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Biochemical Fingerprinting of *Shorea robusta* C.F.Gaertn Leaves: Qualitative Detection and Quantitative Estimation of Medicinally Important Phytoconstituents

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ABSTARCT:
Shorea robusta C.F.Gaertn is a common medicinal tree, popular for its resinous exudates and traditional therapeutic uses. Despite its ethnopharmacological significance, there remains an absence of comprehensive information on solvent-dependent phytochemical diversity, GC–MS based metabolite profiling and antimicrobial activity against clinically important pathogens. Successive extracts of S. robusta *C.F.Gaertn* extract were prepared with methanol, ethanol, hexane and petroleum ether. The function of antimicrobial action was determine by the disc diffusion method using *Bacillus anthracis, Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa*, and phytochemical screening and GC–MS finger printing analysis (SM_MTNGU 3429) were carried out to identify bioactive constituents. Alkaloids was detected in all extract. Flavonoids, saponins, phytosterols and terpenoids were mainly present in the methanol and ethanol fractions. GC–MS profiling revealed a major peak at RT 25.150 min corresponding to terpenoid compounds such as bergamotene and related sesquiterpenes with reported antioxidant and antimicrobial properties. Antibacterial screening of ethanol extract exhibited the greatest suppressive activity (15-17.34mm) towards all the tested pathogens accompanied by methanol extract and hexane and petroleum ether extracts showed comparatively lower activity. Higher inhibition zones (up to 24.33 mm) were observed with streptomycin, thus confirming the assay. The study uncovers *Shorea robusta* C.F.Gaertn resin as a prolific reservoir of secondary metabolites terpenoid-rich metabolites with notable antibacterial potential in a solvent-dependent manner. Thus these observation confirmed its therapeutic use and may provide a future to produce organic antibacterial and antioxidant agents for pharmacological and therapeutic applications.
KEYWORDS: *Shorea robusta* C.F.Gaertn, phytochemical screening, GC–MS analysis, bergamotene, terpenoids, antimicrobial activity, resin extract, solvent extraction, disc diffusion assay

INTRODUCTION

Medicinal plants contains abundant source of secondary metabolites that are crucial to human health and disease management. These phytochemicals like alkaloids, flavonoids, phenolics, tannins, saponins and terpenoids are in change of various medicinal properties. The identified constituents are associated with several pharmacological effects, including protection against oxidative stress, suppression of pathogenic microorganisms, and regulation of inflammation. Phytochemical screening is an valuable techniques for recognition and characterization of these compounds thereby providing scientific pharmacological substantiation of traditional medicinal use. *Shorea robusta* is an important medicinal tree distributed widely throughout the Indian subcontinent and has historically been utilized in indigenous healthcare systems for the control of several disorder. It contain secondary metabolites that could be responsible for its therapeutic effects. However, information on qualitative and quantitative phytochemical composition of the leaves is still limited. Therefore, the present study was undertaken for the biochemical fingerprinting of *Shorea robusta* leaves through qualitative detection and quantitative estimation of medicinally important phytoconstituents (Malviya et al., 2026). Major classes of phytochemicals identified included nitrogen congaing secondary metabolites, flavonoids, phenolic compounds, tannins, saponins, and terpenoids, which are responsible for many of the pharmacological properties exhibited by medicinal plants. These compounds need to be determined qualitatively and

quantitatively for the determination of their medicinal value and quality of herbal materials. *Shorea robusta* is a major representative species of family Dipterocarpaceae and is extensively distributed in Indian sub-continent. Traditionally it is applied associated with treatment of numerous health disorder. The plants many section have been explored for medicinal properties but knowledge on the phytochemical composition of the leaves is limited (Amponsah et al., 2026). In recent years, Therapeutic plants have attracted much prominence in modern health care, because to their availability, cost-effectiveness, cultural acceptability and perceived safety. Therefore, the importance of herbal medicines has increased worldwide, and consequently, more attention has been paid to their quality, efficacy and safety. At the same time, the development and rapid circulation of multi-drug resistant (MDR) microorganisms has become significant public health concern worldwide, with a greater obligation in nations with low and moderate incomes. Despite tremendous progress in antimicrobial drug development and medical research over the last century, the efficacy of conventional antibiotics is compromised driven by the rising prevalence of resistant pathogens. Inappropriate prescription of antibiotics, self-medication, overuse in human and veterinary medicine and the poor regulatory control over the antimicrobial agents are the main causes associated with the widespread prevalence of antimicrobial resistance. In this regard, standardization and scientific validation of plant-derived pharmaceuticals provide promising opportunities for the development of new therapeutic strategies. Most importantly, it is crucial to conserve the traditional knowledge associated with medicinal plants, as this valuable heritage may otherwise be lost. Medicinal plants have gained considerable attention in pharmaceutical and pharmacological investigations due to their promise as reservoirs of innovative antibacterial molecules. The major pathogenic microorganisms that pose a serious health hazard, examine the factors responsible for the emergence and dissemination of antimicrobial resistance, and evaluate the global utilization of bioactive plant resources as alternative antibacterial agents. The various mechanisms by which medicinal plants and their bioactive ingredients exhibit antimicrobial activity, and their potential contribution in the battle against drug-resistant infections (El-Saadony et al., 2025). Secondary metabolites compounds are broadly grouped as polyphenols, terpenoids, alkaloids, sulphur-containing compounds and other secondary metabolites. The phytochemicals contain significant antioxidant, anti-inflammatory, antimicrobial, anti-viral and anticancer activities. They play an important ability to minimise exposure and management of cardiovascular disorders, diabetes, obesity, neurodegenerative diseases and cancer. Phytochemicals are produced by different metabolic pathways and they act as natural defence molecules in plants. Their biological activities are related to the modulation of cellular signaling pathways, immune responses and oxidative stress. More recent work has looked at how they work, how available they are in the body and their therapeutic potential. The interaction of phytochemicals with gut microbiota has also become an important area of research. The role of phytochemicals in modern healthcare. Therefore, more research is needed to explore their full potential in disease prevention, as well as in the creation of new pharmaceuticals (Yang & Ling, 2025).

Shorea robusta is a forest tree of medicinal importance which is abundantly distributed in Indian sub-continent and traditionally used for the management of various ailments. The species is endowed with various bioactive phytoconstitutes, which are attributed to its therapeutic potential. A detailed phytochemical characterization is required to understand the biochemical composition of medicinal plants as well as to establish quality control parameters for their use. The development the biochemical fingerprinting of *Shorea robusta* leaves by qualitative detection and quantitative estimation of medicinally important phytoconstituents. The major classes of natural products and assesses providing scientific evidence in support of the medicinal significance of the species, as well as a basis for future pharmacological, nutraceutical and natural-product research (Mendoza-Calderón et al., 2026). This species has traditionally been used for several therapeutic purposes, but information on the phytochemical composition of its leaves is still scanty. Standard phytochemical screening procedures were used to identify major classes of plant substance and identified of selected bioactive compounds of leaf extracts. Quantitative determination showed appreciable levels of these metabolites with phenolic and flavonoid compounds as the predominant phytochemical groups. The phytochemical analysis showed a unique biochemical fingerprint that indicated the medicinal significance of the species (Yang & Ling, 2025). The medicinal plants to treat diseases has been known since ancient civilizations and continues to

be an important part of traditional medical care worldwide. The possibility of treatment of these medicinal plants is attributed mainly to the existence of various bioactive phytoconstituents. These substance are terpenoids, phenolics, flavonoids, alkaloids and glycosides having multiple bioactives. They have antioxidant, antimicrobial, anti-inflammatory, anticancer and antiviral properties making them potential candidates for drug development. They exert their biological effects by interaction with cellular receptors, membranes, enzymes and nucleic acids. These compounds are of great interest in nutraceutical and pharmaceutical research due to their high biological activity and relatively low toxicity. Many modern drugs are developed from secondary metabolites of plant origin or their derivatives. They also have antioxidant effects that are important for disease prevention and health promotion. Hence, knowledge on phytochemistry, classification and biological activities of bioactive compounds is a prerequisite for the identification of novel lead compounds and for the advancement of discovery of efficacious plant-based therapeutics (Velu et al., 2018).

One another application of ZnO nanoparticles is an efficient alternative to conventional materials in water treatment and biomedical applications, contributing to the achievement of sustainable development and the One Health approach (Lebaka et al., 2025). Several bioactive secondary metabolites such as stilbenes, polymeric resveratrol compounds, oligomers, triterpenoids, coumarins, flavonoids and steroids have been identified from phytochemical investigations. The most important class of compounds are stilbenes and their oligomeric derivatives, which constitute most of the pharmacological importance of the genus. Various *Shorea* species have been commonly employed to treat of diarrhea, toothache, skin disorders, ear infections and wound healing (Martha et al., 2020). Modern research has discovered that different bioactivities of Shorea species indicate their promising potential as sources of natural therapeutic compounds (Velu et al., 2018).

Quantitative data on medicinally important phytoconstituents are prerequisite for standardization, quality assurance and therapeutic validation of medicinal plants. However, precise data on the concentration and distribution of bioactive compounds are still scarce for many medicinal species. A major challenge in pharmacognosy is the knowledge gap, as variation in the effectiveness, safety, and repeatability of herbal remedies can be directly impacted by the concentrations of phytoconstituents like alkaloids, flavonoids, phenolics, tannins, and terpenoids. The effectiveness, safety, and repeatability of herbal remedies can be directly impacted by the concentrations of phytoconstituents like alkaloids, flavonoids, phenolics, tannins, and terpenoids. In addition, lack of adequate quantitative characterization prevents the establishment of standard dosages, quality control parameters and regulatory guidelines for the plant-based products. Therefore, extensive phytochemical profiling and quantitative analysis are important to maintain the uniformity, reliability and acceptance of medicinal plants in modern healthcare and pharmaceutical applications worldwide (Dhansay & Jat, 2025) Comprehensive biochemical characterization is a fundamental approach for understanding the molecular composition, metabolic profiles, and functional properties of biological materials. It involves the systematic analysis of biomolecules, including proteins, carbohydrates, lipids, enzymes, phenolic compounds, and other metabolites that contribute to the biological activity of an organism. Such characterization provides valuable insights into physiological processes, metabolic pathways, and the mechanisms underlying various biological functions. In medicinal plants, biochemical profiling is particularly important for identifying bioactive constituents responsible for therapeutic effects and for establishing quality standards. Furthermore, comprehensive biochemical analyses support drug discovery, nutritional assessment, environmental studies, and the development of functional products. Therefore, detailed biochemical characterization give a crucial tool for assessing the medicinal, nutrition, and environmental importance of biological resources (Sönmez et al., 2025).

MATERIAL AND METHODS:

The plant material utilized in this investigation was was taxonomically identified and authenticated as *Shorea robusta* C.F.Gaertn. (Family: Dipterocarpaceae) by the Herbarium, Department of Botany, Gauhati University, Assam, India. A voucher specimen was prepared following the standard herbarium procedures and was deposited in the university herbarium with Accession No. GUBH20701 (Reference No.

Herb/GU/BH/2025/470). The authenticated specimen was preserved for future reference and verification.

Figure 1:- Voucher herbarium specimen and authentication certificate of *Shorea robusta* C.F.Gaertn. (Dipterocarpaceae) obtained from the Herbarium, Department of Botany, Gauhati University, Assam, India (Accession No. GUBH20701).

Plant parts *Shorea robusta* C.F.Gaertn (leaves) was air-dried under shade conditions to avoid breakdown of bioactive compounds and subsequently grinding into a fine powder with a grinding machine. Solvents with progressively higher polarity were used to extract the powdered substance, such as chloroform, methanol, and distilled water, to ensure efficient extraction of a wide range of phytoconstituents. Isolation was performed out using maceration or Soxhlet apparatus as per standard protocols (Teli et al., 2026). The obtained extract were then concentrated using a rotating evaporator under reduced pressure, while aqueous extracts were further lyophilized to obtain dry residues. Finally, the powdered samples were kept in sealed containers at 4°C for subsequent phytochemical and analytical studies. Preliminary phytochemical analysis of the plant extract was carried out to detect the presence of major bioactive compounds using standard qualitative tests. The extract were prepared by dissolving a predetermined amount of dried plant extract in appropriate solvent (distilled water/ethanol), followed by filtration. The filtrate was used for the following qualitative and quantitative tests.

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Figure 2:-Crude extract of Shorea robusta C.F. Gaertn.

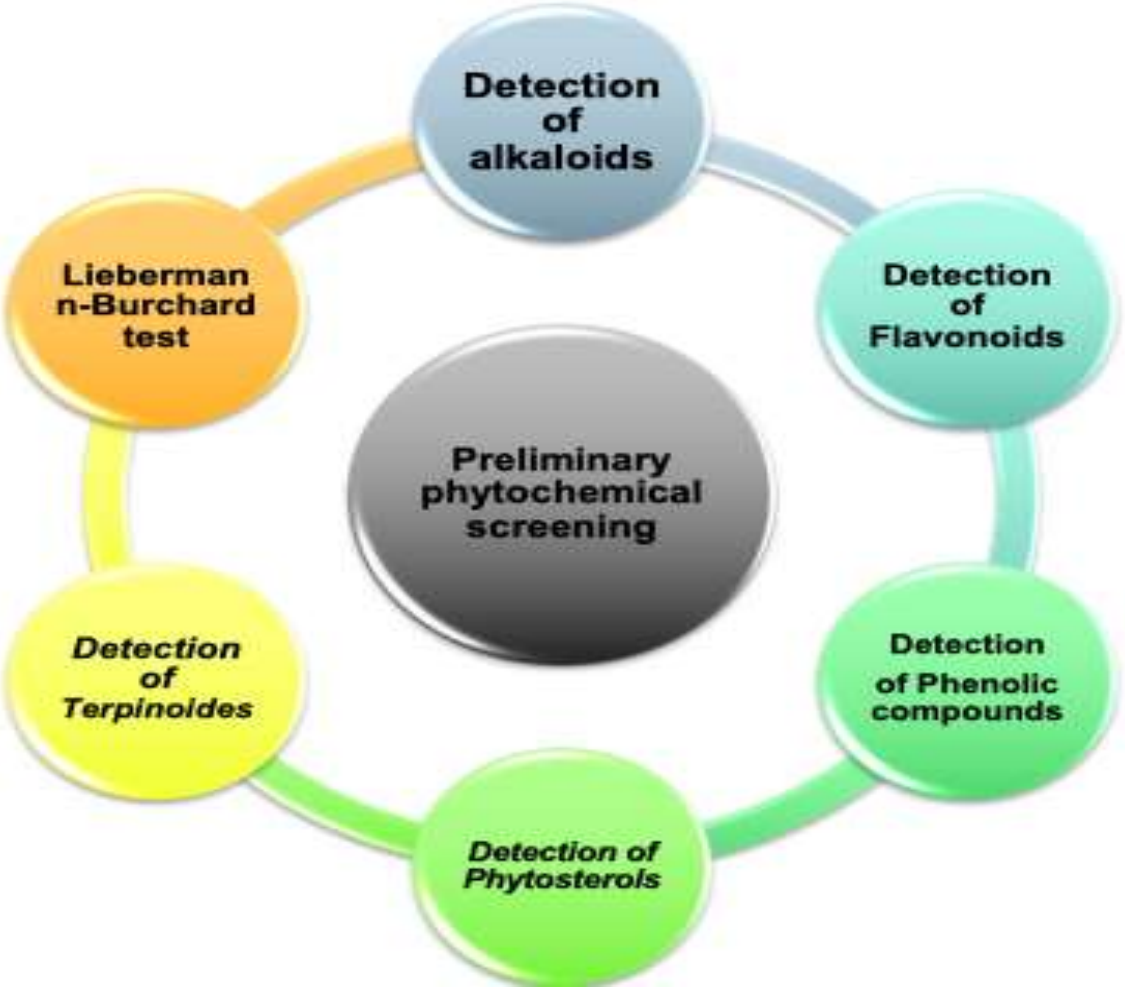


Figure 3: Qualitative phytochemical screening of detection of alkaloids, flavonoids, phenolics, saponins, phytosterols, terpenoids, and tannins using standard colorimetric and precipitation-based assays.

Preliminary phytochemical screening:- The major phytochemical constituents in the plant extract were analyzed using standard qualitative tests. Alkaloids were tested with the Mayer and Wagner's reagents. In Mayer's test few drops of Mayer's reagent were added to extract filtrate and formation of cream or pale-

yellow precipitate considered as positive for alkaloids. Similar results were also obtained by Wagner's test, where the presence of these compounds was confirmed by the appearance of reddish brown precipitate after addition of Wagner's reagent. Flavonoids Few drops of 10 % sodium hydroxide solution was added to the extract. The presence of flavonoid constituents was indicated by appearance of a characteristic yellow colour. The iodine test was used to test for phenolic compounds. The appearance of a reddish color after addition of diluted iodine solution indicated a positive test.
The foam test was carried out for testing the presence of saponins by vigorous shaking of the extract with distilled water. Evidence of saponins was the froth which remained stable for several minutes. Phytosterols were detected by Salkowski's test in which concentrated sulphuric acid was carefully added to the extract. The reddish color developed in the lower layer showed the presence of phytosterols. Terpenoids were tested by Salkowski's and Liebermann-Burchard tests. The extract was mixed with chloroform and concentrated sulfuric acid in Salkowski's test and the formation of a reddish brown ring at the interface of the two layers was considered as positive result. The Liebermann – Burchard test was performed by treating the extract with acetic anhydride and concentrated sulphuric acid. The presence of terpenoid compounds was confirmed by the appearance of reddish violet coloration.
Detection of tannins:- The extract was mixed with 10 % sodium hydroxide solution. Formation of an emulsion on shaking was regarded as an indication of hydrolyzable tannins. These preliminary phytochemical tests provided a general idea of the bioactive constituents present in the plant extract and were the basis for further characterization studies.

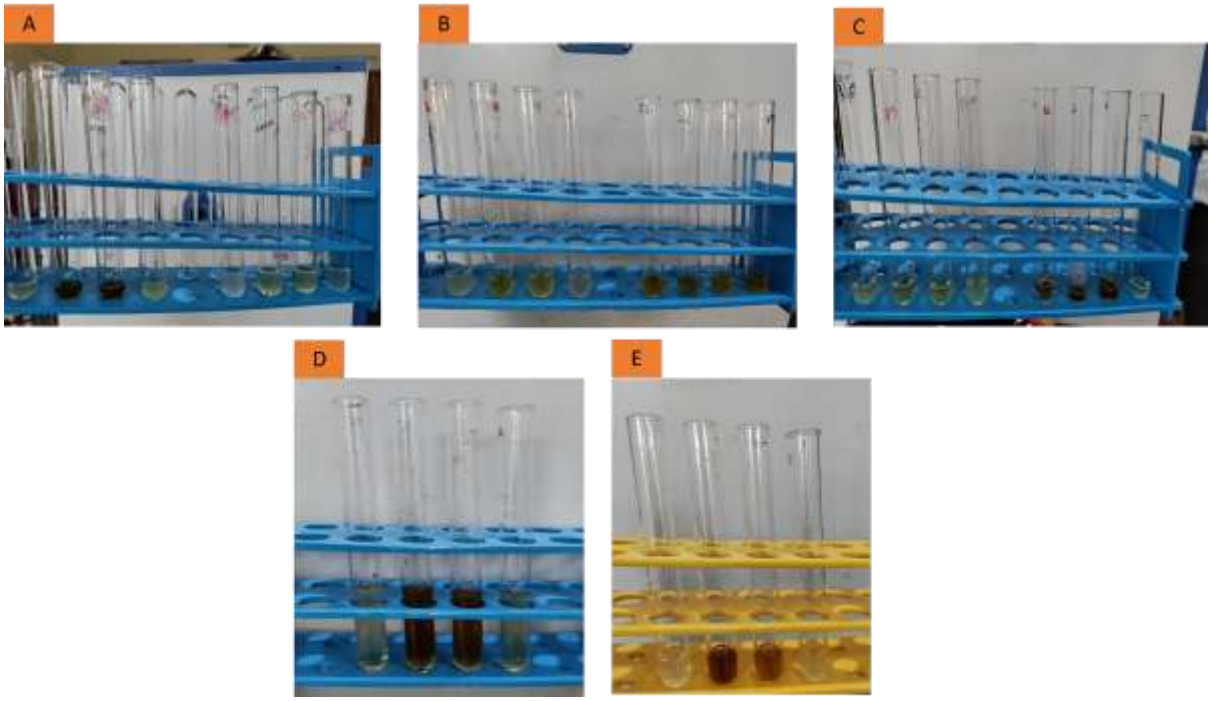


Figure 4:- *Shorea robusta* C.F.Gaertn extracts using various solvents, from left (hexane, ethanol, methanol, and petroleum ether). (A) Alkaline reagent test for flavonoids and foam test for saponins. (B) Mayer's and Wagner's tests for alkaloids. (C) Iodine test for phenolic compounds and Salkowski's test for phytosterols. (D) Salkowski's test for terpenoids. (E) 10% NaOH test for tannins.

Quantitative phytochemical screening:- The chemical components found in the leaf extracts of Shorea robusta C.F.Gaertn, Vitex canescens, and Garcina pedunculata were identified using GC–MS. A PerkinElmer (USA) GC–MS system (Clarus 680 GC and Clarus 600C MS) with an auto-sampler was used for observation. TurboMass software (Version 6.4.2) was applied to process the data, and chemicals were recognised by similar their spectra with the NIST-2014 database. The Elite-5MS capillary column (60 m in length, 0.25 mm in diameter, and 0.25 µm in thickness) was utilized. 95% dimethyl polysiloxane and 5% diphenyl made up the column's stationary phase. The transport gas was 99.99% pure helium gas flowing at a rate of 1 milliliter per minute. One microliter of the sample was administrated in divided

mannr. The ion supply and injector temperatures were maintained at 180°C and 280°C, respectively. After starting at 60°C for one minute, the oven's temperature was raised at a rate of 7°C per minute to 200°C for three minutes, and then it was raised at a rate of 10°C per minute to 300°C for five minutes. It ran for almost 39 minutes in total. There was an eight-minute solvent delay. At 70 eV, mass spectra were captured in the Electron Impact (EI+) mode. The mass range was established between m/z 50 and 600. The mass spectra of unknown peaks were compared with known spectra in the NIST collection to identify the chemicals.

Based on this, the name, molecular weight, and formula of the compounds were determined. For this research support of CAIF, Guwahati Biotech Park Incubation Centre for providing the GC–MS facility, along with technical help and funding support from the DBT project (No. BT/BP/19011/1/2008).

Antibacterial Activity Assessment:- For antimicrobial activity assessment used disc Diffusion, in this method determine zones of inhibition using the Kirby-Bauer disc diffusion method. Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus anthracis*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*)were used to evaluate the antibacterial activity. Isolated colonies were suspended in sterile saline to create the bacterial solution to yield a 0.5 McFarland standard (1.5×10^8 CFU/mL). MHA agar plates were prepared with autoclave sterilized (121℃ for 15 minutes) media. 100ul of bacterial inoculum of each strain was spread over separate MHA agar plate. Vertical wells were punched into each agar plated with a sterile cork borer. Each plate ware introduced with positive control, negative control and 100 ul of extract solutions. Following incubation at 37 ℃ for 24 h, the clear zones were recorded and accurately measured with the help of a digital caliper.

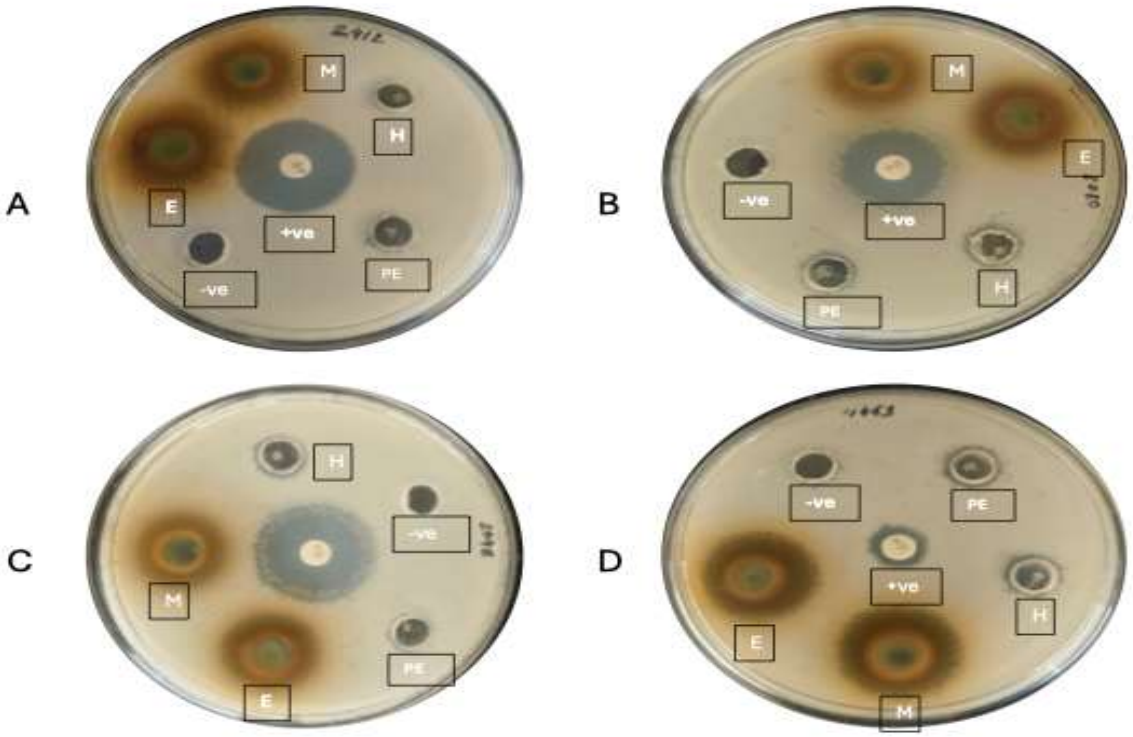


Figure 5:- ***Shorea robusta*** *C.F.Gaertn* ***pedunculata Antibacterial Test*** *Photo plates showing antimicrobial activity of crude extract against four different test pathogens. (A)Escherichia coli (ATCC2412), (B) Pseudomonas aeruginosa (ATCC 2080), (C) Staphylococcus aureus (ATCC 2408) and (D) Bacillus anthracis (ATCC 4453) E= Ethanol, M= Methanol, PE= Petroleum Ether, H= Hexane, -ve =Negative control, +ve = Positive Control.*

RESULT & DISCUSSION

Quantitative phytochemical test result:- Alkaloids were detected in all extracts (methanol, ethanol, hexane, and petroleum ether) using both Mayer's and Wagner's tests, indicating their broad solubility in *Shorea robusta* C.F.Gaertn. Flavonoids were present only in methanol and ethanol extracts, suggesting

their polar nature, while absent in hexane and petroleum ether extracts. Phenolic compounds and tannins were not detected in any extract. Saponins were observed exclusively in methanol and ethanol extracts. Phytosterols were present in methanol, ethanol, and hexane extracts but absent in petroleum ether extract (Table-1). Terpenoids were detected only in methanol and ethanol extracts. So that, ethanol and ethanol extracts showed a higher diversity of phytoconstituents compared to non-polar extracts. (Yadav et al., 2026) study on resin of *Shorea robusta* C.F.Gaertn, *which* contains natural phytoconstituents (mainly terpenoids, resins, and phenolic compounds) which were utilized for nanoparticle synthesis. It is used for enhanced wound-healing, antioxidant, and anti-inflammatory activity, validating their therapeutic potential in diabetic wound management. *Tabernaemontana ventricose* contain alkaloids, flavonoids, saponins, sterols, steroids, phenols, fats, fixed oils, carbohydrates, and amino acids as indicating a broad phytochemical profile (Naidoo et al., 2023).

Table1:- Phytochemical Screening of *Shorea robusta* C.F.Gaertn Extracts

Name of the test		Methanol extract	ethanol extract	hexane extract	petroleum ether extract
Detection of Alkaloids	Mayers test	+	+	+	+
	Wagners test	+	+	+	+
Detection of Flavonoids	Alkaline reagent test	+	+	-	-
Detection of phenolic compounds	Iodine test	-	-	-	-
Detection of saponin	Foam test	+	+	-	-
Detection of phytosterols	Salkowski test	+	+	+	-
Detection of terpenoids	Salkowski test	+	+	-	-
Detection of tannin	10% NaOH test	-	-	-	-

Qualitative GC-MS Test result:- The GC-MS chromatogram of the crude metabolite extract (SM_MTNGU 3429) showed a major peak at retention time (RT) of 25.150 min indicating the presence of various bioactive volatile constituents. Compounds associated with this peak were identified by mass spectral analysis and library matching as Bergamotene, (E,E)-7,11,15-trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene and (1S,5S)-4-methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane . These compounds were mostly terpenoid and sesquiterpene secondary metabolites. Bergamotene was reported to have antioxidant, anti-inflammatory, immunosuppressive, cytotoxic, antimicrobial, antidiabetic and insecticidal activities, suggesting its possible role in the biological potential of the extract(Annaz et al., 2024). (E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene was also reported to exhibit antioxidant activity which can contribute to the improvement of the free radical scavenging activity of the extract (Beraich et al., 2024). In addition, (1S,5S)-4-methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane was linked to cholesterol-modulating activity and may have contributed to the therapeutic importance of the extract (Ongtanasup et al., 2022a). The presence of these terpenoid-rich metabolites at RT 25.150 min, indicated that the extract was rich in bioactive secondary metabolites with diverse pharmacological properties (Câmara et al., 2024). The identified compounds may have synergistic effects due to the combined antioxidant, antimicrobial, anti-inflammatory and cholesterol regulating activities; thereby contributing to the overall medicinal value of the extract. Terpenoids are one of the major classes of bioactive metabolites in *Shorea robusta* C.F.Gaertn and are widely linked to antimicrobial, antioxidant and anti-inflammatory activities. The present GC–MS analysis identified terpenoid-rich compounds that support previous phytochemical studies on S. robusta resin and suggest that these

metabolites may be responsible for the therapeutic efficacy of the species . The presence of these compounds is a biochemical basis for the traditional use of S. robusta in the treatment of skin disorders, gastrointestinal infections and other diseases (Sri et al., 2011). The results proved the scientific evidence of the therapeutic potential of the plant and justified further studies on the bioactive constituents and pharmacological applications.

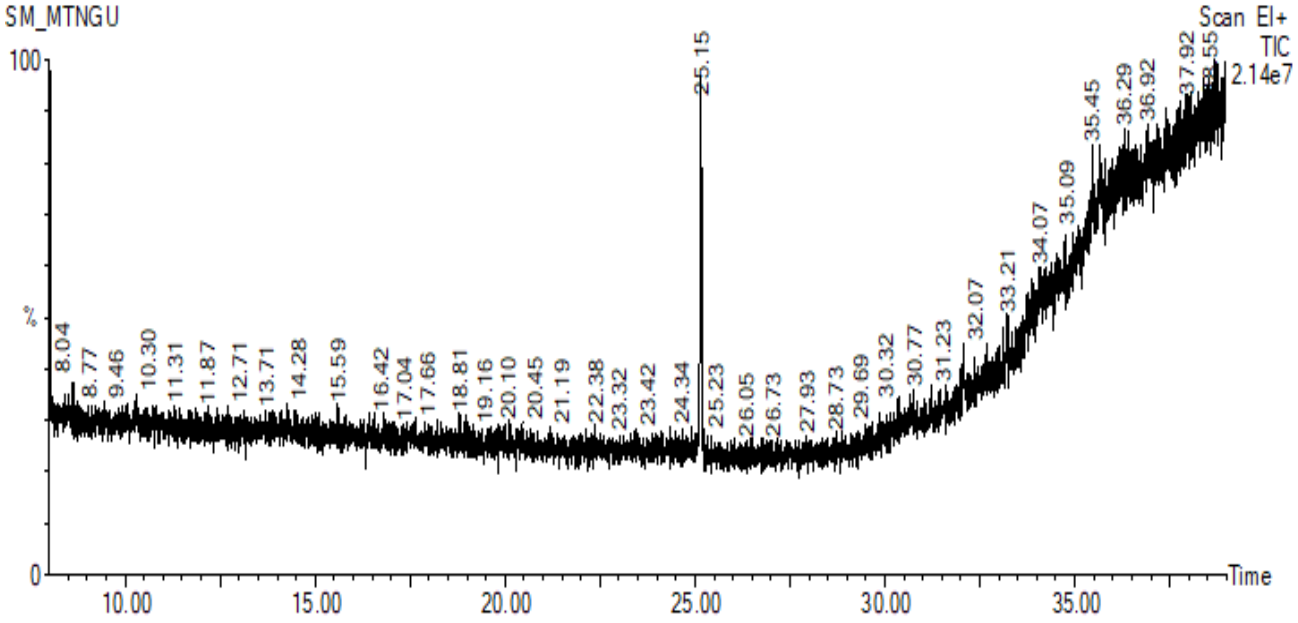
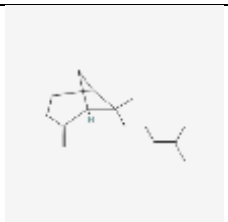
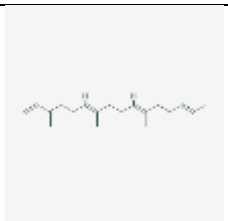
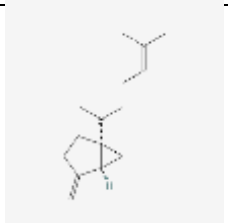


Figure27:- ***GCMS Chromatogram of crude metabolites of the extract SM_MTNGU 3429***

Figure28:- Spectrum of major identified componds in the crude metabolites of the extract SM_MTNGU 3429 at Retention time 25.150

Table-2: Major Compounds identified in the crude metabolites of the extract SM_MTNGU 3429 at Retention time 25.150 with their chemical structure and bioactivity.

Retention time	Compound name with chemical formula	Chemical Structure	Bioactivity	Reference

25.150	Bergamotene (1S,5S,6R)-6-METHYL-2-METHYLENE-6-(4-METHYLPENT-3-EN-1-YL)BICYCLO[3.1		Antioxidant, anti-inflammatory, immunosuppressive, cytotoxic, antimicrobial, antidiabetic, and insecticidal effects.	(Annaz et al., 2024)
	(E,E)-7,11,15-TRIMETHYL-3-METHYLENE-HEXADECA-1,6,10,14-TETRAENE		Antioxidant	(Das et al., 2022)
	(1S,5S)-4-METHYLENE-1-((R)-6-METHYLHEPT-5-EN-2-YL)BICYCLO[3.1.0]HEXANE		Cholesterol-Modulating	(Ongtanasup et al., 2022b)

***Shorea robusta* C.F.Gaertn antibacterial test: -** The crude extracts of *Shorea robusta* C.F.Gaertn exhibit differential antibacterial potential depending on the solvent system and the test organism (*Bacillus anthracis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*). The ethanolic extract emerged as the most consistently active fraction, showing appreciable inhibition across all bacterial strains, with values ranging from 15 ± 1 mm to 17.34 ± 0.95 mm, and the strongest response recorded against *B. anthracis*. In contrast, the methanolic extract demonstrated selective efficacy, with higher inhibition against *B. anthracis* (17 ± 0.82 mm) and *S. aureus* (14 ± 0 mm), but relatively weak action on *E. coli* (7 ± 0 mm). Extracts obtained using non-polar solvents (petroleum ether and hexane) displayed comparatively lower antibacterial activity. Petroleum ether extract was inactive against *E. coli* and showed modest inhibition (≈11–12 mm) against other strains. Similarly, hexane extract did not affect *E. coli* and exhibited only moderate zones of inhibition (≈10.67–11.67 mm) against the remaining organisms. The reference drug *Streptomycin* produced substantially larger inhibition zones, particularly against *E. coli* (24.33 ± 0.48 mm) and *S. aureus* (21.33 ± 0.58 mm), validating the assay sensitivity and highlighting the comparatively lower potency of the plant-derived extracts. The absence of inhibition in the DMSO control confirms that the observed antibacterial effects are attributable solely to the plant extracts. Ethanol appears to be the most effective solvent for extracting antibacterial constituents from *Shorea robusta* C.F.Gaertn, while activity against *E. coli* remains limited in most extract types except the ethanolic fraction. This plant exhibits significant antibacterial potential due to its rich phytochemical profile, including resveratrol oligomers, flavonoids, tannins, and triterpenes. Extracts, particularly methanolic and aqueous fractions, show broad-spectrum activity against pathogenic microbes such as *Staphylococcus aureus* and *Escherichia coli*. The antibacterial mechanisms involve membrane disruption, oxidative stress induction, and inhibition of key bacterial systems. These effects, along with its anti-inflammatory and wound-healing properties, enhance its therapeutic relevance. The methanolic extract of the resin of *Shorea robusta* C.F.Gaertn was reported to have significant antioxidant and antibacterial activities. It was tested for DPPH radical scavenging and FeCl3 reducing power assays disc diffusion antibacterial testing against Gram positive and Gram negative bacteria. The extract exhibited a significant dose-dependent antioxidant activity with the IC 50 value of 35.60 µg/mL, which was similar to ascorbic acid (31.91 µg/mL). Phytochemical screening confirmed the detection of triterpenoids, tannins and flavonoids which might be causing for the bioactivities observed. The *S. robusta* resin is a promising natural source of antioxidants and antibacterial agents and has potential implications in the management of oxidative stress related diseases like diabetes, cardiovascular disorders, cancer and aging (Vashisht et al., 2016).

Table3:-Antimicrobial activity of the *Shorea robusta* C.F.Gaertn crude extract against the test pathogens. Data are mean of three replicates (n=3) ; ±SD; Streptomycin (10µg/ml) was used as positive control; ~ indicates no zone of inhibition.

Extract	4453 (B. Anthracis)	2412 (E. coli)	2408 (S. aureus)	2080 (P. Aeroginosa)
Methanol	17±0.82	7±0	14±0	12±0.81
Ethanol	17.34±0.95	15.67±0.48	15±1	16±0.81
Petroleum Ether	11.67±0.5	~	11±0	12±0.81
Hexane	10.67±0.48	~	11.67±1.6	11.67±0.47
Streptomycin	10.67±0.48	24.33±0.48	21.33±0.58	19±0.47
Dmso	~	~	~	~

CONCLUSION

The present investigation showed that *Shorea robusta* C.F.Gaertn has a rich phytochemical composition and significant biological activity which is highly affected by the polarity of extraction solvents. Polar extracts (particularly ethanol and methanol) contained a higher amount of bioactive secondary metabolites (alkaloids, flavonoids, saponins, phytosterols and terpenoids) than the non-polar fractions. The GC–MS analysis also confirmed the occurrence of terpenoid-rich substance including bergamotene and related sesquiterpenes, which are related to antioxidant, antimicrobial and anti-inflammatory properties. The antimicrobial assay showed that the ethanol extract showed the highest and broad-spectrum inhibitory function on pathogens like *Bacillus anthracis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* while the methanol extract showed selective but significant activity. Whereas, hexane and petroleum ether extracts showed relatively weaker effects. This emphasizes how crucial solvent polarity is to the extraction process of active constituents. The research concludes that *Shorea robusta* C.F.Gaertn is a potential natural source of bioactive phytochemicals exhibiting remarkable antimicrobial activity. The combined action of terpenoids and other secondary metabolites can lead to the disruption of membranes and the inhibition of microbial growth. The present study scientifically validates the traditional medicinal use of S. robusta resin and supports its potential use in the development of natural antimicrobial agents and therapeutic formulations. Further studies on compound isolation, mechanistic evaluation and in vivo validation are recommended to fully explore its pharmacological potential.

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