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Original Article

Antimicrobial Properties of Bridelia Scandens against Oral Pathogens: In vitro study

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Abstract

Objective: It has been well documented that traditional medicinal plants confer considerable antimicrobial activity against various microorganisms. Species of the genus Bridelia scandens is reported to be used traditionally for treating various oral diseases. Its effect against intestinal and other systemic pathogens have been reported However the beneficial effects of this plant materials against oral pathogens is not evaluated. Therefore we have done the present investigation. Aim is to study the antimicrobial properties of alcoholic and aqueous extract of Bridelia scandens against selected oral pathogens by various microbiological assays.

Methods: 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 and alcoholic extract was prepared in Soxhlet apparatus by using ethyl alcohol. The test organisms like Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius Lactobacillus acidophilus and Candida albicans were obtained from Department of Microbiology Yenepoya Medical College, Mangalore. Antimicrobial activity is being determined by various Microbial assays like Kirby Bauer antibiotic sensitivity testing, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)

Results: From our study, it was found that all tested organisms were sensitive to both water and alcoholic extract of leaves of Bridelia scandens at varying concentrations.(200 mg/ml and 400 mg/ml) with zone of inhibition ranging from 20 mm to 48 mm .Even the Minimum Inhibitory Concentration (MIC) was less than 100 mg/ml in most of the organism indicating that extract of leaves of Bridelia scandens is most effective against the oral pathogens. The alcoholic extract is better than aqueous extract.

Conclusions: The extract of leaves of Bridelia scandens is highly effective against selected oral pathogens

Introduction

Oral diseases such as periodontitis and caries have been known to afflict mankind since the dawn of history. In the present age they are of universal occurrence sparing only a few. An effective plaque control is essential for preventing the onset of periodontal diseases and caries and to prevent progression of this disease. The use of plants and plant products as medicines can be traced as far as the beginning of human civilization. A large proportion of the population of African countries still rely on the use of local herbs in management of many ailments ranging from surgical to medical either infectious or non-infectious, with different degree of success or claim of beneficial responses[1] Medicinal plants contain large varieties of chemical substances with important therapeutic properties that can be utilized in the treatment of human diseases.

The tribal and rural populations of India largely depend on medicinal plants for their health care. Several hundreds of plants the world over represent good sources of therapeutic agents, are used traditionally for different

purposes including treatment of bacteria, fungi and viral infections[2 3].Of about 2,50, 000 flowering plants in the world more than 50,000 are used for medicinal purposes[4]. It is widely accepted that the increased availability and the use of antibacterial and antifungal agents in recent years has resulted in the control and even eradication of diseases, but it has also led to the development of resistant strains.

The studies on medicinal plants used as folklore remedies have attracted immense attention in scientific world in an attempt to find possible solutions to the problems of multiple drug resistance due to existing conventional antibiotics.[5]The rate of development of resistance to both old and new drugs calls for active search for more effective as well as affordable anti-infective agents. This problem has prompted tremendous effort to explore for more potent antimicrobial agents especially of natural origin to contrast this resistance.

The therapeutic efficacies of many indigenous plants for several disorders have been described by 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 chew sticks have widely advocated by health agencies where their use is culturally acceptable. The revival of interest in plant derived drugs is mainly due to the current wide spread belief that 'green medicine 'is safe and more dependable than costly synthetic drugs many of which have adverse side effects. The need of the hour is to screen a number of medicinal plants for Promising biological activity. Literature reports and ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry. They may provide natural source of antimicrobial drugs Dakshina Kannada and though used in folk medicine for the treatment of rheumatism, gynaecological condition, vata, lumbago and hemiplegia, its medicinal properties are not elucidated particularly on oral pathogens. Previous studies have revealed that leaves of Bridelia species have many properties such as antibacterial effects against various gram positive and gram negative organisms[6 7]. However the beneficial effects of this plant material against oral pathogens are not evaluated.

Having known the indigenous effect of these leaves, it will be relevant to conduct a systemic study to discover scientific evidence on valuable effects of traditional methods. Research efforts must be directed towards finding cost effective solutions to treatment of various oral infections.

It has been reported that the various species of genus *Bridelia* has Medicinal properties [6 7 8].So we undertook this research work to see the antimicrobial properties of *Bridelia scandens* on oral pathogens that will provide novel compounds that may be employed in controlling some infections globally.[5]Species of genus *Bridelia* belonging to Euphorbiaceae ,are commonly seen in various parts of Indian subcontinent has various properties like antioxidant, anti-inflammatory, purgative, vermifuge, molluscidal activities and were used to treat dysentery, diarrhoea, diabetes, used for removal of urinary concretions and was taken internally for snake bite. It is also reported to be used traditionally for treating dental decay and related pain by people of Dakshina Kannada.

Materials and methods

Study Design

The leaves are collected from local wild plants and are identified and authenticated by a Botanist. The leaves were washed with distilled water to remove dirt and air dried for 5 days

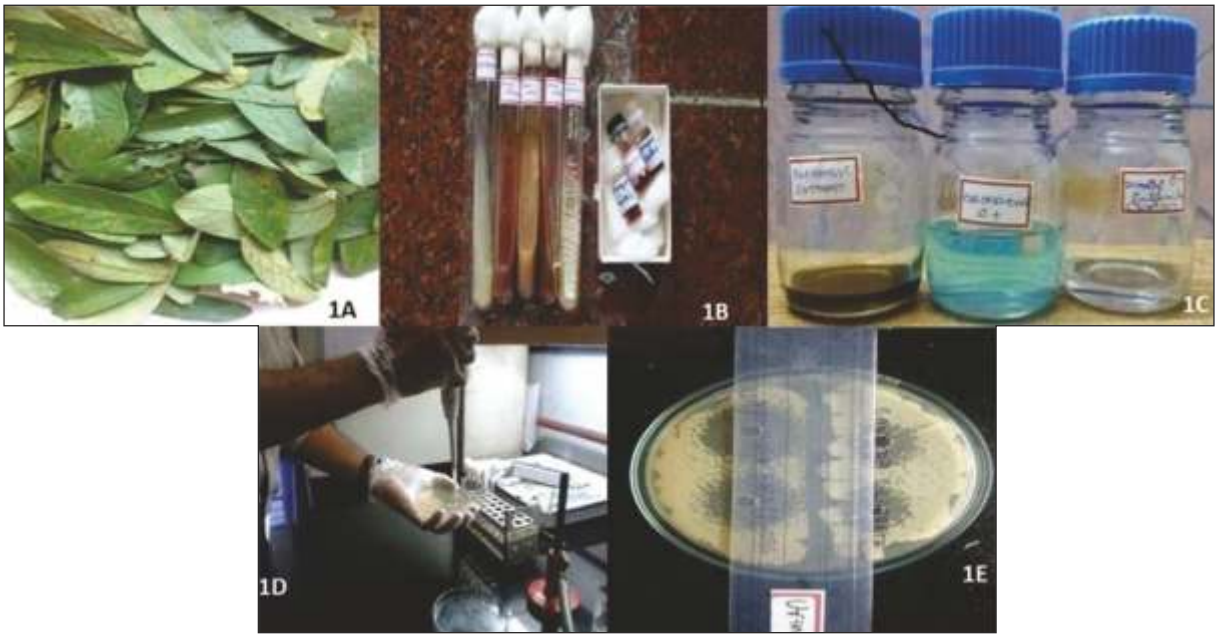

Fig 1 : material & methods, 1A-leaves of Bridelia scandens, 1B- stock culture of organism tested, 1C-Extract, positive control, negative control, 1D- charging wells with extracts, 1E- measuring the zone of inhibition

Preparation of Extracts[9,10]

Aqueous extract

Percolation method:-The leaves were air dried in sun for two weeks and pulverized to fine powder in household blender and stored in air tight bottles.5gm of powder was added to 100ml of distilled water in 250ml bottle. The bottle was kept at room temperature for 48 hours with frequent shaking .The supernatant was taken in a test tube and centrifuged at 2000rpm for 10 minutes .The supernatant in test tube was filtered and stored in sterile penicillin bottle till use.

Boiling method:-5gm of powder was added to 100ml of distilled water in 250 ml bottle. The bottle was kept in water bath and heated at 100° C for 5 minutes and supernatant was centrifuged at 2000rpm for 10 minutes and extract was collected and stored in sterile penicillin bottle.

Alcoholic extract

The collected leaves were air dried at room temperature for two weeks and pulverized to fine powder using household blender and were stored in air tight bottles till use .The alcohol extract was prepared using Soxhlet apparatus 250 ml of ethyl alcohol was taken in the flask of Soxhlet apparatus. 50 gm of powder of leaves of *Bridelia scandens* was taken in muslin cloth and put in the central tube of apparatus .Tap water was allowed to pass continuously through the condenser of apparatus.

The extract in alcohol was obtained after four cycles. This extract was taken in a bottle and bottle was kept in a water bath, heated at 56 ° C till the alcohol evaporates leaving behind the powder form of extract. This powder was transferred into clean vials and stored at 4° C till further use. The yield of alcoholic extract for fifty grams of dried leaves of *Bridelia scandens* was 9.87gms.The alcoholic extract obtained after soxhlet extraction was dissolved in dimethyl sulfoxide (DMSO). Different concentration of the plant extract 200 mg/ml and 400 mg/ml were prepared.

Microbial cultures

The pure culture of oral pathogens tested viz. *Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius ,Candida albicans* and *Lactobacillus acidophilus* were obtained from repository of the Department of Microbiology, Yenepoya Medical College, Mangalore

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was done by Kirby Bauer

Well diffusion method [11,15]

Kirby Bauer diffusion method was a test which used antimicrobial agent impregnated paper disks or wells in medium charged with antimicrobial agents to test whether particular organism were susceptible to specific antimicrobial agents.

If the organisms were susceptible to a particular antimicrobial agent, an area of clearing surrounds the well where organism was not capable of growing. (Zone of Inhibition)

Requirement for Agar disk diffusion method

Mueller Hinton agar, Chocolate agar and Sabouraud's Dextrose Agar (SDA) were used. Divide the plate into three parts or four quadrants by using black glass marking pen .Label C+ for positive control (chlorhexidine) C – for negative control (Dimethyl sulphoxide) and name of the organism tested on third part.

The wells were cut in the medium with help of sterile durhams tube. Antimicrobial testing for bacteria were done in Muller Hinton agar and chocolate agar and Candida on Sabouraud's Dextrose agar.

PH of the medium in bacteria should be 7.4 and fungus should be 5.4.

Procedure

Keep the stock culture of Streptococcus mutans, Streptococcusmitis, Streptococcus salivarius, Candida albicans and lactobacillus acidophilus outside from refrigerator for about half an hour.

Label the three bottles of Robertson's Cooked Meat medium (RCM) -*Streptococcus mutans, Streptococcusmitis* and *Streptococcus salivarius.* Label the two tubes of peptone water or nutrient broth - *Candida albicans* and *Lactobacillus.* Inoculate few colonies of stock culture into respective bottles or tubes. Incubate at 37°Cfor 24 hours

Adjust the turbidity until it was equivalent to McFarland 0.5 standard $5x\ 10^5$ CFU/ml.

Dip a sterile swab into Robertson's Cooked Meat medium or peptone water and release any excess medium by pressing the swab against the side of the tube.

Streaked as a lawn culture onto appropriate solid medium- Chocolate agar for *Streptococcus mutans, Streptococcus mitis Streptococcus salivarius* and Sabouraud's Dextrose Agar for *Candida albicans* and Mueller Hinton Agar for *Lactobacillus* in three directions to ensure confluence. Keep the plate upside down.

By using 100 micro litre pipette the crude extract was charged into the corresponding well, Chlorhexidine into the well-marked C + and dimethyl sulpfoxide (DMSO) into the well-marked C - .

Within 15 minutes of inoculation, the plate was inverted and kept for incubation at 37°C for 18-24 hours. The zone of inhibition was measured with a ruler in mm and results were noted down[16]

The test was repeated for three times.

The positive tests (the organisms showing Zone of Inhibition) were subjected to Minimum Inhibitory Concentration (MIC) by using broth dilution method [12]

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) [13]

To determine MIC various dilutions of the extracts which had antibacterial and antifungal activity in the previous assay was taken in sterile test tubes. Freshly prepared nutrient broth was used as Diluents. Crude extract was diluted by two fold serial dilution method. A tube containing nutrient broth was taken as control. 50ul of the standard culture inoculum was added to each test tube except the control tube. All tubes were incubated at 37° C for 24 hours and then examined for growth by observing turbidity. One ml of culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and sub-cultured onto Muller Hinton Agar and incubated at 37° C for 24 hr. After incubation the concentration at which there was no single colony of

bacteria was taken as MBC.

The inhibitory effect of the extract was studied by Time Kill Assay and determination of Minimum Inhibitory Concentration (MIC). In Time Kill Assay [8]the inoculum containing approximately 5x10 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 24h.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.

Results

The antimicrobial effect of various concentrations of alcoholic and water extract of *Bridelia scandens* was tested against various dental pathogens. Table 1 shows test statistics of all five organisms tested against extract of *Bridelia scandens* by chi-Square using Kruskal Wallis test.

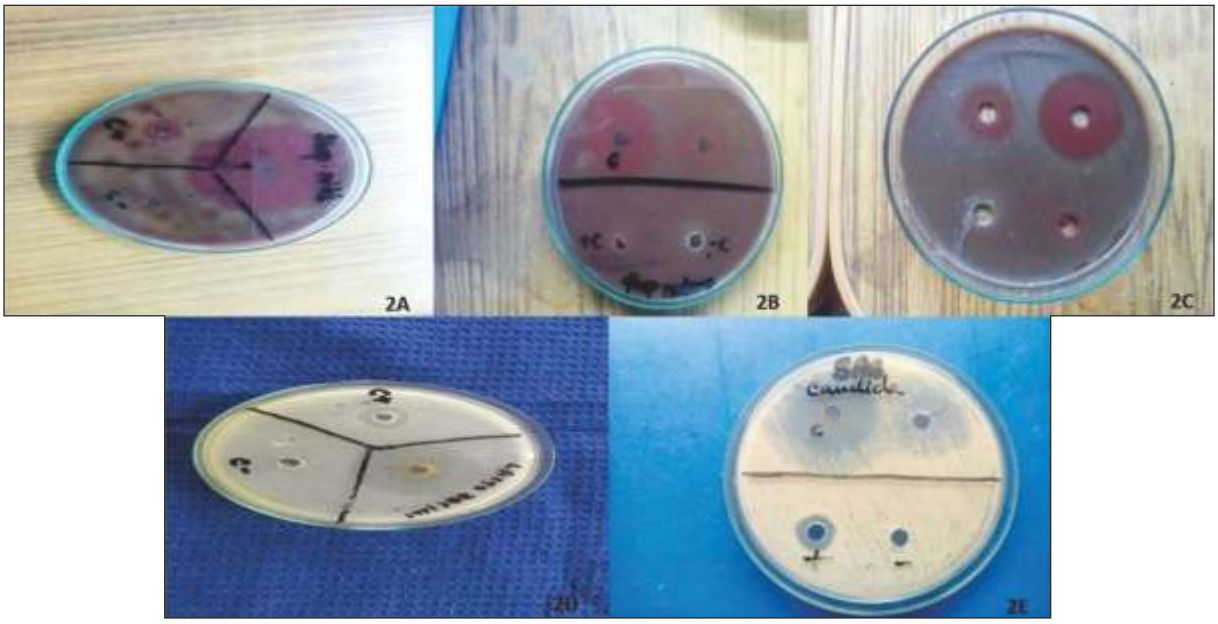

Fig 2 : Culture plates with zone of inhibition, (2A-Streptococcus mitis, Streptococcus mutans, 2C- Streptococcus salivarius, 2D-Lactobacillus acidophilus, 2 E- Candida albicans)

Streptococcus Mutans

When the antimicrobial property of water extract on Streptococcus mutans was tested at concentration of 200 mg/ml a zone of inhibition of 23 mm was obtained .The alcoholic extracts tested against the organism gave a better effect at the same concentration with a zone of 31mm.Positive control 0.2% of chlorhexidine gave a significantly lower value of 12mm as shown in Table 1.

According to Table 1 both the extract were shown to be significantly higher when compared to the control .When the water and alcoholic extracts at the concentration 200 mg/ml, 400 mg /ml and control were Compared, the values showed significance difference with P value of .043 to .046

Alcoholic extracts gave a significantly higher result when compared to water extracts of similar concentrations has revealed in Fig 3.

Streptococcus Mitis-

When the effect of extract of leaves of *Bridelia Scandens* was tested against *Streptococcus mitis* the results were as follows: The water extract and alcoholic extract at the concentration of 200 mg/ml gave a zone of 25 mm and 38mm respectively. The positive control 0.2% chlorhexidine gave a zone of 12mm . The results of the test are shown in table 1

On utilizing the Mann Whitney test and Kruskal Wallistest for comparisons it was found that there is significance

among the test groups,it was observed that both water and alcoholic extract of 200mgm/ml were significantly higher in comparison to the control.It was found that there was a significant difference between the control and the alcoholic extract at the concentration of 200mgm/ml .Alcoholic extract was more effective in both the concentration (200 mg/ml and 400 mg/ml) than water extract with P value of .034 to 1.000. Fig 4 concludes the result.

Streptococcus Salivarius

The water extract of leaves of *Bridelia scandens* against *Streptococcus salivarius* tested at concentration of 200 mg/ml which revealed an inhibition zone of 21mm .The same test organism when tested with alcoholic extracts showed inhibition zone of 25mm.The positive control 0.2% chlorhexidine gave a less effect with a zone of inhibition of 11mm. (Table 2). After confirming significance among the test groups using Kruskal Wallis test the obtained information were analysed using Mann Whitney test for pair wise comparison and it was found that both water and alcoholic extracts had effectiveness of higher significance when compared to the control. When the alcoholic extracts were compared to the water extracts, alcoholic extracts showed significantly higher effect with P value ranging from .o46 to .050. Fig5 explains the result.

Candida Albicans-

According to table 1 the alcoholic and water extracts of the leaves of *Bridelia scandens* were observed to be very effective against this organism with alcoholic extract gave a zone of 28mm and water extract gave a zone of inhibition of24mm.The positive control 0.2% chlorhexidine gave a zone of 13 mm. Kruskal wallis test confirmed significance among the test groups and further Mann Whitney test was done for comparisons.

Both the water and alcoholic extract were found to be significantly higher in comparison to the control and alcoholic extract was found to be of higher significance with the P value of comparison of varying concentration ranging from .036 to .822 as shown in Fig 7.

Lactobacillus Acidophilus-

Table 1 explains that when the antimicrobial property of water extract on *Lactobacillus acidophilus* was tested with a concentration of 200 mg/ml a zone of inhibition of 38 mm was noted. When the same test organism was exposed to alcoholic extract showed an inhibition zone of 46 mm. The positive control 0.2%chlorhexidine showed an inhibitory zone of 13 mm. The above results were analysed using Kruskal Wallis test, it showed that there is significance among the test groups. The alcoholic extract was more effective than water extract at varying concentration with P value ranging from .046 to .050 as given in Fig 6

Thus, the results were further statistically analysed for Pair wise comparison using Mann Witney test [14] and it was found that the water extract at both concentration showed more significance when compared to the control and comparison made between the water and alcoholic extract , alcoholic extract had better inhibitory effect than the water extract.

		N	Mean	Standard Deviation	Min-imum	Max-imum
Streptococcus mutans	AE-200	3	31.000	2.646	28.00	33.00
	AE-400	3	32.000	1.732	30.00	33.00
	WE -200	3	23.667	.577	23.00	24.00
	WE-400	3	20.000	1.000	19.00	21.00
	CONTROL	3	12.333	.577	12.00	13.00
Streptococcus mitis	AE-200	3	38.333	2.082	36.00	40.00
	AE-400	3	33.667	1.528	32.00	35.00
	WE -200	3	25.667	.577	25.00	26.00
	WE-400	3	25.667	.577	25.00	26.00
	CONTROL	3	13.000	.000	13.00	13.00
Streptococcus salivarius	AE-200	3	25.667	.577	25.00	26.00
	AE-400	3	25.000	2.000	23.00	27.00
	WE -200	3	21.667	1.528	20.00	23.00
	WE-400	3	24.333	.577	24.00	25.00
	CONTROL	3	11.667	.577	11.00	12.00
Lactobacillus Acidophilus	AE-200	3	46.333	3.512	43.00	50.00
	AE-400	3	43.000	2.000	41.00	45.00
	WE -200	3	38.333	.577	38.00	39.00
	WE-400	3	35.333	1.528	34.00	37.00
	CONTROL	3	13.000	1.000	12.00	14.00
Candida albicans	AE-200	3	28.000	1.000	23.00	25.00
	AE-400	3	28.667	2.082	27.00	29.00
	WE -200	3	24.333	.577	24.00	25.00
	WE-400	3	24.000	1.000	25.00	27.00
	CONTROL	3	13.667	2.517	11.00	16.00

AE-Alcoholic Extract, WE-Water Extract,
Positive Control-Chlorhexidine, N- Number of test run

Table 1 : Comparison of ZOI of Bridelia scandens by Kruskal Wallis

Test Bacteria /Fungi	Plant extract	Minimum Inhibitory Concentration	Minimum Bactericidal Concentration
Streptococcus Mutans	B.scandens	5	50
Streptococcus Mitis	B. scandens	100	100
Streptococcus Salivarius	B. scandens	12.5	25
Lactobacillus Acidophilus	B. scandens	50	100
Candida Albicans	B. scandens	100	200

Table2 : Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

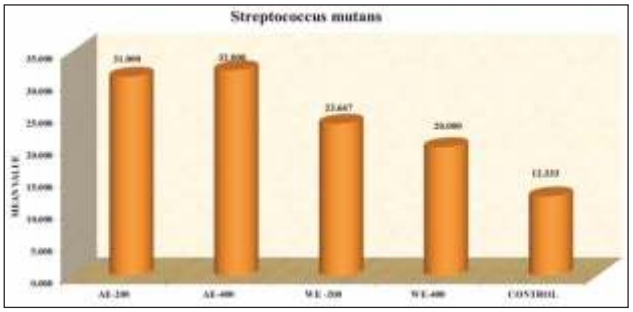

Fig.3 : Mean ZOI of *Streptococcus mutans* against *Bridelia scandens*

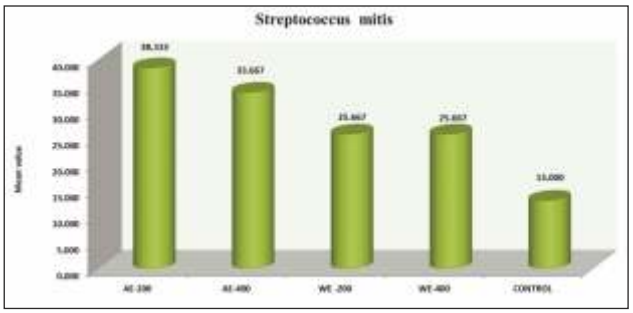

Fig.4 : Mean ZOI of Streptococcus mitis against Bridelia scandens

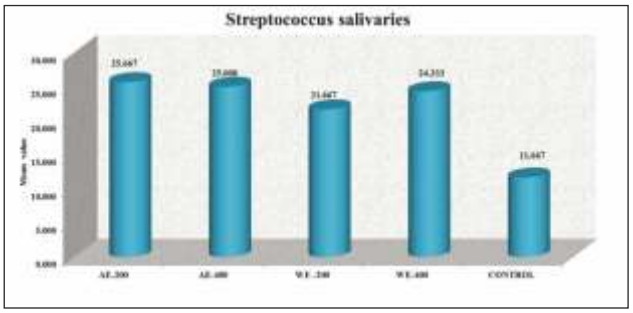

Fig.5. : Mean ZOI of Streptococcus salivarius against Bridelia scandens

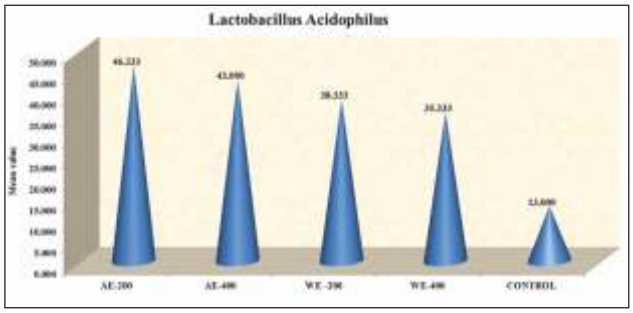

Fig.6 : Mean ZOI of Lactobacillus acidophilus against Bridelia scandens

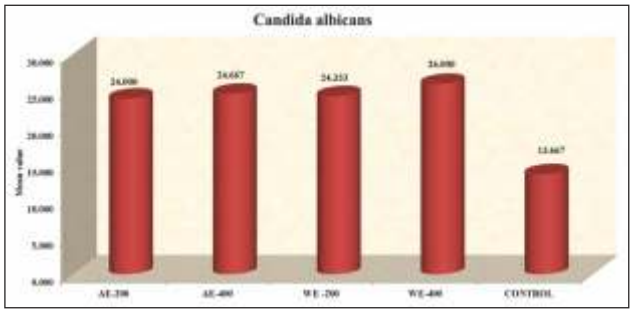

Fig.7 : Mean ZOI of Candida albicans against Bridelia scandens

Minimum Inhibitory Concentration (MIC) (Figure 8) and Minimum Bactericidal Concentration (MBC) (Figure 9)of the plant extracts, that is the lowest concentration that did not permit growth of the test organism were estimated and were tabulated.

(Fig 8 : minimum inhibitory concentration. 3A-(from top to bottom) Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius, Lactobacillus acidophilus, 3B- Candida albicans)

It is noted in our study that *Bridelia scandens* has a better effect against test pathogens.As the Table 2 suggests the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of Bridelia scandens against the test organisms seems to be comparatively less .It is known that lesser the MIC and MBC value more is the efficacy of the extract against the test organisms. Here ,*Bridelia scandens* shows maximum efficacy against *Streptococcus salivarius* with an MIC and MBC of 12.5 mg /ml and 25mgm/ml respectively followed

by *Streptococcus mutans* with an MIC and MBC values of 50 mg/ml each and *Lactobacillus acidophilus* with an MIC and MBC values of 50 mg/ml and 100 mg/ml respectively, *Streptococcus mitis* with MIC and MBC values of 100mgm/ml each and *Candida albicans* with the value of 100 mg/ml for MIC and 200 mg/ml for MBC.

Time Kill Assay showed same results with all test organisms and control. The colony forming unit was almost same even after 24 hours of incubation.

Fig 9 : minimum bactericidal concentration, 4A- Streptococcus mitis, 4B- Streptococcus mutans, 4C-Streptococcus salivarius)

Discussion

According to WHO ,a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and in homeopathic or Ayurvedic medicines, medicinal plants ,their parts and extracts dominate the scenes [17]Even now, more than three-fourths of world's population relies mainly on plants and plant extracts for healthcare.

Dental caries, one of the most common dental diseases seen in Indians ,can be controlled by various preventive and therapeutic measure ,One preventive measure that can be practiced by the individual at home in maintenance of good oral hygiene ,which can be achieved by regular brushing with a tooth brush or natural cleaning aid such as a chewing stick. Due to increased dental health awareness among the public and continuous media publicity, there has been increase in the proportion of people using modern facilities for cleaning their teeth, however in many rural areas, people still depend on natural materials for cleaning their teeth

The increase in bacterial resistance to conventional antibiotics has necessitated the search for new and cost effective ways for the control of infectious diseases. Many studies have shown that medicinal plants constitute a great source for the isolation of active antimicrobial [2,8]

In the present study we have evaluated antimicrobial properties of *Bridelia scandens* which is used commonly for cleaning the teeth by people residing in rural areas of India.

The present study revealed that the bacterial isolates used were susceptible to both the ethanolic and water extracts of leaves of *Bridelia scandens* at varying degree using agar well diffusion method ,although the effect of water extract was not as much as that of ethanol. This indicates that ethanol is the best extractive solvent for extraction of *Bridelia scandens*. This is in agreement with the work of Kareem et al,2008, which stated that ethanol is the best extractive solvent for extracting antimicrobial substances in plants [18].The leaves of *Bridelia scandens* possess thermolabile and thermostable compounds. The cold percolation method is beneficial for such thermolabile compounds and is similar to the method followed by traditional practitioners. The bark and leaves of *Bridelia scandens* is mainly experimented for its other pharmacological activities like antioxidants [19]modulatory [19]analgesic and anti-inflammatory, hypoglycemic, hepato protective and nephro-protective. Little work has been reported on the antimicrobial property of leaves of *Bridelia scandens* on oral pathogens.

For evaluating the antimicrobial properties, the effect of various concentrations of alcoholic and water extracts of the leaves of *Bridelia scandens* was tested on major cariogenic organisms such as *Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius, Lactobacillus acidophilus* and *Candida albicans*. The antimicrobial properties were expressed as the inhibitory zones in agar diffusion method. The results obtained were subjected to Kruskal Wallis test which showed a significance between 5 test groups (AE 200, AE 400,WE 200,WE 400 and Control) and these values were further analysed using Mann Whitney pair wise comparison.

The plant *Bridelia scandens* also known as *Bridelia stipularis* belongs to the family euphorbiaceae which is found in the most part of our country. Many studies on the antibacterial activity of various parts of the *Bridelia scandens* have been carried out on various human pathogens but not on many oral pathogens.

The majority of traditional healers use water to extract active compounds from the plant, because water is not harmful to humans and is generally cheap and easily acquired; however, successful isolation of compounds from plant material is largely dependent on the type of solvent used in the extraction process [20]. Use of water alone leads to difficulties in isolating non-polar active compounds. In this study, ethyl alcohol was quantitatively the best solvent, extracting a greater quantity of plant material than any of the other solvents used. Water and ethanol extracts of *Bridelia scandens* were examined for phytochemical and antimicrobial properties .The extracts which were tested at a final concentration of 200 mg/ml and 400mgm/ml produced in vitro antimicrobial activities in assays against strains of *Streptococcus mutans, Streptococcus mitis .Streptococcus salivarius, Lactobacillus acidiphilus* and *Candida albicans.*

In our study the alcoholic extracts of *Bridelia scandens* showed highest zone of inhibition against *Lactobacillus acidophilus* (38.33mm-46.33mm) followed by *Streptococcus mitis* (33.66mm-38.33mm), *Streptococcus mutans* (31.00mm-32 mm),*Streptococcus salivarius* (25mm-25.66 mm) and *Candida albicans* (24.00mm-24.66 mm).The water extract of leaves of *Bridelia scandens* showed highest zone of inhibition against *Lactobacillus acidophilus* (35.33mm-38.33mm) followed by *Streptococcus mitis* (25 mm-66mm), *Candida albicans* (24.33mm-26.00mm), *Streptococcus salivarius* (21.66mm-24.33 mm) and *Streptococcus mutans* (20.00mm-23.66mm) .

Most of the works have been done on other species of *Bridelia* like *Bridelia ferruginea, Bridelia retusa* and *Bridelia micrantha.* Very few work has been done on *Bridelia scandens* that too on intestinal pathogens.

R.A.Jose et al [21]have shown the effect of extracts of *Bridelia ferruginea* on *Bacillus subtilis ,Escherichia coli, Staphylococcus aureus,Aspergillus niger* and *Fusarium solani.* The results obtained revealed that ethanol extract was most effective against both gram positive bacteria ,gram negative bacteria and fungi. This is in consistent with our finding that ethanol extract is better than water extract.

O N Irobi et al[22]worked on antimicrobial activity of bark extract of *Bridelia ferriginea* on organisms like *Candida albicans, Staphylococcus epidermidis, Streptococcus lactis* and *Streptococcus pyogenes*. The zones of inhibition produced by the extracts in agar diffusion assays against the test organisms ranged from 4 to 20 mm while the chloramphenicol antibiotic control produced zones that measured 15-36 mm. The gram negative bacteria appeared to be more susceptible (4-20mm) to the antimicrobial effect of the extracts than the gram positive organisms (4-18 mm). Chloramphenicol showed maximum effect (15-36 mm) because it is less commonly used drug because of its side effect. In our study the control used is chlorhexidine which gave much poor result (11mm-13mm) .It could be due to frequent use of chlorhexidine as mouth wash which might have led to the development of resistance.

The potency of *Bridelia ferruginea* to inhibit the growth of the pathogens can be traced to the presence of some active ingredients in the plant. This collaborates the work of Sofowara [23]which indicated that *Bridelia ferruginea* contains tannins, glycosides, steroids, saponins, terpenoids, flavonoids and anthraquinones. One of the mechanisms suggested is hydrophobic activity which enables them to partition the lipids of bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Tannins present in the plant extracts have an astringent effect on the mucous membrane and they form a layer over enamel, thus providing protection against dental caries [5]. The bark and the root extracts of *Bridelia ferruginea* were highly effective against *H.influenzae* and *Streptococcus*

pneumoniae and these two organisms are microflora of the oral cavity

This statement is in conformity with the work of Sofowara [24]who reported that the plant has been used as a mouthwash.

In our study it was found that the antimicrobial activity of the alcoholic extract was stronger than the water extract against all organisms This is in accordance with the findings of Doughari et al[24]who revealed a strong antibacterial effect of alcoholic extract of *Bridelia* species against tested organisms, whereas the aqueous extract had lower antimicrobial activity in comparison with alcoholic extract[5]

Conclusion

The results indicated that the bacterial strains were more susceptible than fungal strains to extracts of leaves of *Bridelia scandens*. The present result showed that extracts of the screened plants possess some chemical components which can act against both bacteria and fungi. Antimicrobial properties of these plants can be exploited further in the preparation of natural therapeutic agents against pathogenic bacteria.

After evaluation of antimicrobial properties of leaves of *Bridelia scandens* we came to the following conclusions

The plant extract exhibited high antimicrobial properties against *Lactobacillus acidophilus* and *Streptococcus mitis.*

The alcoholic and aqueous extracts of Bridelia scandens showed affectivity against all five organisms tested Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius, Lacto bacillus acidophilus and Candida albicans.

It was observed that alcoholic extract was more potent than aqueous extract which may be due to varying degrees of solubility of the active constituents in these two solvents. It was also observed that with increase in concentrations the effect against the pathogens also increased.

By way of this study new openings have been made on the use of plants in drug development possibly for the treatment of various infections caused by microbes. The active components from *Bridelia scandens* can be identified and incorporated into modern oral care systems for controlling dental caries and periodontitis.

References

1. AdegbolaD HO,OluwatobaOA,AdebiyiOEand.OdikagbueAN,In vitro evidence of anti infective activity of crude aqueous extract obtained by boiling ripe stem bark of *Bridelia ferruginea*Benth.Journal ofPharmcognosy and Physiotherapy 2010;2:(4):43-8.
2. BessongPO,RojasLB,Obi LC, Igunbor EO. Further screening of Venda medicinal plants for activity against HIV type1 reverse transcriptase and integrase .Afr. J Biotechnol 2006 ;5:526-8.
3. Obafemi CA,Akinpelu DA,Taiwo OO,AdeloyeA.Antimicrobial activity of solvent extracts of Terminaliacatappa Linn leaves, Ife J Sci 2006;8 :29-33.
4. ThorneRF.The classification and geography of the flowering plants,Dicotyledons of the class Angiospermae.Bot Rev 2000;66,441-647
5. Cyriac MB, Pai V,Varghese I, Shantaram M, Jose M. Antimicrobial Properties of Areca Catechu Husk extracts against Common Oral Pathogens.IJRAP 2012;3(1):81-4
6. RashidMA,GustafsonKR,CardellinaJH,BoydMR. Anew podophyllotoxinderivatives from Brideliaferruginea .Nat.Prod.Lett 2000;14 (4):285-92.
7. Orwa C, Mutua A, Kindt R ,Jamnadass R and Antony S.Agrobase tree Database: a tree reference and selection guide version 4.0 http://www.world agro forestry.org 2009
8) SamieA,Obi CL, Bessong PO, Namritha L. Activity profiles of fourteen selected medicinal plants from rural Venda communities in South Africa against fifteen clinical bacterial species.Afr.J .Biotechnol2005; 4(12) :1443-51.
9. Saldhana CJ,Flora of Karnataka vol 1&2,Oxford and IBH Publishers,New Delhi,1984 and 1996.
10. Kokate CK,Practical Pharmacognosy,4th edition 1993,Vallabh Prakashan 106.
11. Bauer AW,Kirby WMM, Sherries JC and Truck M. Antibiotic susceptibility testing by a standardized single disc method,Am J Clin Pathol 1996;36(3):493-6.
12. Rollins DM and Joseph SW.Minimum Inhibitory Concentration(MIC),Pathogenic Microbiology.HTTP://life Umd.edu/classroom/bsci:424
1. Rotomi VO,Lanhon BS, Bartlet JS and Mosadomi HA,Activities of Nigerian Chewing sticks extracts against Bacteroides gingivalis and Bacteroides melaninogenicus ,AntibacterAgents Chemother 1988;32:598-600.
14. Duncan DB,Multiple range and multiple F test Biometrics 1955;11:1-42
15. Jadon A,Badauria M and Shukla S.J of Ethnopharmacology 2007;109(2):215-8.
16. NCCLS (National Committee for Clinical Laboratory Standard). Performance Standards for Antimicrobial susceptibility testing.2002,8th informational Supplement M100S12.
17. Jose M, Bhagya B, Shantaram MV. Ethnomedicinal Herbs used in Oral Health and Hygiene in coastal Dakshina Kannada. J of Oral Health &community Dentistry 2011;5(3):119-23.
18. Kareem SO, Akpan I and OjoO. Antimicrobial activities of Calotropisprocera on Selected Pathogenic Micro organisms. ,Afr J Biomed.Res2008 ; 11:105-10.
19. Tatiya AU,Tapadiya GU, KotechaS,Surana SJ.Effect of solvents on total phenolic, antioxidant and antimicrobial properties of *Bridelia retusa*Spreng stem bark.Indian Journal of Natural products and

Resources 2011; 2(4):442-7.
20. Masoko P, Mmusi TJ,Mogashoa MM,Mokgotho MP,Mampuru LJ, Howard RL. Invitro evaluation of the antifungal activity of *Sclerocaryabirrea* extracts against pathogenic yeasts. Afr .J.Biotechnol 2008;7(20):3521-6.
21. Jose RAand Kayode J. The effect of *Bridelia ferruginea* bark extracts on some pathogenic micro-organisms Ethnobotanical Leaflets 2009;13:1042-6.
22. Irobi ON, MooYoungM, Anderson WA and DaramolaSO.Antimicrobial activity of bark of *Bridelia ferruginea,* Journal of Ethnopharmacology 1994;43(3):185-90.
23. SofowaraEA Medicinal Plants andTraditionalMedicine in Africa 1993; 2.Spectrum Books Ltd:289.
24. Doughari JH, Manzara S. Invitro antibacterial activity of crude leaf extracts of *Mangifera indica*.Afr J of Microbiol 2008;2: 67-72