

Screening for Antibacterial Activity of *Micrococca mercurialis* (L.) Benth an important medicinal plant

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Received: 30 November 2016 / Accepted: 09 December / Published Online: 15 December 2016

<http://www.gayathripublishers.com/ijbt.htm>

Citation: Suresh, J., Saravana Ganthi, A. and Henry Joseph, L. 2016. Screening for Antibacterial Activity of *Micrococca mercurialis* (L.) Benth an important medicinal plant. *Int. J. Biol. Technology*, 7(3):19-21.

Abstract

The ethanol, Acetone, Hexene, Ethyl acetate and aqueous extracts of the leaf of *Micrococca mercurialis* (L.) Benth, were screened for their anti-bacterial sensitivity against 10 human pathogenic bacteria and discussed according to their phytochemical components.

Key words: *Micrococca mercurialis*, leaf, antibacterial activity.

Introduction

The world health organization estimated that 80% of the people living in developing countries almost exclusively use traditional medicine. Most of the traditional medicine relies heavily on medicinal plants (Eloff, 1998). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002). *Micrococca mercurialis* belongs to the family Euphorbiaceae and has been reported to possess powerful diaphoretic, emollient, antimicrobial, anticancer and demulcent properties (Cowan, 1999). This plant contains alpha and gammatocopherols, polyphenols, glucoside, strumarosid, stigmaterol (Dhavan, 1980). A decoction of the root has been used in the treatment of high fevers and to help a woman expel the afterbirth. A decoction of the seeds has been used in the treatment of bladder complaints. They are used internally in the treatment of allergic rhinitis, sinusitis, catarrh, rheumatism, rheumatoid arthritis, constipation, diarrhoea, lumbago, leprosy and pruritis (Ghani, 2003). The fruits are rich in vitamin B and said to be effective in treating small pox, eye ailment as ointment, skin and bladder infections to stop bleeding from cuts, abrasion, herpes and neck gland tuberculosis. Therefore, in the present study, the extracts of *Micrococca mercurialis* were screened for its antimicrobial sensitivity against 10 human pathogenic bacteria and thus ascertain its Bio-efficiency.

Material and Methods

The leaf was air dried and grounded into a coarse powder. For aqueous extraction, 10g of air-dried powder was mixed with double distilled water and boiled on slow heat for 1 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected. The same procedure was repeated twice. After 8 h, the supernatant, collected at an interval of every 2 hours, was pooled and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved (121°C, 15 lbs pressure) and stored at 4°C. For solvent extraction, 10 g of air dried powder was mixed with 100 ml of organic solvent (ethanol, ethyl acetate, acetone, hexene) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190 - 220 rpm for 24 hours. After 24 hours, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatants were collected in 2 ml eppendorf tubes separately and opened for condensation. The filter paper discs were placed in the eppendorf tubes and impregnated well with the extracts for condensation. The impregnated filter paper discs were used to study the sensitivity assay against pathogens.

Phytochemical screening

The freshly prepared crude extract of *M. mercurialis* was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (Brindha *et al.*, 1981).

Preparation of culture media

The petriplates were washed well and dried under hot air oven. Then the petriplates were sterilized at 121°C, 15 lbs pressure. The Muller Hinton Agar was taken and sterilized at 121°C for 15 -20 minutes. After sterilization, the media was poured into petriplates (15-20 ml) under the Laminar Air Flow Hood Chamber and allowed to cool.



Table-1: Effect of *Micrococca mercurialis* (L.) Benth leaf extract against human pathogenic bacteria

Name of the pathogens	Strains type	Zone of inhibition of <i>Micrococca mercurialis</i> leaf extracts (mm)					positive control	Negative control
		Ethanol	Ethyl acetate	Hexene	Acetone	Aqueous		
<i>Escherichia coli</i>	G ^{-ve}	10	-	-	-	-	14	-
<i>Pseudomonas aeruginosa</i>	G ^{-ve}	10	-	-	-	-	23	-
<i>Staphylococcus aureus</i>	G ^{+ve}	8	7	-	-	-	18	-
<i>Salmonella typhi</i>	G ^{-ve}	13	10	-	-	-	22	-
<i>Streptococcus pyogens</i>	G ^{+ve}	10	-	-	-	-	20	-
<i>Serratia marcescens</i>	G ^{-ve}	-	7	7	-	-	22	-
<i>Klebsiella pneumoniae</i>	G ^{-ve}	7	-	-	-	-	15	-
<i>Enterobacter aeruginosa</i>	G ^{-ve}	14	10	-	-	-	18	-
<i>Proteus vulgaris</i>	G ^{-ve}	9	-	9	-	-	21	-
<i>Bacillus subtilis</i>	G ^{+ve}	8	8	10	-	-	18	-

‘-’ Negative

Table -2: Preliminary phytochemical Screening of the crude extract of *Micrococca mercurialis*

S.No	Experiment	Different extract of <i>Micrococca mercurialis</i>				
		Ethyl acetate	Aqueous	Ethanol	Hexene	Acetone
1	Steroid	-	-	-	-	-
2	Triterpenoids	-	-	-	-	-
3	Sugar	+	+	-	+	+
4	Alkaloids	+	-	+	+	+
5	Phenolic groups	+	-	+	+	+
6	Flavonoides	+	-	+	-	+
7	Catachin	-	-	+	-	+
8	Saponin	+	-	+	-	-
9	Antroquinone	-	-	+	-	-
10	Aminoacid	+	+	+	-	-
11	Reducing sugar	-	-	+	-	-
12	Protein	-	-	+	-	+
13	Tannin	+	-	+	-	+

(-) = Absent, (+) = Present

Antimicrobial activity test

Standard bacterial strains were used for screening these were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Streptococcus pyogens*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterobacter aeruginosa*, *Proteus vulgaris*, and *Bacillus subtilis*. These bacterial species were cultured in broth separately. After 12-

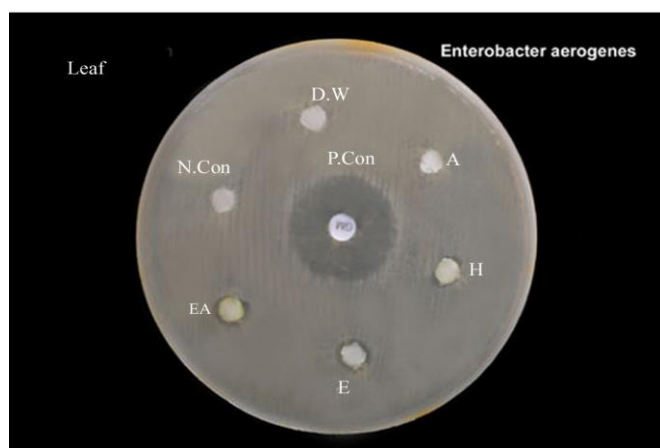
18 hours, they were swapped uniformly on petriplates under the Laminar Air Flow Hood chamber. The impregnated filter paper discs were placed on the culture media swapped by different pathogens. Along with that control discs from the solvent (Negative control) and antibiotic disc (positive control) were screened against pathogenic bacteria. The cultures were incubated at $32 \pm 2^\circ\text{C}$ for 18 hours. After 18 hours, the zone of inhibition was measured by a graduated



scale and tabulated the activity was measured by zone of inhibition in millimeter

Result and Discussion

Plant-based antimicrobial compounds have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Results of the present study are found directly correlated with the observations of previous workers (Okwu and Okwu, 2004). The leaf extracts of ethanol, ethyl acetate and hexene were effective against all the pathogens (Fig.1).



The solvent ethanol extract showed the maximum bio-efficacy when compared with other solvents due to the presence of variety of compounds such as Alkaloids, Phenolic groups, flavonoides, catarchin, aminoacid, protein, tannin (Table- 2). Alkaloids are widely distributed and naturally occurring in plants. Most of the alkaloids from plants are used for medicinal purpose. Alkaloids are chemical constituents from plants that can work on the nervous system of the human body and used for analgesic, antispasmodic and bacterial effects (Jain *et al.*, 2010). Tannic acid has been found to have anti-bacterial, antiseptic, astringent, anti-ulcer and antiviral properties (Wiat *et al.*, 2016). Ethanolic extract of the leaf showed the maximum of 14 mm in diameter was observed against *Enterobacter aeruginosa* and 13 mm in diameter against *Salmonella typhi*. The minimum of 7 mm in diameter was observed against 3 pathogenic bacteria namely *Staphylococcus aureus*, *Serratia marcescens* and *Klebsiella pneumoniae*. Ethyl acetate extract of the leaf showed antibacterial activity against 5 pathogenic bacteria. It had maximum inhibitory action against *Salmonella typhi* (10 mm) and *Enterobacter aeruginosa* (10 mm). Hexene extract of the leaf showed antibacterial activity against three pathogenic bacteria namely *Serratia marcescens* (7mm), *Proteus vulgaris* (9mm) and *Bacillus subtilis* (10mm). Acetone and aqueous extract of the leaf had no inhibitory action against the pathogenic bacteria (Table-1). The activity was measured by zone of inhibition in mm (Shariff *et al.*,

2016). The positive results were compared with that of the reference antibiotic (Gentamycin) and it was found that many extracts in equal proportion with the antibiotic against the pathogenic bacteria. The presence of phenolic compounds in the plant indicates the antimicrobial potential.

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