

# Comprehensive Phytochemical Profiling and Evaluation of Antimicrobial and Antioxidant Activities of *Mesua ferrea* L.: A Multi-Approach Investigation into Its Therapeutic Potential

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## ABSTRACT

The study aimed to determine the total antioxidant activity and total phenolic content of a plant extract from the plant material of *Mesua ferrea*, which was collected from the Western Ghats mountain range. The crude methanolic extract of *M. ferrea* was found to be yellowish-brown in color and had a yield of 11.6556%. The phytochemical properties of the medicinal plant were analyzed to identify drug, lead compounds, and components. The phenolic compounds acting as antioxidants in the plant products were gaining attention due to their health benefits. The antimicrobial activity of the methanolic extract of *M. ferrea* was screened using an agar well diffusion assay. The extract inhibited all test bacteria. The minimum inhibitory concentration (MIC) of the plant extract was determined to be the concentration of the extract that inhibited all tested bacteria. The study concluded that the methanolic extract of *M. ferrea* possesses considerable potential against tested bacteria, with the potential to be used as a potential antimicrobial agent. The results of this study provide valuable insights into the potential of *M. ferrea* as a medicinal plant.

**Keywords:** *Mesua ferrea*, Western ghats, Antimicrobial activity, Minimum inhibitory concentration.

## ARTICLE INFO

Received on	:	31/01/2025
Accepted on	:	14/08/2025
Published online	:	30/09/2025



## INTRODUCTION

Natural products have significantly aided human existence, serving as a foundation for herbal drug discovery. Nature's therapeutic abundance has been utilized to treat ailments like the common cold since the beginning of civilization. Antibiotic resistance is a global issue due to the rise in infections caused by microorganisms. Researchers are exploring plant extracts and compounds to find new drugs to treat these resistant microorganisms, with higher plant products potentially offering novel antimicrobial agents (Kawad et al., 2022).

*Mesua ferrea* is one of the most popular tropical herbal plants, indigenous to Asian countries like India, Sri Lanka, Andaman Islands, Myanmar, Indo-China, Thailand, Peninsular Malaysia and Singapore. *M. ferrea* belonging to, the family Guttiferae. Other vernacular names of *M. ferrea* are Ceylon iron wood, Indian rose chestnut, Cobra's saffron, Nagachampaka or Nagakesar. *M. ferrea*, also known as Ceylon iron wood, Indian rose chestnut, Cobra's saffron, Nagachampaka, or Nagakesar, is a slow-growing tree with attractive red leaves. As it matures, the leaves turn pale green and leathery, dark green on the top but waxy white on the underside. It produces fragrant flowers, cream-colored, ebtactiate, pedicellate, and 2.5-7.5cm in diameter. The buds are subglobose, and the fruit is a capsule, ovoid to ellipsoid with a tough pericarp and persistent woody sepal. The plant's flowers are fragrant and can be solitary or in pairs (Adib et al., 2013).

*M. ferrea*, a medicinal plant, has been used in various cultures globally, particularly in India, for traditional treatments like cough, asthma, scabies, leprosy, dysentery, hemorrhoids, ulcers, impotency, fever, itching, erysipelas, sweating, metrorrhagia, excessive thirst, anti-inflammatory, antipyretic, blood purifier, and cardiac (Thakur et al., 2021). The series of 4-alkyl and 4-phenyl 5,7-dihydroxycoumarins was isolated from *M. ferrea* blossoms and found them to be a potent antibacterial agent on resistant Gram-positive strains (Aruldass et al., 2013). Leaf and flowers are antidotes for snakebite and scorpion sting. *M. ferrea* flowers, especially petals, emit a sweet aroma and essential oil extracted from the entire flower is most suitable for body creams and hair oils, as it masks the base odor of cosmetic products (Thakur et al., 2021).

The study focuses on the research of phyto-constituents in the extract of *Mesua ferrea*, a folklore medicinal plant, to assess its antimicrobial activity. Synthetic antibiotics are ineffective against pathogenic microorganisms, and their development poses health risks. The study highlights the therapeutic potential of plants, as they are readily available, less expensive, and have minimal adverse effects. The findings are crucial for understanding the bioactivities of this plant.

## MATERIALS AND METHODS

### Collection of Plant Sample

The healthy plant sample was collected aseptically with the help of secateurs. Photographs of plant were taken in their

habitat and GPS (Global Positioning System) locations of the sampling site were documented and the collected sample was placed in clean paper bags and brought into the laboratory. The collected plant was identified based on their taxonomic features by referring the standard floras and manuals.

#### Extraction of Phyto-Compounds from the Plant Extract

The crude compound from the plant was extracted by cold maceration technique. The finely pulverized plant material was immersed with analytical grade solvent methanol in the ratio 1:10 in Erlenmeyer's flask and positioned on the rotary shaker and agitated at 200 rpm for 48-72 hours. The Phyto-compounds containing solvent was separated from the extraneous portion by using Whatman No. 1 filter paper with the aid of Buchner funnel connected to the vacuum pump. The filtered phyto-compounds engrossed solvent was decanted in to the petri-dish and evaporated. The crude extract was dried up to get persistent weight. The physical properties of crude plant extract were examined and percentage yield of the plant crude extract was calculated by using the below formula (Hartanti et al., 2019).

$$\text{Yield of crude extract (\%)} = \frac{\text{Weight of the plant crude extract}}{\text{Weight of the pulverized plant material taken}} \times 100$$

#### Qualitative screening of secondary metabolites in plant extract

The groups of secondary metabolites in plant extract were determined by implementing the standard protocol of qualitative tests of Phyto-chemicals.

##### Test for Phenols (Ferric Chloride Test)

About 2 mL of plant extract was treated with 5% of FeCl<sub>3</sub> solution, the formation of deep blue or black colour indicates the presence of phenols.

##### Test for Tannins (Gelatin Test)

About 2 mL of aqueous solution of plant extract was mixed with 1% of gelatin solution to this mixture around 10% NaCl solution was added. The formation of white precipitation specifies the presence of tannins.

##### Test for Alkaloids (Mayer's Test)

To the 2 mL of extract, 2 mL of Mayer's reagent was added. An organic dull white or cream colour precipitate indicates the presence of alkaloids.

##### Test for Flavonoids (Alkaline Reagent Test)

About 2 mL of plant extract was treated with 20% NaOH solution. A formation of intense yellow coloration confirms the presence of flavonoids.

##### Test for Terpenoids (Salkowski's Test)

To 2 mL of extract, 2 mL of chloroform was added. To this mixture around 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added gently along the sides of test tube. The formation of reddish-

brown monolayer coloration at the interface, confirms the presence of terpenoids.

##### Test for Steroids (Liebermann-Burchard Test)

Approximately 2 mL of acetic anhydride was added to 0.5gm of plant extract and then added 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub>. The colour change from violet to blue or green indicates the presence of steroids.

##### Test for Saponins (Foam Test)

The plant extract was agitated with 20 mL of distilled in a graduated cylinder for 15 minutes. The formation of foam layer about 1cm indicates the presence of saponins.

##### Test for Glycosides (Keller-Killiani Test)

Around 2 mL of plant extract was treated with 2 mL of glacial acetic acid to this mixture 2-3 drops of 5% FeCl<sub>3</sub> solution was added to this 1mL concentrated H<sub>2</sub>SO<sub>4</sub> was added gently. Development of dark brown color ring at the interphase indicates the presence of glycosides.

##### Test for Protein and Amino acids (Ninhydrin Test)

To 2 mL of plant extract, 3-5 drops of freshly prepared 2% ninhydrin reagent was added and heated on water bath. The reaction mixture turns to blue color confirms the presence of proteins and amino acids.

##### Test for Carbohydrates (Fehling's Test)

To 2 mL of extract, equal volume Fehling's solution A and Fehling's solution of B was added and then heated on boiling water bath. The formation of brick red precipitate indicates the presence of carbohydrates.

#### Antimicrobial activity of methanol extract of plant

##### Preparation of test pathogenic microorganisms

The test microbial cultures such as *Escherichia coli* (MTCC 1559), *Enterococcus faecalis* (MTCC 439), *Klebsiella pneumonia* (MTCC 7028), *Pseudomonas aeruginosa* (MTCC 1934), *Staphylococcus aureus* (MTCC 902), *Salmonella typhi* (MTCC 734), *Aspergillus brasiliensis* (MTCC 1344) and *Candida albicans* (MTCC 3272) were procured from Microbial Type Culture Collection (MTCC) center, Chandigarh. The assay was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

##### Agar well diffusion assay

The potential of methanolic extract of the plant to inhibit pathogenic microorganisms were determined by Agar well diffusion assay. In this method, the prepared culture broth of the test bacterial and fungal pathogens was swab inoculated on sterile Mueller Hinton Agar and Sabouraud Dextrose Agar (SDA) plates respectively. Standard antibiotics such as Chloramphenicol, Ciprofloxacin and Streptomycin of concentration 1mg/mL and standard antifungals like

Fluconazole and Clotrimazole of concentration 10mg/mL were served as positive control and 10% Dimethyl sulfoxide (DMSO) was used as negative control. 100  $\mu$ L of plant extract of different concentrations (20mg/mL, 10mg/mL, 5mg/mL and 2.5mg/mL prepared in 10% DMSO) along with positive and negative control were pipetted out and transferred aseptically into the labeled wells. Then MHA plates were incubated for 18- 24 hours at 37°C  $\pm$  2°C and SDA plates were incubated at 30°C for 48 hours (Abah and Egwari, 2011).

#### Minimum Inhibitory Concentration (MIC) of plant extract

The effectiveness of plant extract to inhibit the test pathogenic microorganisms was confirmed by evaluating the MIC by modified resazurin 96-well microtitre plate broth dilution assay by using resazurin dye as indicator. 100 $\mu$ L of diluted respective pathogen culture suspension was added to each well and microtitre plates were wrapped with the thin film and incubated aseptically for 18-24 hours at 35°C  $\pm$  2°C for bacterial inoculated microtitre plate and fungi inoculated microtitre plates were incubated at 28°C  $\pm$  2°C. After incubation 10 $\mu$ L of resazurin (0.015%) dye was added to the each well and again plates were incubated for 1-2 hours and observed for the colour change from alamar blue to pink. Ciprofloxacin 1000 $\mu$ g/mL and Clotrimazole 10000 $\mu$ g/mL were used as standard for bacterial and fungal pathogens respectively (Elshikh et al., 2016).

#### Antioxidant activity of plant extract

##### DPPH<sup>+</sup> assay

In this assay, plant extract was dissolved in methanol at concentration ranging from 10-100 $\mu$ g/mL similarly; ascorbic acid (reference standard) was also prepared. To 1 mL of different concentration of plant extract tubes and ascorbic acid tubes exactly 1 mL of DPPH radical solution (0.1mM in methanol) was added. The reaction mixture tubes were then incubated in dark for 30 minutes at 37°C. Then the absorbance was measured in spectrophotometer at the wavelength of 517 nm. Instead of plant extract ascorbic acid or methanol was used as control. The effectiveness of plant extract to scavenge the DPPH<sup>+</sup> radicals was determined by using the bellow equation (Aryal et al., 2019).

$$\text{DPPH}^+ \text{ radical scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

Where,  $A_c$  is absorbance of the control,  $A_s$  is the absorbance of the tested sample

##### ABTS <sup>••</sup> assay

In this assay, 0.2 mL of plant extract of concentration ranging from 10 to 100 $\mu$ g/mL prepared in methanol was mixed with 1.8 mL of the ABTS<sup>••</sup> radical solution. Ascorbic acid (10-100 $\mu$ g/mL concentration) was used as reference standard. The optical density of reaction mixtures was read at 734nm. The percentage of ABTS<sup>••</sup> free radicals scavenged was calculated by using following formula.<sup>15</sup>

Where,  $A_0$  is absorbance of the control and  $A_1$  is the absorbance of the tested sample.

##### FRAP assay

Ferric reducing power Assay (FRAP) of plant extract was determined, to 1 mL plant extract of different concentration (12.5 to 400 $\mu$ g/mL), 2.5 mL of 0.2 M phosphate buffer of pH 6.6 and 2.5 mL of 1% potassium ferricyanide was added and mixed vigorously and incubated at 50°C for 20 minutes in water bath. After incubation, tubes were allowed for cooling and 2.5 mL of 10 % trichloroacetic acid (TCA) was added to the mixture and followed by adding 0.5 mL of 0.1 % ferric chloride and incubate for 10 minutes in room temperature then absorbance of the resulting solution was read at 700 nm against the blank. Ascorbic acid will be used as standard. An increase in the absorbance with increase in concentration of plant extract/standard indicates the increasing capacity of ferric ion reducing power of plant extract (Jeyadevi et al., 2019).

##### Total antioxidant activity

The total antioxidant activity of plant extract was determined by phospho-molybdenum assay. 1 mL of plant extract or standard of concentration ranged from 6.25-200 $\mu$ g/mL was added with 2 mL of phospho-molybdenum reagent (4mM ammonium molybdate, 28mM sodium phosphate and 0.6M sulphuric acid) and mixed vigorously and incubated at 95°C for 90 minutes, then the tubes were allowed to cool and the absorbance was measured at wavelength of 765nm against methanol (blank). Ascorbic acid was used as standard (Batool et al., 2019).

##### Total phenolic content in the plant extract

The total phenolic content of the plant extract was determined by using Folin-Ciocalteu (FC) method. 0.5 mL of plant extract was mixed with 0.5 mL of FC reagent (1:10 diluted) to this mixture, 2% sodium carbonate solution was added and vortexed vigorously. Tubes were incubated at room temperature for 30 minutes and the absorbance was read at 765 nm. Gallic acid was used as standard. Standard graph was plotted for the concentration of 0-1000 $\mu$ g/mL of Gallic acid. The total phenolic content of the plant extract will be express as Gallic acid equivalent in mg/g (GAE mg/g extract) (Baliyan et al., 2022).

## RESULTS AND DISCUSSION

### Collection of plant sample

The plant material of *M. ferrea* was collected from 14.328560oN and 75.107220oW, Soraba, Shimoga District, Karnataka. The plant material area was belonging to Western Ghats. The Western Ghats, also known as Sahyadri mountain range, is a mountain range that covers an area of 160,000km<sup>2</sup>. It harbours number of plants with medicinal properties.

### Extraction of Phyto-compounds from plant

The colour of the obtained crude methanolic extract of *Mesua ferrea* was yellowish-brown colour. The yield of the crude extract was 11.6556%. Hartanti et al. (2019) concluded that, ethanol and ethyl acetate extraction of dried leaves of *M. ferrea* produce 20.5% and 9.6% respectively (Fig. 1).



Fig. 1: Plant sample and dried methanolic extract of *M. ferrea*

### Qualitative screening of secondary metabolites in plant extract

Analysis of the phytochemical properties of the medicinal plants used to show and isolate the drug, lead compounds and components from the parts of the plant. The unique biological activity of the plants can be identified by their phytochemical properties. The results for presence of phyto-constituents in samples were tabulated below in the table 1. Phenolic compounds acting as antioxidants present in the plant products are gaining attention among different people due to their health benefits. In *M. ferrea* methanolic extract we screened phenols, flavonoids, terpenoids, glycosides and carbohydrates. The work of Chakraborty et al. (2023) on phytochemical analysis of ethanolic extract of *M. ferrea* showed the presence of phenols, tannins, saponins, flavonoids, cardiac glycosides, steroids, terpenoids and carbohydrates (Table 1).

Table 1: Preliminary screening of phytochemicals

SI. No	Phytochemicals	Plant Sample
01	Phenols	Present
02	Tannins	Absent
03	Alkaloids	Absent
04	Flavonoids	Present
05	Terpenoids	Present
06	Steroids	Absent
07	Saponins	Absent
08	Glycosides	Present
09	Proteins or Amino acids	Absent
10	Carbohydrates	Present

### Antimicrobial activity of methanol extract of plant

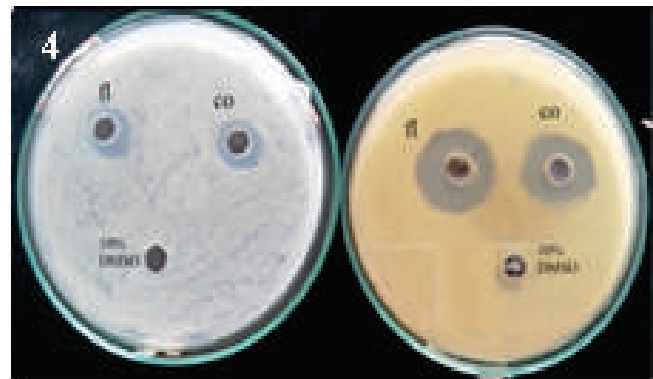
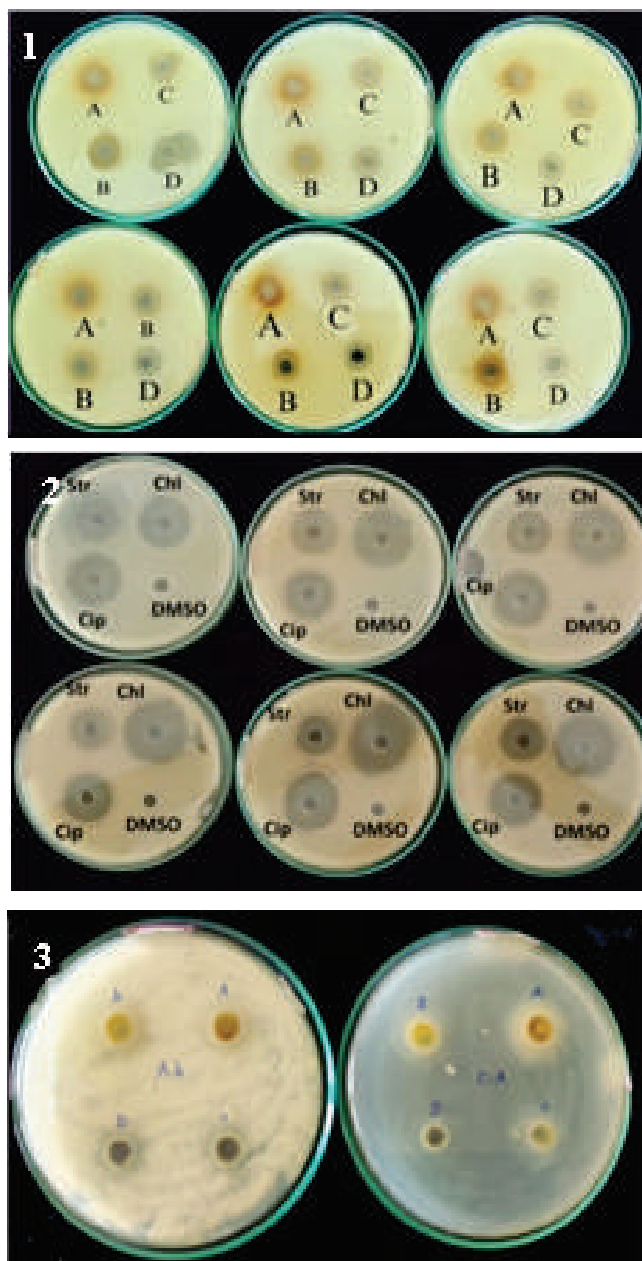
In this study, we screened the efficacy of methanolic extract of *Mesua ferrea* by agar well diffusion assay. The result of the inhibitory activity of extracts against bacteria and fungi are shown in Table 3. The presence of inhibition zone around the well was considered positive for antimicrobial activity. Extract of *M. ferrea* on different concentrations were inhibited all test bacteria. There is no effect on *C. albicans* and *A. brasiliensis* by this extract (Table 2). Abah & Egwari, (2011) worked on antibacterial activity of methanolic extract of *M. ferrea*. The extract possesses considerable potentiality against tested bacteria. *Proteus mirabilis* and *Lactobacillus arabinosus* fairly sensitive, Strains of *Klebsiella pneumoniae* and *Vibrio cholerae* were moderately sensitive to the extract, but the strains of *E. coli* and *Shigella* spp. were much less sensitive to the same (Table 3). Parekh and Chanda (2008) evaluated the methanolic extract of *M. ferrea* seeds for antifungal activity in vitro. The methanolic extract was effective against *C. albicans* (125µg/disc) and *A. candidus* (500µg/disc) (Fig. 2).

Table 2: Timicrobial activity of standard antibiotics and antifungal agents

Test Pathogenic Bacteria	Antibacterial Activity of Standard Antibiotics(1mg/mL)			
	Streptomycin	Chloramphenicol	Ciprofloxacin.	10% DMSO
	Zone of inhibition in diameter (mm)			
<i>E. coli</i>	21	24	25	0
<i>E. faecalis</i>	23	22	28	0
<i>K. pneumoniae</i>	22	25	28	0
<i>P. aeruginosa</i>	19	23	30	0
<i>S. aureus</i>	19	17	29	0
<i>S. typhi</i>	22	24	30	0
Test Pathogenic Fungi	Antifungal Activity of Standard Antifungal Agents(10mg/mL)			
	Fluconazole	Cotrimoxazole	10% DMSO	
	Zone of inhibition in diameter (mm)			
<i>A. brasiliensis</i>	20	17	0	
<i>C. albicans</i>	13	11	0	

**Table 3:** Antimicrobial activity of *M. ferrea*

Test organisms	Zone of inhibition in mm <i>M. ferrea</i>			
	20 mg/mL	10 mg/mL	5mg/mL	2.5mg/mL
<i>E. coli</i>	15	15	13	15
<i>E. faecalis</i>	15	15	15	13
<i>K. pneumonia</i>	14	14	14.5	13
<i>P. aeruginosa</i>	15	14	14	13
<i>S. aureus</i>	16	15	14	13.5
<i>S. typhi</i>	16	16.5	15	14
<i>C. albicans</i>	0	0	0	0
<i>A. brasiliensis</i>	0	0	0	0



**Fig. 2:** Antimicrobial activity. (1) antibacterial activity of plant extract, (2) antibacterial activity of standard antibiotics, (3) antifungal activity of plant extract, (4) antifungal activity of standard antifungal agents [A-20 mg/mL, B-10mg/mL, C-5mg/mL, D-2.5mg/mL, Str-streptomycin, Chl-chloramphenicol, Cip-ciproflaxin, DMSO-10% DMSO, fl-Fluconazole and co-Cotrimoxizole].

**Minimum Inhibitory Concentration (MIC) of plant extract**

The MIC determined through recording of the colour change observed. Active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resorufin (pink) which can be further reduced to hydroresorufin, giving a direct quantifiable measure of bacterial metabolic activity. The methanolic extract of *M. ferrea* reported MIC at  $2.5 \times 10^3$  CFU/mL against all the test bacteria. In the contrast Aruldass *et al.* (2013) revealed that the leaf and fruit extracts of *M. ferrea* showed MIC and MBC values of 0.048 and 0.39 mg/mL against *Staphylococcus aureus*, respectively (Table 4).

**Table 4:** Minimum inhibitory concentration of *M. ferrea*

Sl. No.	Test organisms	MIC ( $\mu\text{g/mL}$ )
01	<i>E. coli</i>	$2.5 \times 10^3$
02	<i>E. faecalis</i>	$2.5 \times 10^3$
03	<i>K. pneumonia</i>	$2.5 \times 10^3$
04	<i>P. aeruginosa</i>	$2.5 \times 10^3$
05	<i>S. aureus</i>	$2.5 \times 10^3$
06	<i>S. typhi</i>	$2.5 \times 10^3$

**Antioxidant activity of plant extract**

**DPPH<sup>•</sup> assay**

Antioxidants react with DPPH, a stable free radical which was reduced to DPPH-H and as consequence the absorbance were decreased from the DPPH radical to the DPPH-H form. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. *M. ferrea* plant extract showed dose independent radical scavenging activity in all tested concentrations. The DPPH radical scavenging activity of this plant extract was depicted in figure 05. The methanolic extract

of *M. ferrea* ( $IC_{50}=5.6263$ ,  $R^2=0.9164$ ) shows similar  $IC_{50}$  value with ascorbic acid ( $IC_{50}=5.01131$ ,  $R^2=0.9448$ ) (Fig. 3). Rajesh *et al.* (2013) revealed that n-hexane extract of *Mesua ferrea* stamens has been reported to possess good free radical scavenging activity with an  $IC_{50}$  value of 66.3 $\mu$ g/mL

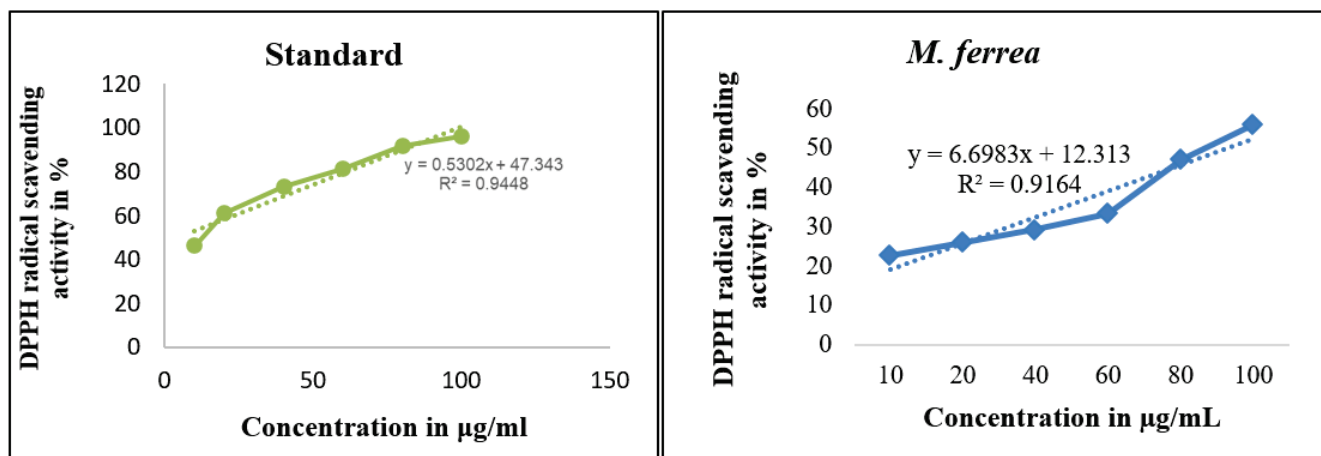


Fig. 3: Graphical representation of scavenging activity of DPPH radicals

#### ABTS<sup>+</sup> assay

ABTS<sup>+</sup> (2, 2'-azinobis-3-ethylbenzothiazoline- 6-sulphonic acid) assays based on the scavenging of light by ABTS<sup>+</sup> radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. The relatively stable ABTS<sup>+</sup> radical was green and it was quantified spectrophotometrically at 734 nm. ABTS<sup>+</sup> radical cations were produced by reacting ABTS<sup>+</sup> and APS. The ABTS<sup>+</sup> scavenging capacity of the extract was compared with ascorbic acid. The ABTS<sup>+</sup> radical scavenging activity of plant extract of *M. ferrea* ( $IC_{50}=3.3905$ ,  $R^2=0.8985$ ) shows similar  $IC_{50}$  value with ascorbic acid ( $IC_{50}=10.532$ ,  $R^2=0.9202$ ) (Fig. 4). Chaitanya *et al.* (2015) worked on the ABTS<sup>+</sup> radical scavenging activity of *M. ferrea* bark extract. The standard, ascorbic acid exhibited 74.29% at 100 $\mu$ g/mL. The  $IC_{50}$  values of MFBHE, MFBEE, MFBME and ascorbic acid were 214.76, 73.69, 108.13 and 4.81 $\mu$ g/mL respectively.

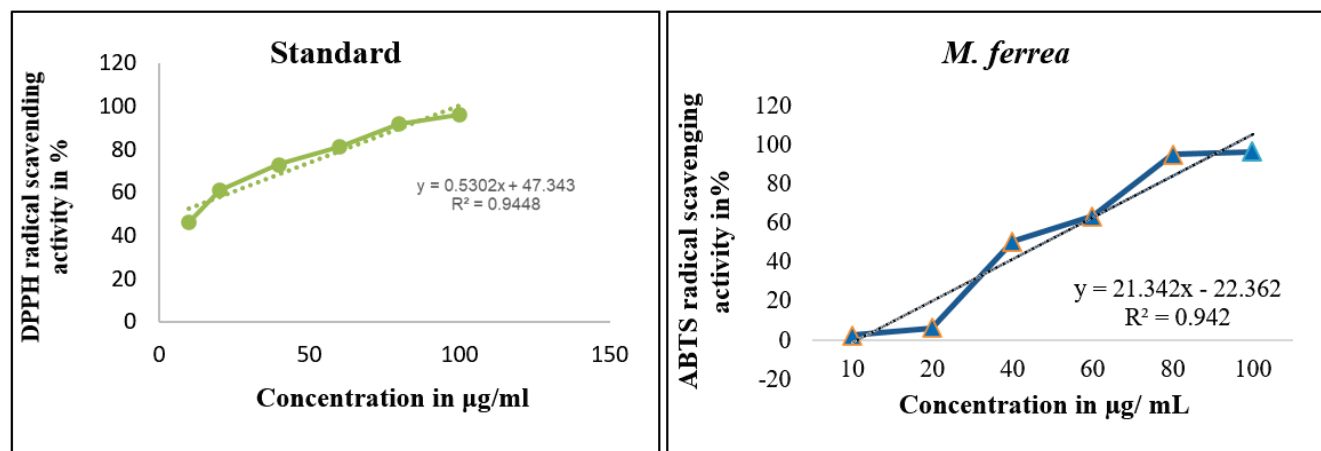


Fig. 4: Graphical representation of scavenging activity of ABTS radicals

#### FRAP assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. FRAP assay measures the reducing potential of an antioxidant reacting with ferric tripyridyltriazine ( $Fe^{3+}$ -TPTZ) complex and producing a coloured ferrous tripyridyltriazine ( $Fe^{2+}$ -TPTZ). The plant extract reduce ferric to ferrous form. This would have the effect of converting free radicals to more stable products and thus terminating free radical initiated chain reaction. The reduction power of methanolic plant extracts were depicted in figure 08. The data showed that reducing power of the extracts increased with increased concentration of extracts. The extract showed potent ferric reducing power (Fig. 5). Murthuza *et al.* (2013) studied the FRAP for plant extract of *M. ferrea*. The methanol extract showed highest activities than petroleum ether extract. These activities are due to the presence of phenolics equivalents.

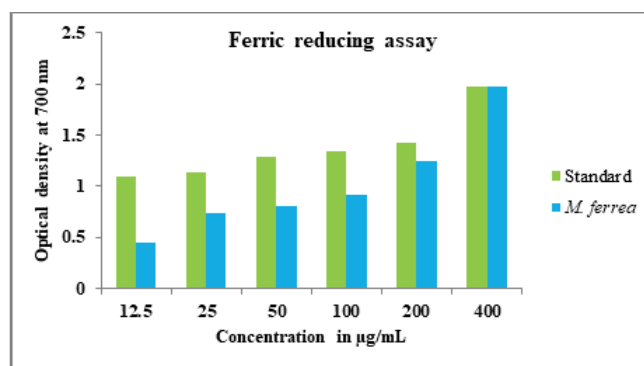


Fig. 5: Graphical representation of Ferric reducing assay of *M. ferrea*

### Total antioxidant activity

The phosphomolybdate method has been used to evaluate the total antioxidant capacity of plant extract. In the presence of extract, Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum complex, which shows a maximum absorbance at 765nm. The sample analyte, and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The Phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. Total antioxidant activity of plant extract increase with increasing concentration of extract (Fig. 6). Murthuza *et al.* (2013) revealed the total antioxidant activity *M. ferrea*. The total antioxidant capacity of petroleum ether and methanol extracts of *M. ferrea* was found to be 200 and 960 mg/g of ascorbic acid.

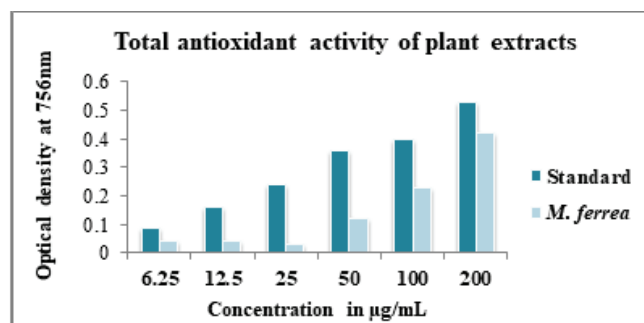


Fig. 6: Graphical representation of total antioxidant activity of plant extract

### Total phenolic content

The FC reaction is an antioxidant assay based on electron transfer, which measures the reductive capacity of an antioxidant. The reduction of the Folin-Ciocalteu reagent in the presence of phenolics resulting in the production of molybdenum-tungsten blue that is measured spectrophotometrically and the intensity increases linearly with the concentration of phenolics in the reaction medium. The absorbance values obtained at different concentrations of Gallic acid were used for the construction of calibration curve. Total phenolic content of the extracts was calculated from the regression equation of calibration curve ( $y = 0.353x + 0.079$ ,  $R^2 = 0.9944$ ) and expressed as mg Gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). TPC value of the *M. ferrea* extract was 3.3739 mg GAE/g (Fig. 7). Teh *et al.* (2013) report the total phenolic contents of *M. ferra* based on the

preliminary phytochemical analysis quantitative estimation of phytoconstituents was carried out. Total phenolic content of *M. ferrea* ethyl acetate and methanol extract was found to be  $206.67 \pm 2.53$  and  $441.3 \pm 3.33$  µg of Gallic acid /mg of crude extracts.

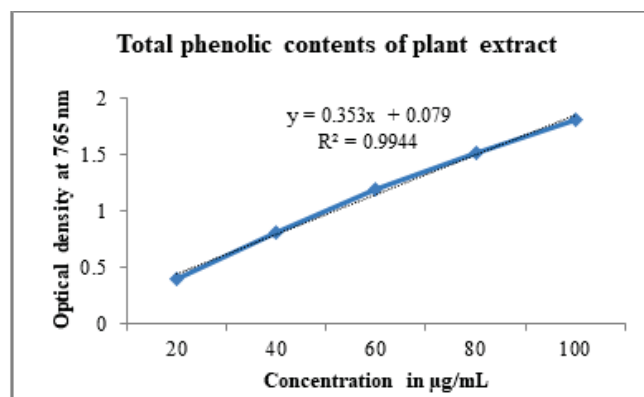


Fig. 7: Gallic acid calibration curve

### CONCLUSION

The results of this study emphasize the considerable therapeutic potential of *Mesua ferrea*. Strong free radical scavenging activity was shown by the plant extract, indicating its efficacy in counteracting dangerous oxidative agents and bolstering the body's natural antioxidant defense mechanisms. The presence of many phytochemicals created by secondary metabolism is responsible for this antioxidant action. Furthermore, *M. ferrea* had strong antibacterial activity, suggesting that it may be used to treat bacterial infections. *Mesua ferrea* may be a promising natural source for the creation of novel medicinal agents, such as antibiotics and antioxidant-based medicines, due to its dual antioxidant and antibacterial qualities.

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**Citation:**

Subhakar A, Shivanand S, Gurubasajar N, Yomakesh D and Basaiah T. 2025. Comprehensive phytochemical profiling and evaluation of antimicrobial and antioxidant activities of *Mesua ferrea* L.: A multi-approach investigation into its therapeutic potential. *Journal of AgriSearch* **12**(3): 185-192.