

Innovations

GC-MS Analysis and Screening of Antimicrobial Potentialities of the Medicinal Plants *Diospyrossylvatica* Roxb. And *Diospyroschloroxylon* Roxb

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Abstract: *This study investigated the phytochemical composition and antimicrobial properties of *Diospyrossylvatica* and *Diospyroschloroxylon* using methanol, acetone, and ethyl acetate extracts. The methanol extracts demonstrated the highest antibacterial activity, with *D. sylvatica* showing an inhibition zone of 15.3 ± 0.57 mm against *Staphylococcus aureus* and 15 mm against *Pseudomonas fluorescens* at a concentration of 10 mg. In contrast, *D. chloroxylon* exhibited stronger activity against *S. aureus* with an inhibition zone of 16.3 ± 0.57 mm. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol extracts revealed 22 bioactive compounds in *D. sylvatica*, with benzoic acid (23.68%) and linoelaidic acid (20.57%) as dominant constituents. For *D. chloroxylon*, Reticuline (46.8%) was the most abundant compound. These findings indicate that both species hold significant therapeutic potential due to their antimicrobial efficacy and diverse bioactive compound profiles.*

Keywords: *Phytochemical components, GC-MS approach, antimicrobial effectiveness, pharmacological activities.*

Introduction:

The rise in human population in large cities, environmental degradation, and insufficient healthcare services have contributed to the increased transmission of infectious diseases [1]. Medicinal plants play a crucial role in pharmacology research, as many pharmaceutical companies depend on these plants for raw materials [2]. Plants are rich in secondary metabolites with notable biological functions, which has led to the traditional use of natural compounds, particularly from microbial sources, to produce antibiotics. However, with the increasing acceptance of herbal medicine as an alternative healthcare option, the screening of medicinal plants for active compounds has become essential. Many plant species

hold significant potential for therapeutic compounds that remain largely unexplored [3]. Phytochemicals, whether acting individually, additively, or synergistically, are effective in treating various diseases and are key to the development of new drugs and medicinal agents in the pharmaceutical industry.

The discovery of active compounds from natural sources is a critical step in drug development. Screening plant extracts is a modern approach for identifying bioactive compounds in various plant species. Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids exhibit diverse biological activities, including antioxidant, anti-inflammatory, anti-diarrheal, anti-ulcer, and anticancer effects [2]. The Gas Chromatography-Mass Spectrometry (GC-MS) technique is increasingly employed to analyze secondary metabolites in medicinal plants, making it a highly effective method for evaluating essential oils, alcohols, acids, esters, alkaloids, steroids, amino, and nitro compounds. The biotechnology industry is particularly interested in medicinal plants, with many pharmaceutical companies relying on plant components for the future development of pharmaceutical compounds [4].

The *Diospyros* species, part of the Ebenaceae family, consist of over 700 species primarily found in tropical regions. *Diospyros* species have been extensively used in traditional medicine to treat conditions like hyperglycemia and hypertension. Additionally, species like *Diospyros malabarica* have been reported to provide medicinal benefits, such as treating wounds and dysentery [5]. *Diospyros chloroxylon* are rich in proteins, carbohydrates, fats, fibers, and minerals highlighting that both its unripe and ripe fruits, along with seeds. The seeds, particularly, contain oleic and palmitic acids, and all extracts show strong antioxidant properties, suggesting potential for therapeutic and edible uses [6]. Literature confirms that *Diospyros* species are effective in treating microbial infections, hemorrhage, and hypertension due to their rich secondary metabolite content [7, 8]. The antifungal, antibacterial, and anti-inflammatory activities observed in multiple studies further emphasize the need to explore these species for therapeutic applications. This study aims to examine the phytochemical composition and antimicrobial activities of *Diospyros sylvatica* and *Diospyros chloroxylon* extracts.

Materials and Methods:

Collection and Identification of Plant material

Fresh bark samples of *Diospyros sylvatica* and *Diospyros chloroxylon* were collected from the Paderu Forest (18°10'10.79" N, 82°46'58.32" E) in Andhra Pradesh, India.

The plant material was identified and authenticated by the Department of Botany at Andhra University, Visakhapatnam. The specimens were deposited in the herbarium at the same department, with voucher numbers 25564 AUV and 25565 AUV assigned for future reference.

Extraction and Phytochemical Analysis

Plant parts were collected, washed with running water, and shade-dried at 32°C. The dried material was finely powdered using an electric blender and sieved with a 0.5 mm sieve to ensure uniform particle size. The powder was stored in sterile containers. Sequential extraction of the powdered material was carried out using methanol, acetone, and petroleum ether through the Soxhlet extraction method. The extracts were stored in sealed jars for 72 hours, and concentrated using a rotary evaporator at 45°C under reduced pressure. The resulting thick extracts were kept in a refrigerator at 4°C for future use. Preliminary phytochemical analysis was performed on each extract following standard protocols [9].

Determination of plant extract yield (%)

The yield of the plant extract was calculated using the following formula:

$$\text{Yield (\%)} = W1/W2 \times 100$$

Where:

- **W1** = Weight of the extract after solvent evaporation.
- **W2** = Dry weight of the plant sample.

Gas Chromatography-Mass Spectrometry (GCMS) analysis

The methanolic bark extracts of *Diospyrossylvatica* and *Diospyroschloroxylon* was undertaken through Gas Chromatography-Mass Spectrometry (GC-MS) at the Sophisticated Analytical Instrument Facility (SAIF) laboratories, IIT Madras, utilizing a standard GCMS model as delineated hereafter. GC-MS system comprising an Agilent 8890 Gas Chromatograph coupled with an Agilent 5977 Mass Selective Detector (MSD). The transportation of samples was facilitated by helium gas maintained at a flow rate of 1.2 ml/min. The injection volume of 1 µl occurred at an elevated temperature of 280 °C. The separation column, HP5, was characterized by dimensions of 30 m x 250 µm x 0.25 µm and exhibited a temperature gradient ranging from 75 °C to 360 °C. The comprehensive runtime for the gas chromatography procedure was established at 53.5 minutes. The ascertained phytochemical entities were characterized by comparing their mass spectrometry spectral patterns against the standard spectra cataloged in the NIST Mass Spectra

Database, specifically employing the licensed NIST 2017 Library, and analyzed using Open Lab CDS 2.5 software[10].

Microbial Strains

The microbial strains used for antimicrobial testing were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh. The bacteria were categorized as Gram-positive and Gram-negative. For Gram-positive bacteria, *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* (MTCC 497) were used, while *Pseudomonas fluorescens* (MTCC 664) and *Salmonella enterica* (MTCC 98) represented the Gram-negative bacteria.

Antimicrobial action

The agar well diffusion method was used to assess the antibacterial activity of plant extracts on Nutrient Broth (NB) agar media. A 100 µL bacterial culture was spread evenly on solidified media in Petri plates using a sterilized L-shaped rod. Wells, 5 mm in diameter, were created using a sterile cork borer and filled with different solvent extracts at three concentrations, as well as positive and negative controls (20 µL each). Plates were incubated at 37°C for 24 hours, after which the inhibition zones (mm) were measured. Each test was performed in triplicate, with streptomycin (30 µg/mL) as the positive control and DMSO (10%) as the negative control. A strict aseptic environment was maintained throughout[11].

Free radical scavenging activity in DPPH assay

The antioxidant potential of bark extracts from the species *Diospyrossylvatica* and *Diospyroschloroxylon* was assessed in terms of its capacity to scavenge free radicals using a reliable DPPH assay [12]. The reaction mixture, which contained 1 ml of the extract at different concentrations of 20, 40, 60, 80, 100, and 120 µg/mL and 3 ml of DPPH (0.2mM), was incubated for 30 minutes in the dark. A UV-visible spectrophotometer (Agilent) was used to detect the absorbance at 517 nm. The standard ascorbic acid's antioxidant capacity was measured using a similar procedure. Methanol was used as a blank. Three duplicates of each experiment were run. The percentage of inhibition of DPPH's capacity to scavenge free radicals was determined using the formula

$$\% \text{ inhibition of DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of the test sample}}{\text{The absorbance of the test sample}} \times 100$$

Results and Discussion

Solvent Extraction Efficiency for Soluble Compounds

The analysis of soluble extracts from *Diospyrossylvatica* and *Diospyroschloroxylon* reveals that methanol is the most effective solvent, yielding the

highest extraction percentages compared to acetone and ethyl acetate (Table 1). *D.sylvatica* produced a 15.2% extraction yield with methanol, followed by 10.5% with acetone and 7.6% with ethyl acetate, while *D. chloroxylon* yielded 13.2% with methanol, 8.28% with acetone, and 7% with ethyl acetate. This demonstrates methanol's superior ability to solubilize bioactive compounds from both species. The lower yields with acetone and ethyl acetate suggest that these solvents are less effective in dissolving the specific compounds present in these plants. Further research could focus on optimizing extraction methods and exploring additional solvents to maximize the yield of bioactive compounds for potential applications in pharmaceuticals and nutrition.

Table 1: percentages of the soluble compounds in various solvent extracts of *Diospyrossylvatica* and *Diospyroschloroxylon*

Name of the plant	Solvent extract	Weight if the powered material	Weight of the soluble extract	Percentage of the soluble extract
<i>Diospyrossylvatica</i>	Methanol	50gm	7.6	15.2
	Acetone	50gm	5.25	10.5
	Ethyl acetate	50gm	3.8	7.6
<i>Diospyroschloroxylon</i>	Methanol	50gm	6.6	13.2
	Acetone	50gm	4.14	8.28
	Ethyl acetate	50gm	3.5	7

Evaluation of Phytochemical compounds in *P. tetraphylla*

The qualitative phytochemical analysis of *D. sylvatica* and *D. chloroxylon* reveals both differences and similarities in their chemical compositions across various solvent extracts, indicating their medicinal potential (Table 2). *D. sylvatica* shows the presence of carbohydrates (in methanol and ethyl acetate), cardiac glycosides, phenols (in acetone and methanol), saponins, flavonoids (in methanol and ethyl acetate), quinones, and terpenoids, highlighting its diverse chemical profile and potential for cytotoxic and antimicrobial activities. In contrast, *D. chloroxylon* exhibits carbohydrates (in all extracts), proteins (in methanol), cardiac glycosides, phenols (in methanol and acetone), saponins, flavonoids (in all solvents), alkaloids (in acetone and methanol), quinones, and terpenoids, with a more consistent presence of carbohydrates and saponins across solvents, suggesting its broad therapeutic applicability. Both plants contain bioactive compounds such as flavonoids and saponins, known for their antioxidative and anti-inflammatory

properties, but *D. sylvatica* exhibits greater variability across solvents, while *D. chloroxylon* shows a stable profile, particularly in saponins and flavonoids.

Table 2: Preliminary qualitative phytochemical assay of various extracts of *Diospyrossylvatica* and *Diospyroschloroxylon*

Plant Constituents	<i>Diospyrossylvatica</i>			<i>Diospyroschloroxylon</i>		
	Methanol	Acetone	Ethyl acetate	Methanol	Acetone	Ethyl acetate
Carbohydrates	++		+	++		+
Proteins			+	+	+	
Glycoside						
Cardiac glycosides	+		+			+
Phenols		+	+	+		
Saponins	+		+	+	+	+
Flavanoids	+	+		+	+	+
alkaloids				+		
quinones	+	+	+		+	
terpenoids	+	+	+	+		
Anthocyanins				+		
Coumarins	+		+	+		+

The qualitative phytochemical analysis of *D. sylvatica* and *D. chloroxylon* reveals a rich variety of bioactive compounds, affirming their medicinal potential. Previous research on *Diospyros* species consistently highlights the presence of diverse phytochemicals, making them important for traditional and modern medicine [7, 8, 13, 14 and 15]. Comparatively, both *D. sylvatica* and *D. chloroxylon* display key phytochemicals like saponins and flavonoids. *D. sylvatica* contains notable amounts of cardiac glycosides and phenolic compounds, which are linked to cardiovascular benefits [16]. In contrast, *D. chloroxylon* exhibits a stronger presence of carbohydrates and saponins, known for their antimicrobial and immune-enhancing properties [17, 18]. Both species also show consistent flavonoid content, reinforcing their antioxidant potential. These findings suggest that both plants could serve as valuable sources for developing therapeutic agents, aligning with the broader research on *Diospyros* species.

Antibacterial Activity

The antibacterial activity of *D. silvatica* and *D. chloroxylon* extracts, including methanol, acetone, and ethyl acetate, was tested against four bacterial strains: *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas fluorescens*, and *Salmonella typhi*, using varying dosages of 10 mg, 5 mg, and 2.5 mg. A positive control, at a dosage of 2 mg, was also included for comparison, showing considerably higher antibacterial activity across all tested bacteria.

Antibacterial Activity of *Diospyrousilvatica*

The methanol extract of *D. silvatica* exhibited highest antibacterial activity against *S. aureus* and *P. fluorescens* with inhibition zones of 15.3 ± 0.57 mm and 15 ± 0 mm at 10 mg and 8.6 mm and 7.3 ± 0.57 at 5 mg, respectively while no activity was detected at the lowest dosage. When compared with the positive control, which produced a much larger zone of 37 mm, and 32 mm respectively the methanol extract was significantly less potent. In contrast, *S. mutans* showed the sensitivity against only methanol extract, with a zone of inhibition measuring 17 ± 1 mm at 10 mg. However, no inhibition was observed at lower concentrations. Though this result was notable, it was still less effective than the positive control, which recorded an inhibition zone of 24 mm. Lastly, for *S. typhi*, the methanol extract produced moderate inhibition, with zones of 12.6 ± 0.57 mm at 10 mg, 10.3 ± 0.57 mm at 5 mg, and 7.6 ± 0.57 mm at 2.5 mg, all of which were considerably lower than the positive control, which measured 38 mm.

The ethyl acetate extract showed no activity against *S. aureus*, *S. mutans*, or *P. fluorescens* at any concentration tested. However, against *S. typhi*, the extract exhibited moderate antibacterial activity, with an inhibition zone of 10.3 ± 0.57 mm at 10 mg and 8.3 ± 0.57 mm at 5 mg, though it was inactive at the lowest concentration. Even at its most effective, the ethyl acetate extract was considerably less potent than the positive control, which produced an inhibition zone of 38 mm for *S. typhi*. The acetone extract demonstrated the highest antibacterial activity against *S. aureus*, with an inhibition zone of 14.6 mm at 10 mg, followed by 11 mm at 5 mg and 8.6 ± 0.57 mm at 2.5 mg. Against *S. mutans* and *P. fluorescens* the acetone extract exhibited no antibacterial activity at any dosage. For *S. typhi*, the acetone extract showed moderate activity, with inhibition zones of 10.6 ± 0.57 mm at 10 mg, 9 ± 1 mm at 5 mg, and 8.6 ± 0.57 mm at 2.5 mg, all of which were lower than the positive control's inhibition zone of 38 mm (Table 3).

Table 3: Antibacterial activity of different extracts (Methanol, Acetone, and Ethyl acetate) of *D. silvatica* at the dosages of 10mg, 5mg and 2.5mg

Bacterial strains	Methanol extract			Ethyl acetate extract			Acetone extract			+ Ve
	10mg	5mg	2.5mg	10mg	5 mg	2.5mg	10m	5 mg	2.5m	2 mg
<i>S. aureus</i>	15.3±0.57	8.6±0.57	-	-	-	-	14.6±0.57	11±0	8.6±0.57	37±1.12
<i>S. mutans</i>	17±1	-	-	-	-	-	-	-	-	24±1.24
<i>P. fluorescens</i>	15±0	7.3±0.57	-	-	-	-	-	-	-	32±1.62
<i>S. typhi</i>	12.6±0.57	10.3±0.57	7.6±0.57	10.3±0.57	8.3±0.57	-	10.6±0.57	9±1	8.6±0.57	38±1.38

Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

Antibacterial Activity of *Diospyros chloroxylon*

The methanol extract of *D. chloroxylon* displayed the highest antibacterial activity against *S. aureus*, with inhibition zones of 16.3±0.57 mm at 10 mg, 12±1 mm at 5 mg, and 10±1 mm at 2.5 mg. In contrast, the ethyl acetate and acetone extracts showed no activity against this strain. When compared with the positive control, which produced a zone of inhibition of 37±1.12 mm, the methanol extract was significantly less potent. Similarly, the methanol extract demonstrated notable activity against *S. mutans*, showing inhibition zones of 17±1 mm at 10 mg, 12.3±0.57 mm at 5 mg, and 9±1 mm at 2.5 mg. The ethyl acetate extract also showed some activity, with inhibition zones of 11.3±0.57 mm at 10 mg and 7±1 mm at 5 mg, but no activity at the lowest concentration. No inhibition was observed with the acetone extract. The positive control recorded a much larger zone of inhibition at 24±1.24 mm.

For *P. fluorescens*, the methanol extract exhibited moderate activity, with inhibition zones of 14±0 mm at 10 mg, 12.3±0.57 mm at 5 mg, and 10.3±0.57 mm at 2.5 mg. Neither the ethyl acetate nor the acetone extracts showed any activity against this strain. The positive control produced a zone of inhibition of 32±1.62 mm, indicating much greater potency compared to the methanol extract. Against *S. typhi*, the methanol extract displayed the highest antibacterial activity, with inhibition

zones of 23.6 ± 0.57 mm at 10 mg, 20 ± 1 mm at 5 mg, and 18.6 ± 0.57 mm at 2.5 mg. The ethyl acetate extract showed moderate activity at 10 mg, with an inhibition zone of 11.3 ± 0.57 mm, but was inactive at lower concentrations. The acetone extract produced inhibition zones of 24.6 ± 0.57 mm at 10 mg, 10 ± 1 mm at 5 mg, and 7.6 ± 0.57 mm at 2.5 mg, which were all lower than the positive control's inhibition zone of 38 ± 1.38 mm (Table 4).

Table 4: Antibacterial activity of different extracts (Methanol, Acetone, and Ethyl acetate) of *D. chloroxylon* at the dosages of 10mg, 5mg and 2.5mg

Bacterial strains	Methanol extract			Ethyl acetate extract			Acetone extract			+ Ve
	10mg	5mg	2.5mg	10mg	5 mg	2.5m g	10m g	5 mg	2.5m g	2 mg
<i>S. aureus</i>	16.3 ± 0.57	12 ± 1	10 ± 1	-	-	-	-	-	-	37 ± 1.12
<i>S. mutans</i>	17 ± 1	12.3 ± 0.57	9 ± 1	11.3 ± 0.57	7 ± 1	-	-	-	-	24 ± 1.24
<i>P. fluorescens</i>	14 ± 0	12.3 ± 0.57	10.3 ± 0.57	-	-	-	-	-	-	32 ± 1.62
<i>S. typhi</i>	23.6 ± 0.57	20 ± 1	18.6 ± 0.57	11.3 ± 0.57	-	-	24.6 ± 0.57	10 ± 1	7.6 ± 0.57	38 ± 1.38

Values represent mean \pm standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

Recent phytochemical studies have focused on the antibacterial properties of *D. sylvatica* and *D. chloroxylon*. The methanol extract of *D. sylvatica* exhibited significant antibacterial activity, with inhibition zones of 15.3 ± 0.57 mm against *S. aureus* and 15 mm against *P. fluorescens* at a concentration of 10 mg. In comparison, a study on *D. chloroxylon* demonstrated similar antibacterial effects against *S. aureus*, with particular emphasis on the variable efficacy based on the solvent used for extraction [19]. Methanol extracts from other *Diospyros* species, such as *D. malabarica*, displayed remarkable antibacterial efficacy, with inhibition zones of 30.25 mm against *S. aureus*, which is significantly higher than that of *D. sylvatica* [5].

The ethyl acetate extract of *D. sylvatica*, however, showed no antibacterial activity against *S. aureus* and *S. mutans*, contrasting with studies of *D. mespiliformis*, which reported broad-spectrum antimicrobial activity against numerous Gram-positive and Gram-negative bacteria [20]. Compounds from *D. lotus*, specifically

dinaphthodiospyrol derivatives, have demonstrated significant antibacterial activity, with inhibition zones reaching up to 35 mm against various bacterial strains [21]. These results suggest that dinaphthodiospyrol compounds are highly effective antibacterial agents, in contrast to the more modest results observed for *D. sylvatica*. Similarly, *D. sylvatica*'s methanol extract produced an inhibition zone of 17 mm against *S. mutans*, while *D. ebenum* showed superior antibacterial efficacy across all tested bacterial strains [22].

The methanol extract of *D. chloroxylon* also demonstrated significant antibacterial activity against *S. aureus*, with inhibition zones of 16.3 ± 0.57 mm at a concentration of 10 mg. This aligns with previous findings that suggest methanol extracts from *Diospyros* species generally exhibit potent effects against a variety of pathogenic bacteria [23]. However, the inhibition was notably weaker compared to the positive control's inhibition zone of 37 ± 1.12 mm, as reported in several studies, which indicate that while *Diospyros* extracts can be effective, they often yield lower activity compared to conventional antibiotics [22]. The ethyl acetate extract showed moderate activity with inhibition zones of 11.3 ± 0.57 mm, contrasting with earlier findings on *D. melanoxylon*, which reported consistently higher concentration-dependent inhibition against similar pathogens [24].

Against *P. fluorescens*, the methanol extract of *D. chloroxylon* showed moderate activity, with inhibition zones of 14 mm at the highest concentration. This is consistent with earlier reports indicating varying degrees of effectiveness by different *Diospyros* species against this organism, with some extracts demonstrating significant inhibition, while others did not [21]. Notably, the ethyl acetate, methanol and acetone extracts lacked activity, reflecting findings from other *Diospyros* studies where solvent choice significantly influenced antibacterial properties [5, 25].

GC-MS chemical profiling

Methanol extracts have been selected for gas chromatography and mass spectrometry (GC-MS) evaluation to investigate the phytochemical composition since they demonstrated the greatest antibacterial and antioxidant activities when compared with the other solvent extracts. This work shows that a number of physiologically active chemicals are found in *D. sylvatica* and *D. chloroxylon* bark methanol extracts and also provides an in-depth knowledge of the phytochemical profile that might be used to develop plant-based medicines.

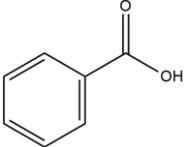
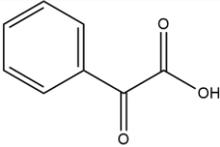
GC-MS chemical profiling of *D. sylvatica*

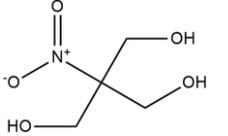
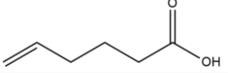
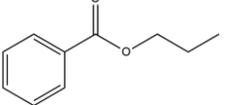
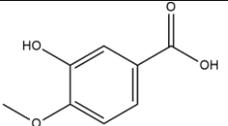
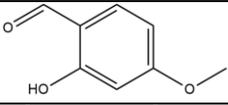
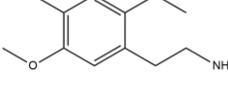
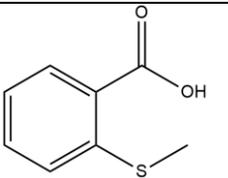
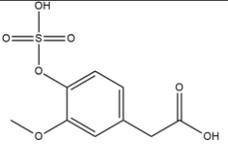
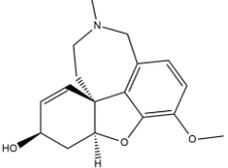
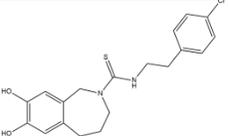
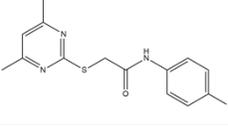
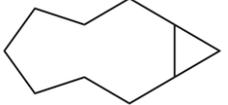
The methanol extracts' GC/MS spectrum data revealed multiple peaks (Figure 1) indicating the presence of 22 distinct chemicals with retention times ranging from 6.802 to 33.259. The GC-MS analysis of the methanol bark extract of *D. sylvatica* revealed a diverse range of compounds classified into various categories

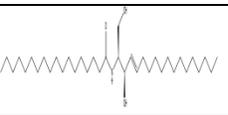
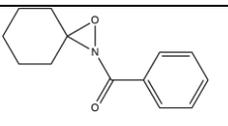
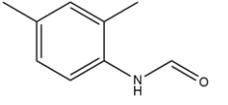
such as carboxylic acids, benzoates, alcohols, and amides. Notably, benzoic acid emerged as the most abundant compound, constituting 23.68% of the area percentage. Other significant constituents included linoelaidic acid and n-hexadecanoic acid, contributing significantly to the overall compound profile, showcasing the extract's rich chemical diversity. The GC-MS analysis identified a total of 22 compounds in the methanol bark extract of *D. sylvatica* (Table 5).

The identified compounds can be classified into several distinct categories. Under the class of carboxylic acids, notable compounds include benzoic acid, which comprises 23.68% of the total composition, followed by Benzoylformic acid at 1.53%, 5-Hexenoic acid at 1.96%, 3-Hydroxy-4-methoxybenzoic acid at 0.52%, and 2-Hydroxy-4-methoxybenzaldehyde at 0.44%. Linoelaidic acid and n-Hexadecanoic acid are also prominent, accounting for 20.57% and 12.46%, respectively, while Octadecanoic acid represents 1.57%. In the category of Esters and Benzoates, n-Propyl benzoate makes up 1.92% of the total composition. Amino acids and derivatives include compounds like Homovanillic acid sulfate, contributing 0.42%, and Capsazepine, at 1.83%. Among the alcohols, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro is a significant compound, accounting for 7.47%. Other notable compounds from various classes include Bicyclo[7.1.0]decane at 0.48%, 2-Cyclopentene-1-tridecanoic acid at 14.27%, N-Stearoylsphingosine at 0.56%, and N-(2,4-Dimethylphenyl)formamide, contributing 0.41% to the composition. The analysis indicates that benzoic acid is the most prominent compound in the extract, with an area percentage of 23.68%. Following this, linoelaidic acid also stands out with a considerable area percentage of 20.57%, while n-hexadecanoic acid accounts for 12.46%. These compounds may play crucial roles in the therapeutic properties attributed to *Diospyrossylvatica*.

Table 5: Bioactive chemical profile of *D. sylvatica* bark methanol extract through GC-MS analysis

S. No	Compound name	Rt minutes	Structure	Molecular weight	Molecular formula	Area %
1	Benzoic acid	6.803		122.12	C ₇ H ₆ O ₂	23.68
2.	Benzoylformic acid	10.663		150.13	C ₈ H ₆ O ₃	1.53

3	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro	13.151		151.12	C ₄ H ₉ NO ₅	7.47
4.	5-Hexenoic acid	14.167		114.14	C ₆ H ₁₀ O ₂	1.96
5.	n-Propyl benzoate	15.860		164.2	C ₁₀ H ₁₂ O ₂	1.92
6.	3-Hydroxy-4-methoxybenzoic acid	16.104		168.15	C ₈ H ₈ O ₄	0.52
7.	2-Hydroxy-4-methoxybenzaldehyde	16.649		152.15	C ₈ H ₈ O ₃	0.44
8.	4-Chloro-2,5-Dimethoxyphenethylamine	17.274		215.67	C ₁₀ H ₁₄ ClNO ₂	1.13
9.	2-(Methylthio)benzoic acid	19.330		168.21	C ₈ H ₈ O ₂ S	0.28
10	Homovanillic acid sulfate	19.716		262.24	C ₉ H ₁₀ O ₇ S	0.42
11	Capsazepine	20.001		376.9	C ₁₉ H ₂₁ ClN ₂ O ₂ S	1.83
12	Galanthamine	21.558		287.35	C ₁₇ H ₂₁ NO ₃	0.67
13	2-((4,6-Dimethyl-2-pyrimidinyl)thio)-N-(4-iodophenyl)acetamide	21.864		399.25	C ₁₄ H ₁₄ IN ₃ OS	0.49
14	n-Hexadecanoic acid	25.371		256.42	C ₁₆ H ₃₂ O ₂	12.46
15	Bicyclo[7.1.0]decane	28.727		138.25	C ₁₀ H ₁₈	0.48

16	Linoelaidic acid	29.587		280.4	C ₁₈ H ₃₂ O ₂	20.57
17	9,12,15-Octadecatrienal	29.712		262.4	C ₁₈ H ₃₀ O	6.96
18	Octadecanoic acid	30.228		284.5	C ₁₈ H ₃₆ O ₂	1.57
19	N-Stearoylsphingosine	30.663		566.0	C ₃₆ H ₇₁ NO ₃	0.56
20	2-Cyclopentene-1-tridecanoic acid, (S)	31.371		280.4	C ₁₈ H ₃₂ O ₂	14.27
21	(1-Oxa-2-aza-spiro[2.5]oct-2-yl)-phenylmethanone	32.859		280.4	C ₁₈ H ₃₂ O ₂	0.38
22	N-(2,4-Dimethylphenyl)formamide	33.259		149.19	C ₉ H ₁₁ NO	0.41

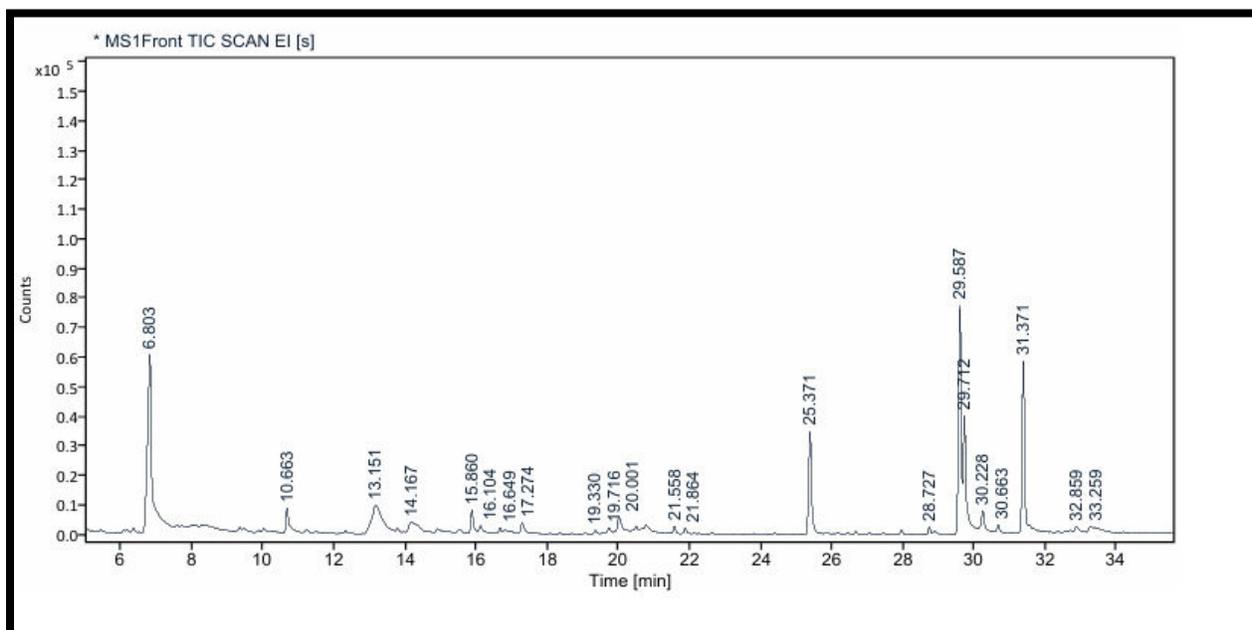


Figure 1: GC-MS Chromatogram of *D. sylvaticabark* methanol extract

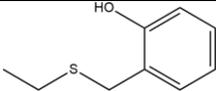
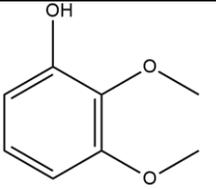
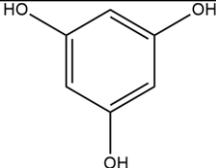
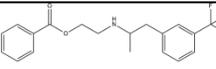
GC-MS chemical profiling of *D. chloroxylon*

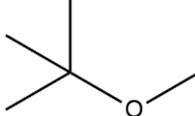
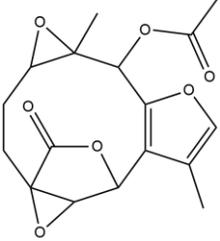
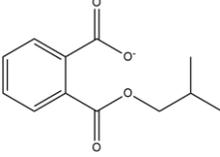
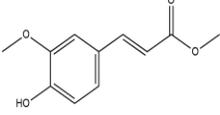
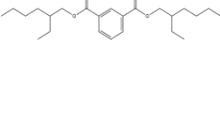
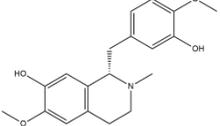
The GC-MS analysis of the methanol bark extract of *D. chloroxylon* reveals a diverse array of compounds, indicating significant phenolic content. A total of ten compounds were identified, with Reticuline emerging as the predominant compound, comprising 46.80% of the analyzed area (Figure 2). This is followed by

1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, which accounts for 27.26% of the total area. Additionally, Zeylanidin constitutes 6.45%, while other compounds such as 2,3-Dimethoxyphenol and Ferulic acid methyl ester contribute to the overall profile at 4.02% and 5.50%, respectively (Table 6). These findings highlight the extract's rich phytochemical profile, which may have implications for its bioactivity.

The identified compounds can be classified into several categories, with a notable emphasis on phenolic compounds. Specifically, 2,3-Dimethoxyphenol, Ferulic acid methyl ester, and Zeylanidin underscore the presence of diverse phenolic derivatives. Furthermore, compounds like Benfluorex and Reticuline indicate potential medicinal uses, adding to the complexity of the extract's chemical composition. The variety of functional groups observed among these compounds suggests that the bark extract may exhibit multiple biological activities, including antioxidant and antimicrobial properties. The significant presence of Reticuline indicates its potential role in the extract's overall bioactivity, making it a key compound of interest. Meanwhile, the high percentage of 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester suggests that it may also contribute substantially to the extract's medicinal properties.

Table 6: Bioactive chemical profile of *D. chloroxylon* bark methanol extract through GC-MS analysis

S. No	Compound name	Rt minutes	Structure	Molecular weight	Molecular formula	Area %
1	Phenol, 2-[(ethylthio)methyl]	5.005		225.31	C ₁₁ H ₁₅ NO ₂ S	3.99
2.	2,3-Dimethoxyphenol	7.329		154.16	C ₈ H ₁₀ O ₃	4.02
3	1,3,5-Benzenetriol	16.866		126.11	C ₆ H ₆ O ₃	2.57
4.	Benfluorex	21.598		351.4	C ₁₉ H ₂₀ F ₃ N ₂ O	0.62

5.	Propane, 2-methoxy-2-methyl	25.338		88.15	C ₅ H ₁₂ O	0.88
6.	Zeylanidin	34.443		334.3	C ₁₇ H ₁₈ O ₇	6.45
7.	Monoisobutyl phthalate	37.264		222.24	C ₁₂ H ₁₄ O ₄	1.91
8.	Ferulic acid methyl ester	39.039		208.21	C ₁₁ H ₁₂ O ₄	5.50
9.	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	40.264		390.6	C ₂₄ H ₃₈ O ₄	27.26
10	Reticuline	40.910		329.4	C ₁₉ H ₂₃ NO ₄	46.80

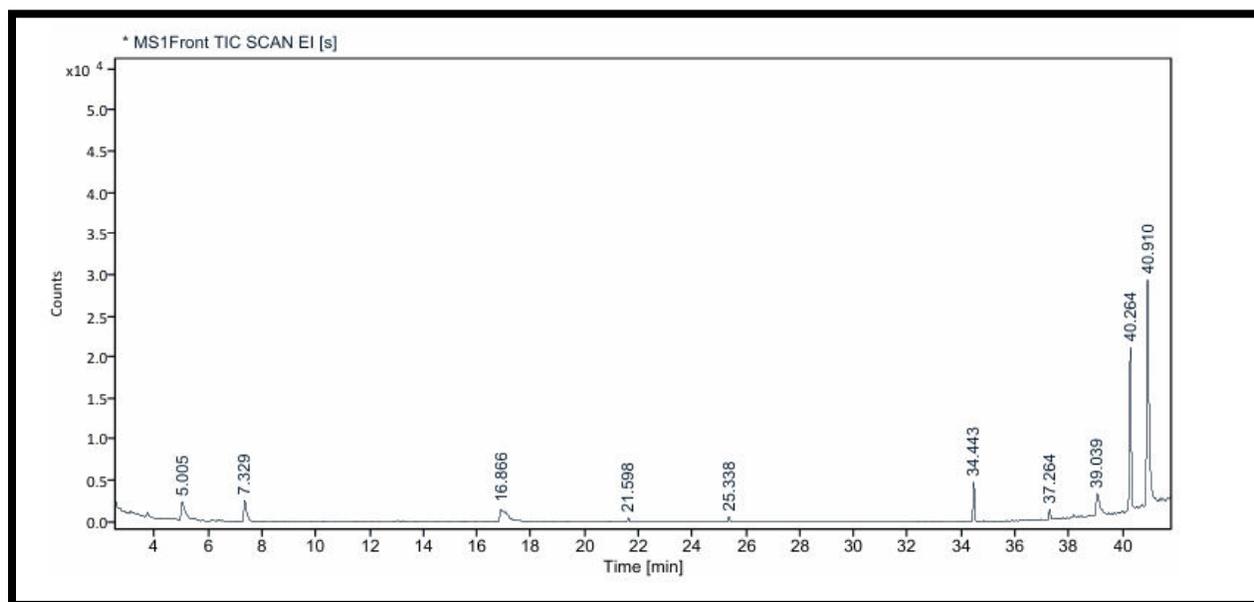


Figure 2: GC-MS Chromatogram of *D. chloroxylon* bark methanol extract

The GCMS analysis of *D. silvatica* has identified several biologically active compounds, with benzoic acid and n-hexadecanoic acid standing out due to their significant area percentages. Benzoic acid, which accounts for 23.68% of the compound profile, is well-regarded for its antimicrobial activity against molds and yeasts [26]. It is commonly used as a preservative in acidic foods, listed under the E-number E210, helping to extend shelf life by inhibiting microbial growth. Additionally, benzoic acid has anti-inflammatory properties, making it useful in topical treatments for skin irritation [27]. Meanwhile, n-hexadecanoic acid, also known as palmitic acid, comprises 12.46% of the compound profile. This fatty acid is known for its antioxidant, anti-inflammatory, and antimicrobial properties. Its inhibition of phospholipase A supports its use as an anti-inflammatory agent, and it has demonstrated effectiveness against bacterial strains such as *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumoniae* [28, 29].

Other notable compounds include linoelaidic acid, with an area percentage of 20.57%, a trans isomer of linoleic acid that promotes lipid accumulation in adipose tissues and exhibits anticancer properties, particularly against breast cancer cells [30, 31]. Additionally, 1,3-propanediol, 2-(hydroxymethyl)-2-nitro, which accounts for 7.47%, is recognized for its antimicrobial activity and is often used in disinfectants and industrial preservatives. However, concerns about the potential toxicity of this compound due to its decomposition into formaldehyde raise safety issues [32]. Together, these compounds contribute to the diverse biological activities and potential therapeutic applications of *D. silvatica*.

The methanol extract of *D. chloroxylon* contains a wide range of bioactive compounds that exhibit significant biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. Key among these is 2,3-Dimethoxyphenol, which has been studied for its antimicrobial effects and its ability to reduce DNA mutagenesis caused by reactive nitrogen species [33]. This compound, along with others from the phenolic group, shows enhanced antioxidant capacities, especially following enzymatic modifications [34]. Similarly, Reticuline, another prominent compound, displays analgesic and anti-inflammatory effects and serves as a precursor to various therapeutic agents, underscoring its importance in pharmacognosy [35]. Additionally, the compound 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester has demonstrated notable anticancer properties, particularly against prostate and colorectal cancers [36, 37].

The biological activities seen in *D. chloroxylon* mirror those documented in other *Diospyros* species, where phenolic compounds are recognized for their antimicrobial properties and antioxidant potential [38]. For instance, 2,3-Dimethoxyphenol's antioxidant activity aligns with studies of similar compounds found in other species [14]. The presence of compounds such as Benfluorex, which is

associated with weight management and hypolipidemic effects, adds to the pharmacological relevance of this species. Research suggests that these bioactive compounds have therapeutic potential, particularly in the management of metabolic disorders, as noted in studies on other *Diospyros* species [39].

D. silvatica and *D. chloroxylon* methanol extract shows considerable promise due to the diversity and potency of its bioactive compounds. These compounds offer potential applications in medicine, especially for antimicrobial, anticancer, and antioxidant therapies. The close similarities with other *Diospyros* species further highlight the potential of this genus in natural product development. Future research should continue to explore the underlying mechanisms of these biological activities and investigate their practical applications in therapeutic contexts.

Conclusion:

The study conclusively demonstrates that *Diospyros sylvatica* and *Diospyros chloroxylon* exhibit significant antimicrobial activity, particularly in their methanol extracts. The methanol extract of *D. sylvatica* showed the highest antibacterial efficacy against *Staphylococcus aureus* (15.3 ± 0.57 mm) and *Pseudomonas fluorescens* (15 mm) at a concentration of 10 mg. Similarly, *D. chloroxylon*'s methanol extract displayed notable inhibition against *S. aureus* (16.3 ± 0.57 mm) and *Salmonella typhi* (23.6 ± 0.57 mm), indicating its superior antimicrobial potential. GC-MS analysis identified 22 bioactive compounds in *D. sylvatica*, with benzoic acid (23.68%) and linoelaidic acid (20.57%) as major constituents, while *D. chloroxylon* contained Reticuline (46.8%) and 13-Benzenedicarboxylic acid bis(2-ethylhexyl) ester (27.26%). These findings highlight the potential of these species in the development of plant-based antimicrobial therapies, particularly given the broad-spectrum activity of their phytochemicals. Future research should focus on optimizing extraction methods and further exploring these bioactive compounds for pharmaceutical applications.

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