



## Anticandidal effect of extract of *Bridelia stipularis*

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### Abstract:

Medicinal and aromatic plants are gift of nature and are being used against various infections and diseases in the world since ages. Species of the genus *Bridelia stipularis* var *scandens* is reported to be used traditionally for treating various oral diseases. However, the antimicrobial effect of these plant materials against oral pathogens is not proved. Therefore, we have done the present study. Aim is to find out the anticandidal effect of water extract of *Bridelia stipularis* against four common oral candidal pathogens. The leaves after identification and authentication by a botanist were collected, air dried, pulverized to fine powder using household blender. The water extract was prepared using cold percolation method. The standard *Candida* species, *Candida albicans*, *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis* obtained from Post Graduate Institute (PGI), Chandigarh was procured. Antifungal activity was determined by Kirby Bauer well diffusion method and Time kill assay.

All four species of *Candida* showed variable results with diameter of zone of inhibition ranging from 12mm to 21mm on Sabouraud's dextrose agar with both 6 hour and 24 hour peptone water subculture. Time kill assay showed inconsistent results even after 24 hours of exposure with the crude extract of *Bridelia stipularis*. All four species of *Candida* causing oral infections are moderately sensitive to crude water extract of dried leaves of *Bridelia stipularis*.

**Key words:** *Bridelia stipularis*, *Candida* species, antifungal activity

### Introduction:

Herbal medicine has been known to man for centuries. The therapeutic efficacies of many indigenous plants for several disorders have been described by the practitioners of traditional medicine. Natural products currently are the leading source in the search for new biologically active compounds. It has been well documented that traditional medicinal plants confer considerable antibacterial activity against various microorganisms. Many plants were reported to inhibit the growth of many oral bacteria particularly *Streptococcus mutans* and control plaque and thus prevent caries. Use of plant based alternatives for oral health has been successfully promoted; for example, the use of antibacterial chewsticks (meswak) have been widely advocated by health agencies where their use is culturally acceptable.

Use of medicinal plants for routine cleaning of teeth and treating various oral diseases has long been a part of Indian culture, and Karnataka has its own tradition.<sup>1,2</sup> Species of the genus *Bridelia* belonging to the family Euphorbiaceae, are commonly seen in various parts of Indian subcontinent and is reported to be used traditionally for treating dental decay and related pain by people of Dakshina Kannada.<sup>3</sup> Previous studies have revealed that this plant has many properties such as antibacterial effects against various gram positive and gram negative organisms.<sup>4,5,6</sup> However, the beneficial effects of this plant materials against oral pathogens is not evaluated. Therefore, we have done the present investigation to examine the antimicrobial activities of extract of leaves

of *Bridelia stipularis* on various *Candida* species.

## Materials & Methods:

### Selection of plant

The leaves were collected from the local wild plants and were identified and authenticated by a botanist (Figure I).



Figure I: Leaves of *Bridelia stipularis*

### Preparation of extract

The collected leaves were washed, air dried at room temperature for two weeks and pulverized to fine powder using house hold blender and were stored in air tight bottles till further use (Figure II).

The aqueous extract was prepared by cold percolation method ( keeping the bottle for 48 hours at room temperature with frequent shaking (Figure III).

Two concentrations were prepared -5gm of powder suspended in 50 ml of distilled water (100 mg/ml), and 2.5 gm of powder in 50 ml of distilled water (50 mg /ml), filtered, centrifuged at 2000rpm for 10 minutes. Extracts were stored in bijou bottle at 4°C.

Four standard species of *Candida* (obtained from PGI Chandigarh) – *Candida albicans* (ATCC 90028), *Candida tropicalis* (ATCC 750), *Candida*

*glabrata* (NCCPF 100018) and *Candida parapsilosis* (ATCC 22019) were tested.

6 hours and 24 hours peptone water culture of *Candida* were used.



Figure II: Powder of dried leaves of *Bridelia stipularis*



Figure III: Extract of leaves prepared by cold percolation method

### In-vitro studies on antimicrobial activity of plant extracts

Antimicrobial activity was determined by Kirby Bauer agar well diffusion technique.<sup>7</sup> The inoculum size of candidal culture was standardized according to the National Committee for Clinical Laboratory Standards.<sup>8</sup> The peptone water culture incubated for 6 hours as well as 24 hours were swabbed on separate agar plates. Wells were charged with extracts (100mg/ml and 50mg/ml).The plates with fungal growth were incubated at 37°C for 48 hours. The antimicrobial activity was

evaluated by measuring the diameter of zone of inhibition expressed in millimeters against the test pathogens (**Figure IV and V**). Chlorhexidine disc was used as positive control and blank disc (impregnated with solvent) as negative control.

The effective plant extract was further subjected for determination of MIC by broth dilution method.<sup>7,8</sup> The inhibitory effect of the extract was studied by Time kill assay<sup>9</sup> and determination of Minimum Inhibitory Concentration (MIC). In Time kill assay<sup>8</sup>, the inoculum containing approximately  $5 \times 10^7$  cfu /ml was introduced into the Mueller Hinton broth containing various extracts and incubated at 37°C. 500µl sample was removed from culture at 6, 12, 18 and 24h, diluted serially and 100µl of the diluted samples were inoculated on Mueller Hinton agar and Sabouraud's dextrose agar plate, incubated at 37°C for 24hours. Control included extract free Mueller Hinton broth seeded with the test inoculum. Viable counts were calculated to give cfu/ml. There was not much difference in test as well as control plate.

MIC determination was performed by broth microdilution method in microtiter plates which were inoculated with  $0.5 \times 10^3$ /ml of *Candida* isolates and incubated at 30°C. Cell density per plate was measured after 48 hours of incubation. MIC was defined as the lowest concentration of extract that inhibits the growth.<sup>7</sup>

### Results:

All four *Candida* species showed varied results in their susceptibility with diameter of zone of inhibition ranging from 12-21mm with both the concentrations of extract as well as in different period of incubation (**Table I and II**). *Candida albicans* showed variable results on plates swabbed with 6 hour peptone water culture in both the concentration of extract. *Candida parapsilosis* showed no growth. There was not much difference in zone size of all *Candida* species with both the concentration of extract. Determination of Minimum Inhibitory Concentration (MIC) and Time kill assay (**Figure VI**) even after 24 hours of incubation showed inconsistent results.

**Table I: Antimicrobial activity of extract (100mg/ml) against *Candida* species**

Candida Species	Zone of Inhibition ( 100mg/ml) (5gm+50 ml)	
	6 hours	24 hours of incubation
<i>Candida albicans</i>	Variable	20 mm
<i>Candida tropicalis</i>	18 mm	20 mm
<i>Candida glabrata</i>	12 mm	15 mm
<i>Candida parapsilosis</i>	No growth	21 mm



**Figure IV: Zone of inhibition with 100mg /ml of extract after 6 hours of incubation**



**Figure V: Zone of inhibition with 100mg/ml of extract after 24 hours of incubation**

**Table II: Antimicrobial activity of extract(50mg/ml) against Candida species**

Candida species	Zone of Inhibition ( 50 mgm/ml ) ( 2.5 gm + 50 ml)	
	6 hours	24 hours of incubation
<i>Candida albicans</i>	Variable	20 mm
<i>Candida tropicalis</i>	18 mm	18 mm
<i>Candida glabrata</i>	16 mm	18 mm
<i>Candida parapsilosis</i>	No growth	19 mm

**Figure VI: Time Kill Assay****Discussion:**

The antibacterial activity of *Bridelia stipularis* var *scandens* has been reported by many workers.

Hebbar et al covered ethnomedicine survey of plants used in oral care in Dharwad district, Karnataka, India. It was revealed that 35 plants belonging to 26 families are being used to treat different

types of oral ailments like toothache, plaque and caries, pyorrhea and aphthae. Sixteen of these plants were new for the treatment of oral ailments and not previously reported. *Basella alba*, *Blepharis repens*, *Capparis sepiaria*, *Oxalis corniculata* and *Ricinus communis* are used for the treatment of aphthae; *Azima tetraantha*, *Caesalpinia coriaria*, *Cleome gynandra*, *Gossypium herbacium*, *Leucas aspera*, *Merremia chryseides*, *Pergularia*

daemia, *Prosopis juliflora* and *Solanum nigrum* are used to treat tooth ache and *Cassia hirsuta* and *Cassia tora* are used in the treatment of plaque and caries.<sup>1</sup>

V H Harsha et al used 45 species of plants used by Kunabi community people based on an ethnomedical field survey. These plants were of 26 families and were used to treat a wide range of discomforts like fever, cough, skin diseases, rheumatism, snakebite, jaundice, dysentery, etc.<sup>2</sup>

Jose M et al reported information on various traditional oral hygiene practices and herbs used for oral health and disease by rural population. They had collected information on the medicinal plants which have been used traditionally for oral health and hygiene. They said that use of high potential medicinal plants will provide easy, cheap healthcare facilities to tackle the increasing rate of oral diseases especially tooth decay and pain.<sup>3</sup>

Dada-Adegabola et al conducted a study on anti-infective activity of crude aqueous extract obtained by boiling ripe stem-bark of *Bridelia ferruginea* benth. Zone of inhibition around extract ranged from 10 to 25 mm in diameter. Thus, the authors validated the activity both for fungal and bacterial agents of the crude extract obtained by boiling of the ripe stem bark of *B.ferruginea* as used traditionally.<sup>4</sup>

Kotigadde et al, conducted a study to evaluate the antibacterial activity of *Bridelia scandens* and had revealed that this plant has significant antibacterial activity against both gram positive and gram negative bacteria. They have shown that the leaves and fruits of *Bridelia scandens* have inhibitory effect on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri* and *Vibrio cholerae*. The authors have suggested that the pure form of extract may be active as antimicrobial agents at low concentrations compared to their observations as their findings were based on the crude extract.<sup>5</sup>

Adetutu et al concluded that the antimicrobial and relatively strong antioxidant activity lend some support to the topical use of *Bridelia ferruginea* leaf for wound healing in the traditional medicine. The effects of leaf extracts against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* showed that extracts had weak antibacterial activity.<sup>6</sup>

Anjum et al carried out analysis of antimicrobial properties of extracts of *Bridelia* species and showed moderate to strong activity against 13 gram positive and gram negative bacterial strains and three fungi. The activity of most of the test samples against *Candida albicans* was most significant with highest inhibition zone being 17.5mm.<sup>7</sup>

But, little work has been done on antimicrobial property on various *Candida* species. Nowadays, herbal drugs are being introduced to find out remedy for most of the diseases. This experiment showed that crude extract obtained by cold percolation method of dried leaves of *Bridelia stipularis* has anti-infective activity against *Candida* species. The bark and leaves of *Bridelia* species were mainly experimented for its other pharmacological activities like antioxidant, immunomodulatory and anti-inflammatory response. The present result showed that the extracts of leaves of *Bridelia stipularis* possess some chemical component which can act against *Candida* species like *Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*. Antimicrobial properties of these plants can be exploited further in the preparation of natural therapeutic agents against fungi. The findings of this study were based on the crude extract. In the pure form, antimicrobial component may be active at very low concentration. The present results raise some expectations on the usefulness of *Bridelia stipularis* as a source of antimicrobial agent. The various methods used for preparation of extracts,

estimation of antimicrobial activity by minimum inhibitory concentration and Time kill assay did not show consistent results which require standardization. Time kill assay has to be done in liquid medium which shows clearing of turbid broth rather than on solid medium. The antimicrobial sensitivity testing for *Candida albicans* and *Candida parapsilosis* should be done using 24 hour liquid culture.

### Conclusion:

The water extract of dried leaves of *Bridelia stipularis* is moderately effective against various *Candida* species which accounts for 80% of oral lesions caused by it.

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