

Potency of *Barleria prionitis* L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin

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Abstract: Oral health is integral to general well being and relates to the quality of life that extends beyond the functions of craniofacial complex. Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. We report in this work for the first time, the potent antifungal activity of *Barleria prionitis* L. bark on two *Candida albicans* strains and *Saccharomyces cerevisiae*, involved in oral diseases of human. Acetone, methanol, ethanol, aqueous (hot and cold) extracts of *Barleria prionitis* bark were screened for *in vitro* activity against four oral bacteria *Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas sp.*, *Bacillus sp.* and three oral fungi *C.albicans* strain 1, *C.albicans* strain 2 and *S.cerevisiae*. This plant was selected due to its traditional use for the treatment of oral infections. Three clinical strains namely *Pseudomonas sp.*, *Bacillus sp.* and *Candida albicans* strain 2 were isolated from dental caries affected patients. The antimicrobial activity of *B.prionitis* extracts on the agar plates varied in different solvents. The methanolic bark extract of *B. prionitis* was the most effective against all the four oral bacteria and the three oral fungi. *Bacillus sp.* was found to be the most sensitive pathogen which survived upto 12.5mg/ml, thus having an MIC of 25mg/ml. The antimicrobial potential of *B.prionitis* bark against *Bacillus sp.* was comparable with the standard antibiotic drug, the positive control, ciprofloxacin which produced a 29.65mm inhibition zone. Interestingly the methanolic extract of *B.prionitis* bark showed much more potent activity against all the tested oral fungi namely *S.cerevisiae*, *C.albicans* strain 1 and *C.albicans* strain 2, than the standard drug amphotericin –B thus having a great potential to control candidiasis and other oral fungal infections. [New York Science Journal 2010;3(11):5-12]. (ISSN: 1554-0200).

Key words: Oral pathogens; *Barleria prionitis*; antimicrobial activity; zone of inhibition; Minimum Inhibitory Concentration (MIC)

1. Introduction

Oral health means more than just good teeth, it also means being free from chronic pain in the mouth or in the facial region, the absence of oral or throat cancer, oral sores, birth defects such as cleft lip and palate, freedom from periodontal disease, tooth decay, and tooth loss, or many other diseases that affect the mouth and oral cavity (Patro et al., 2007). Oral health is integral to general well being and relates to the quality of life that extends beyond the functions of craniofacial complex (Palombo, 2009). There is evidence to prove the interrelationship between oral and general health. Many general health conditions have oral manifestations that increase the risk of oral disease which, in turn, is a risk factor for

many systemic diseases, such as diabetes, cardiovascular diseases etc. However the wider meaning of oral health does not diminish the relevance of the two leading oral afflictions: dental caries and periodontal disease, historically considered an important component of the global disease burden (Patro et al., 2007). Dental caries is a common, complex, chronic disease that results from an imbalance between multiple potential etiological (risk) factors and multiple protective factors over time (Featherstone, 2000; Fejerskov, 2004). Ultimately, this disease process can cause loss of tooth structure through demineralization or cavitation (Crall, 2006). It is the second most common disease in our community after the common cold. It affects more than 98 percent of the world population,

regardless of age, gender, ethnicity and place of residence (Hicks et al., 2004; Tatintcyan, et al., 1997; Wallace and Last, 1992).

Antibiotics have been called miracle drugs, but 60 years of use, underuse and overuse have resulted in increasingly frequent resistance in a growing number of antibiotic-bacteria combinations (DiazGranados et al., 2008; Overbye and Barrett, 2005). The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances' (Wise, 2008). Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe (Clark, 1996). Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. They are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999; Lewis and Ausubel, 2006). The chemical constituents present in plants are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Kamboj, 2000). They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Plant derived medicines have been the first line of defense in maintaining health and combating diseases. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment (Cragg et al., 1997). The World Health Organization (WHO) estimates that 4 billion people (80% of the World's population) use herbal medicines for some aspect of primary healthcare. These evidences contribute to support and quantify the importance of screening natural plants.

Barleria prionitis L. (Family *Acanthaceae*; commonly known as Vajradanti) is an annual shrub, 1–3 feet high, found throughout Africa, India, Sri Lanka and tropical Asia (Ata et al., 2007). In indigenous system of medicine in India, the aerial parts (stem, leaves & flower) are used in catarrhal affections of children, glandular swellings, boils, fever, toothache, inflammation & gastrointestinal disorders; bark in whooping cough as an expectorant; the whole plant and especially the roots are used as tonic and diuretic (Chopra et al., 1956; Nadkarni, 1994; Kirtikar and Basu, 2000; Parrotta, 2001; Mohammed et al., 2004; Kala, 2005; Ediriweera 2007; Ganesan, 2007). Leaves, stem and root of *B. prionitis* possess antibacterial and anti-inflammatory

activities (Singh et al., 2003; Amoo et al., 2009). Iridoid enriched fraction of aerial parts (leaves and stems) was reported for hepatoprotective activity in various acute and chronic animal models (Singh et al., 2005; Jaiswal et al., 2010). The plant showed biological activity against respiratory syncytial virus (Chen et al., 1998) and has also been reported as anti-arthritic, anti-inflammatory and antifertility agent (Gupta et al., 2000). The aqueous bioactive fractions are reported to possess hepatoprotective, antistress, and immunorestorative properties (Suri et al., 2003). Since *Barleria prionitis* has been used traditionally in medicine, the biological evaluation of this plant may lead to the development of safer therapeutic agents. The present communication therefore has been designed to assess the potency of *Barleria prionitis* bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin.

2. Materials and Methods

Bark of *Barleria prionitis* was collected from the local market of Delhi, India. Dr. B.D.Vashishta (Botany Department) Kurukshetra University, Kurukshetra confirmed the identification of the specimens.

Extraction

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (40°C) for five days and pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) often used for the extraction of plant material were used for extraction (Cowan, 1999). A 10g amount of pulverized bark was separately soaked in 100ml of acetone, ethanol, methanol, and cold sterile distilled water for 24h. Also the same amount (i.e. 10g) of pulverized bark was immersed in 100ml of hot sterile distilled water (100°C) and allowed to stand for 30min on a water bath with occasional shaking and kept undisturbed for 24h. Each preparation was filtered through a sterilized Whatman No.1 filter paper and the filtered extract was concentrated under vacuum below 40°C using Heidolph, VE-11 rotaevaporator (Ogundiya et al., 2006; Bag et al., 2009). The dried extract thus obtained was exposed to UV rays for 24h and checked for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4°C until further use (Aneja and Joshi, 2009a).

Test Microorganisms

Clinical strains of *Pseudomonas sp.* (MTCC 10094), *Bacillus sp.* (MTCC 10095) and *Candida albicans* strain 2 (MTCC 10035) were isolated from dental caries affected patients, identified and characterized by conventional biochemical methods. Two oral pathogenic bacteria *S.mutans* (MTCC 497), *S.aureus* (MTCC 740) and two oral pathogenic yeasts *C.albicans* strain 1 (MTCC 227) and *S.cerevisiae* (MTCC 170) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh. The microorganisms were subcultured on the specific media recommended for different microorganisms such as Brain heart infusion agar (*S.mutans*), Nutrient agar (*S.aureus*, *Pseudomonas sp.*, *Bacillus sp.*), Malt yeast agar (*C.albicans* strain 1, strain 2 and *S.cerevisiae*) and incubated aerobically at 37°C. The media were procured from HiMedia Laboratory Pvt. Ltd., Bombay, India. Identification of all the strains was confirmed by standard biochemical and staining methods (Cappuccino and Sharman, 1995; Aneja, 2003; Benson, 2004).

Screening for Antimicrobial Activity

Antimicrobial activity of the five extracts of *Barleria prionitis* bark was determined by following the agar well diffusion method of Okeke (2001). In this method, pure isolate of each microbe was subcultured on the recommended specific media for each microorganism at 37°C for 24h. A plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10⁶cfu/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay. One hundred microlitre (100µl) of inoculum of each test organism was spread onto the specific media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium (Nkere and Iroegbu, 2005). The dried bark extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) for the bioassay analysis (Okeke et al., 2001). A 100µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated specific media agar plates for each test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24h (Khokra et al., 2008; Rajasekaran et al., 2008). Sterile DMSO served as the negative control and

ciprofloxacin (for bacteria) and amphotericin-B (for fungi) served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with \pm standard deviation were calculated (Aneja et al., 2009, 2010).

Determination of Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h (Aneja et al., 2009). The MIC for the acetonetic, methanolic and ethanolic bark extracts was determined by following the modified agar well diffusion method (Cappuccino and Sherman, 1995). A twofold serial dilution of each extract was prepared by first reconstituting the bark extract in 20% DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the specific media agar plates already seeded with 100µl of standardized inoculum (10⁶cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 hrs and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition (>8mm) (in triplicates), considered as the MIC, was recorded for each test organism (Aneja and Joshi, 2009b).

Statistical Analysis

The results are presented as mean \pm SD (Standard deviation). One way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons were used for statistical evaluation. P values less than 0.05 were considered significant.

3. Results and Discussion

The three clinical isolates from dental caries patients showed different morphological and biochemical characteristics. One isolate formed circular colonies with entire margin, slightly raised, opaque, moist surface, showing very light green pigmentation, the cells were Gram negative short rods with a size of 0.5-1 µ and endospore absent, positive catalase test, weak positive oxidase test and negative nitrate utilization test. This colonial type was identified as *Pseudomonas sp.* on the basis of morphological, cultural and biochemical tests for its

identification at the IMTECH, Chandigarh. It was assigned an MTCC No. 10094. Another isolate formed similar circular colonies with entire margin, slightly raised, opaque, moist surface, but there was no green pigmentation instead they were cream coloured. On Gram's reactivity, they were Gram positive rods with a size slightly larger (2-6 μ) than the former isolates and presence of endospores which were subterminal, oval and slightly bulging sporangia. This isolate was identified as *Bacillus sp.* on the basis of identification tests done at the IMTECH, Chandigarh and assigned an MTCC No. 10095. The yeast species formed soft cream coloured colonies with a yeasty odour when grown under aerobic conditions on media having a pH of 7.0 and an incubation temperature of 37°C. Growth was usually detected in 24-48h and subcultures grew more rapidly. The ability of yeasts to grow at 37°C is an important characteristic to be considered for identification from clinical samples because most pathogenic species grow readily at 25°C and 37°C, whereas saprophytes usually fail to grow at higher temperatures. The gross microscopic appearance showed Gram positive large sized colonies. The

isolate was identified as *Candida albicans* strain 2 on the basis of identification tests done at the IMTECH, Chandigarh and assigned an MTCC No. 10035.

The results of antimicrobial potency of ethanol, methanol, acetone and aqueous (hot and cold) bark extracts of *Barleria prionitis*, the positive control ciprofloxacin (for bacteria) and amphotericin-B (for fungi) and the negative control (DMSO) are presented in Table 1 and values of MIC of these extracts against the test pathogens are presented in Table 2. The antimicrobial activity of *B.prionitis* extracts on the agar plates varied in different solvents. Both the positive controls produced significantly sized inhibition zones against the test bacteria (ciprofloxacin) and yeasts (amphotericin-B). However, the negative control produced no observable inhibitory effect. Of the five bark extracts of *B.prionitis*, screened for antibacterial and antifungal activity, acetone, methanol and ethanol showed antibacterial and antifungal activity against all the tested oral pathogens. However, water extracts, both hot and cold, showed no activity against the test strains (Table 1).

Table 1. Antimicrobial activity of *Barleria prionitis* bark extracts against dental caries causing oral pathogens by agar well diffusion method

<i>Barleria prionitis</i> extracts (mg/ml)	Diameter of growth of inhibition zones (mm)						
	<i>Streptococcus mutans</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas sp.</i>	<i>Bacillus sp.</i>	<i>Saccharomyces cerevisiae</i>	<i>Candida albicans</i> strain1	<i>C.albicans</i> strain 2
Acetone	14.95*±1†	14.31±0.57	18.32±0.57	27.32±0.57	11.64±0.57	13.65±0.57	16±0
Ethanol	11.94±1	14.0±0	17.65±0.57	23.97±1	11.31±0.57	12.94±1	11.31±0.57
Methanol	15.65±0.57	16.32±0.57	19.32±0.57	28.65±0.57	13.95±1	15.31±0.57	16.96±1
Hot aqueous	-	-	-	-	-	-	-
Cold aqueous	-	-	-	-	-	-	-
ciprofloxacin	27.32±0.57	34.66±0.57	33.66±0.57	29.65±0.57	Nt	Nt	Nt
amphotericin-B	Nt	Nt	Nt	Nt	11.94±1	13±0	12.94±1
DMSO	-	-	-	-	-	-	-

(-) = no activity, Nt = not tested, * Values, including diameter of the well (8 mm), are means of three replicates, † ± Standard deviation.

A perusal of the data (Table 1) reveals that the methanolic bark extract of *B. prionitis* was the most effective against all the four oral bacteria and the three oral fungi. Among the tested bacteria it showed the highest zone of inhibition against *Bacillus sp.* (28.65mm) followed by *Pseudomonas sp.* (19.32mm), *Staphylococcus aureus* (16.32mm) and *Streptococcus mutans* (15.65mm). Among the tested fungi, *B.prionitis* showed the maximum zone of inhibition against *Candida albicans* strain 2 (16.96mm) followed by *C.albicans* strain 1 (15.31mm) and *Saccharomyces cerevisiae* (13.95mm), in the methanolic bark extracts. The inhibition zones produced by the methanolic extract were followed by acetone and ethanol extracts ranging between 11.94mm and 27.32mm against the tested bacteria and between 11.31mm and 16mm against the tested oral fungi. *Bacillus sp.* was found to be the most sensitive pathogen which survived upto 12.5mg/ml, thus having an MIC of 25mg/ml (Table 2).

Table 2. MIC of *Barleria prionitis* bark extracts against dental caries causing oral pathogens by modified agar well diffusion method

<i>Barleria prionitis</i> extracts	MIC (mg/ml)						
	<i>Streptococcus mutans</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas sp.</i>	<i>Bacillus sp.</i>	<i>Saccharomyces cerevisiae</i>	<i>Candida albicans</i> strain 1	<i>C.albicans</i> strain 2
Acetone	100	100	100	50	100	100	100
Ethanol	100	100	100	50	100	100	100
Methanol	100	100	100	25	100	100	100

The antimicrobial potential of *B.prionitis* bark against *Bacillus sp.* was comparable with the standard antibiotic drug, the positive control, ciprofloxacin which produced a 29.65mm inhibition zone. Interestingly the methanolic extract of *B.prionitis* bark showed much more potent activity against all the tested oral fungi namely *S.cerevisiae*, *C.albicans* strain 1 and *C.albicans* strain 2, than the standard drug amphotericin –B thus having a great potential to control candidiasis and other oral fungal infections. The absence of antibacterial and antifungal activity in the aqueous extracts of *Barleria prionitis* might either be due to the more solubility of the active principles in analytical solvents than the aqueous solvents (Nkere and Iroegbu, 2005; Parekh and Chanda, 2007) or presence of active components in insufficient quantities in the crude extracts to show the activity with the dose levels employed (Taylor et al., 2001).

The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Cowan, 1999). The antimicrobial potency of *Barleria prionitis* may be due to the presence of five iridoid glucoside esters, acetylbarlerin (6, 8-di-O-acetyl shanzhiside methyl ester), barlerin (8-O-acetyl shanzhiside methyl ester), shanzhiside methyl ester and 6-O-acetyl shanzhiside

methyl ester, verbascoside (6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester) (Chen et al., 1998; Suri et al., 2003). It is interesting to note that even crude extracts of *Barleria prionitis* showed good activity against dental caries causing oral pathogens where modern antibiotic therapy has failed. It may, therefore, be concluded from the above investigation that the crude extracts obtained from the bark of the *Barleria prionitis* may be used to treat the bacterial oral infections caused by *Bacillus sp.* which has shown comparable inhibition zone with the standard antibiotic drugs used to treat oral infections and the fungal oral pathogens especially *Candida albicans* and *Saccharomyces cerevisiae* which has shown greater inhibition zones than the antifungal drugs often used to treat fungal pathogens. However, isolation of pure compound and their toxicological study and clinical trials in animal model are to be made before their trials on human.

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