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# PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL ACTIVITY AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *MERREMIA EMARGINATA* (BURM. F.)

S. Parkavi [1], P. Ganesh [*1] and C. Swaminathan [2]

Department of Microbiology [1], Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram - 608002, Tamil Nadu, India.
Department of Microbiology [2], St. Joseph's College of Arts and Science (Autonomous), Cuddalore - 607001, Tamil Nadu, India.

**Keywords:**

*Merremia emarginata*, Phytochemical analyses, Antibacterial activity and Antioxidant activity

**Correspondence to Author:**
**Dr. P. Ganesh**

Assistant Professor,
Department of Microbiology,
Faculty of Science, Annamalai University, Annamalai Nagar,
Chidambaram - 608002,
Tamil Nadu, India.

**E-mail:** drpg1974@gmail.com

**ABSTRACT:** *Merremia emarginata* is a creeping herb, rooting at nodes, and commonly used in folk medicine. The current study was undertaken with the aim of preliminary phytochemical analyses, evaluation of antimicrobial activity, and antioxidant activity by methanol and aqueous extracts of leaves of *Merremia emarginata*. The extracts were subjected to preliminary phytochemical analyses; Antibacterial activities against nine bacterial strains were done by using agar – well diffusion method and free radical DPPH antioxidant activity. Preliminary phytochemical analyses were done by using standard phytochemical methods that showed the presence of Carbohydrates, reducing sugars, phytosterols, fixed oils, saponins, phenolic compounds and flavonoids. Based on the results of phytochemical analyses, methanol and aqueous extracts of the plant were chosen for antibacterial studies. Methanolic extract of leaves showed the highest antibacterial activity followed by the aqueous extract. The present study indicates that the leaves of *Merremia emarginata* possess antibacterial activity and found effective against eight bacterial cultures. Antioxidant activity of the DPPH assay showed that extracts exhibit the scavenging effect of various concentrations.

**INTRODUCTION:** Infectious diseases are a major cause of morbidity and mortality particularly in developing countries. Since the discovery of antibiotics and their uses as chemotherapeutic agents, there was confidence in the medical community that this would lead to the eradication of diseases. However, diseases and causative agents that were once thought to have been controlled by antibiotics are returning in new forms of resistance to antibiotic therapies [1].

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Occurrences of epidemics due to such drug-resistant microorganisms are now a common world-wide problem-posing public health concerns [2]. The worldwide emergence of multi-drug resistant microbial strains is increasingly limiting the efficacy of existing medicines and significantly causing treatment failure of infections [3].

*Merremia emarginata* (Burm.f.) Hallier f. also called Ipomoea reniformis is a procumbent herb belonging to the family Convolvulaceae. The plant is widely distributed in India, Sri Lanka, Malaysia, Philippines, and Tropical Africa. In India, it is commonly found in Tamil Nadu, Kerala, Karnataka, Bihar, Maharashtra, Rajasthan, and Gujarat [4]. It is known by various names in different regions, *viz.*, Kidney leaf morning glory in English, Elikkadhu-keerai in Tamil, and Mooshakarnee in

Sanskrit. It is an uncultivated food crop used as a green leafy vegetable by poor people in India [5]. In the Indigenous system of medicine, the plant has been claimed to be useful for fever, troubles of nose, neuralgia, urinary infection, rheumatism, inflammation, liver, and kidney diseases.

An infusion of the leaves, added with lumps of sugar is used as a remedy for cough, Powder of leaves is used as a snuff during epileptic seizures. Leaf juice acts as a purgative, given internally for headache and migraine and as an ear drop to cure sores, abscesses, and ulcers.

The root is also useful for the treatment of diseases of the eyes and gums and possesses diuretic and laxative actions [4]. The current study was directed for the exploration of phytochemicals from leaves of *Merremia emarginata* for antibacterial potential in order to give credence to its acclaimed ethnomedicinal usage.

## MATERIALS AND METHODS:

**Collection and Processing of Leaves of *Merremia emarginata*:** Fresh leaves of *Merremia emarginata* were collected from Varakkalpattu village, Cuddalore district, Tamil Nadu. The taxonomic identity of the plant was confirmed by the Botanist of Department of Botany at Annamalai University, Chidambaram, and deposit for future references (Acc. No. 355).

The plant materials were washed under tap water, and leaves were separated. The separated leaves were air-dried in the shade, then ground into powder form and stored in airtight containers.

**Preparation of Crude Leaf Extracts:**

**Methanol Extraction:** For alcoholic extraction, 10 g of leaf powder was soaked separately in 100 mL of methanol in a conical flask and kept for 3 days at room temperature and filtered through Whatman no. 1 filter paper.

The filtrate was evaporated in Petri plates at room temperature for 2 - 3 days till volume was reduced to one-fourth of the original volume of the solvent used and stored in airtight bottle [6].

**Aqueous Extraction:** Powdered leaf sample (10 g) was dissolved in 100 ml of distilled water and boiled for 2 h. The residue was removed by filtering through Whatman No. 1 filter paper. The filtrate was further boiled till the volume was reduced to one-fourth of the original volume and stored at 4 °C in airtight bottles [6].

**Preliminary Phytochemical Screening of Leaf Extracts:** The crude extracts are then subjected to preliminary phytochemical screening as per the standard methods of the procedure [6], tested for the presence or absence of primary or secondary metabolites.

**Test for Carbohydrates:** A small quantity of extract was mixed in 2 ml of distilled water. A few drops of Molisch's reagent (which contains α-Naphthol) was added to the thoroughly mixed solution and also added a small amount of concentrated sulphuric acid along the sidewall of the test tube. The presence of carbohydrates was indicated by the appearance of a purple ring at the interface between the acid and test layers.

**Test for Reducing Sugars:** A small quantity of the extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was subjected to Fehling's test and Benedict's test.

**Benedict's Test:** Benedict's reagent (5 ml) was added to 2 ml of extract and boiled for 3 min. The appearance of yellow or red precipitate indicated the presence of reducing sugars.

**Fehling's Test:** To 2 ml of Fehling's solution (1 ml of Fehling's A and 1 ml of Fehling's B solution), 2 ml of extract was added, mixed well, and boiled. The appearance of yellow or red color precipitate indicated the presence of reducing sugars.

**Test for Alkaloids:** A small portion of the alcoholic extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was subjected to Mayer's test and Dragendorff's test.

**Dragendorff's Test:** A small amount of filtrate was treated with Dragendorff's reagent, and the appearance of orange-redd precipitate indicated the presence of alkaloids.

**Mayer's Test:** The filtrate was treated with Mayer's reagent, and the appearance of creamy precipitate indicated the presence of alkaloids.

**Test for Phytosterols:** Salkowski test was done for the detection of phytosterols. The extract was dissolved in 2 ml of chloroform in a test tube. 1 ml of concentrated sulphuric acid was added along the sides of the tube and allowed to stand for 5 min. A reddish-brown color at the interface indicated the presence of phytosterols.

**Test for Fixed Oil:** Spot test was done for the detection of fixed oil. In this test, a small quantity of alcoholic extract was pressed between two filter papers. The appearance of oil strain on the paper indicated the presence of fixed oil.

**Test for Saponins:** Froth test was done for the detection of saponins. Each extract was diluted with 20 ml distilled water and agitated in a graduated cylinder for 15 min. The formation of 1cm layer 'honeycomb' froth indicated the presence of saponins.

**Test for Proteins and Free Amino acids:**
**Biuret Test:** The plant extract was diluted with distilled water and treated with Biuret reagent. Appearances of pink or purple color indicated the presence of proteins and free amino acids.

**Ninhydrin Test:** The diluted extracted was treated with ninhydrin reagent and observed for a characteristic purple color, which indicated the presence of free amino acids.

**Test for Phenolic Compounds:** A small quantity of the extract was dissolved in a few ml of water and subjected to a ferric chloride test. Each extract was dissolved in 5 ml of distilled water and a few drops of 5% ferric chloride solution were added. The appearance of bluish-black color indicated the presence of phenolic compounds.

**Test for Flavonoids:** Alkaline reagent test was done for the detection of flavonoids. The extract was treated with a few drops of sodium hydroxide to give deep yellow color. The disappearance of yellow color after the addition of dilute hydrochloric acid indicated the presence of flavonoids.

**Bacterial Strains:** *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* B, *Acinetobacter baumannii* (MTCC 2366), *Pseudomonas otitidis* (MTCC 2509) and *Enterococcus faecalis* (MTCC 2041) were procured from laboratory Microbial culture collection of St. Joseph's College of Arts and Science (Autonomous), Cuddalore. The strains were maintained on nutrient agar slants at 4 °C and subcultured on nutrient agar slant and incubated at 37 °C for 24 h before doing antimicrobial susceptibility test.

**Determination of Antibacterial Activity of Leaf Extracts:** Antibacterial activity of methanol and aqueous extract of leaves of *Merremia emarginata* was evaluated by the agar well diffusion method [7, 8, 9]. The bacterial test strains were inoculated into Mueller-Hinton broth and incubated at 37 °C for 6 h. After incubation, a sterile cotton swab was immersed in the bacterial suspension and swabbed aseptically on the surface of Mueller-Hinton agar medium and allowed to dry for about 3 min. Wells of 6 mm diameter were punched into the agar medium and filled with 100 µl of crude leaf extracts (100 mg/ml 10% DMSO). Gentamicin (40 mg/ml) was used as a positive control and 10% Dimethyl Sulfoxide (DMSO) was used as a negative control. The plates were incubated in an upright position at 37 °C overnight in an incubator. Antibacterial activity was detected by measuring the zone of inhibition around each well, excluding the diameter of well in mm as low activity (≤ 6 mm), moderate activity (7-10 mm), high activity (11-15 mm), very high activity (≥ 16 mm) and no activity (-) [10].

**Determination of Free Radical Scavenging DPPH Activity:** The DPPH (1, 1 – Diphenyl – 2 – Picrylhydrozyl) free radical scavenging activity of methanol and aqueous extracts of *Merremia emarginata* was determined according to the standard method [11, 12, 13]. The various concentrations of leaf extracts were added to 5 ml of a 0.004% methanol solution of DPPH. Then, the concentrations were kept for 30 min incubation at room temperature.

Absorbance was read against a blank at 517 nm. Ascorbic acid was used as a standard control.

**RESULTS AND DISCUSSION:** Preliminary phytochemical analysis of n-hexane, ethyl acetate, methanol, and aqueous extracts of leaves of

*Merremia emarginata* are shown in **Table 1**. Phytochemical screening of aqueous extract showed the presence of carbohydrates, reducing sugars, phytosterols, fixed oils, saponins, phenolic compounds and flavonoids, where alkaloids and proteins are absent.

Methanol extract revealed the presence of carbohydrates and flavonoids. The presence of these phytochemicals provoked for antibacterial studies on this plant.

**TABLE 1: PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF *MERREMIA EMARGINATA***

| S. no. | Test | Methanol | Aqueous |
|---|---|---|---|
| 1 | Carbohydrates | + | + |
| 2 | Reducing sugars | | |
| | Benedict's | - | + |
| | Fehling's | - | + |
| 3 | Alkaloids | | |
| | Dragendorff's | - | - |
| | Mayer's | - | - |
| 4 | Phytosterols | - | + |
| 5 | Fixed oil | - | + |
| 6 | Saponins | - | + |
| 7 | Protein and Free amino acids | - | - |
| 8 | Phenolic compounds | - | + |
| 9 | Flavonoids | + | + |

**TABLE 2: ANTIBACTERIAL ACTIVITY OF METHANOLIC LEAF EXTRACTS OF *MERREMIA EMARGINATA* (BURM.F.)**

| S. no. | Bacterial pathogens | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | Gentamicin | DMSO | Methanolic leaf extract |
| 1 | *Staphylococcus aureus* | 24 ± 0.95 | - | 13 ± 0.95 |
| 2 | *Escherichia coli* | 19 ± 0.95 | - | 19 ± 0.95 |
| 3 | *Klebsiella pneumonia* | 18 ± 0.95 | - | 14 ± 0.95 |
| 4 | *Proteus mirabilis* | 18 ± 0.95 | - | 14 ± 0.95 |
| 5 | *Pseudomonas aeruginosa* | 29 ± 0.95 | - | 24 ± 0.95 |
| 6 | *Salmonella paratyphi* B | 23 ± 0.95 | - | 13 ± 0.95 |
| 7 | *Acinetobacter baumannii* (MCC 2366) | 22 ± 0.95 | - | 19 ± 0.95 |
| 8 | *Pseudomonas otitidis* (MCC 2509) | 16 ± 0.95 | - | 6 ± 0.95 |
| 9 | *Enterococcus faecalis* (MCC 2041) | 29 ± 0.95 | - | 24 ± 0.95 |

- : No zone of inhibition DMSO: Dimethyl Sulfoxide ± : SEM (Statistical error mean)

The antibacterial activity of Methanolic leaf extract and aqueous extract of leaves of *Merremia emarginata* was tested against nine bacterial pathogens with gentamicin as positive control and DMSO as negative control **Table 2** and **3**.

The antibacterial activity of methanolic leaf extract and aqueous extract of leaves of *Merremia emarginata* was tested against nine bacterial pathogens with gentamicin as positive control and DMSO as negative control **Table 2** and **3**.

Methanolic leaf extract showed low activity against *Pseudomonas otitidis* (6 mm) and high activity against *Staphylococcus aureus* (13 mm), *Klebsiella pneumoniae* (14 mm), *Proteus mirabilis* (14mm) and *Salmonella paratyphi* B. A very high activity was observed against *Escherichia coli* (19 mm), *Pseudomonas aeruginosa* (24 mm), *Acinetobacter baumannii* and *Enterococcus faecalis* (24 mm).

Aqueous leaf extract of the plant exhibited low activity against *Enterococcus faecalis* (6 mm) and moderate activity against Staphylococcus aureus (8 mm), *Escherichia coli* (7 mm), *Klebsiella pneumoniae* (9 mm), *Proteus mirabilis* (8 mm), *Pseudomonas aeruginosa* (9 mm), *Salmonella paratyphi* B (8 mm), *Acinetobacter baumannii* (9 mm) and *Pseudomonas otitidis* (7 mm).

Results of the determination of antibacterial activity indicated that the methanolic leaf extract showed the highest zone of inhibition compared with aqueous leaf extract against all the tested bacterial pathogens. Gentamicin displayed superior antagonistic activity against the bacterial pathogens with a zone of inhibition ranging between 13 mm and 29 mm. On the other hand, DMSO did not exhibit any effect on all the tested microorganisms.

The best method to determine the antioxidant activity of plant extracts is by using free radical scavenging DPPH assay [13, 14, 15]. The free radical scavenging DPPH of *Merremia emarginata* leaves extracts was recorded and compared with ascorbic acid **Table 4**. The highest free radical activity in methanolic leaves extract. The results obtained in this study suggest that methanol and aqueous leaf extracts showed free radical scavenging activity by their electron transfer or hydrogen atom donating ability.

**TABLE 4: ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS OF *MERREMIA EMARGINATA* (BURM.F.)**

| Concentration of Sample | 100 µg | 200 µg | 300 µg |
|---|---|---|---|
| Standard (Ascorbic acid) | 87.6% | 93.10% | 95.03% |
| Methanol Extract | 30.42% | 50.94% | 71.95% |
| Aqueous Extract | 13.57% | 15.6% | 24.64% |

## Antioxidant DPPH Method % Activity:

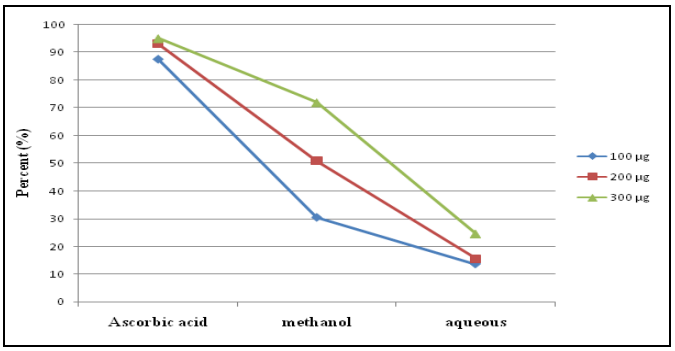


**FIG. 1: GRAPHICAL REPRESENTATION OF ANTI-OXIDANT ACTIVITY OF LEAVES EXTRACTS OF *MERREMIA EMARGINATA* (BURM.F.)**

**CONCLUSION:** The present study indicates that the leaves of *Merremia emarginata* possess antibacterial activity. The leaf extract of *Merremia emarginata* was found to be effective against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* B, *Acinetobacter baumannii* and *Enterococcus faecalis*. However, its effectiveness against *Pseudomonas otitidis* is very less. Methanolic extract of leaves of this plant showed the highest free radical scavenging activity in a dose-dependent manner. Further study is necessary to explore the lead molecules responsible for aforesaid activity from this plant.

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**CONFLICTS OF INTEREST:** Nil

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