

## ASSESSMENT OF PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *Barleria prionitis* LEAVES AGAINST ORAL MICROFLORA TO IMPROVE DENTAL HYGIENE

P.D. DIWAN<sup>1</sup> AND Y.A.GADHIKAR\*<sup>2</sup>

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

Received:13 February 2012, Revised and Accepted:14 April 2012

### 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 *Barleria prionitis* (Family-Acanthaceae) 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. Similarly leaves of *Barleria prionitis* were evaluated for its phytochemical composition. 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 almost all the test organism from the present study were found to be effected by all the four type of extract of *Barleria prionitis* leaves but pronounced inhibition of the four extract was observed for bacterial species, *Lactobacillus rhamnosus*. Chloroform extract was found to be more effective against the entire test microorganism.

**Keywords:** *Barleria prionitis*, 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 sticks Almas and Al Lafi (1995). Studies by Danielsen et al. (1989), Aderinokun et al. (1999) and Almas and Al Zeid(2004) have demonstrated chewing sticks are at least as effective as toothpaste in maintaining oral hygiene. Sathananthan et al. (1996) reported that Africans that use chewing sticks have fewer carious lesions than those that use toothbrushes. In a related development, Enwogu (1997) reported that chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes; these advantages of the chewing sticks over the conventional toothpaste and brushes have been attributed to the strong teeth of Africans Ugoji et al. (2000).

The vast biodiversity of Indian forest provides several plants which are mention in Ayurveda for dental care. Ganesan S. (2008) reported root of plant species *Barleria prionitis* L. used against toothache and dentrifices. Hence in the present investigation antibacterial activity of aqueous, pet ether, chloroform and acetone extract of leaves of *Barleria prionitis* (Family -Acanthaceae) was carried out against seven bacterial species which are known to cause oral diseases in human being. *Barleria prionitis* L. is commonly known as Porcupine flower, Barleria, Vajradanti, Kundan, Mulla goranti, Pilikantashelio, etc. It is a herb common in India. *Barleria prionitis* has numerous medicinal properties including treating fever, respiratory diseases,

toothache, joint pains and a variety of other ailments; and it has several cosmetic uses. A mouthwash made from root tissue is used to relieve toothache and treat bleeding gums. The leaves are used to promote healing of wounds and to relieve joint pains and toothache. Tribals of Melghat region use this plant species for medicinal purpose.

### MATERIAL AND METHOD

#### Plant collection and identification

Leaves of *Barleria prionitis* L. 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 Soxhlet 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.

#### 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 of Bauer et al. (1966) as described by More G. et al.(2008). 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<sup>o</sup> 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.

Table 1: List of Bacterial Strains

S.N	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

#### Analysis of data

##### % Zone of inhibition

% Zone of inhibition of 4 extracts of *Barleria prionitis* L. plant 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 *Barleria prionitis* L. (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: Zone of inhibition of 4 extract of *Barleria prionitis* L. leaves against test microorganisms

Plant and control	L.r	S.m	S.e	E.c	S.a
<b>Aqueous extract zone of inhibition in mm</b>					
<i>Barleria prionitis</i>	5	0	1.5	0.1	0
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	20%	0%	6%	0.35%	0%
<b>Petroleum extract zone of inhibition in mm</b>					
<i>Barleria prionitis</i>	5	0.5	3	0.3	0
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	20%	2.77%	12%	1.07%	0%
<b>Chloroform extract zone of inhibition in mm</b>					
<i>Barleria prionitis</i>	10	0.5	2	0.3	0.5
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	40%	2.77%	8%	1.07%	0.33%
<b>Acetone extract zone of inhibition in mm</b>					
<i>Barleria prionitis</i>	10	0	1.1	1.1	0.1
Standard drug (Ciprofloxacin)	25	18	25	28	30
% zone of inhibition	40%	0%	4.4%	0.35%	0.33%

Table 2 depicts the result of Zone of inhibition of 4 extract of *Barleria prionitis* L. leaves against test microorganisms. From the results, it is observed that chloroform extract showed maximum inhibitory action against the test microorganism i.e. 40% for against *Lactobacillus rhamnosus*, 8% for *Staphylococcus epidermidis*, 2.77% for against *Streptococcus mutans*, 1.07% for *Escherichia coli* and 0.33% for *Staphylococcus aureus*. Although all test organism from the present study were found to be affected by the four types of

extract of *Barleria prionitis* L 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 *Barleria prionitis* L. leaves are equally effective where as bacterial strains showed varied zone of inhibition at 5% level of significance.

Table 3: Phytochemical analysis of *Barleria prionitis* L. leaves.

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

Table 3 depicts the result of phytochemical analysis of *Barleria prionitis* L. 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. Deshpande R. et al. (2011) evaluated antibacterial activity of different extract of *J. regia* against oral micro flora and found that acetone extract was found to be more effective against oral microflora. Similar results were observed in the present investigation where chloroform extract of *Barleria prionitis* L. leaves was found to be more effective.

Ogundiya M.O. et al. (2009) carried out antimicrobial activity of acetone and ethanol extract of stem, root of *Terminalia glaucescens* and reported that ethanol extract exhibited significantly higher effect. 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 out by Nazia M. et al. (2006). More G. et al. (2008) studied 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 activity.

This study has confirmed antimicrobial potential of the plant *Barleria prionitis* L., 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.

## REFERENCES

1. Aderinokun GA, Lawoyin JO and Onyeano CO. Effect of two common Nigerian chewing sticks on gingival health and oral hygiene. *Odontostomatol Trop*. 1999, 22:13-18.
2. Almas K and Al Lafi TR. The natural toothbrush. *World Health Forum*. 1995, 16: 206-210.
3. Almas K and Al-Zeid Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: a clinical study. *J. Contemp. Dent. Pract.* 2004,5:105-114.
4. Bauer AW, Kirby WM, Sherris JC and Turck M. *Am. J. Clin. Pathol.* 1966, 45(4): 493- 496.
5. Danielsen B, Baelum V and Manji F. Chewing sticks, toothpaste and plaque removal. *Acta Odontol Scand.* 1989, 47:121-125.
6. Deshpande RR, Kale AA, Ruikar Ad, Panvalkar RS, Kulkarni AA, Deshpande NR and Salvekar JP. Antimicrobial activity of Different extract of *Juglans regia* L. against oral microflora. *Int J Pharm Pharm Sci.* 2011, 3(2):200-201.
7. Enwogu CO. Socio-economic factors in the dental caries prevalence and frequency. *Nigerian caries research.* 1997, 8: 155-177.
8. Ganesan S. Traditional oral care medicinal plants survey of Tamil Nadu. *J. Natural product radiance.* 2008, 7 (2):166-172.
9. More G, Shikalangea TE, Lall N, Bontha F. and Meyer JM. Antimicrobial activity of medicinal plants against oral microorganisms. *Journal of Ethno pharmacology.* 2008,119:473-477.
10. Nazia Masood AC and perween T. Bactericidal activity of Black paper, Bay leaf, Aniseed and Coriander against oral isolates. *Pak. J. Pharm. Sci.* 2006, 19 (3):214-218.
11. Ogundiya MO, Kolapo AL, Okunade MB. and Adejumbi JA. Assessment of Phytochemical composition and antimicrobial activity of *Terminalia glaucescens* against oral pathogens. *EJEAF Che.* 2009, 8 (7):466-471.
12. Sathananthan K, Vos T and Bango G. Dental caries, fluoride levels and oral hygiene practices of school children in Matebeleland South Zimbabwe. *Community Dent. Oral Epidemiol.* 1996, 24:21-24.
13. Ugoji E, Egwari, LO and Obisesan B. Antibacterial activities of aqueous extracts of ten African chewing sticks on oral pathogens. *Nig. Journal of Internal Medicine.* 2000, 3 (1):7-11.