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GC–MS-Based Metabolite Profiling and Evaluation of Antioxidant and Antimicrobial Activities of Litsea glutinosa (Lour.) C.B. Rob. Bark from Garo Hills, Meghalaya

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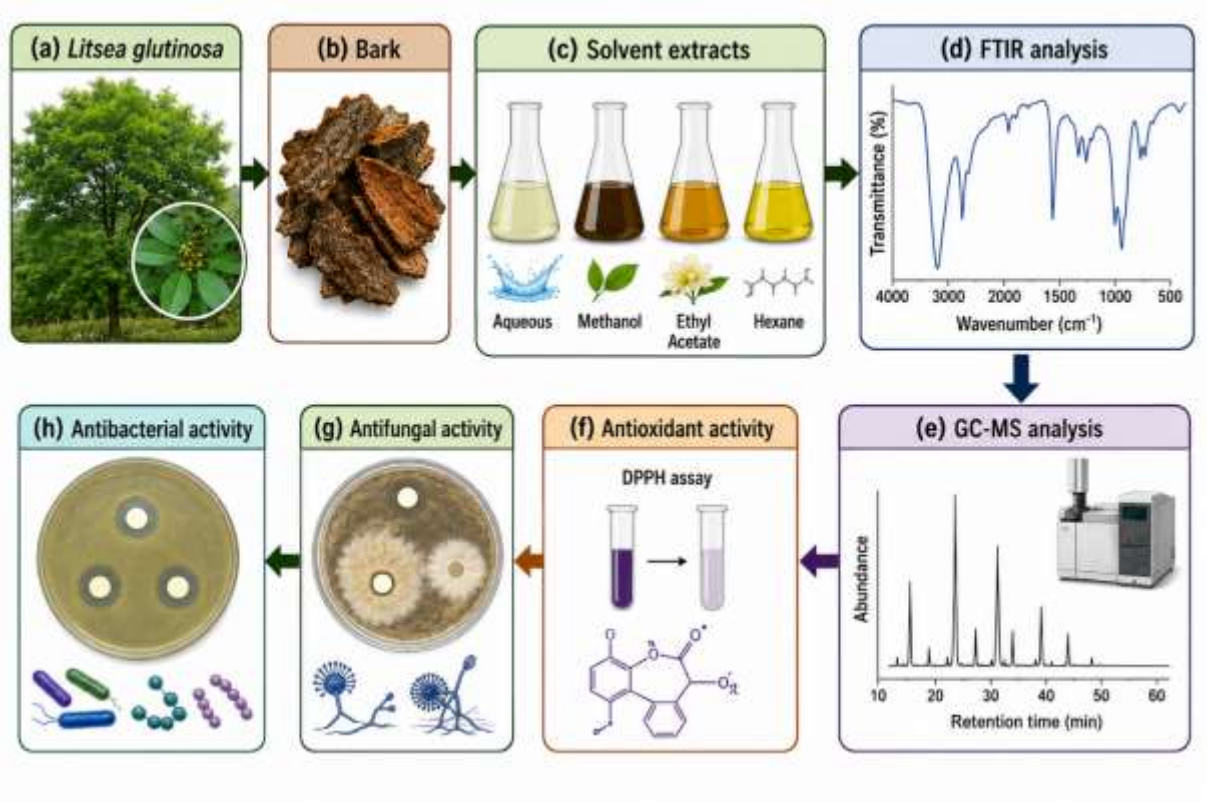
ABSTRACT:

Litsea glutinosa (Lour.) C.B. Rob., a species in the family Lauraceae, is an underutilized medicinal plant having considerable ethnobotanical significance in traditional therapeutic practices. The current study was undertaken to assess the antioxidant and phytochemical characterization and investigate the pharmacological potential of *L. glutinosa* using the extract of root bark. The solvents of varying polarity were used for performing the phytochemical characterization, such as methanol (MeOH), ethanol (EtOH), hexane (Hex), ethyl acetate (Et Ac), and water (Aq). Initial phytochemical analysis has indicated a significant existence of phenolics and flavonoids, implying the substantial antioxidant capacity. The TPC was found to be highest in Et Ac solvent (0.165 ± 0.01 (mg GAE/g dry weight ± SD)) and the least in n-Hex (0.045 ± 0.04 (mg GAE/g dry weight ± SD)), whereas TFC was found to be highest in MeOH extract (283.5 ± 0.001 (mg QE/g dry weight ± SD)) and lowest in (47.8 ± 0.001 (mg QE/g dry weight ± SD)) quantified using the Folin-Ciocalteu reagent and $AlCl_3$ colorimetric techniques, respectively, suggesting a significant presence of bioactive phytochemicals. Moreover, the antioxidant study using DPPH radical scavenging activity was found to be highest in the MeOH extract with the IC_{50} value of 15.26 µg/ml and the least in the Hex extract with the IC_{50} value of 99.72 µg/ml. Furthermore, GC-MS profiling of the bark extract revealed a wide spectrum of bioactive components, viz., naphthalene; myrtenyl angelate; Z,Z-6,28-heptatriactontadien-2-one; ButanoicAcid,2-Methyl-,2-Methyl-2-Propenyl Ester; Hentriacontane; heptacosanoic acid, 25-Methyl-, Methyl Ester, etc., many of which were previously linked to pharmacological activity in the past. These compounds highlighted the therapeutic potentiality of L. glutinosa, particularly in its anti-inflammatory, antibacterial, antioxidant, and potentially anticancer qualities. In addition, the FTIR analysis for methanol extract has revealed various different types of functional groups, viz., alcohols, phenols, alkanes, alkyls, carboxylic acids, esters, ethers, and halo compounds. The Minimum Inhibition Concentration (MIC) performed using 2 bacterial and 2 fungal strains has shown an MIC % of inhibition variations in 3 concentrations taken, i.e., 1000-250 µg/ml, showing the potentiality of inhibiting the growth of bacteria and fungi tested. The results of this study provide support to the traditional applications of L. glutinosa bark and encourage more research into the plant as a possible source of new bioactive compounds to be used in pharmaceutics.

KEYWORDS: *Litsea glutinosa* (Lour.) C.B. Rob., medicinal herb, phytochemistry, GC-MS, FTIR.

Graphical Abstract



INTRODUCTION:

Humans and plants have been linked since the ancient times as plants has been acknowledged as humanity's earliest healing source owing to the presence of most valuable source of chemicals with therapeutic potential in human history [1]. It is utilized extensively for food, medicine, shelter, and other purposes, rendering bioactive chemicals obtained from them potentially helpful for both human and animal welfare [2,3]. It also still serves as an essential pool for identifying innovative drug leads today [4]. Plants used for traditional medicine contain a wide range of substances that plays a significant role in treating chronic as well as infectious diseases such as skin diseases, stomach ache, diarrhoea, diabetes, vomiting, bone pain, inflammation, central nervous system disorders etc. [5].

Taxonomic Plant Description

L. glutinosa (Lour.) C.B. Rob. (Lauraceae) reaches a height of 3 to 15 meters and is either deciduous or evergreen. This medicinal plant is also referred to as sycamore, Bollywood, Indian laural, soft/brown bollygum, or beech/bolly beech. It has long been thought of as an emollient, semen-generator, and longevity enhancer. The plant is small to medium in size, with stems that can be either straight or bent and up to 60 cm in diameter. According to [6] this 3 typically grows to an average height of about 13 meters, with a clear trunk reaching nearly 5 meters before the first branches appear. The trunk measures around 63 cm in girth, while the crown spreads just over 5 meters across and rises to a height of about 8.6 meters. Each tree usually develops five to six main branches. Its leaves are fairly large, with an average area of 91 cm^2, weighing around half a gram each, and showing a specific leaf area of 175.7 cm^2 per gram. The bark is relatively heavy, with about 2.75 g per unit area. The young branchlets are covered

in a silky grayish-yellow coat, carrying oval leaves that range from 7 to 15 cm long and 3 to 7 cm wide. These leaves are arranged alternately, supported by short stalks about 1–2.6 cm long. Their bases are wedge-shaped, while the tips are usually blunt or slightly curved. In contrast, the fruits are small and round, only 5–7 mm in size. The species has a high seed germination rate about 85% which occurs within 15 to 45 days. Flowering takes place from May to June, with male flowers being easy to spot because they often have reduced or missing petals. Each fertile stamen is productive, typically carrying at least 15 flowers [7].

World Distribution

L. glutinosa (Lour.) C.B. Rob. has a broad natural range, occurring across South and Southeast Asia, parts of East Asia, and into northern Australia and the Pacific Islands. It is native to regions such as the Andaman and Nicobar Islands, Bangladesh, India (including Assam, the Himalayas, and other states), Nepal, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia (Java, Borneo, Sulawesi, Lesser Sunda Islands, Maluku), the Philippines, Papua New Guinea, the Solomon Islands, and areas of China (South-Central, Southeast, and Hainan). Its range also extends to Queensland, the Northern Territory, and Western Australia. Beyond its native distribution, the species has been introduced to several islands and coastal regions, including the Comoros, Mauritius, Rodrigues, Reunion, Seychelles, KwaZulu-Natal in South Africa, New Caledonia, Vanuatu, and the Caribbean islands of Trinidad and Tobago (Plants of the World Online).

Table 1. The Taxonomical classification of *L. glutinosa*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Laurales
Family	Lauraceae
Genus	*Litsea*
Species	*L. glutinosa* (Lour.) C.B. Rob.

Plant Taxonomical Description

(Ref. CABI Compendium)

Vernacular name: Boldokaki (Garo)

Figure 1. *L. glutinosa* (Lour.) C.B. Rob.

Medicinal Significance

Litsea species have long been used from time immemorial to cure multiple diseases all over the world. Ailments such as including stomach ache, diarrhoea, vomiting, influenza, diabetes, bone pain, inflammation, central nervous system disorders, skin diseases and others. This species' mildly astringent bark is used externally to treat aches and bruises. To cure bruises, aches and manage contusions, powdered leaves, bark, and roots are applied externally [8,9]. In Ayurveda, the bark of this plant is renowned for its stimulant, astringent, spasmolytic, and anti-diarrheal qualities [8]. *L. glutinosa* is used to treat diarrhoea, dysentery, stomach ache, indigestion, gastroenteritis, swelling, traumatic injuries, colds, arthritis, asthma, diabetes, pain alleviation, sprains and bruises, rheumatism, and strong sexual strength [7,10,11]. The mucilaginous leaves are considered antispasmodic and emollient. In addition, a paste prepared by grinding bark with water is used as a plaster in cases of sprain, bruises, wounds, inflammation, back pain, rheumatic and gouty joints, bone fractures, etc. It has analgesic, antiseptic and emollient effects [11]. The bark and leaves of this plant are harnessed for their soothing and mildly astringent attributes, particularly in cases of diarrhoea and dysentery [10]. *L. glutinosa* flourishes on elevated terrains in India at great heights [12].

Phytochemical analysis

In recent decades, natural diets abundant in phenolics and flavonoids with antioxidant properties sparked interest in nutrition and food science. Natural phenolics and flavonoids are plant secondary metabolites characterized by an aromatic ring containing at least one -OH group. Phenolic compounds serve as effective electron donors due to the direct involvement of their -OH groups in antioxidant activity [13].

MATERIALS AND METHODS:

Chemicals and reagents

Folin-Ciocalteu reagent (FCR), Gallic acid, Sodium carbonate (Na_2CO_3), Aluminium Trichloride ($AlCl_3$), Sodium Nitrate ($NaNO_3$), Sodium Hydroxide (NaOH), Quercetin (QE).

Plant samples collection

Root barks of *L. glutinosa* were collected from Ramchengga village located under South Garo Hills District, Meghalaya. It is then dried under room temperature, grounded to fine power for future use. They were processed for herbarium and for identification submitted to Botanical Survey of India (BSI), Shillong bearing the Voucher No. 102189.

Preparation of macerate and extraction

The mature leaves were collected from the studied locations and packed in polythene bags. Later, the bark was sun-dried or kept in an oven at a temperature of about 60-70˚C and grinned to fine powder. Dried powder of weight 5-10g was taken for hydro distillation sing Soxhlet apparatus for 5-6 hours, using solvents mentioned above. The extract was concentrated using rotary evaporator to the desired amount; they were kept in Freeze (4˚C) for further use.

Qualitative Phytochemical Screening

The qualitative phytochemical screening was performed in order to test for the presence of Alkaloids, Flavonoids, Saponins, Steroids, Tannins, Terpenoids and Phenolic Compounds by following the methods of [14,15].

Determination of Total Phenolic Content

The Total Phenolic Content of *L. glutinosa* (Lour.) C.B. Rob. extract was determined by using Folin-Ciocalteu reagent as oxidizing agent and gallic acid as a standard [3]. Different concentrations of gallic acid solution having a concentration ranging from 0-100 µg/ml was prepared. To the 0.5 ml of gallic acid solution, a 2.5 ml of Folin- Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5% w/v) solution was added. A standard curve was prepared after the mixture was incubated for 20 minutes and absorbance was measured at 760 nm. The Total Phenol Content of the sample and the standard curve was prepared from gallic acid solution with different concentration and the was measured as mg of GAE (gallic acid equivalent)/gm of the extract.

Determination of Total Flavonoid Content

The Total Flavonoid Content of *L. glutinosa* (Lour.) C.B. Rob. bark was determined by using the $AlCl_3$ colorimetric method with slight modifications [16]. In a test tube of 4 mL distilled water a volume of 1 mL of a known concentration of different extract was added and it was allowed to stand for 5 minutes after the addition of 0.3 mL 5% (w/v) sodium nitrate solution. Later, 0.3 mL 10% (w/v) Aluminium Chloride was added to the mixture and allowed to face for 1 minute. Afterwards, 2 mL of 1 M sodium hydroxide solution was aliquoted into the mixture, and the final volume was adjusted to 10 mL by adding water, it was properly shaken for 15 seconds, and allowed to react for a further half-hour. The absorbance of the mixture was recorded at 510 nm against the blank by using an equivalent spectrophotometer with the use of triplicates. The total flavonoid content value was expressed as mg of quercetin equivalent (QE) per gram of dry extract and was compared with a typical curve of Quercetin solution in Methanol in various concentrations.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

The free RSA of the dilute leaf extract of *L. glutinosa* was tested using a 1,1-diphenyl 2-picryl hydrazyl (DPPH) technique with few modifications [17]. For making the stock solution, a total of 24 mg of DPPH was dissolved in 100 mL of methanol. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 L of leaf extract. 3 ml of solution containing DPPH in 100 L of

methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. The following formula was used to compute the percentage of antioxidants or RSA:

% of antioxidant activity= [(Ac-As) ÷ Ac] × 100

where: Ac-Control reaction absorbance; As-Testing specimen absorbance.

Gas Chromatography Mass Spectrometry (GC-MS)

The MeOH, EtOH, Hex, Et Ac and Aq extracts of *L. glutinosa* (Lour.) C.B. Rob. bark was analyzed for the presence of different volatile compounds by the technique of GCMS. First, the plant powder of weight 5-10g was taken and kept in Soxhlet apparatus for 5-6 hours, using solvents such as MeOH, EtOH, Hex, Et Ac and Aq used in subsequent order of polarity. It is then concentrated by using rotary evaporator to the desired amount. About 1-1.5ml of the extraction is then taken in a tube for further Gas Chromatography-Mass Spectrometry analysis. The GCMS analysis of biologically active compounds of *L. glutinosa* (Lour.) C.B. Rob. were performed at the Guwahati Biotech Park, Assam, India [18]. It was done using Perkin Elmer Clarus 680/600C Mass Spectrometer MS which is a sophisticated instrument used for analytical chemistry applications, particularly in the fields of environmental analysis, food safety, pharmaceuticals, and more. It operates on the fundamental principle of ionization, mass analysis and detection to provide accurate and sensitive analysis of chemical compounds in a wide range of applications [19].

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The methanol extract (30 ml) of the plant (2g) was mixed with dry potassium bromide (KBr) salt, using a mortar and pestle, and compressed into a thin pellet. To identify and characterize the compounds or functional groups present, the FTIR spectra of the extract were collected by a Bruker model ALPHA II, and operating at the basic of 600-4000 wavenumber [20].

Antimicrobial Activity

The study employed two bacterial and two fungal strains to evaluate the antimicrobial activity of the selected two plants. The bacterial strains included *Staphylococcus aureus* and Coagulase-negative *Staphylococcus aureus* (both Gram-positive), while the fungal strains consisted of *Candida albicans* and *Candida tropicalis*. All the test organisms were obtained from the Microbiology Laboratory of P.A. Sangma International Medical College (PIMC), Meghalaya, India and performed at the University of Science and Technology Meghalaya, Department of Applied Sciences.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of a drug that prevents inhibition of growth as measured by observed turbidity in the test tube (CLSI, 2016). The MIC was determined for the antibacterial most efficient extracts, using the method described by [21].

For the experiment, 6 sterile test tubes were set up in four rows, with each row assigned to a different plant extract. The antimicrobial activity of each extract was measured using the micro-broth dilution method. 100 µL of sterile nutrient broth was pipetted into all the tubes. 200 mg of dried extract was dissolved in 0.4 mL of dimethyl sulfoxide (DMSO), giving a concentration of 500 mg/mL. From this solution, 100 µL (equivalent to 250 mg) was taken for use. The extract was then serially diluted in the test tubes to prepare concentrations of 125, 62.5, 31.25, 15.62, and 7.81 mg/mL. After dilution, 100 µL of bacterial suspension (106 CFU/mL) was added to each tube, and the tubes were incubated at 37°C for 24 hours. Two control tubes were prepared: one containing nutrient broth with bacteria, serving as the

positive control, and another containing nutrient broth with the plant extract, serving as the negative control [21].

Statistical Analysis

The data were analyzed using simple descriptive statistics in Microsoft Excel. All experiments were performed with at least three replications (n=3) and the results were expressed as mean ± standard deviation (SD). One-way ANOVA determined the difference between groups such as TPC, TFC and DPPH. The p-values below 0.05 were considered statistically significant. PCA is an unsupervised statistical method that identifies patterns in a dataset, revealing underlying similarities and/or differences. The Principal Component Analysis (PCA) was performed using the PAST 4.03 Software which shows the relationships between phytochemicals compositions and antioxidant activity among different species and classify them [22].

RESULT:

Qualitative Phytochemical Screening

Table 2. Qualitative Phytochemical constituents of *L. glutinosa* bark extracts

Phytochemical Constituents	Different Solvents				
	Methanol	Ethanol	Hexane	Ethyl Acetate	Aqueous
Alkaloids	+	+	+	+	+
Flavonoids	+	+	-	+	+
Saponins	+	+	+	+	+
Steroids	+	+	-	+	-
Tannins	+	+	-	-	+
Terpenoids	-	+	+	+	+
Phenolic Compounds	+	+	-	+	+

Total Phenolic and Flavonoid Content

The antioxidant activity correlates to the number of phenolics present which are capable of free radical scavenging. Therefore, to extract phenolic and flavonoid compounds, suitable solvents must be used [23]. Table 3 shows the capacity of studied solvents for extract phenolics and flavonoids from *L. glutinosa* (Lour.) C.B. Rob. Among the studied solvents. Ethyl acetate extract has produced the highest (0.165 ± 0.01(mg GAE/g dry weight ± SD) phenolic compounds among the 5 solvents tested and the flavonoid content was found to be highest (283.5 ± 0.001(mg QE/g dry weight ± SD) concentration in solvent MeOH.

Table 3. Total phenolic, flavonoid, DPPH radical scavenging activity and One-Way ANOVA of *L. glutinosa* (Lour.) C.B. Rob. bark extracted with different solvents. Values are the means of three replicates ± SD.

Solvent	Total Phenolic Content (mg GAE/g dry weight ± SD)	Total Flavonoid Content (mg QE/g dry weight ± SD)	DPPH radical Scavenging Activity (IC_{50})
LG_MeOH	0.085 ± 0.006	283.5±0.001	15.26

LG_EtOH	0.069 ± 0.001	159.1±0.0005	57.07
LG_Hex	0.045 ± 0.04	47.8 ± 0.001	99.72
LG_Et Ac	0.165 ± 0.01	245.7 ± 0.005	54.92
LG_Aq	0.077 ± 0.0001	111.6 ± 0.001	-
F-Value	0.0002	8.57E-07	0.001
***p*-value**	0.99978	1	0.9994

LG_MeOH= *L. glutinosa* Methanol extract; LG_EtOH= *L. glutinosa* Ethanol extract; LG_Hex= *L. glutinosa* Hexane extract; LG_Et Ac= *L. glutinosa* Ethyl Acetate extract; LG_Aq= *L. glutinosa* Aqueous extract.

Antioxidant activity based on 1,1-diphenyl-2 picryl-hydrazyl (DPPH) assay

The antioxidant assay of *L. glutinosa* bark extracts performed using a 1,1-diphenyl-2-picryl hydrazyl (DPPH) technique have revealed that MeOH extract is more potent at scavenging DPPH radicals with the IC_{50} value of 15.26 µg/ml and n-hexane has the less ability to scavenge DPPH radicals with IC_{50} 99.72 µg/ml (**Table 3**).

Characterization of phytocompounds through GC-MS

GC-MS profiling of bark extract in MeOH solvent

Table 4 and Fig 2 represent the 5 compounds extracted from *L. glutinosa* (Lour.) C.B. Rob. with the MeOH solvent. The maor compounds identified based on relative contents were Phenol, 2-Methyl- (6.616%), Methyl 2,6-Anhydro-Alpha. -D-Altroside (3.443%), Succinic Acid, Di (But-2-en-1-yl) Ester (1.164%), Myrtenyl Angelate (0.559%) and Naphthalene (0.384%). 2-Methylphenol, commonly known as o-cresol, is a phenol substituted by a Methyl group at position 2. It is a minor urinary metabolite of toluene. It has a role as a human xenobiotic metabolite. Naphthalene exhibits a number of antagonistic properties, including anticancer, antimicrobial, anti-inflammatory, antiviral, antitubercular, antihypertensive, antidiabetic, anti-neurodegenerative, antipsychotic, anticonvulsant, antidepressant [24].

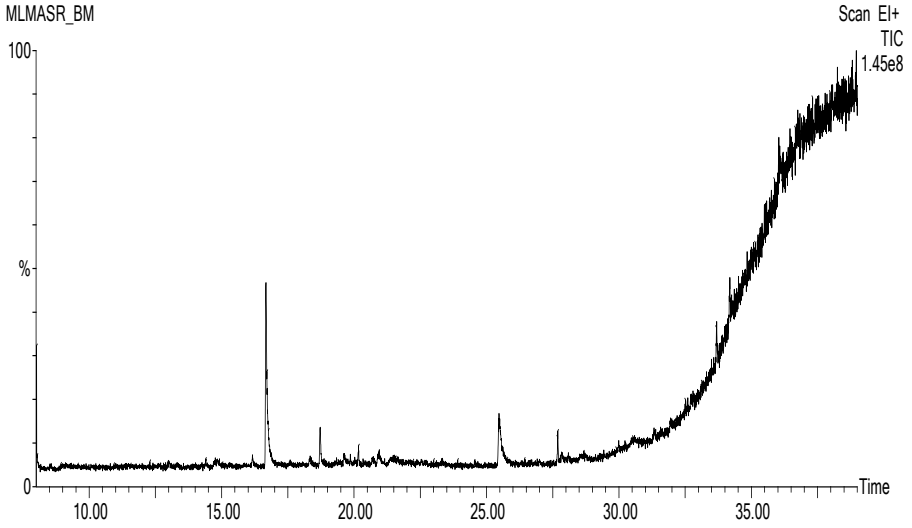


Figure 2. GC-MS chromatogram of *L. glutinosa* (Lour.) C.B. Rob. extracted with MeOH solvent.

GC-MS profiling of bark extract in EtOH solvent

Table 4 and Fig 3 present the 5 compounds extracted from *L. glutinosa* (Lour.) C.B. Rob. with the solvent EtOH. The major compounds characterized were Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl- (15.194%), Tris (Tert-Butyldimethylsilyloxy) Arsane (1.231%), Dodecane, 1-Fluoro- (0.521%),2-Ethyl-1-Hexanol, Heptafluorobutyrate (0.406%) and Z,Z-6,28-Heptatriactontadien-2-One (0.403%) etc.

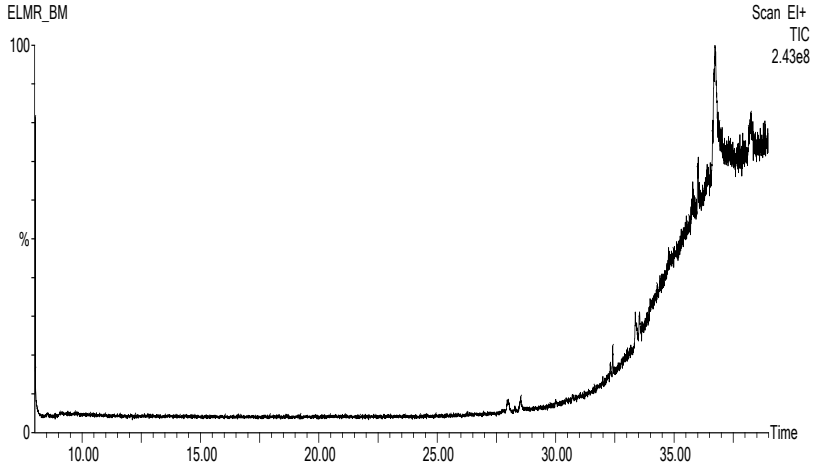


Figure 3. GC-MS chromatogram of *L. glutinosa* (Lour.) C.B. Rob. extracted with EtOH solvent.

GC-MS profiling of bark extract in Hex solvent

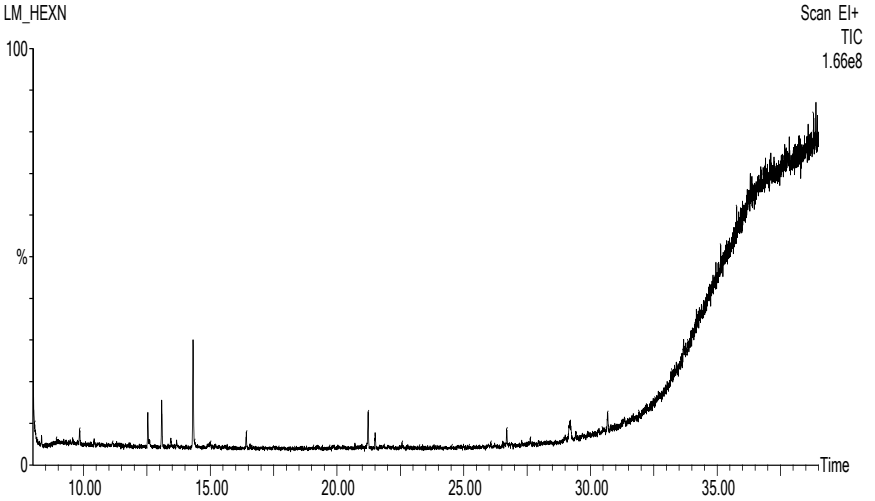


Figure 4. GC-MS chromatogram of *L. glutinosa* (Lour.) C.B. Rob. Extracted with Hex solvent.

Table 4 and Figure 4 present the 5 compounds extracted from *L. glutinosa* (Lour.) C.B. Rob. with the Ethanol solvent. The main compounds identified based on relative contents were Cyclobutanecarboxylic Acid, 2-Propenyl Ester (2.949%), Butanoic Acid, 2-Methyl-, 2-Methyl-2-Propenyl Ester (1.177%), Benzene, 1,3-Bis(1,1-Dimethylethyl)- (1.134%), 1-Butoxypropan-2-Yl Pentanoate (0.827%), Hentriacontane (0.425%), Hentriacontane is a long-chain alkane. It has a role as an antitubercular agent. Hentriacontane, is a solid long chain alkane with various pharmacological effects including anti-inflammatory, antitumor and antimicrobial activities [25–28].

GC-MS profiling of bark extract in Et Ac solvent

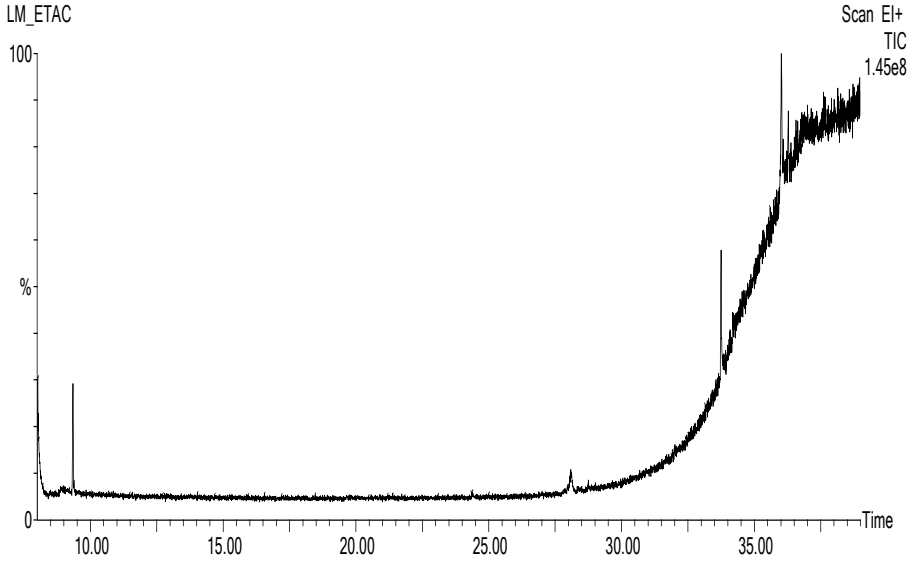
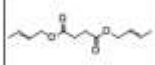
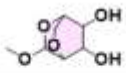
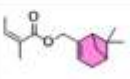


Figure 5. GC-MS chromatogram of *L. glutinosa* (Lour.) C.B. Rob. extracted with Et Ac solvent.

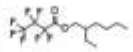
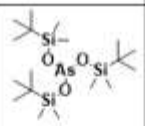
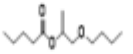
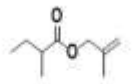
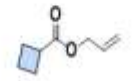
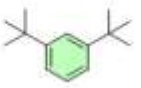
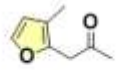
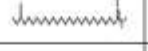
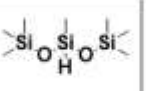
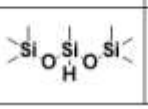
Table 4 and Fig 5 present the 5 compounds extracted from *L. glutinosa* (Lour.) C.B. Rob. with the EtOH solvent. The compounds characterized in high concentration were 1,1,1,3,5,5,5-Heptamethyltrisiloxane (4.128%),Heptacosanoic Acid,25-Methyl-, Methyl Ester (2.752%),1,6-Heptadien-3-Yne(2.156%), 1,1,1,3,5,5,5-Heptamethyltrisiloxane(1.522%) and3-Methyl-2-(2-Oxopropyl) Furan (1.318%).The Z,Z 6,28 Heptatriactontadien 2 one made up ~25.9% of the bioactive compounds, suggesting it may significantly influence the observed bioactivities [29]. It is also found to have anti-diabetic properties [30].

Table 4. Phytochemical profiling in *L. glutinosa* (Lour.) C.B. Rob. bark extracts in different solvents.

A. GC-MS profiling of *L. glutinosa* (Lour.) C.B. Rob. Bark in MeOH solvent.

Sl. No	Identified Compound Name	Molecular Weight (MW) g/mol	Molecular Formula	Retention Time (min)	Peak Area (%)	Biological Properties	Structure of Compound	Reference
1.	Phenol, 2-Methyl-	108.14	C_7H_8O	16.677	6.616	No properties found		
2.	Succinic Acid, Di (But-2-En-1-yl) Ester	226.27	$C_{12}H_{18}O_4$	18.723	1.164	Not yet reported		
3.	Naphthalene	128.169	$C_{10}H_8$	20.168	0.384	Anticancer, antimicrobial, anti-inflammatory, antiviral, antituberculer, antihypertensive, antidiabetic, anti-neurodegenerative, antipsychotic, anticonvulsant, antidepressant		24
4.	Methyl 2,6-Anhydro- Alpha -D-Altroside	176.17	$C_7H_{12}O_6$	25.470	3.443	Not yet reported		
5.	Myrtenyl Angelate		$C_{15}H_{22}O_2$	27.691	0.559	Antimicrobial/antibiofilm; Antioxidant/Anti-inflammatory; Antidiabetic effects; Anticancer activity; Cardiovascular/CNS		

B. GC-MS profiling of *L. glutinosa* (Lour.) C.B. Rob. Bark in EtOH solvent.

6.	2-Ethyl-1-Hexanol, Heptafluorobutyrate	326.25	$C_{12}H_{17}F_7O_2$	27.997	0.406	Not yet reported		
7.	Dodecane, 1-Fluoro-	188.32	$C_{12}H_{25}F$	28.527	0.521	Not yet reported		
8.	Z,Z-6,28-Heptatriacontadien-2-One	530.9	$C_{37}H_{70}O$	32.408	0.403	Antidiabetic activity		30
9.	Tris (Tert-Butyldimethylsilyloxy)Arsane	468.7	$C_{18}H_{45}AsO_3Si_3$	33.359	1.231	Not yet reported		
10.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-	577.2	$C_{16}H_{50}O_7Si_8$	36.725	15.194	Not yet reported		
C. GC-MS profiling of *L. glutinosa* (Lour.) C.B. Rob. Bark in Hex solvent.								
11.	1-Butoxypropan-2-YL Pentanoate	216.32	$C_{12}H_{24}O_3$	12.540	0.827	Not yet reported		
12.	Butanoic Acid, 2-Methyl-, 2-Methyl-2-Propenyl Ester	156.22	$C_8H_{14}O_2$	13.085	1.177	Not yet reported		
13.	Cyclobutanecarboxylic Acid, 2-Propenyl Ester	140.18	$C_8H_{12}O_2$	14.321	2.949	Not yet reported		
14.	Hentriacontane	436.8	$C_{31}H_{64}$	16.422	0.425	A solid long chain alkane with various pharmacological effects including anti-inflammatory, antitumor and antimicrobial activities, antibacterial and antifungal		25-28
15.	Benzene, 1,3-Bis(1,1-Dimethylethyl)-	190.32	$C_{14}H_{22}$	21.229	1.134	Not yet reported		
D. GC-MS profiling of *L. glutinosa* (Lour.) C.B. Rob. Bark in Et Ac solvent.								
16.	1,6-Heptadien-3-Yne	92.14	C_7H_8	9.349	2.156	Not yet reported		
17.	3-Methyl-2-(2-Oxopropyl) Furan	138.16	$C_8H_{10}O_2$	28.087	1.318	Not yet reported		
18.	Heptacosanoic Acid, 25-Methyl-, Methyl Ester	438.8	$C_{29}H_{58}O_2$	55.744	2.752	Larvicidal\insecticidal, Broad-spectrum antibacterial & antioxidant (correlative), Putative anticancer		31-33
19.	1,1,1,3,5,5,5-Heptamethyltrisiloxane	221.5	$C_7H_{22}O_2Si_3$	36.010	4.128	Not yet reported		
20.	1,1,1,3,5,5,5-Heptamethyltrisiloxane	221.5	$C_7H_{22}O_2Si_3$	36.275	1.522	Not yet reported		

Chemical characterization through FTIR profiling in L. glutinosa root bark in MeOH solvent

The FTIR spectrum is an important tool for characterization of the functional groups of unknown plant samples and to determine the active components based on their peak value in theIR region. The absorption spectra of *L. glutinosa* bark extract in MeOH solvent are presented **Fig 6**. From the FTIR peak values and identified functional groups are represented in **Fig 6** and **Table 5**. The functional groups of bio-active compounds arise in the bark powdered plant sample have revealed the following functional groups in which the frequency ranges are 3500-3200 cm^{-1}, 3000-2900 cm^{-1}, 2900-2800 cm^{-1}, 1500-1400 cm^{-1}, 1200-1110 cm^{-1}, 1100-1000 cm^{-1} and 900-600 cm^{-1}. The analysis has revealed various different types of functional groups viz., Alcohol, phenols, alkanes, alkyls, carboxylic acids, esters, Ethers, and Halo compound. The IR spectrum at a peak 3327.54 cm^{-1} is represented as the O-H or N-H stretching vibration, that confirms the presence of alcohols and phenols. The bands at 2943.37 cm^{-1}, 2831.46 cm^{-1} and 1449.16 cm^{-1} under frequency ranges 3000-2900 cm^{-1}, 2900-2800 cm^{-1} and 1500-1400 cm^{-1} represented C-H stretching vibration of Alkanes, Alkyls containing Aliphatic compounds. The peak range 1200-1110 cm^{-1} indicated the C-O or C-N stretching vibration, confirming the presence of alcohol. The frequency range 1100-1000 cm^{-1} is represented the C-O stretching vibration, confirming the presence of Alcohol, Carboxylic Acid, esters, ethers at 1022.74 cm^{-1} peak value. The peak at 652.04 cm^{-1} at a range of 900-600 showed C-Br stretching vibration, which confirmed the presence of Halo compound.

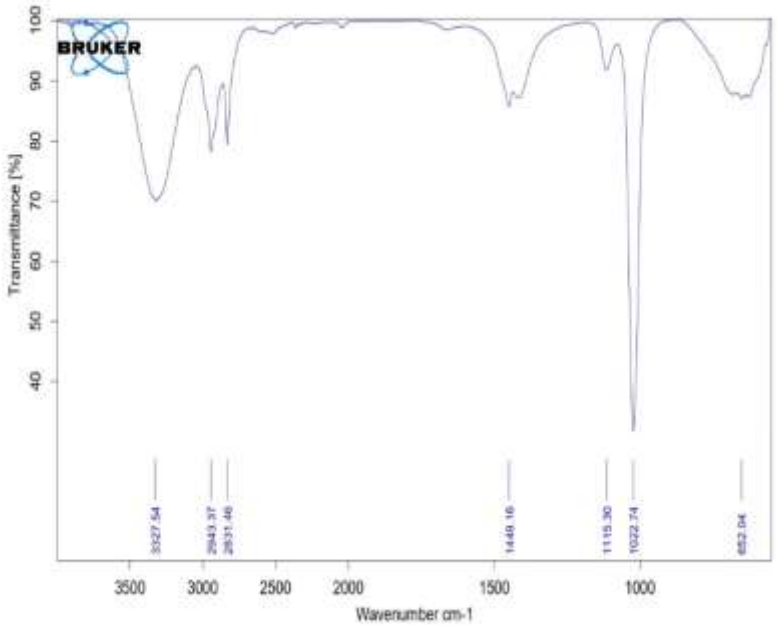


Figure 6. FTIR Spectrum of *L. glutinosa* bark extract in MeOH

Table 5. FTIR peak values and functional groups in *L. glutinosa* bark extract in MeOH solvent

Sl.No.	Frequency (cm^{-1})	Wave Number (cm^{-1})	Bond/Mode of vibration	Functional Group	Peak details
1.	3500-3200	3327.54	O-H or N-H	Alcohol, Phenols	Medium
2.	3000-2900	2943.37	C-H	Alkanes, alkyls	Weak
3.	2900-2800	2831.46	C-H	Alkanes, alkyls	Weak

4.	1500-1400	1449.16	C-H	Alkanes, Alkyl	Weak
5.	1200-1110	1115.30	C-O or C-N	Alcohol	Weak
6.	1100-1000	1022.74	C-O	Alcohol, carboxylic acid, esters, ethers	Strong
7.	600-900	652.04	C-Br	Halo compound	Weak

Antimicrobial Activity

The Minimum Inhibition Concentration (MIC) performed against two bacterial strains i.e., *Staphylococcus aureus* and *Coagulase-negative Staphylococcus aureus* (both Gram-positive), and the fungal strains i.e., *Candida albicans* and *Candida tropicalis* have shown the MIC % of inhibition variations in three concentrations studied ranging from 1000-250 µg/ml. These results shows that the studied strains have the ability of inhibiting the growth of bacteria and fungi tested (Table 6).

Table 6. MIC of *L. glutinosa* bark extracts against tested bacteria and fungi (in µg/ml)

Test Organisms	MeOH	EtOH	Hex	Et Ac	Aq
S. aureus	1000	500	250	250	250
CONS	250	1000	250	250	250
C. tropicalis	250	250	250	250	250
C. albicans	500	250	250	250	250

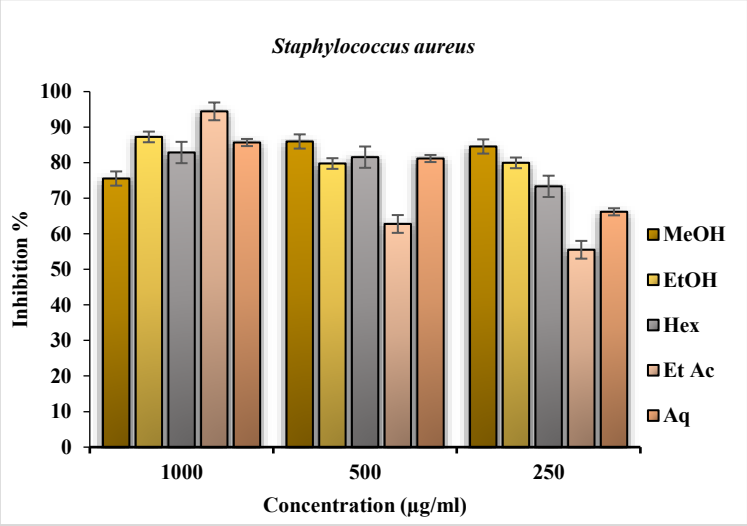


Figure 7. Antibacterial activity of *L. glutinosa* against *S. aureus*

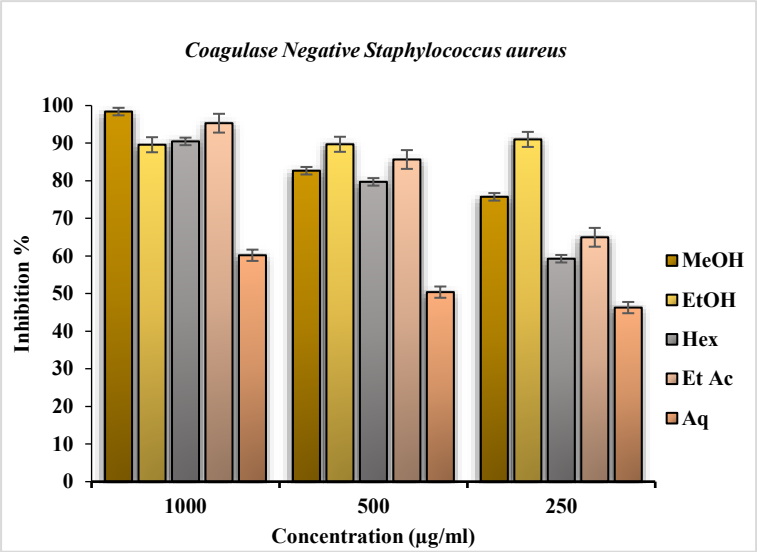


Figure 8. Antibacterial activity of *L. glutinosa* against *Coagulase Negative S. aureus*

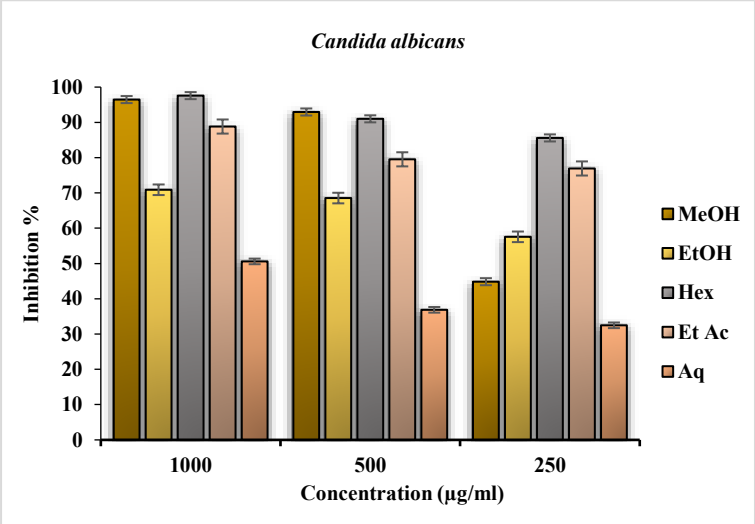


Figure 9. Antifungal activity of *L. glutinosa* against *C. albicans*

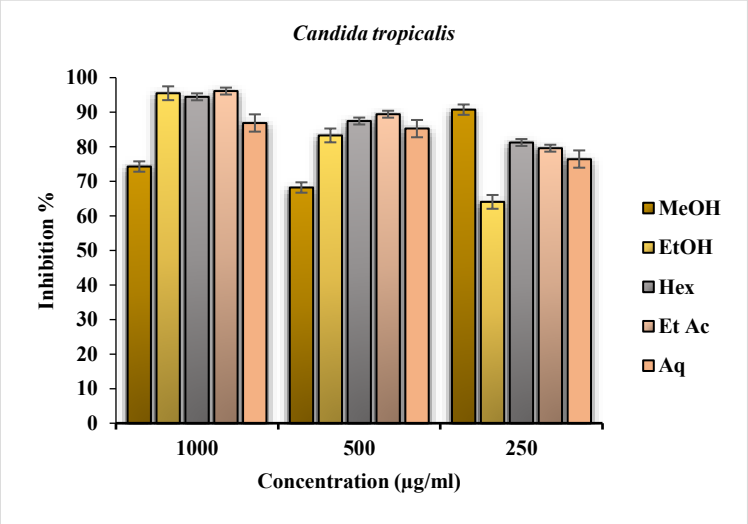


Figure 10. Antifungal activity of *L. glutinosa* against *C. tropicalis*

Statistical Analysis

Table 7. Factor loading of variables explained with eigen % and variance % for *L. glutinosa.*

Factor Loadings	PC 1	PC 2
TPC	-2.2014	-0.77678
TFC	2.2969	-0.68507
DPPH	-0.08486	1.4976
MeOH	0.46474	-0.38718
EtOH	0.50993	-0.10822
Hex	0.23443	0.80932
Et Ac	0.49465	-0.24473
Aq	0.47366	0.35141
Eigenvalue	3.7911	1.20853
% variance	75.822	24.171

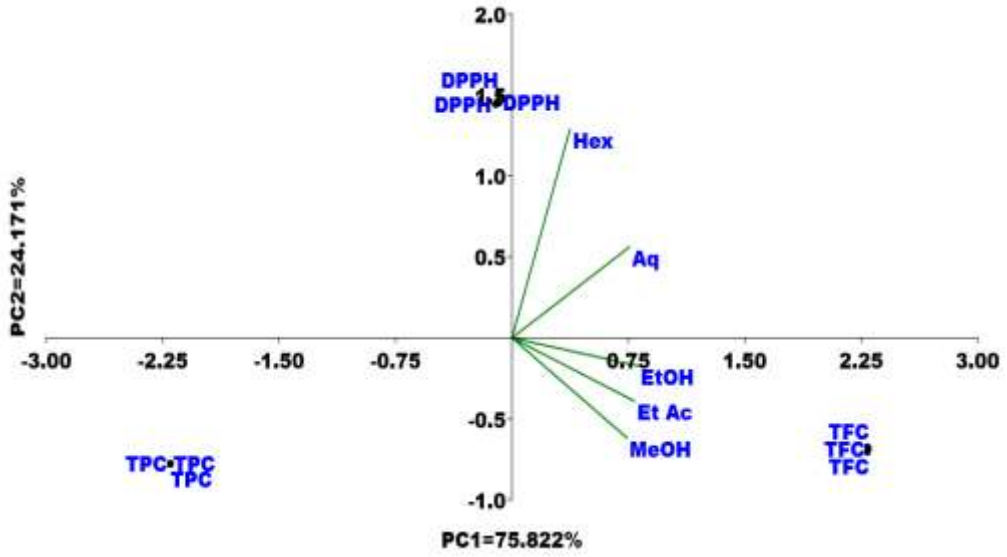


Table 8. List of variables with their code indicated in PCA ordination diagram

Code	Variables
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
MeOH	Methanol
EtOH	Ethanol
Hex	Hexane
Et Ac	Ethyl Acetate
Aq	Aqueous

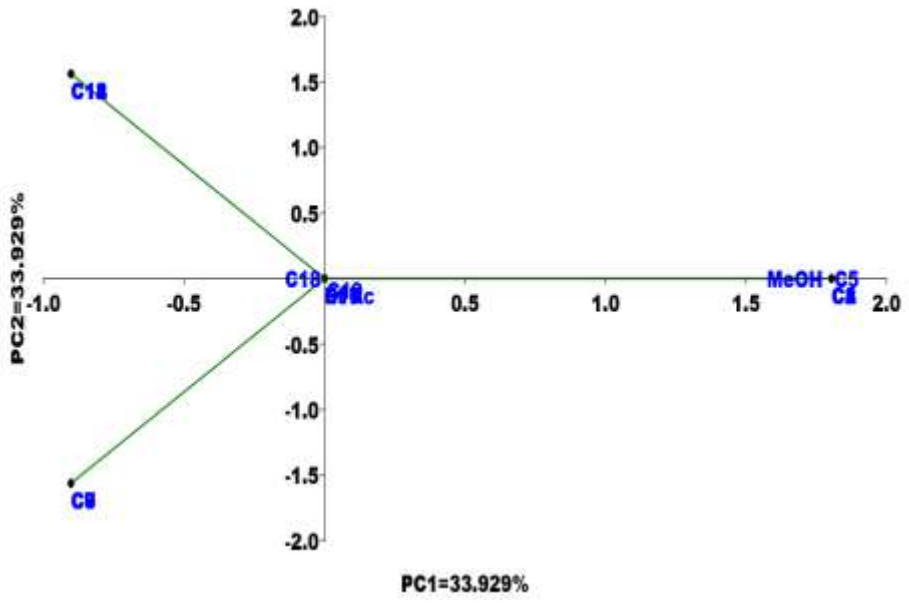


Figure 12. Biplot graph based on the principal component analysis (PCA) depicting the Bioactive compounds of *L. glutinosa* bark extracts. The abbreviations used are as follows: C1-Phenol, 2-Methyl-, C2-Succinic Acid, Di(But-2-En-1-yl) Ester, C3-Naphthalene, C4-Methyl 2,6-Anhydro-.Alpha.-D-Altroside, C5-Myrtenyl Angelate, C6-2-Ethyl-1-Hexanol, Heptafluorobutyrate, C7-Dodecane, 1-Fluoro-, C8-Z,Z-6,28-Heptatriacontadien-2-One, C9-Tris (Tert-Butyldimethylsilyloxy)Arsane,C10-Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-,C11-1-Butoxypropan-2-YL Pentanoate, C12-Butanoic Acid, 2-Methyl-, 2-Methyl-2-Propenyl Ester, C13-Cyclobutanecarboxylic Acid, 2-Propenyl Ester, C14-Hentriacontane, C15-Benzene, 1,3-Bis(1,1-Dimethylethyl)- C16-1,6-Heptadien-3-Yne C17-3-Methyl-2-(2-Oxopropyl)Furan C18-Heptacosanoic Acid, 25-Methyl-, Methyl Ester C19- 1,1,1,3,5,5,5-Heptamethyltrisiloxane MeOH-Methanol, EtOH-Ethanol, Hex-Hexane, Et Ac-Ethyl Acetate.

DISCUSSION:

In a current study, the phytochemical analysis of *L. glutinosa* was performed. It is known that the phytochemicals are important for various biological activities. Phenolics and flavonoids, have been linked to analgesic effects and may alter pain pathways by interacting with important neurotransmitters or receptors involved in pain perception. Phenolics are known to have antipyretic properties, which are probably caused by their impact on the hypothalamic control of body temperature. Furthermore, tannins and saponins have demonstrated antibacterial action against a range of pathogens, underscoring their possible functions in the defensive mechanisms of the plant [34]. Several analytical tests were performed on *L. glutinosa* extract and its different organic-soluble fractions to confirm the presence of physiologically active phytochemicals. The medicinal potential of the phytochemicals found in this study, such as alkaloids, flavonoids, phenols, and saponins, has been extensively established in the scientific literature. In the field of medical sciences, these substances are found to possess a variety of beneficial properties, including anti-inflammatory, antibacterial, and antioxidant actions. The results of the current investigation might strengthen our knowledge of *L. glutinosa* as a source of bio-active chemicals with possible pharmaceutical uses and offer insightful information for the future development of new therapeutic medicines [34]. Extract in Et Ac solvent produced the highest concentration of phenolic compounds among the five solvents tested (0.165 ± 0.01(mg GAE/g dry weight ± STDEV)). This finding aligns with the previous studies on *L. glutinosa* leaf extract, where Et Ac was reported to have

the highest TPC 200.61 ± 0.47 (mg of GAE/g of extract ± SD) [35]. These could be due to the fact that the solvent's semi-polar nature allows it to selectively extract moderately polar phenolics more effectively than more polar solvents such as MeOH or EtOH or non-polar such as Hex solvents. The flavonoid content was found to be highest in MeOH solvent concentration with (283.5 ± 0.001(mg QE/g dry weight ± SD). Our studies are in line / aligns with the other study carried out earlier on *L. glutinosa* which confirmed high flavonoid content in MeOH leaf extract [35]. This could be certainly because MeOH extract has the tendency to yield the highest amounts of flavonoids. The results also demonstrate that the extraction efficiency for polyphenols and flavonoids is highly influenced by solvent polarity, as indicated by the variations in flavonoid content across the different solvents [35]. The DPPH radical scavenging activity showed that all the plant extracts showed concentration-dependent increases in radical scavenging capacity [13,36]. The greatest DPPH radical scavenging potency with a minimum IC_{50} value was recorded for methanol extract with the IC_{50} value of 15.26 µg/ml. In the extract of Hex has the less ability to scavenge DPPH radicals with IC_{50} 99.72 µg/ml. In agreeing to the report of other researchers, where MeOH extract was found to have the highest DPPH radical scavenging activity [37]. This may be owing to its superior extraction of polar phenolics and flavonoids, which are essential antioxidants, whereas Hex being the non-polar solvent extracts few antioxidants binding efficient.

A number of compounds were characterized in the GC-MS profiling in *L. glutinosa* (Lour.) C.B. Rob. bark extracts using various solvents have identified all together 100 bioactive compounds. The compounds identified are Phenol, 2-Methyl-; Succinic Acid, Di(But-2-En-1-yl) Ester; Naphthalene; Methyl 2,6-Anhydro-Alpha.-D-Altroside; Myrtenyl Angelate; 2-Ethyl-1-Hexanol, Heptafluorobutyrate; Dodecane, 1-Fluoro-; Z,Z-6,28-Heptatriactontadien-2-One; Tris (Tert-Butyldimethylsilyloxy) Arsane; Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-; 1,6-Heptadien-3-Yne; 3-Methyl-2-(2-Oxopropyl)Furan; Heptacosanoic Acid, 25-Methyl-, Methyl Ester; 1,1,1,3,5,5,5-Heptamethyltrisiloxane; 1-Butoxypropan-2-YL Pentanoate; Butanoic Acid, 2-Methyl-, 2-Methyl-2-Propenyl Ester; Cyclobutanecarboxylic Acid, 2-Propenyl Ester; Hentriacontane; Benzene, 1,3-Bis(1,1-Dimethylethyl)-. Some of these compounds were found to have pharmacological properties. Naphthalene was found to have anticancer, antimicrobial, anti-inflammatory, antiviral, antitubercular antihypertensive, antidiabetic, anti-neurodegenerative, antipsychotic, anticonvulsant, antidepressant [24]. Myrtenal is a naturally occurring monoterpene that was extracted from the essential oils of several plants. Its derivatives such as Myrtenyl Angelate have been demonstrated to possess a number of biological characteristics, including cytotoxicity. Potential anticancer medicines are being developed by analyzing the cytotoxic activity of these derivatives to ascertain their antitumor effect [38]. Z, Z-6,28-Heptatriactontadien-2-One is found to have antidiabetic activity. Heptacosanoic Acid, 25-Methyl-, Methyl Ester have been reported in a study done on Chloroxylon swietenia Methanolic leaf extract & Au nanoparticles, which highlights its larvicidal/insecticidal characteristics [32]. The findings matched with the research conducted in other areas where a broad-spectrum antibacterial & antioxidant (correlative) property is found in *Cleistopholis patens* Ethyl Acetate bark extract [31]. The compound Hentriacontane was also present in MeOH extract of *Opuntia ficus-indica* pulp extract. The study underlines the anti-inflammatory and antimicrobial property [25]. Similar findings were obtained in other research that investigated the pharmacological effects such as its anti-inflammatory, anticancer, and antibacterial activities, with peritoneal macrophages demonstrating its anti-inflammatory capabilities [26]. The results were similarly consistent with the study conducted in South Arabia, which also highlighted that the substance Hentriacontane is a solid long chain alkane with good anti-tumour properties, found in

Artemisia arborescens L petroleum ether extract [28]. However, bio activities of majority of the compounds are not found may be because it has other uses different from biological properties; and further studies will be required to ascertain in order to know their functions.

FTIR analysis conducted from the bark powder of *L. glutinosa* plant revealed various types of functional groups such as alcohol, phenols, alkanes, alkyls, carboxylic acids, esters, ethers, and halo compounds. The study on the Natural Surfactant Saponin from *L. glutinosa* is quite similar having functional groups such as (C-H) in methanol extract of *L. glutinosa* [39]. FTIR spectroscopy has proven to be a reliable and sensitive approach for detecting the molecular composition of medicinal plants with therapeutic qualities.

The antimicrobial investigation on *L. glutinosa* demonstrated that the extracts show inhibitory activities against bacteria such as *S. aureus* and Coagulase-Negative *S. aureus*, and fungi such as *C. albicans* and *C. tropicalis*, suggesting its potential in the treatment of different ailments, notably skin problems. This corresponds with the other study conducted on *Moringa oleifera* L. leaves and *Matricaria recutita* L. flowers, examining their effects on antibiotic-resistant and sensitive bacterial strains isolated from patients with wound infections. The examined extracts of *M. oleifera* and *M. recutita* shown various degrees of antibacterial activity against *Proteus mirabilis* isolates, along with bacteria such as *Klebsiella* sps, *P. aeruginosa*, and *Staphylococcus* sps that were both antibiotic-sensitive and resistant. According to additional research by Sultana et al. (2015) and Mohammed et al. (2017), the most prevalent bacterium discovered in wound infections was *S. aureus* [21]. Another research done on the 4 medicinal plants (*Drimia sanguinea*, *Elephantorrhiza elephantina*, *Helichrysum paronychioides*, and *Senecio longiflorus*) against 3 bacterial and 3 fungal strains *Bacillus cereus*, *Shigella flexneri*, *Candida glabrata*, *Candida krusei*, *Trichophyton rubrum* and *Trichophyton tonsurans have demonstrat*ed varying degrees of antimicrobial potentiates against the studied bacteria and fungi [40].

CONCLUSION:

This study conducted on the phytochemical screening with the bark extracts from *L. glutinosa* using various solvents, identified compounds such as alkaloids, flavonoids, saponins, steroids, tannins, saponins, steroids, terpenoids and Phenolic compounds. They have demonstrated significant anti-inflammatory, antimicrobial, antioxidant effects, antidiabetic, antifungal, analgesic, antipyretic, and antibacterial activities, supporting their traditional medicinal use. The study emphasizes the necessity of more research to determine and characterize the significant components causing these effects. These results further proof of the uses of *L. glutinosa* in ethno-medicine and illustrate potential source for novel medications and nutraceuticals, however more researches, including clinical trials, is required to validate these further therapeutic uses.

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