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## Qualitative phytochemical screening of leaf extract of *Gmelina philippensis* Cham and assessment of its antioxidant and antibacterial activity in different solvents

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### Abstract

The phytochemicals that are present in plants are responsible for their medicinal properties. These phytochemicals play an important role in the treatment of various diseases. The current paper is designed to investigate the bioactive compounds, antioxidant activity, and antimicrobial activity of *Gmelina philippensis* Cham leaf extract in various solvents. Standard methods were used to screen *Gmelina philippensis* leaf extract for the presence of carbohydrates, flavonoids, coumarins, tannins, terpenoids, saponins, steroids, alkaloids, and proteins in various organic solvents in the order of increasing polarity. The antioxidant activity of *Gmelina philippensis* Cham leaf extract in various solvents was tested using the DPPH assay. The two gram-negative and gram-positive bacteria were used for assessing the antimicrobial activity of leaf extract. So, this plant can be used to identify natural bioactive and novel compounds that could serve as a starting point for new pharmaceutical research activities.

**Keywords:** *Gmelina philippensis* Cham, phytochemical screening, FT-IR, antioxidant activity, antibacterial activity

### Introduction

More than 80% of people in the world consume natural remedies for various purposes<sup>[1]</sup>. Plant species have remained a reliable source as they have had low-cost, low-side-effect anti-infective medications. Many developing countries have enhanced efforts to gather traditional medicinal information and conduct scientific research on medicinal plants as they are used in the production of herbal remedies<sup>[2]</sup>. Plants are thought to contain bioactive chemicals that could be used to create novel "leads" to combat a variety of diseases<sup>[3-6]</sup>.

The plant chemical contents is required not only for discovery of medicinal agents but also for the finding new sources of economically viable phytochemical compounds for the synthesis of complex chemicals and for determining the true meaning of folk medicines. Because various phytochemicals have complementary and overlapping modes of action. Basic validation of herbal medicines is emerging as a new discipline of study that highlights and prioritizes the standardization of natural medicines and goods.

In India, *Gmelina* species are used for Medicinal purposes. *Gmelina arborea* is utilized for stomachic, laxative, and abdominal pain<sup>[7]</sup>. The leaf, root, and bark of *Gmelina arborea* have antioxidant activity<sup>[8, 9]</sup>, anti-inflammatory<sup>[10]</sup>, antimicrobial<sup>[11, 12]</sup> and. The root and leaves of *Gmelina asiatica* are used to treat jaundice and as expectorants and demulcents<sup>[13]</sup>. The fruits are combined with lime and applied externally to the throat like a cough medicine in Peninsular Malaysia, whereas the leaves and roots of *Gmelina philippensis* Cham were employed as a rheumatism remedy. The fruit juice of *Gmelina philippensis* Cham is used to treat foot eczema. The root juice is used to cure fatigue and as a purgative. Internally, the root extract is used as a stimulant, resolvent, and in the treatment of joint and nerve problems<sup>[14]</sup>. Methanolic leaf extract of *Gmelina philippensis* Cham has an antioxidant property.

*Gmelina philippensis* Cham commonly called as parrot's peak or badhara is a plant genus that belongs to the Lamiaceae family, which was previously known as Verbenaceae<sup>[15]</sup>. *Gmelina* is best recognized as a tree species which is currently considered a beautiful ornamental plant. It is a climbing shrub reaching upto 3 to 8 m in height with pendant branches. The leaves are about 5 to 7.5 cm in length and elliptic to obovate in shape, smooth surface having thickness of 3 to 4 cm. The inflorescence has yellow flowers that are mildly aromatic and grow from a hanging framework of overlapping bracts. The stem is monopodially branched and cylindrical in shape.

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The flowering takes place in April-July. The Fruits are fleshy, smooth, yellow, and ovoid to obovoid, drupe, and about 2 cm long and appear in June-August. This plant is inherent in the Philippine islands, S.E. Asia and India also distributed in Australia, United States, Malaysia, Vietnam, Thailand, Bangladesh Indonesia and Myanmar [16, 17].

Phytochemical screening is critical in discovering novel sources of medicinally and industrially useful chemicals with medicinal relevance to make the best and most sensible use of available natural resources [18-20]. *K. pneumoniae*, *E. Coli*, *S. aureus* and *S. faecalis* were used for testing the antimicrobial activity of plant extract. The radical scavenging activity of plant extracts was assessed by DPPH free radical assay. Production of aromatic substances is one of the speciality of this plant, most of which are oxygen and phenols substituted derivatives. Majority of them are secondary metabolites and around 12,000 among them already been isolated. A percentage of the total was anticipated to be less than 10%. Secondary metabolites such as this are important in plant defences [21].

According to the World Health Organization, traditional medicines, primarily plant remedies, are still used by 80 percent of the population in developing nations for basic health care. Antibacterial properties have been studied in hundreds of plant species, however the enormous majority have received little attention [22, 23]. Plant-based antimicrobials offer enormous therapeutic promise, are less toxic, environmentally friendly, and do not have the adverse effects that synthetic medicines have [24]. Antioxidants, on the other hand, have the capability to capture free radicals produced during normal metabolic processes and provide protection from oxidative stress. Antioxidants also slow the progression of a variety of chronic disorders. As a result of their therapeutic and physiological benefits, medicinal plants with antibacterial and antioxidant potential are gaining popularity [25].

As far as we know, no previous studies were reported on the phytochemical screening of leaf extract of *Gmelina philippensis* Cham and its antioxidant and antibacterial activity in different solvents. We were therefore interested in looking at the phytochemical screening, antioxidant activity, and antibacterial activity of *Gmelina philippensis* Cham leaf extract in various solvents.

## 2 Experimental

### 2.1 Chemicals and reagents

Ethanol, ethyl acetate, acetone, petroleum ether, n-hexane, double distilled water, lead acetate, sodium hydroxide, DPPH, HCl, sulphuric acid, nitric acid, chloroform, ferric chloride, benedict's reagents, wagner's reagent was used in this research.

### 2.2 Collection of Plant Materials

The healthy and fresh plant leaves (fig 1) were collected from the Botanical Garden of the Govt. Vidarbha Institute of Science and Humanities Amravati (M.S., India) during the rainy season. The collected plant leaves were cleaned and thoroughly washed with double distilled water before being dried for 15 days at room temperature. The dried leaves were crushed to a coarse powder and kept in an airtight container for future use.



Fig 1: Plant of *Gmelina philippensis* Cham.

### 2.3 Preparation of the *Gmelina Philippensis* Cham. Extract

A mixer grinder is used to powder the air-dried plant leaves. Using a Soxhlet extractor, 10 g of powdered leaves were extracted in increasing order of polarity of solvent. For extraction, 250 ml of each solvent (n-hexane, ethyl acetate, acetone, pet ether, ethanol and distilled water) was mixed with 10gm of powdered leaves and heated for 7 hours at about 50-60 °C. Following complete solvent evaporation, each of these solvent extracts was dried, weighed, and stored at room temperature in an airtight bottle until further analysis. Table 1 shows the colour and consistency of each extract.

Preliminary phytochemical screening was performed on all of these extracts in order to identify various chemical ingredients.

Table 1: Colour and consistency of each extracts

Sr. No	Extract prepared in different solvent	Colour	Consistency
1.	Aqueous/Water	Dark Brown	Dried
2.	Ethanol	Green	Dried
3.	Ethyl Acetate	Dark Green	Sticky
4.	Acetone	Light pink	Dried
5.	Pet. Ether	Green	Dried
6.	n-Hexane	Green	Dried

### 2.4 Preliminary screening of secondary metabolites

To observe common phyto-constituents, a qualitative phytochemical analysis of the *Gmelina philippensis* Cham was done. The extract was performed using standard experimental procedures. Alkaloids were analyzed using Wagner's reagents, while flavonoids were analyzed using the Shinoda alkaline reagent. Similarly, lead acetate and alkaline reagent were used to test phenolic compounds. Furthermore, the presence of saponins was determined using a foam test, and tannins were determined using a gelatin test.

### 2.5 DPPH Assay for antioxidant Activity

The leaf extract's free radical scavenging activity of *Gmelina philippensis* Cham in aqueous, ethanol, ethyl acetate, and petroleum ether extracts were investigated using the 1-diphenyl-2-picrylhydrazyl. To make a stock solution, 1.083 mg of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was dissolved in 10 ml of ethanol. The stock solution (100 g/ml) for each extract was made by dissolving 1 ml of the extract in 10 ml of ethanol. Similarly, 10 mg of ascorbic acid was dissolved in 10 mL of ethanol to produce a stock solution of standard ascorbic

acid (1 mg/ml). The absorbance of blank solution (5 ml ethanol + 1 ml of DPPH solution) was recorded at 420 nm. Similarly, the absorbance of each extract and comparative standard ascorbic acid were taken at 420 nm were recorded. Since the color of each extract after the addition of DPPH was interfering with the absorbance, to remove the error subtract the absorbance value before and after the addition of DPPH.

### 2.5 Antimicrobial Activity

The antimicrobial activity of leaf extract of *Gmelina philippensis* Cham in aqueous, ethanol, ethyl acetate and petroleum ether extracts were investigated against gram-positive and gram-negative pathogens by using disc diffusion method. *E. coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus faecalis* were used for evaluating the antibacterial activity.

### 2.6 FT-IR

The functional groups that are present in the leaf of *Gmelina philippensis* Cham was determined using the fourier transform infrared spectroscopy technique in which ethanol was used as a solvent along with water extract. Shimadzu IR Prestige<sup>21</sup> FTIR instrument was used to record the FTIR spectra of the samples. The spectrum was captured in the 500–4000 cm<sup>-1</sup> wavelength region [26].

## 3 Result and Discussion

The results thus obtained for phytochemical screening, functional group, antioxidant, and antibacterial activity of leaf extract of *Gmelina philippensis* Cham were discussed as follows:-

### 3.1 Phytochemical Screening of Secondary metabolites

The phytochemical screening of leaf extract of *Gmelina philippensis* Cham were carried out in different solvent in increasing polarity using the standard procedures as described

by Harborne [27], Trease and Evans [28]. The results of these tests were indicated as positive (+) or negative (-) as shown in Table 2.

The carbohydrates, coumarin, tannins, terpenoids and protein were found only in the aqueous and ethanolic extract whereas Saponins were found in aqueous extract only. Aqueous and ethyl acetate extracts showed a favourable reactivity to flavonoids. Steroids were detected in acetone, pet ether and n-hexane. Alkaloids or glycosides were not found in any of the extracts. In aqueous extract total 7 bioactive molecules are present as water is universal solvent it shows maximum positive results and compare to other solvent extract except aqueous ethanol extract shows maximum bioactive components so from this bioactive molecules can easily be isolated. The visual observation after adding certain reagents are shown in fig.2.

The flavonoids have a number of biological activities which include antimicrobial, anticancer, antiallergic, and antitumor properties. Flavonoids are also excellent water-soluble antioxidants and free radical scavengers that protect the cells from oxidative damage while simultaneously having strong anticancer activity [29, 30]. Believes that flavonoid present in plant leaves are an essential part of a balance. Furthermore [31], stated that saponins has the property to coagulate the red blood cells and to bind the cholesterol. According to [32], these properties confer high medicinal activity on *Gmelina philippensis* Cham extract. Another important metabolite and active compound in *Gmelina philippensis* Cham is tannins that may be responsible for antifungal which has astringent and detergent properties [33, 34]. The terpenoids present in the leaf extract of *Gmelina philippensis* Cham supports its use in the treatment of cancer, ulcer and malaria [35].

### Phytochemical Tests Carbohydrate

**Table 2:** Phytochemical screening of leaf extract of *Gmelina philippensis* Cham. in various solvent

Secondary Metabolites	Leaf extract of <i>Gmelina philippensis</i> Cham. in different solvents					
	n-Hexane	Petroleum Ether	Acetone	Ethyl Acetate	Ethanol	Aqueous
Flavonoids	-	-	-	+	+	+
Carbohydrates	-	-	-	-	+	+
Tannins	-	-	-	-	+	+
Coumarins	-	-	-	-	+	+
Saponin	-	-	-	-	-	+
Steroids	+	+	+	-	-	-
Terpenoids	-	-	-	-	+	+
Amino Acids	-	-	-	+	+	+
Glycosides	-	-	-	-	-	-
Protein	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-

### Benedicts Test

5 ml of Benedict's reagent was added to 1 ml of each extract, then boiled in a water bath for 5-7 min. The presence of red precipitate indicated the presence of carbohydrates.

### Flavonoids

#### Lead Acetate Test

To 2-3 ml of leaf extract, a 10% lead acetate solution was added. The appearance of a yellow precipitate indicated the presence of flavonoids.

### Alkaline reagent test

When a few drops of sodium hydroxide were added to 2ml of extract, a yellowish-orange colour appeared, which turned colourless when dil. HCL was added, indicating the presence of flavonoids.

### Coumarins

To the 2ml of each plant extract 1-2ml of 10% sodium hydroxide was added. The presence of coumarins was indicated by the yellow colour.

### Tannins

To each extract, 2-4 drops of 0.1% FeCl<sub>3</sub> solution were added. The presence of tannins was indicated by the appearance of a brownish-green colour.

### Terpenoids

2 ml of chloroform was added to 5 ml of each extract along with 3 ml of concentrated sulphuric acid. The appearance of reddish-brown colour indicated the presence of terpenoids.

### Saponins

#### Foam Test

Each extract was shaken vigorously for 5-10 min. appearance of foam indicated the presence of saponins.

### Steroids

#### Salkowski Test

To the 1 ml of each leaf extract equal volume of chloroform and few drops of conc. Sulphuric acid was added appearance of brown ring indicates the presence of steroids.

### Amino Acids

1 ml of concentrated nitric acid was added to 3 ml of each extract and heated for 3 minutes. 0.5 ml of sodium hydroxide was added after cooling. The presence of aromatic amino acids was indicated by the reddish-orange colour.

### 3.2 FT-IR

The functional groups of active components found in the aqueous and ethanol leaf extract of *Gmelina philippensis* Cham. were identified using FT-IR spectra.

#### Aqueous Extract

The broad absorption peak attributed at 3387.00 cm<sup>-1</sup> was due to the –NH amide stretching of CH<sub>2</sub>, while the C–H stretching of CH<sub>2</sub> produced a moderate absorption band at 2924.09 cm-

1. The medium broad peak at 1602.86 cm<sup>-1</sup> was due to C=C stretching. The peak at 1452 and 1415.70 cm<sup>-1</sup> have been attributed due to asymmetric bending of methyl group of the proteins. The absorption peak at 1114.86 cm<sup>-1</sup> was due to the vibrations of C–O–C whereas the peak appeared at 910.40 cm<sup>-1</sup> denoting the vibration of C–O–H [27, 36, 37] as shown in Fig.3

#### Ethanolic Extract

The ethanolic extract of *Gmelina philippensis* Cham. depicted characteristic absorption peak at 3387 cm<sup>-1</sup> was due to the –NH amide stretching of CH<sub>2</sub>, while the C–H stretching of CH<sub>2</sub> produced a moderate absorption band at 2922.09 cm<sup>-1</sup>, The medium broad peak at 1600.92 cm<sup>-1</sup> was due to C=C stretching, The peak at 1454 cm<sup>-1</sup> have been attributed due to asymmetric bending of methyl group of the proteins, The peak appeared at 1109.07 was due to presence of carbohydrate as shown in fig.4. We observed the maximum number of positive secondary metabolites in aqueous and ethanolic extract. As a result, we have used only aqueous and ethanolic extract for FT- IR spectra [38].

### 3.3 Antioxidant Activity

A DPPH scavenging assay was used to analyze the antioxidant activity of leaf extracts in aqueous, ethanol, ethyl acetate, and petroleum ether extracts. The aqueous extract in this study had the highest antioxidant activity of 76.48 percent, 54.07 percent for ethanolic extract, 51.66 percent for ethyl acetate, mentioned in table 3 which could be responsible for increasing antioxidant activity [39, 40].

### 3.4 Antimicrobial Activity

The antibacterial properties of leaf extract of *Gmelina Philippensis* Cham. were investigated in water, ethyl acetate, and petroleum ether against two

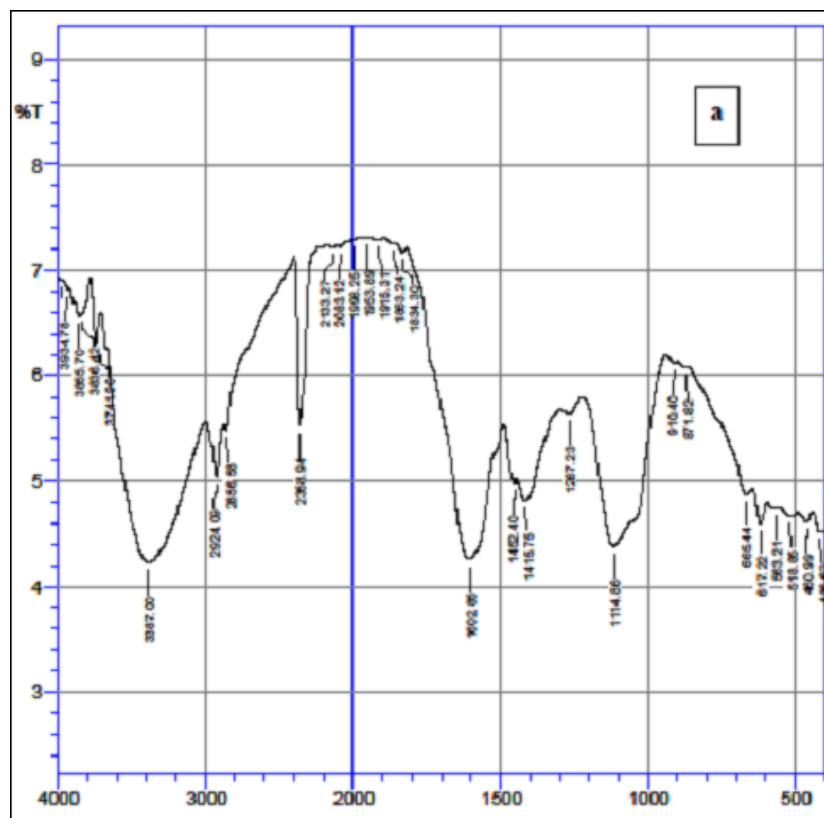
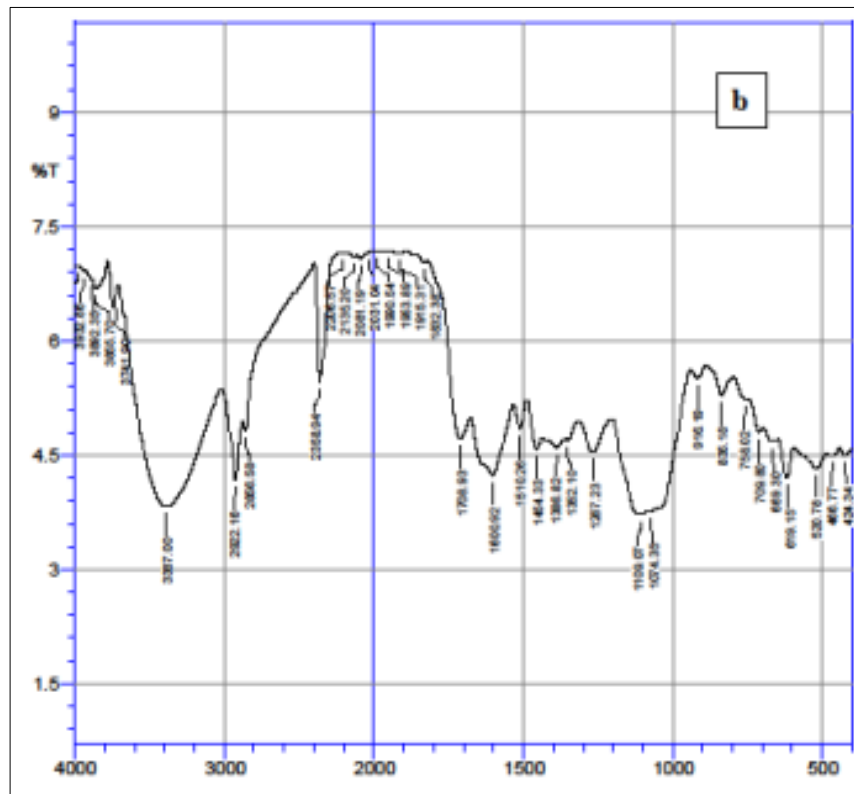


Fig 2: FTIR Spectra of aqueous extract of *Gmelina philippensis* Cham.



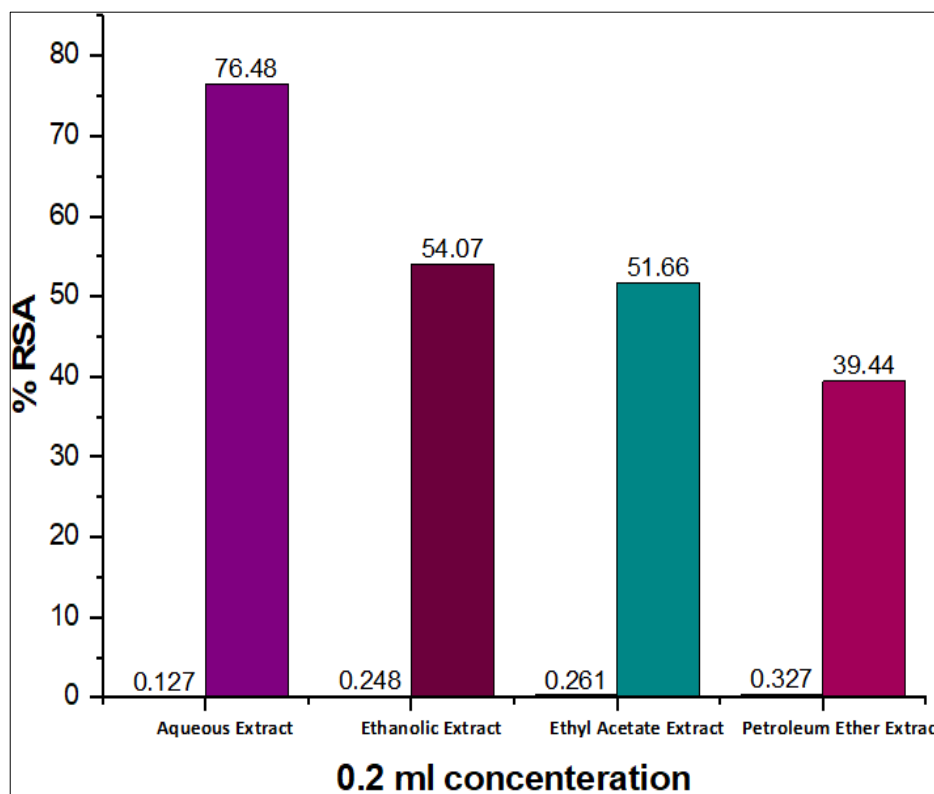


**Fig 3:** FTIR Spectra of ethanolic extract of *Gmelina philippensis* Cham.

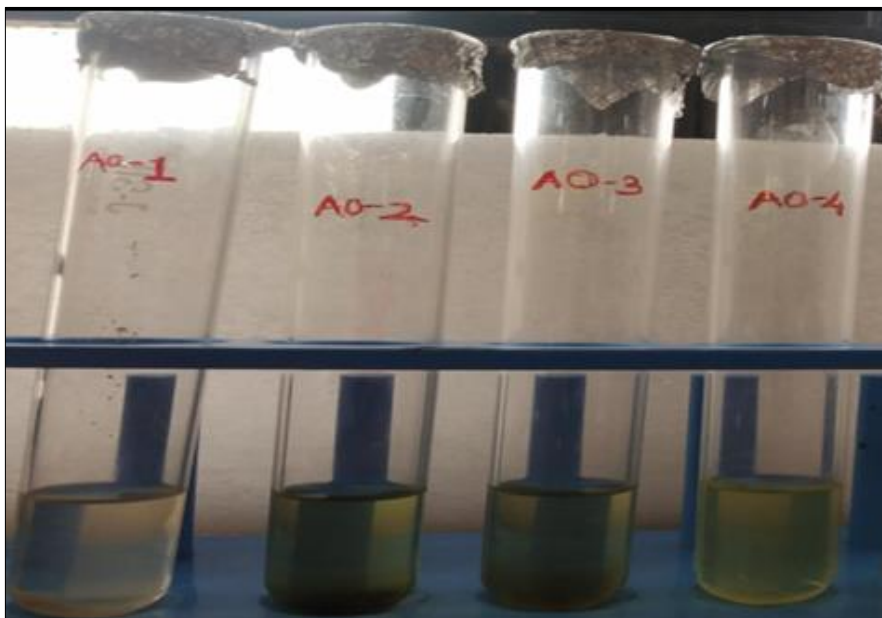
Gram-negative and two Gram-positive pathogens. The presence or absence of inhibitory zones was used to determine the antibacterial potency.

The results revealed that the poor inhibition activity was shown by aqueous extract against all bacterial cultures. Whereas ethanolic extracts have antibacterial inhibition

activity against *S. faecalis* only. Whereas extract of petroleum ether showed antibacterial activity against *K. pneumoniae*, *E. coli*, and *S. faecalis* as shown in table 4. The previous work reported the antimicrobial activity of *G. philippensis* Cham against methanolic extract only<sup>[41]</sup>.



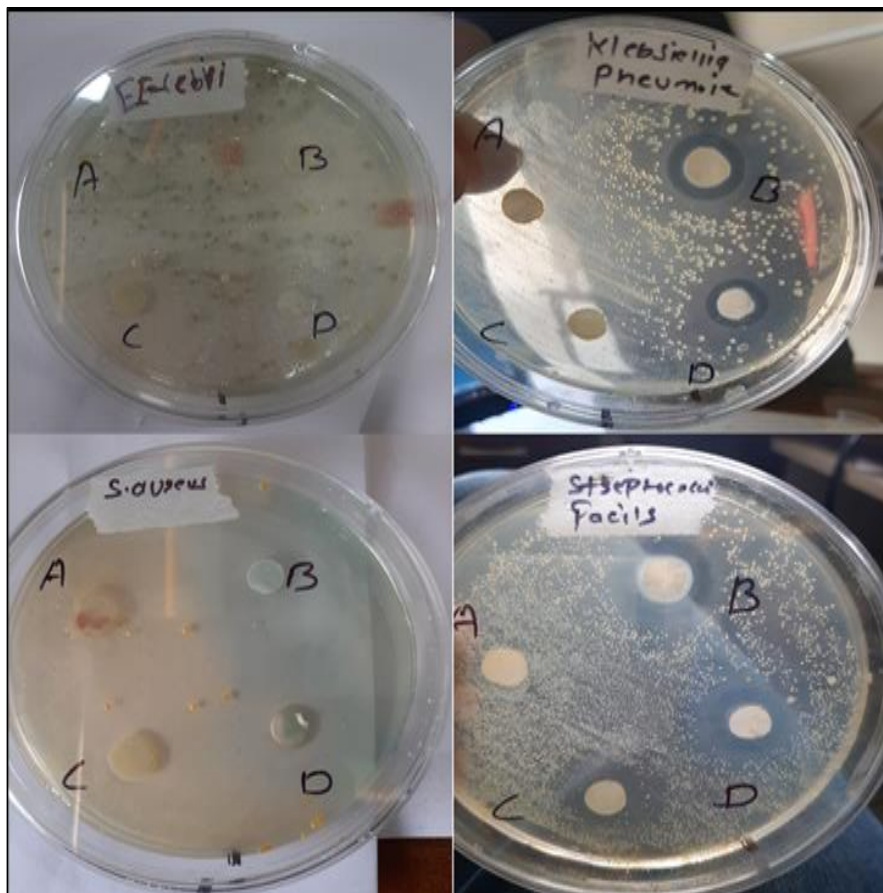
**Fig 4:** % Radical scavenging activity of leaf extract of *G. philippensis* Cham. in different solvents



**Fig 5:** Color solution of each leaf extract after addition of DPPH (a) Aqueous extract (b) ethanol extract (c) Ethyl acetate extract (d) Pet-ether extract by DPPH radical scavenging

**Table 3:** Absorbance and percentage radical scavenging activity of *G. philippensis* Cham. leaf extract in various solvents

Extract	Conc	Absorbance	% RSA	Control
Aqueous	0.2ml	0.127	76.48	0.54
Ethanol	0.2ml	0.248	54.07	0.54
Ethyl Acetate	0.2ml	0.261	51.66	0.54
Pet.ether	0.2ml	0.327	39.44	0.54



**Fig 6:** Inhibition zone of leaf extract of *Gmelina philippensis* Cham. in a) aqueous extract b) Ethanol extract c) Ethyl acetate extract and d) pet-ether extract against *E. coli*, *K. pneumoniae*, *S. aureus*, *S. faecalis*

**Table 4:** Inhibition zone of leaf extract of *Gmelina philippensis* Cham. in a) aqueous extract b) Ethanol extract c) Ethyl acetate extract and d) pet-ether extract against *E. coli*, *K. pneumoniae*, *S. aureus*, *S. faecalis*

Pathogens Extract	<i>E. Coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>S. faecalis</i>
Aqueous	---	---	---	---
Ethanol	17 mm	8 mm	4 mm	17 mm
Ethyl acetate	---	---	---	16 mm
Petroleum ether	11 mm	14 mm	---	11 mm

#### 4. Conclusion

The study reveals the presence of bioactive substances such as carbohydrates, flavonoids, coumarins, tannins, terpenoids, saponins, steroids and amino acids in the leaves of *Gmelina philippensis* Cham. This plant appears to be a promising drug for new drug discoveries. This study also verifies the plant's antibacterial and antioxidant properties. Leaf extracts of *Gmelina philippensis* Cham. have antibacterial activity, making them a potential source of antimicrobial agents to combat pathogenic infections that are rapidly becoming resistant to antimicrobial medicines. The DPPH assay revealed that the plant extract has strong antioxidant activity, making it a good choice for biological and chemical investigation, as well as for isolating therapeutically active components.

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##### Conflict of Interests

The authors declare that they have no competing financial interests.

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