

ASSESSMENT OF PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *Merremia emerginata* LEAVES AGAINST ORAL MICROFLORA TO IMPROVE DENTAL HYGIENE

PRITI D DIWAN¹, YASHASHREE.A.GADHIKAR^{*2}

Department of Zoology, Govt.Vidarbha Institute of Science and Humanities, Amravati, (M.S.). Email: *yash.gadhikar@rediffmail.com

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ABSTRACT

Oral diseases are major health problem with dental caries. Oral health influence the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. Hence there is an urgent need to find out an alternative way, instead of antibiotic and chemicals for the treatment of oral diseases which show more side effects. Present investigation is focused on antibacterial activity of aqueous, petroleum ether, chloroform and acetone extract of leaves of *Merremia emerginata* (Family-Convolvulaceae) against seven bacterial species (*Lactobacillus rhamnosus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Actinomyces viscosus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Streptococcus mutans*) which are known to cause oral diseases in human being. Antibacterial activity of the extracts was determined by paper disc diffusion method using 200mg/ml concentration of extract and there % zone of diffusion was calculated. Result showed that all the four extracts are equally effective at 5% level of significance. Although test organism from the present study were found to be effected by all the four type of extract of *Merremia emerginata* leaves but pronounced inhibition for four extract was observed for bacterial species, *Lactobacillus rhamnosus*. Acetone extract was found to be more effective against the entire test microorganism.

Keywords: *Merremia emerginata*, Oral micro-organisms, Antibacterial activity, Oral diseases, Medicinal plants.

INTRODUCTION

Since an ancient time, mankind has used plants to treat common diseases and some of these traditional medicines are still included as a part of habitual treatment of many communities. Folk medicines mainly based on plants, have a respectable position today, specially in developing countries where availability of modern health services are limited. In the present days there has been renewed interest in improving health and fitness through the use of natural product.

Oral diseases are major health problem with dental caries. Oral health influence the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. Periodontal diseases are recognized as a major public health problem throughout the world and are the most common cause of tooth loss in adult. These periodontal diseases are associated with bacterial infection; therefore antibacterial treatment seems to be an appropriate method for improving the conditions of inflamed tissue. Hence there is an urgent need to find out an alternative way for use of antibiotic and chemicals for treatment of oral diseases which show more side effects. The use of medicinal plants to treat dental problem has been discussed from time to time by many researchers. The World Health Organization has recommended and encouraged the use of chewing sticks¹. Chewing sticks are at least as effective as toothpaste in maintaining oral hygiene^{2,3,4}. Africans that use chewing sticks have fewer carious lesions than those that use toothbrushes⁵. In a related development, Enwogu (1997) reported that Chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes⁶; advantages of the chewing sticks over the conventional toothpaste and brushes have been attributed to the strong teeth of Africans⁷. *In vitro* antibacterial activity of traditional plants like *J. curcus* and *F. benghalensis* against oral microorganisms and found that latex of *J. curcus* and aqueous arial root extract of *F. benghalensis* shows more inhibitory action against six oral bacterial species⁸.

The vast biodiversity of Indian forest provides several plants which are mention in Ayurveda for dental care. Root of plant species *Merremia tridentata* L. used against toothache and dentriferices⁹. Hence in the present investigation antibacterial activity of aqueous, pet ether, chloroform and acetone extract of leaves of *Merremia emerginata* (Family -Convolvulaceae) was carried out against seven bacterial species which are known to cause oral diseases in human being. *Merremia emerginata* is a common herb; posses yellow colored flowers and commonly called as *Undiramani* in Marathi. Tribals of Melghat region use this plant species for medicinal purpose as well as food stuff.

MATERIAL AND METHOD

Plant collection and identification

Leaves of *Merremia emerginata* was collected from Amravati and Melghat region. Authentication and identification was performed at department of botany Govt. Vidarbha Institute of Science and Humanities, Amravati. Collected material was shade dried and stored into airtight container.

Preparation of extracts

Shade dried powdered extract of leaves was subjected to successive Sox let extraction using solvent of varying polarity such as water, petroleum ether, chloroform and acetone. After extraction solvent was removed under reduced pressure. Extracted material was stored in airtight container till use.

Test organism / Microbial flora

Seven lyophilized bacterial strains were procured from Institute of Microbial Technology (IMTECH), Microbial Type Culture Collection (MTCC) Chandigarh.

Table 1: List of Bacterial Strains

S. No.	Bacterial Strain	MTCC Code	Growth Medium (Agar and Broth)	Incubation time in hours
1.	<i>Lactobacillus rhamnosus</i>	*1408	MRS agar	24
2.	<i>Streptococcus mutans</i>	890	Brain Heart Infusion(BHI)	48
3.	<i>Staphylococcus aureus</i>	3408	Soyabene Casein Digest	24
4.	<i>Actinomyces viscosus</i>	7345	Pikoskaya's agar	24
5.	<i>Staphylococcus epidermidis</i>	3639	Nutrient agar and broth	24
6.	<i>Escherichia coli</i>	732	Nutrient agar and broth	24
7.	<i>Bacillus subtilis</i>	3160	Nutrient agar and broth	24

All the agars and broth medium are of HI-MEDIA.

Antibacterial activity by disc diffusion assay

Antibacterial activity of 4 extracts i.e. aqueous, petroleum ether, chloroform and acetone were determine by paper disc diffusion method^{10, 11}. Sterilized Whatman filter paper no. 1 discs of 5 mm diameter were soaked in respective 200 mg/ml extract solution. 0.2 ml inoculums of test organism was spread on surface of respective bacterial agar plates. Previously soaked discs were placed on surface of inoculated plates. Ciprofloxacin is used as positive control and water, DMSO was used as negative control. Bacterial plates were initially transferred to refrigerator for 40-45 min to allow diffusion and then transferred to incubator set at 37° c. and incubated for given incubation period. All the tests were performed in triplicates and under the sterile condition. Zone of inhibition in mm were measured from edge of disc after incubation.

Analysis of data

% Zone of inhibition

% Zone of inhibition of 4 extracts of plant *Merremia emerginata* leaves against seven bacterial strains were calculated by formula-

$$\% \text{ Zone of inhibition in mm} = \frac{\text{Zone of inhibition of experimental plant extract in mm}}{\text{Zone of inhibition of positive control (standard drug) in mm}}$$

Statistical analysis

Data obtained was subjected to two way ANOVA test.

Phytochemical analysis

RESULTS

The results for zone of inhibition against test organisms (oral bacteria) were observed for all the four extracts of leaves of *Merremia emerginata* (aqueous, pet. ether, chloroform and acetone extract). Percent zone of inhibition was calculated by comparing zone of inhibition of plant extract with the zone of inhibition of standard drug used (Ciprofloxacin).

Table 2 depicts the result of Zone of inhibition of 4 extract of *Merremia emerginata* leaves against test microorganisms. From the results, it is observed that acetone extract showed maximum inhibitory action against the test microorganism i.e. 24% for *Staphylococcus epidermidis*, 20% for against *Lactobacillus rhamnosus*, 11.11% for against *Streptococcus mutans*, 3.57% for *Escherichia coli* and 1.66% for *Staphylococcus aureus*. Although all test organism from the present study were found to be affected by the four types of extract of *Merremia emerginata* leaves but pronounced inhibition of all the extract was observed for bacterial species *Lactobacillus rhamnosus*. Minimum inhibitory action of all extracts was found against *Staphylococcus aureus* and no antibacterial effect was found against *Bacillus subtilis* and *Actinomyces viscosus*. Two way ANOVA test applied to the present data, revealed that four extracts of *Merremia emerginata* leaves are equally effective where as bacterial strains showed varied zone of inhibition at 5% level of significance.

Table 2: Zone of inhibition of 4 extract of *Merremia emerginata* leaves against test microorganisms

Plant and control	L.r	S.m	S.e	E.c	S.a
Aqueous extract zone of inhibition in mm					
<i>Merremia emerginata</i>	5	1	3	0.7	0.5
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	20%	12%	5.5%	2.5%	1.6%
Petroleum extract zone of inhibition in mm					
<i>Merremia emerginata</i>	5	1.5	2.1	1	0.5
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	20%	8.3%	8.4%	3.5%	1.7%
Chloroform extract zone of inhibition in mm					
<i>Merremia emerginata</i>	5	1	2.1	1	0.5
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	20%	5.5%	8.4%	3.5%	1.7%
Acetone extract zone of inhibition in mm					
<i>Merremia emerginata</i>	5	2	6	1	0.5
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	20%	11.1%	24%	3.5%	1.7%

L.r- *Lactobacillus rhamnosus*, S.m-*Streptococcus mutans*, S.e- *Staphylococcus epidermidis*, E.c- *Escherichia coli*, S.a- *Staphylococcus aureus*.

Table 3: Phytochemical analysis of *Merremia emerginata* L. leaves.

Test	Inference
Alkaloid	++
Anthraquinones	-
Flavonoids	++
Simple Phenolecs	+++
Steroid	+
Tanine	++
Saponins	+++

Table 3 depicts the result of phytochemical analysis of *Merremia emerginata* leaves which shows the presence of Alkaloid, Flavonoids, Simple Phenolecs, Steroid, Tanine, and Saponins.

DISCUSSION

Numerous antibacterial screening has been performed with respect to location of microorganism such as skin infections, uterine infections etc. As oral bacterial infection are linked with various chronic diseases, screening of antibacterial activity of medicinally important plant against bacteria found in oral cavity are also important. Various plants have being already evaluated for its

efficacy in oral hygiene. Antibacterial activity of different extracts of *J. regia* against oral micro flora and found that acetone extract was found to be more effective against oral micro flora¹². Similar results were observed in the present investigation where acetone extract of *Merremia emerginata* leaves was found to be more effective.

The in vitro antibacterial activity of *P. alba* against Gram positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative *Escherichia*

coli, *Pseudomonas aeruginosa*, *Salmonella typhi* was carried out and efficient result was observed against *Staphylococcus aureus* and *Bacillus subtilis*¹³. Similar study was conducted using solvent extract of *Merremia emerginata* leaves.

An antimicrobial activity of acetone and ethanol extract of stem, root of *Terminalia glaucescens* and reported that ethanol extract exhibited significantly higher effect¹⁴. Antibacterial potential of aqueous decoction of *Piper nigrum L.*, *Laurus nobilis L.*, *Pimpinella anisum L.* and *Coriandrum sativum L.* against 176 bacteria isolated from oral cavity of 200 individuals has been carried out¹⁵. An ethanol extract of eight plant species used traditionally in South Africa were investigated against oral pathogens such as *Actinomyces* and *Candida* species and got resistance activity¹¹. The curative properties of medicinal plants are perhaps due to the several aromatic compounds or secondary metabolites of plants which are serve as defense mechanism against predation of many microorganisms, insects and herbivores etc. Specifically phenolic compounds are known for antimicrobial activity¹⁶.

This study has confirmed antimicrobial potential of the plant *Merremia emerginata*, thus supporting its folklore application as preventive remedy against oral microbial diseases. The present investigation is an attempt to give herbal products against the drugs used today.

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