

Antimicrobial Efficacy of Emblica Officinalis Fruit Extracts on S.Mutans, E.Faecalis and C.Albicans

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ABSTRACT

Objective: Emblica officinalis fruit is used in Indian traditional medicine since ages as an antimicrobial agent. Review of literature yielded a paucity of studies related to the effect of Emblica officinalis fruit extract against Streptococcus mutans, Enterococcus faecalis and Candida albicans, so the present study was designed. Our aim was to determine the antimicrobial efficacy of ethanol, acetone and distilled water extracts of Emblica officinalis against Streptococcus mutans, Enterococcus faecalis and Candida albicans.

Materials and Method: Extract of Emblica officinalis in ethanol, acetone and distilled water were obtained by cold maceration. The crude residue was obtained by evaporation at room temperature. The antimicrobial efficacy of Emblica officinalis in all extracts were assessed against oral microorganisms by finding out zone of inhibition and minimal inhibitory concentration.

Results: Emblica officinalis in ethanol, acetone and distilled water were found to be effective against Streptococcus mutans, Enterococcus faecalis and Candida albicans by highest zone of inhibition being 38 mm for acetic extract of emblica officinalis against Enterococcus faecalis. Minimum inhibitory concentration of ethanolic and acetic emblica officinalis extracts for Candida albicans was established at 0.09% and distilled water extract at 1.56%. Minimum inhibitory concentration for ethanolic extract of emblica officinalis for Enterococcus faecalis was 3.12%, acetic extract of Emblica officinalis was established at 0.39% and distilled water extract of emblica officinalis was established at 12.5%.

Conclusion: Emblica officinalis could be considered as an effective antimicrobial agent. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration.

Keywords: Emblica officinalis, Streptococcus mutans, Enterococcus faecalis, Candida albicans.

INTRODUCTION



The number of multi-drug resistant microbial strains and emergence of such strains with a reduced proclivity to antibiotics is continuously increasing. This

increase in drug resistance is attributed to the unsystematic use of broad-spectrum antibiotics and immunosuppressive agents¹. As various side-effects are attributed to the conventional medicine, the use of herbs or natural products as an alternative to conventional medicine in healing and treatment of various diseases has been on the rise in the last few decades. Use of herbal medicines began with the

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earliest man and they are considered as nature's gift, which can provide a healthy life to human beings. Herbs constitute the most important part of human diet in certain cultures. The use of herbal medicine for treatment of various diseases is practiced even today in many countries around the world. India is one such country which is blessed with a rich heritage of traditional medical systems including Ayurveda, Siddha and Unani which use herbs and minerals in the formulation of drugs. The main reason why herbs are used is because of their cost effectiveness, accessibility, acceptability and availability compared to the formulated drugs².

Emblica officinalis is a time tested herb which is used in the Indian traditional medicine system. It belongs to family euphorbiaceae, which is commonly called by several names such as amalaka, aavala, amla, amlaki and Indian gooseberry. It is rich in phenols, tannins, polyphenols, flavonoids, kaempferol, ellagic acid and gallic acid³. Amla is extensively found all over India, as well as Srilanka, Malayasia, China, Pakistan and Bangladesh⁴. The fruits of the plant have been used in Ayurveda as a potent herbal agent. *Emblica officinalis* is used in the prevention and treatment of various diseases. It is used as an anticancer agent, analgesic, antipyretic, antioxidant, cardiogenic, cerebral tonic, intestinal tonic, antimicrobial and also in the treatment of hemorrhage and diarrhoea⁵.

Dental caries is an infectious microbial disease affecting quality of life of individuals and its high morbidity potential has brought it to the focus of dental health professionals. *Streptococcus mutans* is one of the microbes which initiate dental caries. Oropharyngeal candidiasis is a frequent problem within immunocompromised and elderly populations⁶. *Candida albicans* species are implicated in the development of candidiasis. *Enterococcus faecalis* has proved to be a potentially important microorganism in the colonization or overgrowth in endodontic infections. It is the dominant microorganism in post-treatment apical periodontitis and has often been isolated from the root canal in pure culture which is considered to be a cause for endodontic failure⁷. To eliminate or to reduce the number of such microorganisms in the oral cavity is a challenging task. Herbal antimicrobial agents like *Emblica officinalis* can be tried to counteract such problems.

There is limited literature to support the in vitro antimicrobial activity of *Emblica officinalis* against oral pathogens because not much emphasis is laid on the use of herbal products in dentistry. In this regard, the present study aims to assess and compare the in vitro antimicrobial activity of ethanol, acetone and distilled water extract of *Emblica officinalis* against *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*.

MATERIALS AND METHODS

Materials: *Emblica officinalis* 150g, Sterilised mixer grinder, weighing machine, three air tight bottles, sterile cloth, Solvents: Acetone, Distilled water, Ethanol, Positive controls: Chlorhexidine, sodium hypochlorite, tested micro organisms were *Streptococcus mutans* [ATCC 25175], *Enterococcus faecalis* [ATCC 35550] and *Candida albicans* [ATCC 2091]. Brain Heart Infusion [BHI] broth, Sabouraud Dextrose broth, sterile MIC tubes, Micropipettes and dimethyl sulfoxide.

Collection of the sample and drying the plant material: Fresh fruits of *Emblica officinalis* were obtained from the local market of Davangere city. Confirmation of the identified sample was done from the Department of pharmacy, Bapuji educational association. The obtained *Emblica officinalis* fruits were washed under running tap water and they were chopped to fine pieces, seeds were removed and were air dried under room temperature. The dried fruits were pulverised to fine powder using a sterilised mixer grinder and was stored in air tight bottle. Extract preparation: 50g of *Emblica officinalis* powder was separately soaked in three sterile containers containing 200 ml of acetone, ethanol and also in sterile distilled water. All the three containers were subjected to cold maceration (Occasional shaking for all three containers and it was kept undisturbed for 48 hours). All three preparations were filtered through a sterile cloth. The filtered extract obtained was dried for five days by concentrating under vacuum below 40 degree Celsius using an evaporator. Preparation of stock solution: 2g of each dried extracts obtained were dissolved in 4ml of dimethyl sulfoxide (which acts as a carrier agent) to obtain 50% extracts.

Antimicrobial assay: The acetone, ethanol and distilled water extracts were screened for

Table 1: Zone of inhibition (in millimeters) for different volumes of Distilled water, ethanolic and acetonic extracts of *Emblca officinalis* compared to positive controls.

Organisms	Extract	Diameter of inhibition zones in mm					Positive controls
		75µl	50µl	25µl	10µl	5µl	
C.albicans	Distilled water	24	22	14	R	R	14mm
	Ethanol	36	36	30	28	23	[Flucanazole]
	Acetone	34	32	28	12	12	
E. faecalis	Distilled water	28	26	17	12	R	
	Ethanol	33	30	24	14	14	16 mm
	Acetone	38	36	30	18	15	[4.2% NaOCl]
S.mutans	Distilled water	26	25	16	12	R	
	Ethanol	30	29	27	20	19	15 mm
	Acetone	33	32	28	16	15	[0.2%Chlorhexidine solution]

Table 2: Minimum Inhibitory Concentration of fruit extracts of *Emblca officinalis* against common oral microorganisms on specific media for each microorganism, determined by modified agar well diffusion method.

		50%	25%	12.5%	6.25%	3.12%	1.56%	.78%	.39%	.19%	.09%
C.albicans	Ethanol	S	S	S	S	S	S	S	S	S	S
	Acetone	S	S	S	S	S	S	S	S	S	S
	Distilled water	S	S	S	S	S	S	R	R	R	R
E. faecalis	Ethanol	S	S	S	S	S	R	R	R	R	R
	Acetone	S	S	S	S	S	S	S	S	R	R
	Distilled water	S	S	S	R	R	R	R	R	R	R
S.mutans	Ethanol	S	S	S	S	S	S	R	R	R	R
	Acetone	S	S	S	S	S	S	S	S	R	R
	Distilled water	S	S	S	S	R	R	R	R	R	R

S= Sensitive, R=Resistant

antimicrobial properties. Nutrient broth was used to get the viable growth of microbes.

Determination of zone of inhibition: The antibacterial potential of distilled water, acetonic and ethanolic extracts of *Emblca officinalis* were assessed against *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans* by disc diffusion method. Brain heart infusion agar media was employed to culture *Streptococcus mutans* and *Enterococcus faecalis*. Sabouraud Dextrose broth was employed to culture *Candida albicans*. The

concentration of the organism was adjusted to 10,000 colony forming units/ml by using 0.5 McFarland Standards and was applied on the surface of the plate. The agar plates were incubated over night at 37° Celsius. Four such plates were prepared. In each plate, five wells of 8 mm diameter each were punched and were filled with 5 µl, 10 µl, 25 µl, 50 µl, 75 µl of distilled water extract of *Emblca officinalis*. In the second and third agar plate, similar volumes of ethanolic extract and acetone extract of *Emblca officinalis* were filled in the wells and in the last plate, a fixed volume of the

control agents were filled in the wells. The antibacterial activity was interpreted from the size (diameter) of inhibition zone observed as clear zone surrounding each well on the agar plates. The zone of inhibition was measured in millimeters using vernier caliper.

Determination of minimum inhibitory concentration: Minimum inhibitory concentration is defined as the lowest concentration of the extract capable of inhibiting the growth of bacterium tested in 24 hours. The minimum inhibitory concentration was determined by serial tube dilution technique. The tubes of the broth medium, containing graded doses of compounds were inoculated with test organisms. After incubation, growth occurred in the tubes where the concentration of the compound was below the inhibitory level.

RESULTS

The results of antimicrobial activity of the three extracts of *Emblica officinalis* have been shown in Table 1 and Table 2 respectively. From the data presented in the Table 1, it is evident that all the three extracts of *Emblica officinalis* showed antimicrobial inhibitory activity against all the three microorganisms with highest zone of inhibition being 38 mm for acetonetic extract of *emblica officinalis* against *Enterococcus faecalis*. From the data presented in Table 2, Minimum inhibitory concentration of ethanolic and acetonetic *emblica officinalis* extracts for *Candida albicans* was established at 0.09% and distilled water extract at 1.56%. Minimum inhibitory concentration for Ethanolic extract of *emblica officinalis* for *E.faecalis* was 3.12%, acetonetic extract was 0.39% and distilled water extract was 12.5% and ethanolic extract of *emblica officinalis* was 1.56%, acetonetic extract 0.39% and distilled water extract at 6.25% respectively.

DISCUSSION

Ethnobotanical approach is one of the common methods that are employed in choosing the plants for pharmacological study. India is one of the twelve mega biodiversity centres having more than 45000 plant species⁸. Use of plants as a source of medicine has been inherited and is an important component of health care system. The systemic screening of plant extracts for antibacterial activity

is a continuous effort to find new antibacterial compounds. In the present study the invitro antimicrobial property of distilled water, ethanolic and acetonetic extracts of *E.officinalis* was assessed against *E.faecalis*, *C.albicans* and *S.mutans*. All the three *E.officinalis* extracts were effective against all the three microorganisms.

The possible reason for the antibacterial activity of *E.officinalis* might be due to the tannins present in its fruits. The fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B, which has antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid and glucose. The fruit also contains Phyllembelin^{9,10}.

The result of the present study is in line with a study conducted by Aneja et al which was conducted to check antimicrobial activity of *Emblica officinalis* fruit extracts against *Streptococcus Mutans*, zone of inhibition for *Streptococcus mutans* and *candida albicans* was 11.94mm. Minimum inhibitory concentrations (MIC) of the extracts were also determined against the selected microorganisms showing zones of inhibition $\geq 8\text{mm}$ ¹¹.

The results of the present study were also in line with another study conducted by Saeed et al which checked the antimicrobial potential of aqueous infusions and aqueous decoctions of *Emblica officinalis* against 186 bacterial isolates belonging to 10 different genera of Gram +ve bacterial population and 2 isolates of *candida albicans* isolated from urine specimens³.

The antimicrobial efficacy of *E.officinalis* in distilled water, ethanolic, acetonetic extracts showed zone of inhibition of 26mm, 30mm and 33mm respectively against *S.mutans* when compared to positive control [0.2% Chlorhexidine solution] which showed zone of inhibition of 15 mm, indicating *Emblica officinalis* extracts were more potent than positive control. Distilled water, ethanolic, acetonetic extracts of *Emblica* showed zone of inhibition of 24mm, 36mm, 34mm against *C.albicans* when compared to positive control [Flucanazole] which showed zone of inhibition of 14mm, indicating *Emblica officinalis* extracts more potent than positive control. Distilled water, ethanolic, acetonetic extracts of *Emblica* showed zone

of inhibition of 28mm, 33mm and 38mm against *E. faecalis* when compared to positive control [4.2% NaOCl] which showed zone of inhibition of 16 mm, indicating they were more effective than positive control.

Since the ethanolic, acetonetic and distilled water extracts of fruits of *Emblica officinalis* were most effective against *Enterococcus faecalis*, *Candida albicans* and *Streptococcus mutans*, purification and toxicological studies of the plants and in vivo trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against oral microbials. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. Further in vivo studies should be conducted to know the effectiveness of *Emblica officinalis* as a tooth paste, gel, or remineralising agent for incipient caries lesion. *Emblica officinalis* as a mouth rinse should also be tried in vivo to know the ability of *Emblica officinalis* to prevent oral infections.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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