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Unravelling the phytochemical complexity of medicinal tree: Analytical approaches and quantitative determination of bark of *Symplocos cochinchinensis* Moore var. *laurina*

D. Suwethaasri*, S.Manivasakan*, K. Baranidharan*[◆], R. Ravi*, P. Hemalatha*, P. Radha***, R. Nandha Kumar* and S. Bargavi*

*Department of Forest Products and Wildlife, Forest College and Research Institute, Mettupalayam-641301, Coimbatore, Tamil Nadu, India

**Department of Forest Biology and Tree Improvement, Forest College and Research Institute, Mettupalayam-641301, Coimbatore, Tamil Nadu, India

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Abstract

This study investigated the antioxidant properties, GC-MS analysis, brine shrimp lethality, and preliminary phytochemical composition of the bark of *Symplocos cochinchinensis* Moore var. *laurina*. The bark was analysed to assess its free radical scavenging activity, toxicity levels, and medicinal potential. The findings revealed a high radical scavenging activity and low toxicity, highlighting the therapeutic potential of the bark. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, sterols, carbohydrates, cardiac glycosides, and volatile oils. These compounds endow the bark with diverse pharmacological properties, including antibacterial, antifungal, antioxidant, antidote, and central nervous system stimulant activities. The results underscore the medicinal significance of *S. cochinchinensis* bark, supporting its potential applications in pharmaceutical and therapeutic contexts.

1. Introduction

Traditional medicine relies heavily on medicinal plants and trees. Because of its widespread use, it also contributes significantly to the primary healthcare of several people. The tribal and rural inhabitants of India are primarily dependent on medicinal plants not only to address their healthcare needs but also for their livelihood. According to the World Health Organization, around 80% of the global populace relies on traditional medicine as their sole health care resource (Sri *et al.*, 2022). Thousands of plants are used in traditional medicine by different cultural groups worldwide. Most of these indigenous therapeutic plants have not yet been thoroughly investigated. Although, it is widely believed that plant medicines are non-toxic, it is recognized that most plant products induce neurotoxicity, reproductive toxicity, hepatotoxicity, *etc.* (Subramoniam, 2014). In developed countries, 25% of pharmaceuticals are based on plant medicine and plant derivatives (Senthilkumar *et al.*, 2013).

Alkaloids, terpenoids, saponins, phenols, coumarins, tannins, anthocyanins, sugars, quinones, glucosides, and more are examples

of secondary metabolites found in medicinal plants. Many researchers have been interested in these secondary metabolites because of their interesting bioactivities, such as antibacterial, antimicrobial, anti-inflammatory, and special structural antiviral, antifungal, and antitumour measures (Shastrakar *et al.*, 2023). These are the sources that can be isolated from the leaves, bark, fruits, roots, and seeds (Musini *et al.*, 2013). The pharmaceutical sector uses several secondary metabolites that are obtained from natural sources as medications (Panner Selvam *et al.*, 2024; Vasanthkumar *et al.*, 2024).

The scientific proof of bioactive chemicals existence and their therapeutic applications is aided by phytochemical analysis. Phytoconstituents in medicinal plants are primarily analysed directly using gas chromatography and mass spectrometry. It can be used for a wide range of applications. Due to the technique's shown value, GC-MS investigations have been used more and more in recent years for the analysis of medicinal plants. These days, natural antioxidants derived from plant sources are of particular interest Anjalam *et al.* (2016). Screening for natural antioxidants has received a lot of interest, and attempts have been made to find chemicals that are good substitutes for synthetic antioxidants (Srinivasarao *et al.*, 2015). There is greater evidence that phytochemicals with antioxidant qualities are linked to a decreased risk of mortality from a variety of illnesses (Rice-Evans, 2004; Dixon *et al.*, 2005). Indian medicinal plants have a long history of being used to cure a wide range of illnesses. Although, ethnomedicine is widespread in India, most of the plants have not been investigated for toxicity. To ensure user

Corresponding author: Dr. Baranidharan Krishnamurthy

Professor and Head, Department of Forest Products and Wildlife Forest, College and Research Institute, Mettupalayam-641301, Coimbatore, Tamil Nadu, India

E-mail: baranidharan.k@tnau.ac.in

Tel.: + 91-9894424686

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safety as the usage of these medicinal plants grows, experimental toxicity screening is essential (Otang *et al.*, 2017). Brine shrimp lethality tests are now frequently employed to evaluate the cytotoxic potential of bioactive substances. It is an initial screening for plant extract toxicity (Sara *et al.*, 2017). Medicinal plants are increasingly being studied for their antioxidant potential in decreasing free radical-induced tissue harm (Pourmorad *et al.*, 2006). Plant-based antioxidants are currently gaining popularity. More research suggests that phytochemicals with antioxidant capabilities are linked to many diseases and carry a lower chance of mortality (Rice-Evans, 2004; Dixon *et al.*, 2005).

In the last few years, people have become more interested in herbal and traditional medicines because of their natural origin and remarkable efficacy in the treatment of various ailments. This study focused on *Symplocos cochinchinensis* Moore var. *laurina*, also referred to as kabli-vetti or Lodh tree. It is a flowering plant from the sapphire berry family of Symplocaceae that grows in tropical and subtropical Asia. It is characterized by its elliptical, serrated leaves, fragrant white flowers, and distinctive blue-purple drupes. The tree can grow to a height of 35 metres. Locals periodically cut down trees in the wild for use as food, dye, beads, tannins, and wood. Their primary means of multiplication is by seeds. Traditionally, its bark and leaves have been used in Ayurveda and folk medicine for their astringent, anti-inflammatory, and antimicrobial properties, particularly in treating diarrhoea, dysentery, and oral ailments (Jain and Singh, 2010). The bark used in traditional medicines holds a significant commercial value that meets a growing global demand for natural health products, also used for curing diabetes, giddiness, and vomiting control (Vadivu and Lakshmi, 2008). The bark of *S. cochinchinensis* has been scientifically validated for its antidiabetic, antioxidant, and antilipidemic activities that contribute to the therapeutic properties. Ecologically, the plant supports biodiversity by serving as a larval host for butterflies and a food source for birds (Robinson *et al.*, 2010). Recent studies highlight its potential in antioxidant and hepatoprotective applications, though further pharmacological validation is needed (Rao *et al.*, 2017).



Figure 1: *S. cochinchinensis*.

2. Materials and Methods

The bark sample of *S. cochinchinensis* were collected from the Kollu hills inside the reserve forest area latitude 11°15'53.46"N and Longitude 78°20'44.90"E. As it is collected from the natural forest the approximate age of tree was nearly 20 years. Disease free fresh barks were collected in huge amounts from the field are washed under the running water to remove the dirt and dust of the plant parts (Figure 1 and 2). After cleaning it was shade dried for 2 weeks and crushed into powder for further analysis, like phytochemical screening, antioxidant assay, and Brine shrimp test. The methanol is used as an extract (Table 1). The collected plant was identified from the Botanical Survey of India (BSI) No.: BSI/SRC/5/23/2025-26/Tech/440.



Figure 2: Bark of *S. cochinchinensis*.

2.1 Antioxidant assay

2.1.1 Antioxidant (DPPH) assay

The scavenging ability of the natural antioxidants of the bark towards the stable free radical DPPH was measured by the method of Mensor *et al.* (2001).

Reagents

1. DPPH – 2,2-diphenyl-2-picryl hydrazyl hydrate (0.3mm in methanol)
2. Methanol

Procedure

The extracts (20 µl) were added to 0.5 ml of methanolic solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol, without the extracts, served as the positive control. After 30 min of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity (\%)} = 100 - [(AC - AS)/AC] \times 100$$

where

AC = control is the absorbance and

AS = sample is the absorbance of reaction mixture (in the presence of sample).

Table 1: Phytochemical analysis methodology

Test	Method	References
Alkaloid	Testing for alkaloids presence using Dragendroff's test and Mayer's reagent.	Morsy, 2014
Flavonoid	Flavonoids can be found using the Shinoda, Alkaline Reagent, Alkaline, and Lead Acetate tests.	Shaikh and Patil, 2020
Sterols	The Libermann test is used to identify sterols.	Banu and Cathrine, 2015
Terpenoids	Detection of terpenoids using Libermann test.	Yadav <i>et al.</i> 2011
Anthraquinones	Testing for anthraquinones presence <i>via</i> Bontrager's test and 10% aqueous sulphuric acid test.	Morsy, 2014
Anthocyanin	Identification of anthocyanins through HCL test.	Shaikh and Patil, 2020
Proteins	Detection of proteins using Ninhydrin test.	Yadav <i>et al.</i> (2011)
Phenolic compounds	Phenolic substances are identified using the ellagic acid, gelatin, and Fecl3 tests.	Shaikh and Patil, 2020
Quinones	Quinones are detected using the alcoholic KOH and Conc. HCl assays.	Shaikh and Patil, 2020
Carbohydrates	Testing for carbohydrates using Molisch's test.	Shaikh and Patil, 2020
Tannin	Detection of tannins presence through Braymer's test, Gelatin test, and 10% NaOH test.	Shaikh and Patil, 2020
Saponin	Identification of saponins <i>via</i> Froth test.	Morsy, 2014
Cardiac glycosides	Detection of cardiac glycosides presence through Baljet's test, Bromine water test, and Keller-Kiliani's test.	Shaikh and Patil, 2020
Glycosides	Glycosides are identified using the Aqueous NaOH test and Bontrager's test.	Shaikh and Patil, 2020
Lignin	Labat test is used for the lignin detection	Shaikh and Patil, 2020
Coumarins	The presence of coumarins is determined by the ethanol extract test.	Morsy, 2014
Volatile oils	The fluorescence test for volatile oil detection.	Shaikh and Patil, 2020

2.2 Brine shrimp test

Preparation of samples

The sample was taken as such for the study with the different volume 100, 250, 500, 1000, 1500 μ l is added to each beaker containing saline solution respectively.

Procedure

- 30 shrimps were introduced into the sample solution of various concentration.
- The movement of shrimp is monitored at intervals of 2, 4, 6, 24 h.
- Blank solution: 30 shrimps in Brine solution
- Positive control: Potassium dichromate (1mg/ml).
- The mortality of shrimp is calculated after 24 h.

$$\text{Death} = \frac{\text{Number of dead shrimps}}{\text{Number of dead shrimp} + \text{Number of live shrimps}} \times 100$$

For each of the sample, 30 shrimps were added to 25 ml of the solution. The mortality of the shrimps was monitored as that of blank and positive control (Oladimeji *et al.*, 2006).

2.3 GC-MS analysis

Gas chromatography and mass spectrometer analysis was carried out in the methanolic extract of bark by using Perkin elmer clarus

SQ8C. This instrument was set as follows, injector port temperature set to 220°C, interface temperature set as 250°C, source kept at 220°C. The oven temperature programmed as available, 75°C for 2 min, 150°C @ 10°C/min, up to 250°C @ 10°C/min. Split ratio was set to 1:12 and splitless injector mode was employed. The DB-5 MS capillary standard non polar column was used whose dimensions were 0.25 mm OD \times 0.25 μ m ID \times 30 meter length procured from Aligent Co., USA. As a carrier gas, helium was used at a rate of 1 ml/min. The range of the MS's scan was 50 to 600 Da. The source was maintained at 220°C and 4.5e-6 motor vacuum pressure. The ionization energy was - 70eV. Additionally, the MS had an internal pre-filter that reduced neutral particles. The data system has built-in libraries for searching and matching the spectrum. NIAT MS Search 2.2v contains more than five lakh references.

Identification of compounds

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST14). The spectrum of the unknown component was compared with the spectrum of the known component stored in the inbuilt library. The component was identified with the peak area % (Painuli *et al.*, 2016).

3. Results

3.1 Preliminary phytochemical analysis

The bark of *S. cochinchinensis* contains a variety of phytochemicals, including alkaloids, flavonoids, sterols, polysaccharides, cardiac glycosides, and volatile oils (Table 2).

Table 2: Preliminary phytochemical analysis of *S. cochinchinensis*

Phytochemical constituents	<i>S. cochinchinensis</i> bark
Alkaloids	Present
Flavonoids	Present
Sterols	Present
Anthraquinone	Absent
Phenolic Compounds	Absent
Carbohydrates	Present
Tannin	Absent
Cardiac Glycosides	Present
Glycosides	Absent
Volatile Oils	Present
Terpenoids	Absent
Anthocyanin	Absent
Proteins	Absent
Quinones	Absent
Saponin	Absent
Lignin	Absent
Coumarins	Absent

3.2 GC-MS analysis

3.2.1 Identification of bioactive compounds present in *S. cochinchinensis* bark extract by GC-MS analysis

The GC-MS study of *S. cochinchinensis* bark extract revealed the presence of thirty-two bioactive chemicals (Tables 3 and 4) (Figure 3).

Table 3: GC-MS analysis of *S. cochinchinensis* bark

S.No.	Rt	Area	Compound name	Molecular formula	Molecular weight
1.	3.018	1.618	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	C ₁₆ H ₂₈ O ₃	268.39 g/mol
2.	3.113	2.572	Picrotoxin	C ₃₀ H ₃₄ O ₁₃	602.6 g/mol
3.	3.379	3.036	Dihydroxyacetone	C ₃ H ₆ O ₃	90.08 g/mol
4.	3.764	0.566	(4-Methoxyphenyl)-[2-methyl-1-(2-morpholin-4-ylethyl) indol-3-yl] methanone	C ₂₃ H ₂₆ N ₂ O ₃	378.5 g/mol
5.	4.41	0.589	(2-Mercaptoethyl) guanidine	C ₃ H ₉ N ₃ S	119.19 g/mol
6.	6.905	0.414	DL-Arabinose	C ₅ H ₁₀ O ₅	150.13 g/mol
7.	7.05	0.833	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144.12 g/mol
8.	7.685	0.545	1-Dodecene	C ₁₂ H ₂₄	168.32 g/mol
9.	8.23	8.404	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11 g/mol
10.	8.551	0.521	Maltol	C ₆ H ₆ O ₃	126.11 g/mol
11.	9.501	0.353	Cyclohexanone, 2-(2-butynyl)-	C ₁₀ H ₁₄ O	150.22 g/mol
12.	10.071	0.382	Aristol-1(10)-en-9-yl isovalerate	C ₂₀ H ₃₂ O ₂	304.5 g/mol
13.	10.581	0.555	Cyclotetradecane	C ₁₄ H ₂₈	196.37 g/mol
14.	10.836	0.359	Benzaldehyde, 4-(methylthio)	C ₉ H ₇ F ₃ OS	220.21 g/mol
15.	11.402	6.301	Sucrose	C ₁₂ H ₂₂ O ₁₁	342.3 g/mol
16.	11.652	0.862	a-Guaiene	C ₁₅ H ₂₄	204.35 g/mol

17.	12.422	1.391	a-D-Glucopyranose, 1,6-anhydro-	C ₁₃ H ₁₆ O ₇ S	316.33 g/mol
18.	12.997	0.746	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32 g/mol
19.	13.407	0.689	trans-Calamenene	C ₁₅ H ₂₂	202.33 g/mol
20.	14.493	0.885	3-Deoxy-d-mannonic lactone	C ₆ H ₁₀ O ₅	162.14 g/mol
21.	14.633	0.441	9-Nonadecene	C ₁₉ H ₃₈	266.5 g/mol
22.	15.103	0.988	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	279.31 g/mol
23.	16.224	0.433	Ethanol, 2-(9,12-octadecadienyloxy)-,(Z, Z)-	C ₂₀ H ₃₈ O ₂	310.5 g/mol
24.	21.481	0.425	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270.5 g/mol
25.	22.111	1.01	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42 g/mol
26.	22.241	0.629	Diethyl phthalate	C ₁₂ H ₁₄ O ₄	222.24 g/mol
27.	23.797	13.965	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390.6 g/mol
28.	25.387	0.45	7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-, 2,4,7,10,11-pentaacetate	C ₃₀ H ₄₄ O ₁₁	580.7 g/mol
29.	26.182	0.357	Demecolcine	C ₂₁ H ₂₅ O ₅	371.4 g/mol
30.	27.293	3.613	1-Docosene	C ₂₂ H ₄₄	308.6 g/mol
31.	29.569	0.739	Hexadecanoic acid, 1-[[[(2- aminoethoxy)hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester	C ₃₇ H ₇₄ NO ₈ P	692 g/mol
32.	29.694	0.76	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9bis(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-7b hydroxy-1,1,6,8-tetramethyl-, [1aR-(1aá,1bá,4aá,7aá,7bá,8á,9á, 9aá)]-	C ₂₆ H ₃₄ O ₁₀	506.5 g/mol

Table 4: Bioactive compounds in *S. cochichinensis* bark extract

Compound name	Reported uses
Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	Antibacterial
n-Hexadecanoic acid	Antioxidant, antifungal
Dihydroxyacetone	Antifungal
Maltol	Antimicrobial
Picrotoxin	Central nervous system stimulant, antidote

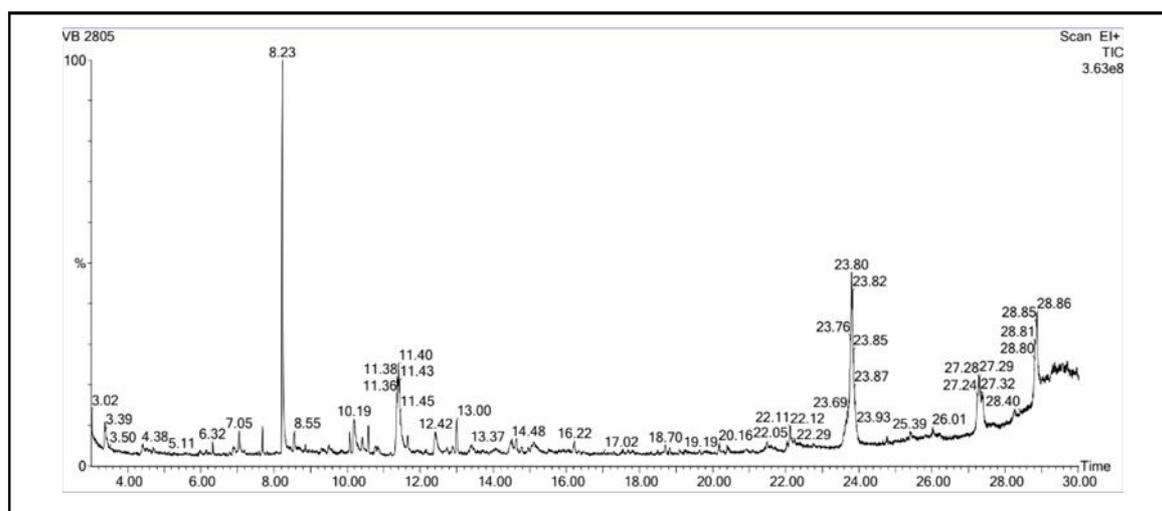


Figure 3: GC-MS analysis of *S. cochichinensis* bark.

3.3 Antioxidant assay

3.3.1 DPPH radical scavenging activity of *S. cochichinensis* bark (VB)

The ability of the bark extracts to scavenge DPPH was quantified using a spectrophotometric assay. The antioxidant assay was done to evaluate the free radical scavenging activity of medicinal plants against the standard ascorbic acid and the results revealed that the inhibition was tested at different concentration, viz., 10, 50, 100, 150, 200 and 250 µg/ml. The VB has maximum inhibition rate as 52.46 per cent when compared to standard ascorbic acid 45.88 per cent at 10 µg/ml followed by 50 µg/ml with the maximum VB inhibition

value of 62.30 per cent whereas, the standard had minimum inhibition of 57.38 per cent. The sample VB has the maximum inhibition of 72.13 per cent at 100 µg/ml when compared to the standard of 64.75 per cent which was followed by 150 µg/ml which had the maximum inhibition at VB 77.87 per cent when compared to standard 75.41 per cent. At 200 µg/ml the VB has the minimum inhibition of 78.69 per cent and the maximum was observed in ascorbic acid 90.16 per cent followed by 250 µg/ml and the VB sample has minimum inhibition 89.34 per cent compared to Ascorbic acid 92.62 per cent. The IC₅₀ value of VB was recorded as maximum (32.14 per cent) and the standard had 22.12 per cent were observed (Table 5) (Figure 4).

Table 5: DPPH radical scavenging activity of VB (*S. cochichinensis* bark)

Concentration (µg/ml)	VB	Standard (Ascorbic acid)
10	52.46 ± 0.64G ¹	45.88 ± 0.03C ¹
50	62.30 ± 1.91G ⁰	57.38 ± 1.56C ⁰
100	72.13 ± 1.72C ^α	64.75 ± 1.50C ^α
150	77.87 ± 0.05G ^{..}	75.41 ± 0.77C ^{..}
200	78.69 ± 2.46C ^{..}	90.16 ± 0.86G ^{..}
250	89.34 ± 1.88G ^{..}	92.62 ± 1.13C ^{..}
IC ₅₀ value (µg/ml)	32.14 ± 0.33	22.12 ± 0.57

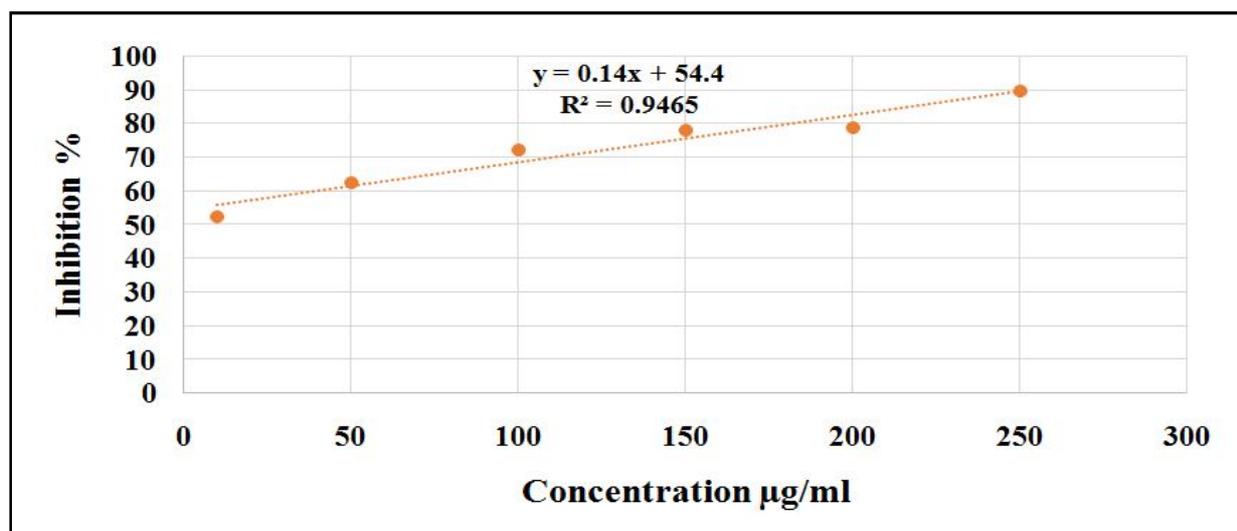


Figure 4: Antioxidant activity of *S. cochichinensis* bark extract.

3.4 Brine shrimp analysis

The brine shrimp lethality assay was used to determine the level of cytotoxicity contained in the selected medicinal tree. The *S. cochichinensis* (Bark) was tested for brine shrimp lethality assay at different concentration, viz., 100, 250, 500, 1000, 1500 µg/ml with control K₂Cr₂O₇ (Potassium dichromate) and was monitored for 24 h to check the mortality rate after 1, 2, 4, 6 and 24 h. Out of the total 30 brine shrimp taken for the analysis at 100 µg/ml, the minimum mortality of 33% was observed, (with 10 brine shrimps dead) after 24 h when compared to the control K₂Cr₂O₇ in which the maximum mortality 100% was noted as all the 30 brine shrimp were died even

at very low concentration. At 250 µg/ml, after 24 h the mortality rate was 47% in which 14 brine shrimp were dead, at 500 µg/ml the mortality rate was 70% in which 21 brine shrimp was dead totally. At 1000 µg/ml, the mortality rate was 90% in which 27 brine shrimp were died after 24 h and at 1500 µg/ml after 24 h the mortality rate was 100% in which all the 30 brine shrimp were dead. The results indicated that the bark of *S. cochichinensis* was comparatively less toxic than K₂Cr₂O₇ which showed a maximum lethality of shrimps at higher concentration. The LC₅₀ value for *S. cochichinensis* recorded was 285.84 µg/ml from the present study. The toxicity level of *S. cochichinensis* was found to be very less toxic (Table 6) (Figure 5 and 6).

Table 6: Brine shrimp lethality assay of *S. cochichinensis* (bark)

Sample name	Concentration (µg/ml)	(No. of shrimps dead) #					Mortality of brine shrimp (%)
		1 h	2 h	4 h	6 h	24 h	
<i>S. cochichinensis</i> (bark)	100	0.0	0.0	0.0	1.0	10.0	33.00 ^a
	250	0.0	0.0	1.0	1.0	14.0	47.00 ^b
	500	0.0	0.0	1.0	2.0	21.0	70.00 ^c
	1000	0.0	1.0	2.0	2.0	27.0	90.00 ^d
	1500	0.0	2.0	30.0	-	-	100.00 ^e
Control K ₂ Cr ₂ O ₇	1 (mg/ml)	30.0	-	-	-	-	100.00 ^e
						LC ₅₀	285.84 µg/ml
						S. Ed	0.84
						CD (=0.05)	1.76



Figure 5: Brine shrimp lethality assessment of *S. cochichinensis* bark.

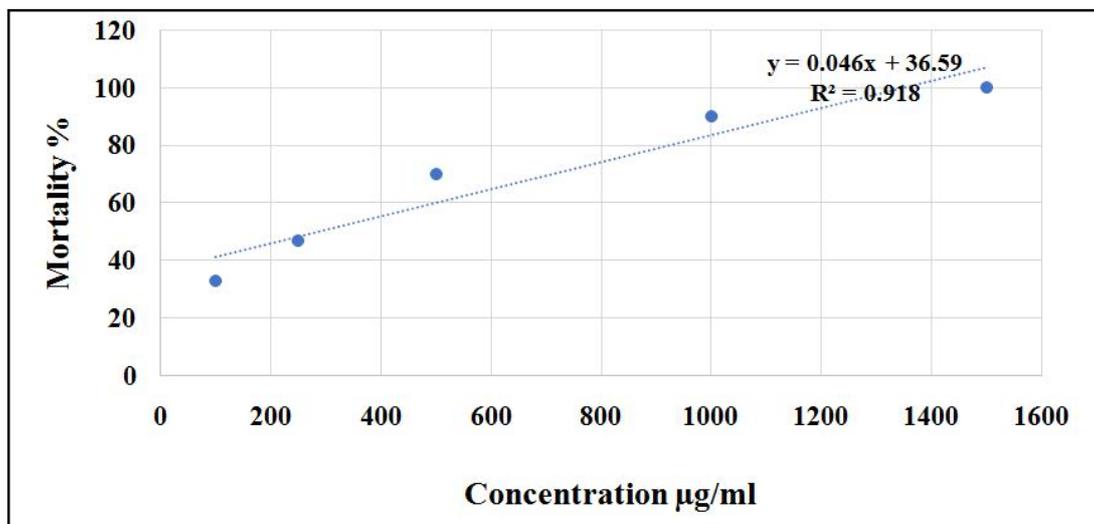


Figure 6: Brine shrimp lethality assay of *S. cochichinensis* bark extract.

4. Discussion

4.1 Phytochemical

The phytochemical compounds are well-known for their medicinal significance. Alkaloids, identified by Wadood *et al.* (2013), are recognized for their potent therapeutic activities, including analgesic and antibacterial effects (Eleazu *et al.*, 2012; Singh *et al.*, 2023). Flavonoids are plant-derived secondary metabolites that can act as reducing agents in chemical processes and bind to metals. Harborne, 2000 stated that flavonoids were the secondary metabolism of plants and that serves as the defence mechanism against predation by many microorganisms. Sterols, which have antibacterial properties (Epan *et al.*, 2007), contribute to plant medicinal value (Arora, 2013). Carbohydrates are one of the significant phytochemicals which had hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory and detoxifying effects (Zhang *et al.*, 2018; Chen *et al.*, 2019), were the uses of carbohydrates were consistently observed by (Victor and Chidi, 2009) in their phytochemical studies of various medicinal plants. The detection of cardiac glycosides, known stimulants in cardiac failure (Nandagopalan *et al.* 2016). Additionally, the presence of volatile oils, which possess strong antimicrobial activity (Akthar *et al.*, 2014; Sharma *et al.*, 2020), highlights the therapeutic properties of the bark extract. (Kalpana *et al.*, 2016) stated that findings of *S. cochinchinensis* bark supports that these components were utilized by the industry for several purposes, including cancer prevention, pesticide, antimicrobial, anti-inflammatory, and antioxidant. Therefore, this analysis implies that additional research can be conducted to assess the therapeutic potential of the ethanolic extracts of *S. cochinchinensis* bark for traditional medicinal applications.

4.2 GC-MS analysis

Major constituents including Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate, n-hexadecanoic acid, dihydroxyacetone, maltol, and picrotoxin. These chemicals have pharmacological effects that support the plant's traditional therapeutic uses. Specifically, Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate has demonstrated antibacterial effects (Balamurugan *et al.*, 2017), n-hexadecanoic acid is reported to have antioxidant and antifungal properties (Varsha *et al.*, 2015), dihydroxyacetone exhibits antifungal properties (Stopiglia *et al.*, 2011), maltol is recognized for its antimicrobial action (Saud *et al.*, 2019), and picrotoxin is known as a central nervous system stimulant and antidote (Straub *et al.*, 1994). These findings validate the ethnomedicinal relevance of the plant and suggest that further isolation and biological evaluation of these individual phytochemicals could provide significant therapeutic applications (Tables 6 and Figure 4).

4.3 Antioxidant assay

The DPPH radical scavenging experiment of *S. cochinchinensis* bark (VB) showed strong antioxidant activity, with inhibition percentages increasing with concentration. The VB extract showed higher scavenging activity than the standard ascorbic acid at lower concentrations (10 to 150 µg/ml), with the highest inhibition of 77.87% at 150 µg/ml compared to the standard's 75.41%. However, at higher concentrations (200 and 250 µg/ml), ascorbic acid exhibited greater inhibition (90.16% and 92.62%, respectively) than VB (78.69%

and 89.34%). The IC₅₀ value of VB was higher (32.14 µg/ml) than that of ascorbic acid (22.12 µg/ml), indicating that while VB has strong antioxidant potential, the standard remains more potent. VB's antioxidant action is linked to the inclusion of phytochemicals such as alkaloids and flavonoids, which are renowned for their free radical scavenging properties (Motaleb, 2005). These results suggest that *S. cochinchinensis* bark possesses promising antioxidant potential and could be explored further for applications in health and pharmaceutical sectors Ansari *et al.* (2019) (Table 5) (Figure 5).

4.4 Brine shrimp analysis

The methanolic bark extract of *S. cochinchinensis* showed a dose-dependent increase in cytotoxicity in brine shrimp, with mortality rates ranging from 33% at 100 µg/ml to 100% at 1500 µg/ml after 24 h of exposure. Tanamatayarat *et al.* (2016) classified the extract as "less toxic" based on its LC₅₀ value of 285.84 µg/ml. *S. cochinchinensis* was found to be significantly less toxic than the control K₂Cr₂O₇, which caused 100% mortality even at low concentrations. These findings suggest that while the extract has cytotoxic effects at higher concentrations, it is relatively safe and may be suitable for further pharmacological applications. The results align with previous studies (Sharmin *et al.*, 2018 and Asoso *et al.*, 2019) in *Aporosa wallichii* and *Calotropis procera* showing increased brine shrimp mortality with rising concentrations of plant extracts, reinforcing the utility of this assay in preliminary toxicity screening of medicinal plants (Table 6) (Figure 6).

5. Conclusion

The bark of *S. cochinchinensis* holds remarkable medicinal potential, supported by its diverse bioactive compounds and therapeutic properties. Studies reveal that it contains valuable phytochemicals such as alkaloids, flavonoids, sterols, cardiac glycosides, carbohydrates, and essential oils, which contribute to its traditional medicinal uses. Advanced GC-MS analysis identified 32 bioactive compounds, including n-hexadecanoic acid and Picrotoxin, recognized for their antimicrobial, antioxidant, and neuroactive effects. Tests evaluating the bark's antioxidant capacity, particularly through the DPPH radical scavenging method, showed that its effectiveness improves with higher concentrations, with significant activity even at lower doses likely due to its flavonoid and alkaloid content. Additionally, the brine shrimp lethality assay suggested that the extract has relatively low toxicity (LC₅₀ = 285.84 µg/ml), indicating its safety for further pharmacological research. These findings highlight the therapeutic promise of *S. cochinchinensis* bark, supporting its potential use in developing treatments for oxidative stress, microbial infections, and possibly other health disorders. Its rich phytochemical composition and demonstrated bioactivity reinforce its value in both traditional and modern medicine.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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