Original Article

Antimicrobial Activity Of Bael, Cinnamon And Neem Extracts Against Common Oral Microorganisms – An In-Vitro Comparative Study

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Abstract

Aims: To assess the in-vitro antimicrobial activity of Bael, Cinnamon, and Neem extracts on Streptococcus mitis, Streptococcus mutans, Enterococcus faecalis, Campylobacter rectus , Porphyromonas gingivalis and Candida albicans and to compare it with the gold standard Chlorhexidine (0.2%).

Methods and Material: Leaves of Bael, Neem and Cinnamon barks were washed, shade dried and powdered individually. Cold maceration technique was used to prepare the ethanolic extracts of the test products. The standard strains of the selected test pathogens were procured and incubated in their respective selective media for upto 48 hours. The antimicrobial property of the test products at three different concentrations (0.1%, 0.2% and 0.3%) was assessed using the Zone of inhibition by Agar Well diffusion method.

Results: Bael leaf extract (0.3%) had the highest zone of inhibition against Streptococcus mutans, Enterococcus faecalis, Campylobacter rectus and Porphyromonas gingivalis compared to 0.3% Neem, 0.3% cinnamon and 0.2% chlorhexidine. With regard to Streptococcus mitis, 0.2% Chlorhexidine had the highest zone of inhibition compared to the herbal extracts. Among the tested products, only 0.2% Chlorhexidine and 0.3% Cinnamon extract had an antimicrobial activity against Candida albicans.

Conclusions: Bael extract (0.3%) had the highest antimicrobial property against common oral microorganisms when compared to cinnamon extract (0.3%), neem extract (0.3%) and Chlorhexidine (0.2%). However, Bael and neem did not exhibit any antifungal property

Key-words: Bael extract, In-vitro study, Cinnamon extract, Neem extract, oral pathogens

Introduction:

The World Health Organization (WHO) states that around 80% of the total population in world relies chiefly on traditional herbal medicine and a major part of it involves the use of plant extracts (active constituents) to treat the common diseases.[1] In India, about 2,500 plant species belonging to more than 1000 genera are being used in the indigenous systems of medicine.[2]

Herbal preparations have been used since time immemorial for curing various ailments including dental disorders such as prevention of carious lesions, gingivitis, ulcers and xerostomia.[3] In the recent times, interest in herbal alternatives has increased manifolds especially with respect to antimicrobial agents since indiscriminate use of synthetic antimicrobial agents has resulted in development of drug resistance. This drives the need to screen medicinal plants for novel bioactive compounds as it is regarded safe with fewer side effects.[4,5]

Bael (Aegle marmelos), a herb used since ancient system of Indian medicine, belongs to Rutaceae family and is cultivated widely for its medicinal properties.[6,7,8] Limonene, the chief constituent of bael leaf extract, possess analgesic, anti-inflammatory, antibacterial, antipyretic, hypoglycaemic, immunomodulatory, and wound healing property [8]. Though the antimicrobial property of bael leaf extract has been documented in literature but its potential effect against dental pathogens is not yet documented.

Literature reveals that among the various herbal products tested for antimicrobial property, Cinammon and neem extracts are considered to be the most extensively used.[9] **Cinnamon** (Cinnamomum cassia) is one of the most commonly used spices worldwide not only for cooking but also in traditional and modern medicines.[10] The U.S. Department of Agriculture scientists has revealed that cinnamon contains the highest level of antioxidants (procyanidins).[11]

Further, cinnamon barks has antibacterial, anti-inflammatory and antifungal properties.[12] **Neem** (Azadirachta indica), a member of the Meliaceae family, is referred to as 'A tree for solving global problems.'[13] Chewing sticks of neem is advocated for optimum maintenance of healthy periodontium.[14] Nimbidin, Azadirachtin and nimbinin are active compounds present in Neem which is responsible for its antibacterial activity.[15,16]

Chlorhexidine (CHX) is recognized as the gold standard agent for chemical plaque control but on the other hand, side effects like brown discoloration, taste perturbation, supra gingival plaque formation and even unacceptable taste.[17] Hence an in-vitro experimental study was designed with an aim to assess and compare the antimicrobial activity of Bael extract with Cinnamon, Neem extracts and CHX against oral microorganisms as a preliminary step towards the development of Bael mouthwash.

Subjects and Methods:

Ethical

Clearance

The nature and purpose of the study was explained to the Institutional Review Board of the affiliated institution and ethical clearance was obtained (MADC/IRB- XII/ 2017/ 272).

Botanical verification and Quality considerations

The taxonomic identification of the bael and neem leaves was performed at the Regional Ayurveda Drug

Development Instititute under the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India, Chennai, TamilNadu.

Preparation of herbal extract:

Fresh leaves of Bael and Neem and the barks of cinnamon were collected and shade dried for two weeks, powdered and transferred to sterile air tight containers. About 25g each of powdered Bael leaves, Neem leaves and cinnamon barks were individually dissolved in 75ml of solvent (ethanol), labelled appropriately and were kept in the orbital shaking incubator at 37^{o}C for 72 hours with intermittent mixing. At the end of 72 hours, mixtures were filtered with the Whatman filter paper. The filtrate obtained was subjected to distillation process for four days to yield the respective herbal extract. The herbal extracts obtained was dissolved in Dimethyl Sulfoxide (universal solvent).

Five concentrations of each herbal extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were prepared to assess its antimicrobial activity. Since the herbal extracts were intended to be used as a mouthwash, its palatability was assessed among ten randomly selected people (10 for each herbal extract) using a 9 point hedonic scale which is the standard method to assess the palatability.[18] Based on the results of the palatability, microbial analysis was confined to three concentrations namely, 0.1%, 0.2% and 0.3% as the higher concentrations (0.4% and 0.5%) had an unacceptable taste.

Test Microorganisms:

The standard MTCC (Microbial Type Culture Collection) strains of the following organisms were procured: Streptococcus mitis (MTCC No: 2695), Streptococcus mutans (MTCC No: 890), Enterococcus faecalis (MTCC No: 439), Campylobacter rectus (MTCC No: 3297), Porphyromonas gingivalis (ATCC No: 33277) and Candida albicans (MTCC No: 227)

Agar-Well Diffusion Test

Nutrient agar and Chloramphenicol yeast agar were prepared and the cultures were spread on the respective agar plates. Wells of 5mm diameter were punched in agar plates. The prepared extracts of Bael, Neem and Cinnamon each with 0.1%, 0.2% and 0.3% and Chlorhexidine (0.2%) was poured into the wells in the agar plates. The plates were allowed to stand for 1 hour for diffusion of extracts to take place and was incubated at 37°C for 24 hours. The antimicrobial activity was measured based on the diameter of inhibition zone formed around the wells. After incubation period, plates were removed and zones of inhibition were measured using a vernier caliper in millimetre (mm) for 7 consecutive days.

STATISTICAL ANALYSIS

Descriptive statistics on zone of inhibition was expressed in terms of mean and standard deviation. Between groups comparison of zone of inhibition was carried out using Kruskall Wallis test followed by Mann Whitney U test as post hoc test. P value < 0.05 was considered statistically significant.

Results:

COMPARISON OF ANTIMICROBIAL EFFICACY OF BAEL, CINNAMON AND NEEM EXTRACTS AND CHLORHEXIDINE AGAINST THE TEST PATHOGENS AT THE END OF SEVEN DAYS (Table 1, 2 and 3)

Bael extract of 0.3% concentration showed highest zone of inhibition against Enterococcus faecalis, Campylobacter rectus, Streptococcus mutans and Porphyromonas gingivalis compared to the various tested

concentrations of 0.1%, 0.2% and 0.3% each of Bael extract, Neem extract, Cinnamon extracts and 0.2% Chlorhexidine. A higher zone of inhibition against Streptococcus mitis was showed by 0.2% Chlorhexidine compared to various tested concentrations of the herbal extracts. Antifungal activity was shown only by Chlorhexidine and Cinnamon.

COMPARISON OF THE MEAN ZONE OF INHIBITION (IN MM) OF 0.3% BAEL WITH OTHER 0.3% HERBAL PRODUCTS AND 0.2% CHLORHEXIDINE FOR SEVEN DAYS (Table 4)

Bael extract of 0.3% showed a higher mean zone of inhibition compared to Cinnamon, Neem and Chlorhexidine against Enterococcus faecalis, Campylobacter rectus, Porphyromonas gingivalis and Streptococcus mutans. These differences noted in the means zone of inhibition were statistically very highly significant ($p<0.001$). But with regard to Streptococcus mitis, 0.2% Chlorhexidine had a higher zone of inhibition followed by 0.3% Cinnamon, 0.3% Bael and 0.3% Neem extracts.

Discussion:

In the recent times, the use of herbal products is on the rise due to the spread in the awareness of the effect of complementary and alternative medicine. [19] Herbal products are abundant and easily available, has various medicinal properties and may possess minimal/no side effects to the general as well as the oral health compared to the synthetic alternatives. The present study was conducted to assess and compare the antimicrobial activity of Bael, Cinnamon and Neem extracts against the common oral pathogens.

The six test pathogens were selected aiming to represent the wide variety of microbial species present in the common dental diseases like gingivitis, dental caries, periodontal diseases, endodontic re-infections and also opportunistic infections. In this study, agar well diffusion method was employed as it is a well-established technique commonly used in in screening the antimicrobial efficacy of chemicals before in vivo testing. [20,21] All the herbal extracts in this study exhibited antibacterial effects but did not exhibit antifungal property.

Firstly, Bael extracts had the highest inhibitory effect against Streptococcus mutans, Enterococcus faecalis, Campylobacter rectus and Porphyromonas gingivalis which was likely due to the presence Coumarins, alkaloids, polysaccharides, tannins and carotenoids.[22] Similarly it had an inhibitory effect against Streptococcus mitis, but was lesser than that of the Chlorhexidine. Although the literature reveals that bael has proven antifungal property[22], our study revealed that there was no effect against Candida albicans.

Neem extracts had the second highest inhibitory effect after bael against Campylobacter rectus but compared to other herbal extracts in the study, it had lesser inhibitory effect against other test pathogens. A meta-analysis study stated that among the herbal products that have been successful, neem (Azadirachta indica) was one among them.[23]

Cinnamon extracts had an antifungal effect and had the second highest inhibitory effect against Streptococcus mitis, Streptococcus mutans, Enterococcus faecalis, Porphyromonas gingivalis and Candida albicans compared to the bael and neem extracts. This effect could be due to the presence of Cinnamaldehyde, tannins and flavanols which has proven to have antibacterial and antifungal properties in previously conducted studies.[24-26] However, it has been demonstrated that, even not having an isolated action against specific bacterial species, phytotherapic products may act as an important adjuvant agents potentializing the effect of other medications. [27]

Herbal medicine is still the mainstream of about 75-80% of the whole population, mainly in developing countries for primary health care because of better cultural acceptability, better compatibility with fewer side effects. Hence efforts have been made for development and use of the natural herbal products to be safe, easily available and substitute the standard pharmaceutical remedies.[28]

In view of the limitations of the present in vitro study, it is worth mentioning that these results may not correspond to the actual behaviors of tinctures in-vivo because they are not exposed to the same conditions found in the oral cavity. The result obtained in this study can serve as a guide for selecting a kind of herbal mouth rinses as well as providing information to the dental professionals about the efficacy of these products.

The in-vitro study has concluded that, Bael leaves had a significant antimicrobial effect against the common oral pathogens like Streptococcus mitis, Streptococcus mutans, Enterococcus faecalis and Porphyromonas gingivalis. Hence, it has opened up new vista for Bael to be developed as a mouthwash for promotion of oral health.

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Table 1 – Zone of inhibition exhibited by Bael extract and Chlorhexidine (CHX) against the test pathogens

ORGANISMS	BAEL EXTRACTS CONCENTRATIONS			CHX
	0.1 %	**0.2%**	**0.3%**	**0.2%**
Streptococcus mitis	25mm	28mm	32mm	47mm
Enterococcus faecalis	29mm	37mm	42mm	28mm
Candida albicans	No activity	No activity	No activity	15mm
Campylobacter rectus	26mm	27mm	30mm	16mm
Streptococcus mutans	24mm	31mm	35mm	18mm
Porphyromonas gingivalis	20mm	25mm	32mm	15mm

Table 2 – Zone of inhibition exhibited by Neem extract and Chlorhexidine (CHX) against the test pathogens

ORGANISMS	NEEM EXTRACTS CONCENTRATIONS			CHX
	0.1 %	**0.2%**	**0.3%**	**0.2%**
Streptococcus mitis	15 mm	19mm	25mm	47mm
Enterococcus faecalis	18mm	25mm	29mm	28mm
Candida albicans	No activity	No activity	No activity	15mm
Campylobacter rectus	15mm	21mm	27mm	16mm
Streptococcus mutans	12mm	19mm	25mm	18mm
Porphyromonas gingivalis	14mm	19mm	27mm	15mm

Table 3 – Zone of inhibition exhibited by Cinnamon extract and Chlorhexidine (CHX) against the test pathogens

ORGANISMS	CINNAMON EXTRACTS CONCENTRATIONS			CHX
	0.1 %	**0.2%**	**0.3%**	**0.2%**
Streptococcus mitis	22mm	30mm	33mm	47mm
Enterococcus faecalis	18mm	21mm	24mm	28mm
Candida albicans	10mm	14mm	18mm	15mm
Campylobacter rectus	10mm	18mm	26mm	16mm
Streptococcus mutans	10mm	19mm	28mm	18mm
Porphyromonas gingivalis	20mm	26mm	30mm	15mm

Table 4: Comparison of the antimicrobial activity (Zone of inhibition in mm) of 0.3% Bael with other 0.3% herbal products and 0.2% Chlorhexidine

Organisms	Bael	Cinnamon	Neem	CHX	p value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Enterococcus faecalis	40.857± 0.899^{A}	33.000± 0.816^{B}	28.287± 0.48^{C}	25.287± 3.19^{D}	<0.001
Streptococcus mitis	31.142± 0.899^{A}	32.714± 0.487^{B}	23.285±1.25^{C}	46.000± 0.81^{D}	<0.001
Campylobacter rectus	29.287 ± 0.755^{A}	25.857± 0.377^{B}	26.714 ± 0.48^{C}	13.285± 1.97^{D}	<0.001
Porphyromonas gingivalis	31.142± 0.690^{A}	29.428± 0.786^{B}	26.571±0.534^{C}	13.857± 2.03^{D}	<0.001
Streptococcus mutans	34.571± 0.534^{A}	27.571± 0.786^{B}	23.714±1.253^{C}	18.000± 0.00^{D}	<0.001

*Kruskall Wallis Test

^Different alphabets as superscript refers to significant difference between groups using Mann whitney U test.

ANTIMICROBIAL EFFICACY OF BAEL LEAVES EXTRACTS AGAINST TEST PATHOGENS

DAY 1 **DAY 7**

Streptococcus mitis

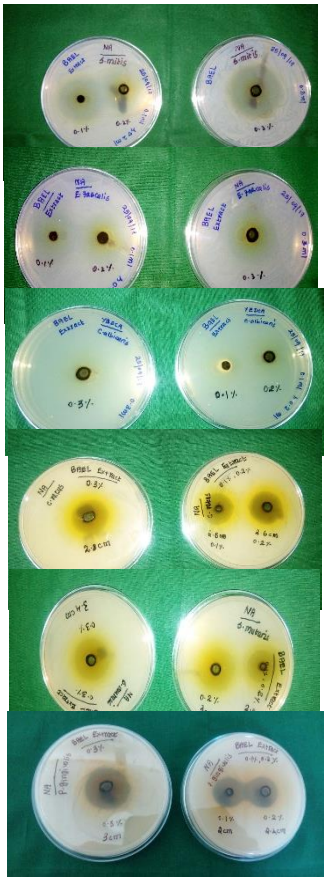
Enterococcus faecalis

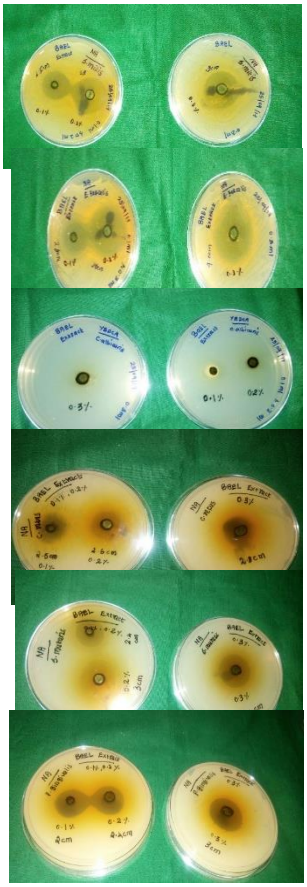
Candida albicans

Campylobacter rectus

Streptococcus mutans

Porphyromonas gingivalis





ANTIMICROBIAL EFFICACY OF NEEM LEAVES EXTRACTS AGAINST TEST PATHOGENS

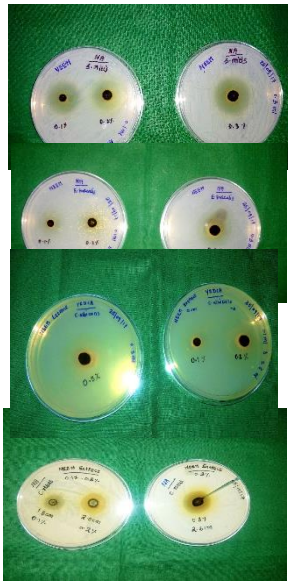
DAY 1 **DAY 7**

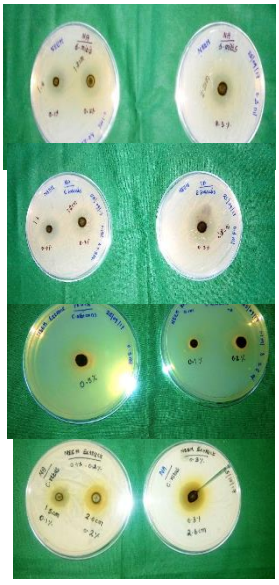
Streptococcus mitis

Enterococcus faecalis

Candida albicans

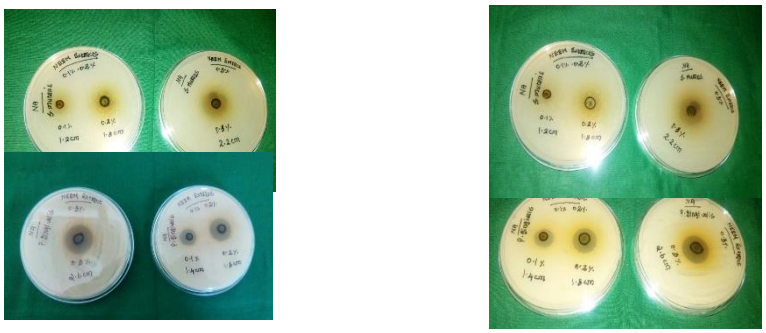
Campylobacter rectus





Streptococcus mutans

Porphyromonas gingivalis



ANTIMICROBIAL EFFICACY OF CINNAMON LEAVES EXTRACTS AGAINST TEST PATHOGENS

DAY 1 **DAY 7**

Streptococcus mitis

Enterococcus faecalis

Candida albicans

Campylobacter rectus

Streptococcus mutans

Porphyromonas gingivalis