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Isolation and Characterization of Phytol Compound from the *Lagerstroemia reginae* plant Extract

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ABSTRACT

Herbal medications have been found to have the same pharmacological efficacy as pharmaceutical medications. Analysing the bioactive components found in herbal extracts is made easier by preliminary screening. The bioactive components in the methanolic extract of *Lagerstroemia reginae* were examined, as well as spectral investigations of the substance. The bioactive chemical components included in the active methanol extract were also identified via GC-MS analysis. The outcomes showed that the methanol extracts included a phytol component. The isolated pure Phytol compound is characterized by SEM, EDAX, FTIR, UV, H^1NMR, C^{13}NMR and GC-MS. The study came to the conclusion that active phytol compounds found in leaf extracts of *Lagerstroemia reginae* might be used to create plant-based drugs.

1. Introduction

Since they contain several phytochemicals that prevent and treat a variety of diseases, plants are strong and effective biochemists (Yadav and Dixit, 2008). From ancient times, traditional healers have used medicinal plants and their components, such as stems, leaves, bark, roots, and seeds, to treat a variety of illnesses. The "active components" or "active component" of herbal medicines are phytochemicals found in plants that have therapeutic advantages. These substances are the main source for new medication development (Balandrin *et al*., 1993). India is the world's greatest producer of medicinal plants and has been referred to as the botanical paradise of the globe due to its diverse flora. Active components in medicinal plants have been discovered to be an effective source as therapeutic compounds. As the majority of pharmaceutical chemicals are made from medicinal plants, experts in the field of biotechnology are very interested in these plants.

People are now considering traditional healing practises like Ayurveda, Siddha and Unani after decades of major issues with Western medicine. This occurs as a result of the negative consequences of synthetic medications. In health care initiatives, especially in underdeveloped nations, herbal medicines are significant. The concept of a medical plant in ancient Indian literature is extraordinarily broad and all plant parts are seen to be potential sources of medicinal compounds (Shankar and Ved, 2003). Due to insufficient quality control, there is a lack of awareness of alternative medicines in wealthy nations. The results of research on conventional medications need to be documented (Dahanukar *et al*., 2000).

The presence of a variety of complex chemical compounds with various compositions that arise as secondary metabolites is primarily responsible for the medicinal capabilities of medicinal plants. Alkaloids, glycosides, tannins, proteins, phenolic compounds and flavonoids are the most prominent of these bioactive components of plants (Cragg and Newman, 2001).

A detailed understanding of the primary chemical components found in plants is fascinating not just for the discovery of therapeutic agents, but also because it may be useful for identifying new sources of economically viable phytocompounds for the synthesis of novel complex chemicals and for understanding the true value of folk remedies. Hence, today, the standardisation of herbal medications and goods depends greatly on the validation of herbal pharmaceuticals. Gas chromatography (GC) and mass spectrometry (MS) were employed to analyse the components found in natural plants. For the investigation of non-polar components, volatile essential oils, fatty acids, lipids and alkaloids, GC/MS has recently become frequently employed.

Several plant products are assessed based on their historic applications in the modern era of medication research and the identification of novel pharmacological compounds. In order to provide a solid scientific foundation for the traditional use of medicinal herbs, biological screening is required. Throughout, there are several screening procedures being conducted for novel plant-based bioactive compounds.

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Traditional medicines can be examined using Gas Chromatography (GC) and Mass Spectroscopy (MS), which can also be used to describe the component of interest. In the essential oils of several aromatic plants, such as *Cleome serrata* and *Lantana radula*, there is an acyclic diterpene alcohol known as phytol (Passos *et al.,* 2012). Phytol has been shown to have a number of medicinal properties, including efficacy against mycobacteria (Saikia *et al.,* 2010), anticonvulsant, antispasmodic, and anticancer properties (Costa *et al.,* 2012; Lee *et al*., 1999). Diterpenes have shown some promise pharmacological anti-inflammatory properties, although few investigations have specifically focused on phytol (Fernandez *et al*., 2001; Demetzos *et al.,* 2001).

Lagerstroemia reginae was the plant utilised for the investigation a magnificent medium-sized deciduous tree. Bark is smooth or thinly flaking, pale-brown in colour. Simple, big, elliptical, elongated and short-stalked leaves that become blood red before dropping are present. The most beautiful blossoms are huge, tall clusters of rose-like flowers (approximately 4.5–5 cm across), which have crinkly petals and many stamens. These are found at the extremities of branches. The fruit wall of these woody, spherical (2 cm in diameter) and dehiscent structures have five or more spreading lobes. The study's primary goal is to identify and quantify the bioactive phytol compounds in the methanolic extract of *Lagerstroemia reginae* using gas chromatogram phy-mass spectrometry analysis and other spectral techniques.

2. Materials and Methods

2.1 Collection of Plant Materials

The fresh *L. reginae* samples were collected at random in the Yercaurd Hills of Tamil Nadu. The sample parts were washed under running water, left to air dry, homogenised into a fine powder, and then kept in airtight containers in the refrigerator.

2.2 Preparation of Extracts

The unprocessed sample extract was produced using the Soxhlet extraction procedure. A thimble was used to collect an evenly distributed 20gm of powdered sample material before a separate extraction with 250ml of methanol. Until the solvent in the extractor's syphon tube turns colourless, the extraction process must be maintained for 24 hours. Once in a beaker, the extract was then heated to between 30 and 40 degrees Celsius on a hot plate until the solvent had completely evaporated. Dried extract was stored in a 4°C refrigerator until usage.

2.3 GC-MS analysis

A Perkin-Elmer GC Clarus 500 system and GC interfaced to an MS equipped with an Elite-I, fused silica capillary column (30 mm × 0.25 mm 1D × 1 µ mdf, made of 100% dimethyl poly siloxane) were utilised for the GC-MS analysis of the methanolic extract of *L. reginae*. An electron ionisation device with an ionising energy of 70 eV was employed for GC-MS detection. The injector temperature was 250°C, while the ion-source temperature was 280°C. Helium gas (99.999%) was utilised as the carrier gas at a constant flow rate of 1 ml/minute and an injection volume of 2 µl (split ratio of 10:1). The oven temperature was set to start at 110°C (isothermal for 2 minutes), grow at a rate of 10°C/minute to 200°C, then decrease at a rate of 5°C/minute to 280°C, and terminate with an isothermal period of 9 minutes at 280°C. At 70 eV, a scan period of 0.5 seconds, and fragments between 45 and 450 Da, mass spectra were recorded. The GC continued for 36 minutes in total. By comparing each component's average peak area to the sum of all peak areas, the relative percent quantity of each component was determined. Software called turbomass was used to handle mass spectra and chromatograms.

2.4 Identification of phytocomponents

Analysis of the mass spectrum the National Institute of Standard and Technology (NIST) database, which contains more than 62,000 patterns, was used for the GC-MS experiment. A comparison was made between the spectra of the unknown component and the spectrum of the known components kept in the NIST collection. The components of the test materials' names, molecular weights, and structures were determined.

2.5 Structural characterization of an isolated compound

These are some of the spectrum investigations used to characterise the structure of an isolated pure chemical;

2.5.1 FTIR Analysis

For the purpose of examining the existence of functional groups, the Fourier-transformation-(FTIR) spectra of the isolated molecule was captured using an FT-IR spectrophotometer. Using a disc of potassium bromide (KBr), the sample was fixed and the spectra was then analysed at wave numbers ranging from (400-4000 cm^{-1}). On the basis of their wave number, the numerous bands are documented.

2.5.2 NMR spectroscopy Analysis

The study employed a Bruker Avance II 400 NMR spectrometer with a cryomagnet with a 9.4 T field strength and a 400 MHZ H^1 frequency to record the H^1 and C^{13}NMR spectra. Chemical changes are measured and represented in ppm.

2.5.3 Mass spectra Analysis

The Shimadzu GC-MS-QP 2010 gas chromatography equipped with a DBI (methyl phenyl siloxane, 30 m × 0.25mm i.d) capillary column was used to perform the GC-MS analysis carries helium at a flow rate of 0.7 mL/min; the column oven temperature is 70°C, 5 min in 180˚C, 180-260˚C at 3˚ C/min, 5 min in 260˚C, 260-280˚C at 0.2˚C/min and finally 5 min in 280˚C; the injector temperature is 280 °C; the detector temperature is 290°C; the volume of TMS or its derivatives is injected at a rate of 1 µl in n-hexane (2%); the split ratio is 3:0. These are the MS operational parameters: quadrupole temperature of 100 °C, ion source temperature of 200°C, solvent delay of 6.0 min., scan speed of 2000 amu/s, scan range of 0-600 amu, and eV voltage of 3000 volts (Kemp, 1991).

2.5.4 Scanning Electron Microscopy

Scanning electron microscopy is one of the electron microscopy methods and it provides a different image on surface of the samples by focusing of high energy beam of electrons. Scanning electron microscopy detects the signal from sample by interaction of electrons into the surface of sample. SEM detecting different type of signals and that signals not only coming from samples also comes from some other interactions of nearby the sample surface (Gilbert et al. 1978). SEM images have a good depth of providing three dimensional images on the sample surface. This good depth and wide range magnification helps to find out the structure on surface of sample and these properties are help to familiar the imaging mode of SEM.

2.5.5 EDAX

EDAX, also known as Energy Dispersive X-ray Spectroscopy (EDS) or Energy Dispersive X-ray Analysis,

is an analytical technique used to identify the elemental composition of a material. It works by bombarding a sample with a focused beam of electrons, which causes the emission of characteristic X-rays from the atoms within the sample. By analysing the energy of these emitted X-rays, the elements present in the sample can be identified and quantified. EDAX is often used in conjunction with electron microscopy techniques like SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy).

2.5.6 Antimicrobial studies

One of the most potent and effective contributions of modern science and technology to the management of infectious diseases is the discovery and development of antibiotics. However, there has been alarming increase in the rate of pathogenic microorganism resistance to commonly used antimicrobial medicines (Ge et al., 2002; Nair and Chanda, 2005; Neogi et al., 2008). Numerous phytochemicals found in medicinal plants, including flavonoids, alkaloids, tannins, and terpenoids, have antibacterial and antioxidant activities (Talib and Mahasneh, 2010). Antibiotic resistance is a subject that continues to provide difficulties for the healthcare industry in both developed and developing nations all over the world. The existing antibiotic therapy is significantly threatened by the emergence and spread of multidrug resistant organisms

3. Results and Discussion

Nowadays, medicinal plants are regarded as substantial supplies of components for brand-new, cutting-edge medications. Several contemporary medications are created and made using curative herbs. The creation, updating, use and quality assurance of herbal formulations depend heavily on the analysis and extraction of various plant materials and their constituents (Heinrich, 2000). In the present study the bioactive compound present in the methanol extract of *L. reginae* were screened and characterized.

This plant's leaves contain a number of significant bioactive substances. Moreover, the leaves contain the poison pennyroyal. This dangerous substance can harm the liver (Cragg and Newman, 2001). Yet, this plant's essential oil is used topically to the skin to ward off insects and stop their biting (Georgiev and Stoyanova, 2006). Scientists and researchers have recently shown an increasing interest in developing novel medications derived from medicinal plants. In addition, they are interested in the plant's stems and leaves since they may contain certain bioactive substances that may be isolated and utilised as drugs to cure illnesses. They hold the view that medication made from stems and leaves is reliable and safe. The separated medications from this plant can compete with expensive, unfavourable synthetic pharmaceuticals (Iwu *et al*., 2009; Georgiev and Stoyanova, 2006).

3.1 FE-SEM Studies

Field emission scanning electron microscopy (FESEM) was used to analyze the materials' surface morphology. At both low and high magnifications (ranging from 10 nm to 10 µm), the results demonstrated the Phytol on a nano-flake structure. The Phytol have a distinct porosity structure and are organized in a flake-like shape, as seen in Fig. 1. Several evenly spaced Phytol compound combine to form flake-like configurations to form these nano-flakes, which increase the surface area overall.

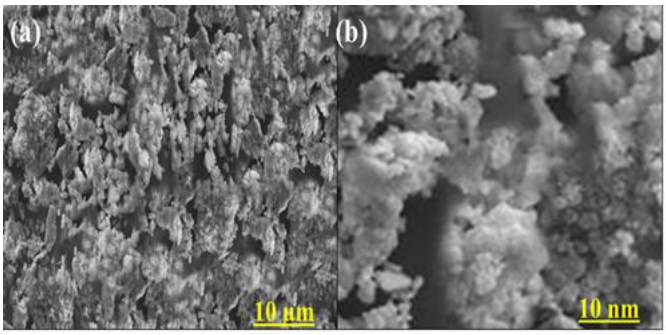
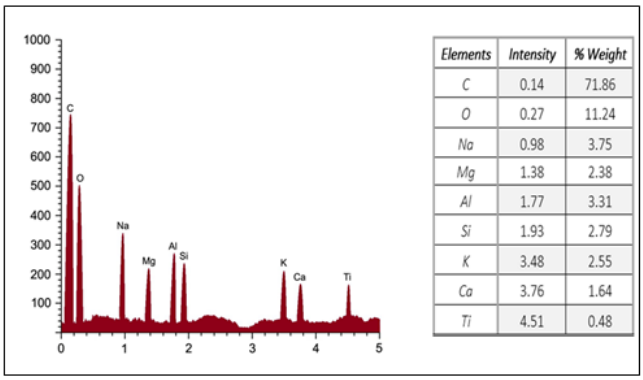


Figure 1: FESEM Analysis of Phytol compound

3.2 EDAX Analysis

The elemental composition of the Phytol was verified by energy-dispersive X-ray spectroscopy (EDAX) analysis, as illustrated in Fig. 2f. As shown in Table 1, the atomic percentages of carbon (C), oxygen (O), and sodium (Na) were confirmed by the EDAX spectrum to be 71.86%, 11.24%, and 3.75%, respectively. The significant carbon content shows that the Phytol extract was successfully converted to activated carbon. The atomic fraction of carbon (71.86%) was notably larger than that of oxygen, indicating that the produced substance was carbon-rich.



Elements	Intensity	% Weight
C	0.14	71.86
O	0.27	11.24
Na	0.98	3.75
Mg	1.38	2.38
Al	1.77	3.31
Si	1.93	2.79
K	3.48	2.55
Ca	3.76	1.64
Ti	4.51	0.48

Figure 2: EDX Analysis of Phytol compound

3.3 GCMS Analysis

The results of the GC-MS analysis of the methanolic extract of *L. reginae* were presented in Table. 1, with a total of 11 identified chemicals from the chromatogram. For *L. reginae*, the active principle (name), retention time (RT), molecular composition and molecular weight were determined in the methanol extract. The existence of multiple components with varied retention durations, as shown in Figure. 1, was verified by the GC-MS spectra.

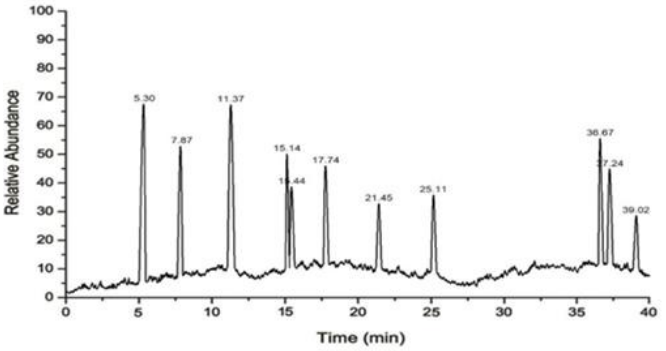


Figure 3: GCMS Analysis of L. *reginae* Methanolic extract

Phytol molecule has been discovered as the bioactive substance. Acyclic diterpene alcohol phytol is a part of chlorophyll. Precursors like phytol are frequently utilised to

create synthetic versions of vitamins E and K1. Comparing phytol to other chemicals, it exhibits the highest biological activity. Anxiolytic, metabolism-regulating, cytotoxic, antioxidant, autophagy and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating and antibacterial properties of phytol have all been researched (Rontani *et al*., 2003). Here is the phytol compound figure 2.

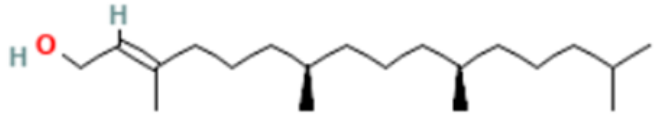


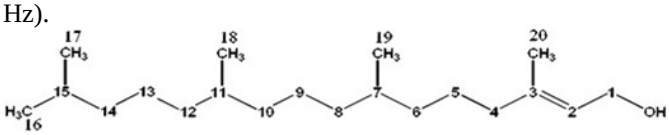
Figure 4: Structure of Phytol Compound

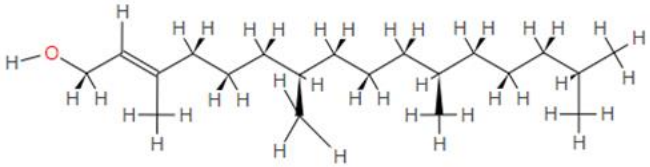
3.4 Mass spectroscopy analysis

The best method for determining the relative molecular mass (molecular weight) of a bioactive compound was mass spectrometry, which was only applicable to organic compounds due to the electron bombardment that results in energetic charged ions, which in turn determines the molecular mass of the compound. The molecule was identified and its main molecular ion fragmentations were based on the mass spectrum that was acquired. The most prevalent value, which corresponds to the base peak, was m/z value 71. The molecular ion peak, which indicates the molecular mass, was at m/z 296. The other fragments obtained were m/z 43, m/z 57, m/z 81, m/z 95, m/z 111 and m/z 123. Major spectral peaks obtained during the fractionation of phytol are shown in Figure 3.

3.5 H^1NMR and C^{13}NMR Spectroscopy

One key method used by researchers to detect unidentified substances is nuclear magnetic resonance (NMR). The fundamentals of NMR are mostly based on the magnetic characteristics of atomic nuclei such as protons, hydrogen atoms, carbon isotopes, and carbon atoms (Christophoridou *et al*., 2005). H^1NMR: δ 0.61-0.86 (16H, 0.68 (td, J = 7.7, 7.2 Hz), 0.68 (td, J = 7.7, 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.68 (q, J = 7.2 Hz), 0.80 (d, J = 6.6 Hz), 0.80 (d, J = 6.6 Hz)), 0.92-1.05 (6H, 0.97 (d, J = 6.6 Hz), 0.99 (d, J = 6.6 Hz)), 1.19-1.54 (9H, 1.25 (quint, J = 7.2 Hz), 1.25 (quint, J = 7.2 Hz), 1.25 (quint, J = 7.2 Hz), 1.25 (quint, J = 7.2 Hz), 1.37 (quintq, J = 7.2, 6.6 Hz), 1.37 (quintq, J = 7.2, 6.6 Hz), 1.46 (tt, J = 7.7, 7.4 Hz), 1.46 (tt, J = 7.7, 7.4 Hz), 1.47 (tsept, J = 7.2, 6.6 Hz)), 1.68 (3H, s), 1.88-2.00 (2H, 1.94 (t, J = 7.4 Hz), 1.94 (t, J = 7.4 Hz)), 3.72-3.84 (2H, 3.78 (d, J = 7.3 Hz), 3.78 (d, J = 7.3 Hz)), 5.20 (1H, t, J = 7.3 Hz).





C^{13}NMR: δ 16.2 (1C, s), 19.9-20.1 (2C, 20.0 (s), 20.0 (s)), 22.5-22.6 (2C, 22.6 (s), 22.6 (s)), 24.3-24.4 (2C, 24.4 (s), 24.4 (s)), 25.4 (1C, s), 28.0 (1C, s), 31.8-31.9 (2C, 31.8 (s), 31.8 (s)), 36.5-36.5 (4C, 36.5 (s), 36.5 (s), 36.5 (s), 36.5 (s)), 39.3 (1C, s), 40.6 (1C, s), 59.2 (1C, s), 123.5 (1C, s), 140.9 (1C, s).

3.6 FTIR Analysis

The compound phytol exhibited a characteristic band at 3546.91 cm^{-1} and 3387.74 cm^{-1} indicating the presence of O-H stretching intermolecular hydrogen bonded OH. 2991. cm^{-1} and 2874.38 cm^{-1} indicating the presence of C-H stretching Vibration. 1617.56 cm^{-1} indicate the presence C=C bond. 1372.79 cm^{-1} indicate the presence of CH and CH_2 bending vibration. 1071.18 cm^{-1} indicating the presence of C-OH stretching vibration. Below 900 cm^{-1} indicating the finger print region.

3.7 UV Analysis

All plant crude extracts have very high concentrations of a few physiologically active substances. It is possible to think of the high concentration of main chemicals as a component of a plant's defence mechanisms. They are a part of a sizable class of defensive compounds called phytoanticipins or phytoprotectants that have been discovered in this plant (Hossain and Nagooru, 2011; Hashmi *et al*., 2013). Antioxidant, neuroprotective, antibacterial, anticancer, anti-inflammatory and anti-diuretic properties of phytol have been documented (Kumar *et al*., 2010; Banjare *et al*., 2017).

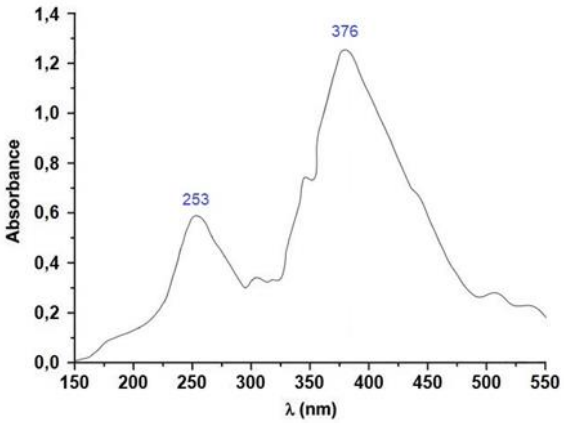


Figure 8: UV Analysis of Phytol compound

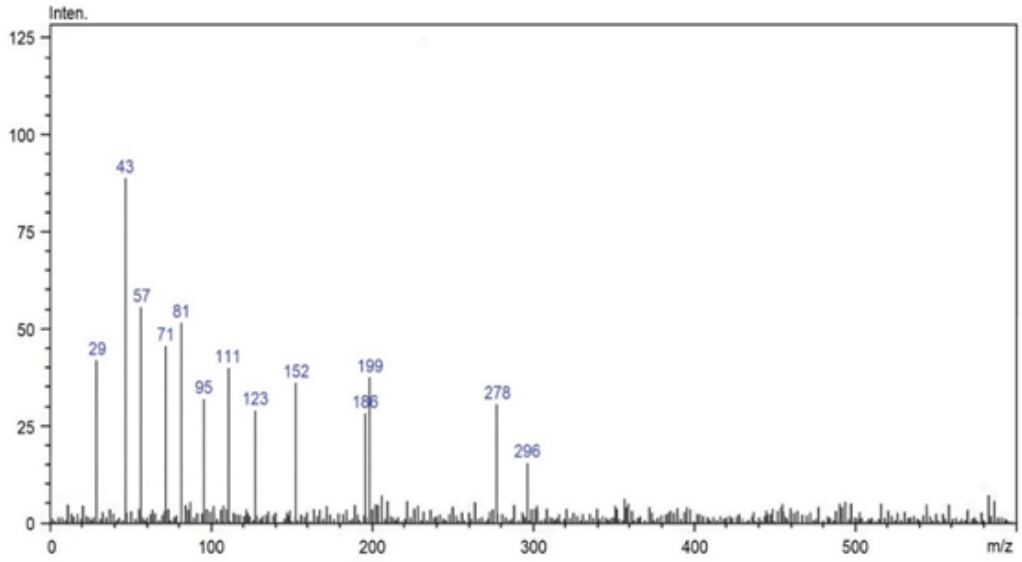


Figure 5: MASS Analysis of Phytol compound

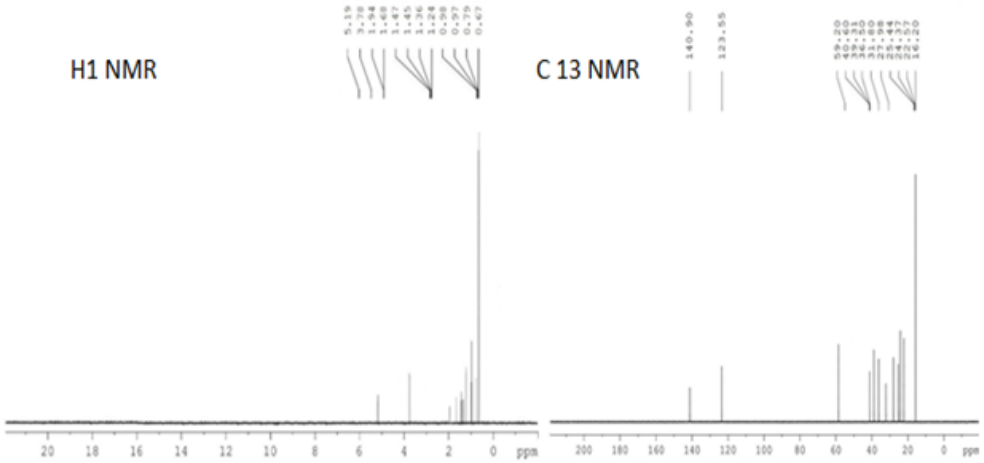


Figure 6: H^{1}NMR and C^{13}NMR Analysis of Phytol compound

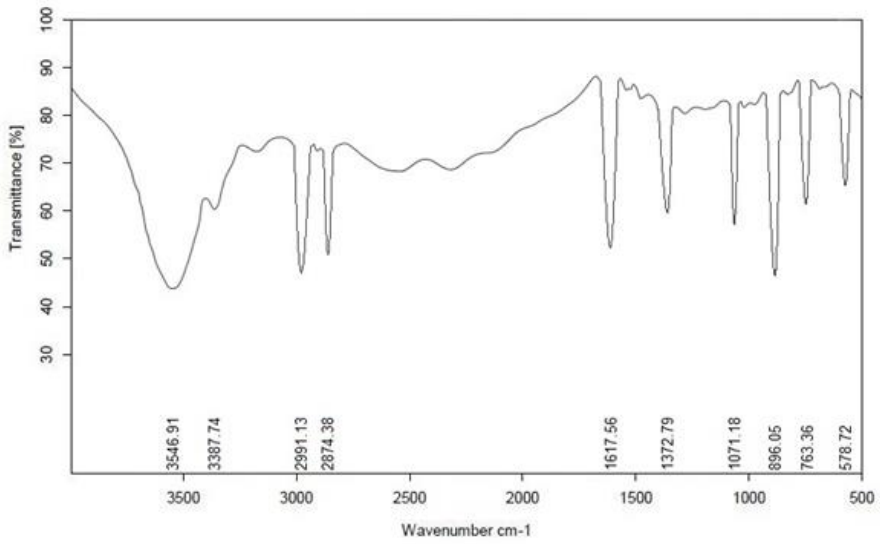


Figure 7: FTIR Analysis of Phytol compound

Table 1: GCMS Analysis of L. reginae Methanolic extract

S.No	RT	Compound Name	Molecular Formula	Molecular Weight	Biological activity
1	5.30	D-Limonene	$C_{10}H_{16}$	136.23	Antimicrobial, Antioxidant and Anti-inflammatory activity
2	7.87	1-Isopropyl-4-methyl-3-propoxy-1-cyclohexen-4-ol	$C_{13}H_{24}O_2$	212.33	Antimicrobial activity
3	11.37	Tetradecane	$C_{14}H_{30}$	198.39	Antimicrobial, Antifungal, Antioxidant and Cytotoxicity activity
4	15.14	Neophytadiene	$C_{20}H_{38}$	278.5	Antimicrobial, Antifungal and Anti-inflammatory activity
5	15.44	Heptadecane	$C_{17}H_{36}$	240.5	Antioxidant, Anti-inflammatory activity and Antineoplastic activity.
6	17.74	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antioxidant, Anti-inflammatory, hypocholesterolemic and Cancer prevention activities
7	21.45	9-octadecenoic acid	$C_{18}H_{34}O_2$	282.5	Anti-inflammatory, Antiandrogenic, Anemiagenic Antioxidant, Hypocholesterolemic and Cancer prevention activities
8	25.11	Phytol	$C_{20}H_{40}O$	296.5	Anxiolytic, Metabolism modulating, Cytotoxic, Antioxidant, Autophagy and Apoptosis-inducing, Antinociceptive, Anti-inflammatory, Immune-modulating and antimicrobial effects.
9	36.67	Stigmast-5-en-3-ol	$C_{29}H_{50}O$	414.7	Antimicrobial, Anticancer and Pharmacological activity.
10	37.27	beta-Amyrin	$C_{30}H_{50}O$	426.7	Antimicrobial and Anti-inflammatory activity
11	39.02	Alpha - Amyrin	$C_{30}H_{50}O$	426.7	Antimicrobial and Anti-inflammatory activity

Table 2: H^1NMR and C^{13}NMR Analysis of Phytol compound

Carbon No.	Chromophore	H^1NMR Chem Shifts[a] (ppm) Phytol	C^{13}NMR Chem Shifts[b] (ppm) Phytol
1	-C-OH	4.13 (d)	59.20
2	=CH	5.404 (t)	123.55
3	=C<	-	140.90
4	$>CH_2$	1.986 (t)	40.60
5	$>CH_2$	1.44 (m)	25.44
6	$>CH_2$	1.30 (m)	-
7	>CH	1.44 (m)	31.80
8	$>CH_2$	1.30 (m)	-
9	$>CH_2$	1.30 (m)	24.37
10	$>CH_2$	1.30 (m)	-
11	>CH	1.44 (m)	-
12	$>CH_2$	1.30 (m)	-
13	$>CH_2$	1.30 (m)	-
14	$>CH_2$	1.14 (m)	39.31
15	>CH	0.87 (m)	24.37
16	$-CH_3$	0.87 (d)	22.57
17	$-CH_3$	0.84 (d)	-
18	$-CH_3$	0.83 (d)	-
19	$-CH_3$	0.82 (d)	-
20	$-CH_3$	1.65 (s)	16.20

Table 3. Antimicrobial Activity of Phytol in Methanol Extract

Organism Type	Organism Name	Control	Concentration (µl)			
			30	40	40	40
Bacteria	*Salmonella Typhi*	21	18	21	23	26
	Bacillus cereus	22	19	22	24	29
Fungal	*Aspergillus niger*	21	17	20	22	21
	Trichophyton rubrum	20	16	19	22	24

4. Conclusion

It was discovered that all of the plant's crude extracts included certain physiologically potent chemicals in very high concentrations. It is possible to think of the high concentration of main chemicals as a component of a plant's defence mechanisms. The methanolic extract of L. reginae contained the chemical phytol, which was extracted. Research on the methanolic extract of L. reginae has enabled the identification of active phytochemicals and the comprehension of the therapeutic advantages of the plant that are connected to its traditional applications. The bioactive substance was examined using mass spectrometry (MS), ultraviolet, SEM, EDAX, Infrared, C^{13}, and H^{1}NMR. Based on the information, Phytol ($C_{20}H_{40}O$), a compound with a molecular weight of 296.6, was identified as the component. The entire screening process produced molecular proof of the chemical. The development of innovative plant-based medications has been a recent goal of the global pharmaceutical industry. This goal is based on the traditional applications of such plants throughout history. In order to participate in biological activity experiments, the phytoconstituents that are genuinely accountable for the observed bioactivities must first be separated using various experimental techniques.

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