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**EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND *IN-VITRO*  
ANTIBACTERIAL ACTIVITY OF *MESUA FERREA* LEAVES**

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**Abstract:**

In this study we investigated phytochemical constituents screening and *In-vitro* antibacterial activity of hexane, ethyl acetate, ethanol (70% v/v) and methanol extracts of *Mesua ferrea* leaves. The results were revealed the presence of steroids, terpenoids, glycosides, tannins, alkaloids, saponins, phenols and carbohydrates but the all extracts are do not contain flavonoids, quinines and amino acids. The selected plant extracts were produced concentration dependent zone of inhibition against tested bacterial strains. The extracts showed more activity against gram negative organisms than gram positive organisms. The extracts showed better activity at highest concentrations i.e. at 600 and 1200  $\mu\text{g}/100\mu\text{l}$ . Among the four types of *Mesua ferrea* leaves extracts, the methanol extract showed better activity than remaining extracts at 1200  $\mu\text{g}$  concentrations against gram negative bacteria.

**Key Words:** *Mesua ferrea* leaves, phytochemical screening, *In-vitro* Antibacterial activity.

**Introduction**

Plants are the base for the development of a new medicine or they may be used as phyto-medicine for the treatment of diseases. The increasing prevalence of different diseases adds urgency to the search for novel drugs. For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new drugs. There are

several screening studies have been reported in different parts of the world on the different plants<sup>1</sup>. The natural products have received considerable attention in recent years due to their diverse pharmacological properties including antimicrobial activity<sup>2-4</sup>. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. Systematic screening of plants may result in the discovery of novel effective compounds<sup>5</sup>. The increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new ant infectious agents.

*Mesua ferrea* is a Succulent shrub with milky latex; leaves alternate, simple, and fleshy; flowers small and inconspicuous, in a small cup with a red rim of glands belongs to the family Euphorbiaceae. The aim of the present work was to study antibacterial activity of *Mesua ferrea* leaves in different solvents like hydro-alcohol (ethanol 70%), methanol, ethyl acetate and hexane against eight bacterial strains and these extracts were found to be potent antibacterial activity.

## **Materials and Methods**

### **Chemicals:**

Muller Hinton agar media was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

### **Test Organisms:**

Eight bacterial species were used. The bacterial species were purchased from National collection of industrial micro organisms (NCIM), Pune. The Bacterial species were maintained in the nutrient broth medium on placing shaker in separate culture tubes for each species separately. Out of eight, four are Gram positive Organisms (*Bacillus pumillis*, *Bacillus megaterium*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*) four are Gram Negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhimurium*)

### **Culture Media:**

For Anti bacterial activity Muller-Hinton Agar media (Solid and Broth) was used. For maintaining the bacterial species Nutrient both was used.

### **Preparation of Extracts:**

The plant material used in present study was collected from Araku valley, Visakhapatnam district, Andhra Pradesh and authenticated by the taxonomist Dr. Prayaga Murthy Pragada, Depart of Botany, Andhra University. Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. The powdered material was separately extracted in a soxhlet apparatus for 6 hrs successively with hexane, ethyl acetate, Hydro-alcoholic (ethanol 70% v/v) and methanol was concentrated to dryness under vacuum by using Rota-vapor.

### **Phytochemical Analysis:**

Phytochemical studies were carried out for hexane, ethyl acetate, hydro alcoholic and methanol extracts of *Mesua ferrea* leaves to detect the presence of different phytochemical constituents like steroids, terpenoids, tannins, flavanoids, saponins, glycosides, amino acids etc by using standard procedures<sup>6-8</sup>.

### **Antibacterial Activity:**

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds (extracts)<sup>9</sup>. The dilutions were prepared at a concentration of 150, 300, 600, 1200 µg/100µl. A sterile borer was used to prepare the cups of 4 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculums. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity.

**Results and Discussion**

**Phytochemical screening:** Qualitative Phytochemical screening for different extracts of *Mesua ferrea* leaves revealed the presence of steroids, terpenoids, glycosides, tannins, alkaloids, phenols and carbohydrates. The methanol, hydro-alcoholic extracts contain saponins but hexane, ethyl acetate extracts do not contain saponins and the all extracts are not containing flavonoids, quinines, amino acids and oils. The results of phytochemical screening were showed in the Table 1.

**Table 1: Phytochemical constituents present in different extracts of *Mesua ferrea* leaves.**

Name of the Phytochemicals	Extracts of <i>Mesua ferrea</i> leaves			
	Hexane extract	Ethyl acetate extract	Methanol extract	Ethanol (70%)
Phenols	+	+	+	+
Phytosterols	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	+	+	+
Saponins	-	-	-	+
Flavonoids	-	-	-	-
Tannins	-	+	+	+
Carbohydrates	+	+	+	+
Alkaloids	+	+	+	+
Amino acids	-	-	-	-
Oils	-	-	-	-
Quinones	-	-	-	-

+ = Present, - = Absent

**Evaluation of antibacterial activity:**

Among all the tested *Mesua ferrea* leaves extracts 70% ethanol and methanol extracts have shown significant antibacterial activity as compared to that of hexane and ethyl acetate extracts. All the extracts showed good zone of inhibition against gram negative bacteria than gram positive bacteria. All the extracts of *Mesua ferrea* leaves had produced a minimum zone of inhibition against some tested bacterial species at a dose of 150 and 300 µg/100µl. The hexane extract do not showed zone of inhibition against *Bacillus pumillis*. All extracts showed good zone of inhibition at a dose of 600 and 1200 µg/100µl. The highest zone of inhibition was showed by hexane and methanol extracts against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at the dose of 1200 µg/100µl. further study will be carrying out to isolate the active compounds from the extracts through column chromatography. The results of Antibacterial activity were showed in Table 2.

**Table 2: Antibacterial activity of *Mesua ferrea* leaves extracts.**

Name of the <i>Mesua ferrea</i> leaves Extract	dose (µg/cup)	zone of inhibition <sup>#</sup> (diameter in mm)							
		gram +ve				gram -ve			
		<i>B.p.</i>	<i>B.m</i>	<i>S.e</i>	<i>S.p</i>	<i>E.c</i>	<i>P.a</i>	<i>K.p</i>	<i>S.t</i>
<b>Hexane</b>	150	-	-	-	-	6	7	-	-
	300	-	6	-	6	8	9	6	6
	600	-	8	6	8	10	12	8	8
	1200	-	11	8	10	12	16	10	9
<b>Ethyl acetate</b>	150	-	-	5	-	6	7	5	5
	300	-	5	7	6	9	9	7	6
	600	6	7	9	8	12	12	9	8
	1200	8	10	12	10	15	14	12	10
<b>Ethanol (70%)</b>	150	-	-	-	-	-	6	5	-
	300	-	-	5	6	5	8	7	6
	600	7	6	7	8	8	10	9	8
	1200	10	9	10	11	10	13	12	10
<b>Methanolic</b>	150	-	-	5	-	-	6	-	-
	300	7	-	7	-	-	9	6	7

	600	10	-	9	-	6	12	8	10
	1200	14	7	12	6	8	16	10	12
<b>Chloramphenicol</b>	10	14	15	15	14	15	16	16	17
<b>DMSO</b>		-	-	-	-	-	-	-	-

B.a=Bacillus pumillis, B.m=Bacillus megaterium, S.m= Staphylococcus epidermidis, S.p=Streptococcus pneumonia; E.c=Escherichia coli, P.a= Pseudomonas aeruginosa, K.p= Klebsiella pneumonia, S.t=Salmonella typhimurium

-=No activity #Values are the average of triplicate; Includes the cup diameter (4mm)

### Conclusion

The data clearly indicated that the hexane, ethyl acetate, hydro-alcoholic and methanol extracts of *Mesua ferrea* leaves showed good antibacterial activity. Among the all the hexane and methanol extracts showed highest zone of inhibition on *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at a concentration of 1200 µg/100µl i.e. 16mm.

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