrene exhibits cytotoxic activity, linoleic acid ethyl ester serves as a potent antioxidant, and squalene contributes to cancer prevention and immune system modulation. These findings highlight the potential of *E. nimmonii* seed extract as a valuable source for developing

pharmaceutical and nutraceutical formulations aimed at disease prevention and therapeutic applications. Further pharmacological investigations are warranted to explore its full therapeutic potential.

3.2.3 GC-MS Profile of Cow Urine Extracts

The GC-MS analysis of cow urine extracts from *E. nimmonii* leaves and seeds identified a range of bioactive compounds with significant pharmacological properties (**Tables 5 and 6**). Notably, benzoic acid, benzyl benzoate, neophytadiene, phytol and L- alpha-terpineol were among the major constituents, indicating the therapeutic potential of these extracts.

Benzoic acid ($C_7H_6O_2$), a prominent compound found in both leaf and seed extracts, exhibited a high area percentage of 39.02% and 41.42%, respectively. It is well known for its antimicrobial and antifungal properties, supporting its traditional use in treating infections and skin conditions[40,41]. Benzylbenzoate ($C_{14}H_{12}O_2$),identified at retention times of 29.515 minutes (leaf extract) and 31.035 minutes (seed extract), is widely recognized for its antifungal and antiparasitic activities, commonly used in topical treatments for scabies and lice[42].

Neophytadiene ($C_{20}H_{38}$), found in the leaf extract with an area percentage of 8.60%, is a bioactive diterpene known for its anti-inflammatory and antimicrobial effects, suggesting potential applications in managing inflammatory disorders and infection of skin diseases[24]. The presence of phytol ($C_{20}H_{40}O$), a diterpene alcohol with antioxidant, anti-inflammatory, immune modulator and anti caner properties, further enhances the therapeutic potential of the extract. L-alpha-terpineol ($C_{10}H_{18}O$), detected in both extracts, contributes to their medicinal value due to its known antimicrobial and antioxidant activities[39].

Additionally, the presence of n-hexadecanoic acid *(*$C_{16}H_{32}O_2$), commonly found in medicinal plants, reinforces the anti-inflammatory and antimicrobial potential of the cow urine extracts. Other significant compounds, such as linoleic acid ethyl ester and oleic acid play essential roles in cellular protection against oxidative stress, making these extracts valuable candidates for pharmaceutical and nutraceutical applications.

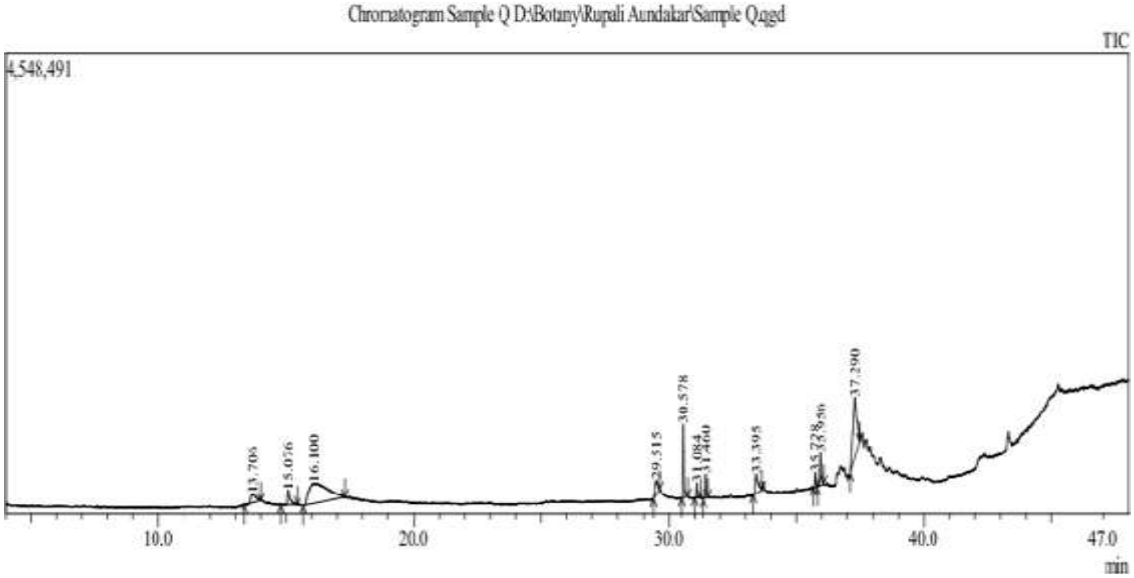


Figure 5: GC-MS chromatogram of cow urine extract of Leaf of *E.nimmonii*.

Table 5: Phytocomponents Identified in the Cow Urine Extract of *E. nimmonii* Leaves by GC-MS Analysis.

Sr. No	CompoundName	Molecular Formula	Molecular Weight (g/mol)	Retention Time (R.T.) (min)	Reported Biological Activity
1	Cyclohexane-carboxylicacid	$C_7H_{12}O_2$	128	13.706	Antimicrobial, anti-inflammatory[38]
2	L-alpha-Terpineol	$C_{10}H_{18}O$	154.29	15.076	Antioxidant, antimicrobial[39]
3	Benzoicacid	$C_7H_6O_2$	122	16.100	Antifungal, antimicrobial[40,41]
4	Benzylbenzoate	$C_{14}H_{12}O_2$	212.248	29.515	Antifungal, antiparasitic, scabies[42]
5	Neophytadiene	$C_{20}H_{38}$	278.59	30.578	Anti-inflammatory, antimicrobial[24]
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	-	31.460	Antioxidant, anti-inflammatory[43]

7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	33.395	Anti-inflammatory, antimicrobial[44]
8	g-Octadecenoic acid methyl ester (E)	$C_{19}H_{36}O_2$	296	35.728	Antioxidant, cardioprotective
9	Phytol	$C_{20}H_{40}O$	296	35.956	Antioxidant, anti-inflammatory[45]
10	Choleston-3-amine N,N,4,4-tetramethyl	$C_{31}H_{57}N$	443	37.290	Potentialbioactive lipid[46]

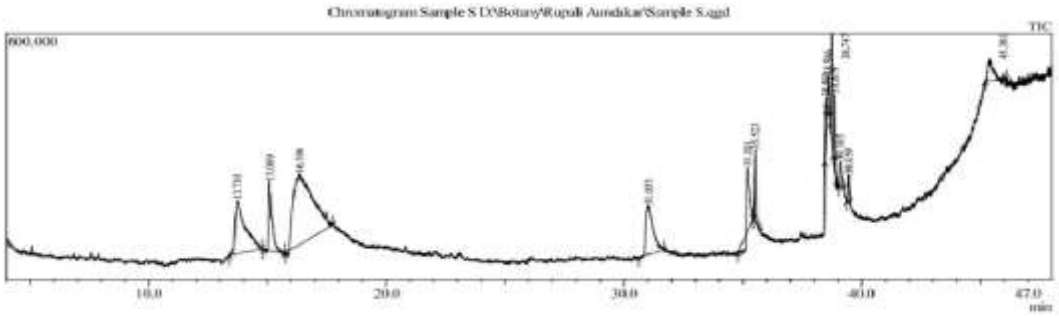


Figure 6: GC-MS chromatogram of cow urine extract of seeds of *E.nimmonii*.

Table6: Phytocomponents Identified inthe CowUrineExtract of *E.nimmonii*Seeds by GC-MS Analysis

Sr. No	CompoundName	Molecular Formula	Molecular Weight (g/mol)	Retention Time(R.T.) (min)	Reported Biological Activity
1	Cyclohexane-carboxylic acid	$C_7H_{12}O_2$	128	13.710	Antimicrobial, anti-inflammatory[38]
2	L-alpha-Terpineol	$C_{10}H_{18}O$	154.29	15.089	Antioxidant, antimicrobial[39]
3	Benzoic acid	$C_7H_6O_2$	122	16.306	Antifungal, antimicrobial[40,41]
4	Benzyl benzoate	$C_{14}H_{12}O_2$	212.248	31.035	Antifungal, antiparasitic [42]
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	35.201	Anti-inflammatory, antimicrobial[44]
6	Hexadecenoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	35.923	Antioxidant, lipid metabolism support[30]
7	9,12-Octadecadienoic acid (2,2)	$C_{18}H_{32}O_2$	280	38.501	Antioxidant, Anti-inflammatory, cardio protective[47]
8	Oleic acid	$C_{18}H_{34}O_2$	282.46	38.586	Anti-inflammatory, anti tumor, cardiovascular
					health[48]
9	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	38.747	Antioxidant, anti-inflammatory[36]
10	Ethyloleate	$C_{20}H_{38}O_2$	310	38.879	Antioxidant, lipid-lowering agent[49]
11	Octadecanoicacid	$C_{18}H_{34}O_4$	314.5	39.105	Antioxidant, antimicrobial[49]
12	Octadecanoic acid, 17-methyl methyl ester	$C_{20}H_{40}O_2$	312.53	39.459	Anti-inflammatory, antimicrobial[49]

The GC-MS analysis of cow urine extracts from *E. nimmonii* leaves and seeds highlights the presence of several bioactive compounds with antimicrobial, antioxidant, anti-inflammatory, and antifungal properties. Benzoic acid and benzyl benzoate, identified as major constituents, validate the traditional medicinal use of cow urine extracts in treating infections and skin ailments. Other components, such as phytol, neophytadiene, and L-alpha-terpineol, further contribute to their therapeutic potential, particularly in oxidative stress-related disorders. The presence of essential fatty acids, such as linoleic acid ethyl ester and oleic acid, underscores the extract's potential benefits in cardiovascular and immune health. These findings suggest that *E. nimmonii* cow urine extracts could serve as valuable candidates for pharmaceutical and nutraceutical applications, warranting further research into their full therapeutic potential.

4. Discussion

The FTIR analysis of *Erinocorpus nimmonii* extracts confirms the presence of various bioactive functional groups, highlighting their medicinal potential. The detection of hydroxyl (-OH) and carboxyl (-COOH) groups in dicates the presence of phenolic acids and flavonoids, known for their antioxidant and antimicrobial properties [19]. The presence of alkane and alkene bonds suggests the involvement of terpenoids, which are often linked with cytotoxicactivity [50], while phosphateion (PO_3) stretching further supports the antimicrobial potential of the extracts [18]. These findings align with previous studies on medicinal plant extracts exhibiting similar FTIR spectral characteristics [20]. Notably, the presence of aromatic ring structures, indicated by C=C stretching at1440.61cm^{-1}and1653.20cm^{-1},confirms the existence of flavonoids, which contribute to radical scavenging and cytotoxic activities[19]. These spectral data further validate the therapeutic potential of *E. nimmonii* extracts and their application in pharmaceutical and nutraceutical formulations.

FTIR spectroscopy is a powerful tool for identifying functional groups based on characteristic peak values within the infrared spectrum. The ethanolic extract of *E. nimmonii* leaves revealed 36 functional groups (Table 1, Figure 1), with prominent peaks at 3352.99 cm^{-1} and 3286.43 cm^{-1}, indicating hydroxyl (O-H) stretching from alcohols and phenols. Peaks at 2916.80 cm^{-1} and 2849.26 cm^{-1} correspond to asymmetric and symmetric stretching of –$CH(CH_2)$ vibrations, suggesting the presence of lipids and fatty acids. A peak at 1606.35 cm^{-1} indicates C=O stretching, characteristic of ketones, while 1028.40 cm^{-1} represents PO_3 stretching, confirming phosphate ions. Additional peaks suggest the presence of carboxylic acids, nitriles, terminal alkynes, aromatic compounds, and amides [17]. Similarly, the FTIR spectrum of *E. nimmonii* seed extract (Table 2, Figure 2) confirms a diverse range of functional groups. Peaks between 3926.90cm^{-1} and 3672.27cm^{-1} correspond to hydroxyl (O-H) stretching, signifying alcohols, carboxylic acids, and phenols, while peaks at 3336.65 cm^{-1} and 3292.47 cm^{-1} further indicate hydroxyl compounds associated with phenols and carboxyl acids. Peaks at 2972.97 cm^{-1} and 2887.62 cm^{-1} confirm the presence of C-H stretching from aromatic and alkane compounds. Strong PO_3 stretching, observed at 1087.24 cm^{-1} and 1044.86 cm^{-1},confirms the presence of phosphate ions, where as peaks at 2351.94 cm^{-1}, 2248.32 cm^{-1}, and 2185.10 cm^{-1} indicate nitrile compounds. Metal carbonyl groups are identified through peaks at 2095.69 cm^{-1} and 1924.47 cm^{-1}, while peaks at 1772.23 cm^{-1}, 1653.20 cm^{-1}, and 1554.52 cm^{-1} highlight the presence of functional groups such as C=O (carbonyl), C=C (alkene), and N-H bending (amide).Additionally ,halogen compounds are confirmed through peaks at 880.31 cm^{-1}, 800.29 cm^{-1}, and 661.75 cm^{-1}, which correspond to =C-H bending, C-I, and C-Cl stretching. These spectral characteristics provide a comprehensive chemical profile of *E. nimmonii* extracts, reinforcing their potential pharmacological applications.

The GC-MS analysis of *Erinocorpus nimmonii* extracts confirmed the presence of multiple bioactive compounds, reinforcing their potential medicinal applications. The leaf extract revealed key phytoconstituents such as catechol, neophytadiene, and phytol acetate, which have been widely studied for their strong antioxidant and antimicrobial activities. Catechol, a well-known radical scavenger, plays a crucial role in reducing oxidative stress, while neophytadiene and phytol acetate contribute to antimicrobial and anti-inflammatory properties, aligning with previous findings on medicinal plant extracts [29,34]. These compounds suggest the suitability of *E. nimmonii* leaf extract for therapeutic formulations targeting oxidative stress-related diseases and microbial infections.

The seed extract contained notable bioactive components, including styrene, linoleic acid ethyl ester, and squalene. Squalene has been recognized for its cytotoxic effects on cancer cells, indicating potential anticancer applications [37]. Linoleic acid ethyl ester is a powerful antioxidant that prevents lipid peroxidation, reducing cellular damage linked to oxidative stress, while squalene, a known immune-modulatory compound, has demonstrated significant potential in inhibiting tumor progression and enhancing immune response [45,48]. The presence of these compounds suggests that *E. nimmonii* seed extract could be explored further for its role in oncology and preventive medicine.

The cow urine extracts of *E. nimmonii* leaves and seeds also exhibited a diverse range of bioactive constituents, including antimicrobial agents such as benzoic acid, benzyl benzoate, and L-alpha-terpineol. Benzoic acid and benzyl benzoate are well- documented for their antifungal and antimicrobial activities, reinforcing their traditional use in Ayurvedic medicine for treating infections and skin disorders[24]. Additionally,L-alpha-terpineol, known for its antioxidant and anti-inflammatory properties, contributes to the therapeutic potential of cow urine extracts, suggesting applications in managing inflammatory and microbial infections. The presence of essential fatty acids, including linoleic acid ethyl ester and oleic acid, further enhances the pharmacological relevance of these extracts, particularly in cardiovascular and immune health.

Overall, the GC-MS profiling of *E. nimmonii* extracts underscores their pharmacological potential in antioxidant, antimicrobial, and anticancer therapies. The identified bioactive compounds support their use in traditional medicine while also presenting opportunities for modern pharmaceutical and nutraceutical development. Further in vitro and in vivo studies are warranted to validate the therapeutic efficacy and potential applications of these extracts in clinical settings.

5. Conclusion

This study systematically evaluated the phytochemical composition, bioactive potential, and medicinal properties of *Erinocarpus nimmonii* using GC-MS, FTIR and bioactivity assays. GC-MS identified bioactive compounds like catechol, neophytadiene,

squalene, and benzyl benzoate, contributing to its antioxidant, antimicrobial, and anti- inflammatory effects. FTIR confirmed the presence of key functional groups linked to polyphenols, flavonoids, and terpenoids. These findings validate *E. nimmonii* as a promising candidate for herbal medicine, nutraceuticals, and pharmaceutical applications. Its rich phytochemical profile, strong bioactivity, and antimicrobial potential support its traditional medicinal use and highlight its potential in treating oxidative stress-related diseases and microbial infections. Future research should focus on isolating active compounds, conducting in vivo and clinical trials, and developing herbal formulations such as ointments, dietary supplements, and functional foods. Mechanistic studies are needed to understand its pharmacological pathways, and sustainable conservation efforts shouldbeprioritizedtoensureitsavailabilityformedicinal use.Inconclusion,*E.nimmonii* holds significant therapeuticpotential, encouraging further exploration for herbal drug development and alternative medicine applications.

Author Information Authors and affiliations
Department of Botany Shivaji University, Kolhapur, Maharashtra, India-416004. Rupali Aundhakar, D.K.Gaikwad

Authors Contribution
Mrs. Rupali Aundhakar carried out the experiments, assisted in designing the study, and drafted the manuscript designed the graphical abstract. D.K.Gaikwad was involved in study design, manuscript preparation ,and provided funding support. The final draft of the work has been thoroughly read and approved by all authors.

Declarations
Declaration of competing interest
The authors declare tha they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

Conflict of Interest
The authors declare no conflict of interest.
Ethics Approval Not applicable.

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