International Journal of Applied Dental Sciences 2019; 5(4): 243-247

ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2019; 5(4): 243-247

www.oraljournal.com
Received: 25-08-2019
Accepted: 27-09-2019

Dr. Soniya Adyanthaya
Reader, Dept. of Oral Pathology
Yenepoya Dental College
Yenepoya University
Nithyananda Nagar, Derlakatte
Mangalore, Karnataka, India

Dr. Vidya Pai
Professor and Head of the Department, Dept of Microbiology Yenepoya Medical College Yenepoya University
Nithyananda Nagar, Derlakatte
Mangalore, Karnataka, India

Dr. Maji Jose
Professor and Head of the Department, Dept of Oral Pathology & Microbiology
Yenepoya Dental College
Yenepoya University,
Nithyananda Nagar, Derlakatte
Mangalore, Karnataka, India

Corresponding Author:
Dr. Soniya Adyanthaya
Reader, Dept. of Oral Pathology
Yenepoya Dental College
Yenepoya University
Nithyananda Nagar, Erlakatte
Mangalore, Karnataka, India

Evaluation of antimicrobial activity of *Emblica officinalis* against common oral pathogens

Dr. Soniya Adyanthaya, Dr. Vidya Pai and Dr. Maji Jose

Abstract
Background: In the last few decades, field of herbal medicine is gaining importance in developing and developed countries due to its natural origin, lesser side effects and many other advantages. The scientific evidence has brought about the possibility of utilization of herbal plant in the treatment of fungal and bacterial infections and the development of anti-bacterial and anti-fungal products.
Objective: To evaluate the in vitro antimicrobial activity of *Emblica officinalis* bark extracts against dental caries pathogens namely *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Lactobacillus species*, *Prevotella intermedia* which is a periodontal pathogen and a fungal organism *Candida albicans*.
Methods: The barks of *E. officinalis* were collected and different solvents were used for the preparation of the extract namely petroleum ether, dichloro methane, ethyl acetate, methanol and aqueous solvent. The antibacterial potential was evaluated using the agar well diffusion assay.
Result: The largest zone of inhibition was obtained with the methanol extract of *Emblica officinalis* against *Lactobacillus species* (16mm) followed by *Prevotella intermedia*(14mm) and *Streptococcus mitis*(12mm). Antifungal activity was exhibited by methanol and ethyl acetate extract with zone of inhibition of 10mm and 8mm respectively.
Conclusion: *Emblica officinalis* enjoys good antimicrobial activity and can be used as a potential source of antimicrobial agents against pathogens effecting oral health.

Keywords: Antimicrobial**,** cariogenic, *Emblica officinalis,* periodontal

Introduction
Emblica officinalis (*E. officinalis*) Gaertn. (synonym *Phyllanthus emblica* Linn.) or Indian gooseberry, commonly known as, Amla, Nelli and Amalaki, belongs to the family Euphorbiaceae [1]. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. It is a deciduous tree, 8-15 m tall, with alternate, subsessile leaves and greenish to creamy-yellow, unisexual, actinomorphic, trimerous flowers [2, 3]. Two varieties of *E. officinalis* are reported worldwide, the wild ones with smaller fruits and the cultivated ones, also known as 'Banarasi', with larger fruits [4]. Extracts of various plant parts such as leaves, stem, root, seeds and fruits have been widely used in treatment of various diseases [5]. The plant is known to possess anti-viral, anti- bacterial, anticancer, anti allergic, and anti-mutagenic properties [6]. Fruits are known to exhibit anti-inflammatory, analgesic, antidiarrhoeal and anti-pyretic properties [7, 8]. *E. officinalis* is also reported to have antioxidant, immunomodulatory, cytoprotective, antitussive and gastroprotective and memory enhancing properties. It is used in the treatment of ophthalmic disorders, liver diseases and lowering cholesterol level [2]. The potent antibacterial activity of *E. officinalis* against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *S. paratyphi A*, *S. paratyphi B* and *Serratia marcescens* has been reported. The active ingredients of E. officinalis which are protective against microbes include flavonoids (quercetin), ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine) and hydrolysable tannins (emblicanin A and B) [9].
Oral diseases including dental caries and periodontal diseases are the important health problems in the worldwide. Oral health influence the general quality of life and poor oral health is linked to chronic conditions and systemic diseases [10-14]. The occurrence of dental caries is attributed to acidogenic and aciduric Gram-positive bacteria, primarily the mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*), *Lactobacilli* and

Actinomycetes [15] and periodontal diseases have been linked to anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus species*, *Prevotella species* and *Fusobacterium species* [16, 17]. Due to increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immunocompromized individuals, there is need for alternative prevention and treatment options for oral diseases that are safe, effective and economical [18, 19]. The traditional medicinal plants have been evaluated for their potential application in the prevention or treatment of oral diseases particularly those of microbial origin [20, 21]. The objective of present study was to investigate the antimicrobial properties of *E. officinalis* on selected cariogenic and periodontal pathogens.

Materials and methods

Preparation of plant extract

Preparation of petroleum ether extract

The fresh barks *of E. officinalis* were collected locally and authenticated. After collection it was washed with distilled water to remove dirt. The barks were cut into very small pieces and dried at room temperature under shade for 21 days. The barks were ground into a coarse powder with the help of a suitable grinder and the powder was then stored in an airtight container and stored in a cool, dark and dry place till the use. 100 grams of the plant powder was soaked in 1000ml of petroleum ether and stirred at room temperature for 24 hours. The mixture was filtered through Whatman filter paper and filtrate was concentrated to dryness at 45 ^{0}C on a rotary evaporator. The extract prepared was refrigerated to be used as such for further studies. The residual precipitate was extracted further as described below.

Preparation of dichloromethane extract

Residue obtained after extraction with petroleum ether was further soaked in dichloromethane and stirred at room temperature for 24 hours. The mixture was filtered through Whatman filter paper and filtrate was concentrated to dryness at 45 ^{0}C on a rotary evaporator. The extract prepared was refrigerated to be used as such for further studies. The residual precipitate was extracted further as described below.

Preparation of ethyl acetate extract

Residue obtained after extraction with dichloromethane was further soaked in ethyl acetate and stirred at room temperature for 24 hours. The mixture was filtered through Whatman filter paper and filtrate was concentrated to dryness at 45 ^{0}C on a rotary evaporator. The extract prepared was refrigerated to be used as such for further studies. The residual precipitate was extracted further as described below.

Preparation of Methanol extract

Residue obtained after extraction with ethyl acetate was further soaked in methanol and stirred at room temperature for 24 hours. The mixture was filtered through Whatman filter paper and filtrate was concentrated to dryness at 45 ^{0}C on a rotary evaporator. The extract used was refrigerated to be used as such for further studies. The residual precipitate was extracted further as described below.

Preparation of Aqueous extract

Residue obtained after extraction with methanol was further soaked in distilled water and stirred at room temperature for 24 hours. The mixture was filtered through Whatman filter paper and filtrate was concentrated to dryness at 45 ^{0}C on a rotary evaporator. The extract prepared was refrigerated to be used as such for further studies.

Collection of test organisms

Test organisms were collected from carious cavities of affected teeth by scraping soft caries using excavator and from periodontal pockets using paper points. After collection the paper points were dropped into 20 ml of brain heart infusion broth (BHI broth) which was used as transport media.

The inoculated Sheep blood agar plates were incubated at 37 ^{0}C for 48 hrs aerobically and anaerobically (media streaked in duplicate – one for aerobic and the other for anaerobic culture). The different types of colonies were picked up, isolated and subcultured onto sheep blood agar plates for further identification. Colonies of different test organisms were identified by colony morphology, gram staining, catalase test, pigment production, aerotolerance and sugar fermentation tests. The organisms isolated from the samples included *Streptococcus mutans* (*S. mutans*), *Streptococcus salivarius* (*S. salivarius*), *Streptococcus mitis* (*S. mitis*), *Lactobacillus species*, *Prevotella intermedia* (*P. intermedia*), and *Candida albicans* (*C. albicans*). These organisms were preserved for studies by repeated subculturing on blood agar slants and maintained in deep freezer at -80 ^{0}C. For antibacterial activity studies, fresh subcultures were done in BHI broth and used as inocula.

Determination of antimicrobial activity

The extracts of *E. officinalis* prepared using different solvents were dissolved in dimethyl sulfoxide (DMSO), in the concentration of 5G/10 ml and then filtered using whatman filter paper no.1. The dissolved extract was then diluted using DMSO to obtain a concentration of 500 mg/ml, 250 mg/ml and 125 mg/ml.

The susceptibility of the test bacteria to plant extracts was determined using an agar well diffusion assay on 5% sheep blood agar plates. Fresh 24 hour old broth cultures of bacteria were adjusted to 0.5 McFarland turbidity (1-2 x 10^6CFU mL) and spread evenly over the entire surface of the agar plates using a sterile cotton swab. The plates were allowed to air-dry for approximately 10 min following which 5 wells (6 mm holes) were cut into the agar using sterile steel borer.

Individual wells were filled with plant extracts (50 µL). Three wells were filled with the different concentrations of extract 500mg/ml, 250 mg/ml and 125 mg/ml. 2.5% Sodium hypochlorite and DMSO were pipetted into the other two wells and used as positive and negative/solvent control respectively. The plates were incubated at 37ºC for 48 hours period. For each microorganism tested, zones of inhibition of growth were examined, and the diameter of each zone was measured and recorded. Each concentration was tested in triplicate and the results are the average of three independent experiments.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the different extracts of *E. officinalis* was determined by broth dilution method. Sterile Brain Heart Infusion broth, 1 ml was taken in test tubes to which 10 microlitres of the fresh bacterial inoculums were added. Then the extract was added in the concentrations of 125, 60.5, 30.25, 15.13, 7.56 mg/ml to each tube. Tube containing only bacterial inoculums served as

growth control and sterile BHI broth served as negative control. The tubes were incubated at 37 ºC for 24 hours. The tubes were checked for turbidity and the highest concentration showing turbidity was taken as MIC. Subcultures were done on Blood agar from each of the tubes and the plates incubated for 24 hours at 37 ^{0}C. The highest concentration that did not grow any colony was taken as MBC.

Results

Sodium hypochlorite which was used as positive control was effective against all test strains of bacteria and *Candida albicans*. DMSO did not show any zone of inhibition against test organisms which acted as negative control. All the examined extracts of *E. officinalis* showed varying degrees of antibacterial activities against the tested pathogens.

At a concentration of 125 mg/ml and 250mg/ml, the 5 different extracts of *E. officinalis* did not show zone of inhibition against any of tested bacteria and fungi. The results of antimicrobial activity of *E. officinalis* at 500mg/ml concentration are given in table-1. The methanol extracts of *E. officinalis* showed antimicrobial activity against *S. mutans*, *S. mitis*, *Lactobacilli* and *P. intermedia*. It did not show zone of inhibition against *S. salivarius* and *C. albicans*. The extract of petroleum ether exhibited zone of inhibition against *Lactobacillus* and *P. intermedia*. The extract of ethyl acetate was effective against *S. mitis* and *P. intermedia*. The dichloromethane extract and aqueous extract were ineffective against all the tested pathogens.

MIC was carried out for methanolic extract of *E. officinalis* as maximum inhibitory effect was exhibited by this extract. The results are shown in the table-2. All the concentrations tested showed growth in MIC tubes of *S. mutans*, *S. salivarius* and *C. albicans*. Hence the MIC values for these organisms was more than the highest concentration tested (125 mg / ml).

Discussion

Plants have provided man kind with many medicinally useful compounds from ancient time and are considered as the oldest source of pharmacologically active compounds. Many valuable drugs have found their origin from the traditional medicinal practices [22-24]. Bacterial resistance to antibiotics due to resistant strains is increasingly becoming a global concern to public health [25]. Therefore, it is essential to undertake studies to analyse biological activities of medicinal plants against various pathogenic organisms and to explore new antimicrobial compounds. *E. officinalis* has found numerous applications in treating various diseases affecting human beings and it is also considered to possess potent antimicrobial activities against pathogens [2]. In this study, extracts of *E. officinalis* were screened against selected bacterial and fungal strains affecting the oral health.

The antibacterial activity in aqueous extracts of fruits, seed, stem, leaves and root of *E. officinalis*, except the root extract all other parts showed antimicrobial effect against all the eight strains of bacteria. The study also observed the superiority of extracts of *E. officinalis* against major antibacterial antibiotics [5]. The bactericidal activity of *E. officinalis* could be attributed to the phytochemical components present in it namely flavonoids (quercetin), ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine) and hydrolysable tannins (emblicanin A and B) [26]. The ethanol extract of *E. officinalis* bark was found to exhibit significant zone of inhibition against only the gram negative bacteria such as *E. coli*, *S. typhi* and *Vibrio cholerae* and it failed to show antibacterial activity against the gram positive bacterias tested [27].

Since ancient time, different parts of medicinal plants are being used in oral hygiene practices in many parts of the world. The chewing sticks and their extracts are increasingly gaining importance because of their effects on organisms that are involved in oral infections.[28,29] It has been suggested that chewing sticks may provide locally available antimicrobial agents in a manner similar to antimicrobial polymers and applications and could provide suitable substitute therapy if they can be shown to be efficacious [29]. Since parts of *E. officinalis* are used in the oral hygiene practices, it is important to establish the antimicrobial property of this medicinal plant against oral pathogens and to know the bioactive compounds responsible for this action.

In the present study, methanol extract of *E. officinalis* was found to be most effective against test pathogens. It showed effective zone of inhibition against all the organisms except *Streptococcus salivarius*. Methanol exrtract showed highest zone of inhibition against *Lactobacillus species* (16mm), followed by *P. intermedia* (14mm) and *S. mitis* (12mm). All the 5 different extracts did not show zone of inhibition against *S. salivarius*. In one of the study on antimicrobial effect of fruit extracts of *E. officinalis* on dental caries pathogens, all the five extracts namely acetone, ethanol, methanol, hot water and cold water of *E. officinalis* showed inhibitory activity against *S. mutans* while the acetonic, hot and cold aqueous extracts showed inhibitory activity against *S. aureus* also. These extracts did not show inhibitory activity against *Lactobacillus acidophilus*, *C. albicans* and *Saccharomyce cerevisiae*. In this study, fruit extract of *E. officinalis* was used whereas in our study fresh barks of the plant were used. The fruit, seed, stem and leaf extracts of *E. officinalis* is known to possess varying antimicrobial activity against different pathogens [5].

In the present study, methanol extract has shown maximum antifungal activity against *C. albicans* with zone of inhibition 10mm followed by ethyl acetate extract which showed 8mm zone of inhibition. Other 3 extracts failed to exhibit antifungal activity. Ethanol extract of *E. officinalis* was found to have less antifungal activity compared to Mayaca and garlic extract and significant zone of inhibition against wine, coconut oil and pomegranate extract against *C. albicans* [30].

E. officinalis definitely possesses potent antimicrobial activities, thus serving as an important pathway for the development of new antimicrobial agent which can be inexpensive, safe and effective. The results of this study offers an insight into the antibacterial properties of *E. officinalis* which is used traditionally for the treatment of oral problems and other ailments, as well as opportunity for selection of bioactive extracts for initial fractionation and further studies in antibacterial assays.

Table 1: showing the zone of inhibition in mm for **Emblica officinalis** extract at 500mg/ml concentration

***Emblica officinalis* extracts**	***S. mutans***	***S. mitis***	***S. salivarius***	***Lactobacillus* species**	***Prevotella* species**	Candida albicans
Petroleum ether	6	6	6	10	8	6
Dichloro methane	6	6	6	6	6	6
Ethyl acetate	10	6	6	10	10	8
Methanol	10	12	6	16	14	10
Aqueous	8	10	6	6	6	6
Positive control (Sodium hypochlorite)	16	18	12	20	16	10

Table 2: showing MIC and MBC value of methanolic extract of *Azadirachta indica*

Microorganisms	**MIC (mg/ml)**	**MBC (mg/ml)**
S. mitis	125	>125
Lactobacillus species	30.5	125
Prevotella species	30.5	125
S. mutans	>125	>125
S. salivarius	>125	>125
Candida albicans	>125	>125

Acknowledgement
The authors are thankful to Yenepoya deemed to be University, Mangalore, Karnataka for providing research facilities and financial support to carryout this study.

References

1. Joy PP, Thomas J, Mathew S, Shakaria BP. Medicinal plants. Kerala agricultural University, Aromatic and Medicinal Plant Research Station Odakkali, Kerala, India, 1998, 50-51.
2. Khan KH. Roles of *Emblica officinalis* in Medicine- A Review. Botany Research International. 2009; 2(4):218-228.
3. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants, Orient Longman Ltd., Madras. Wealth of Asia, 1998, CD-ROM, NISCOM, New Delhi, 1995.
4. Ghosal S, Tripathi VK, Chouhan S. Active constituents of *Emblica officinalis*: Part 1. The chemistry and antioxidant effect of two new hydrolysable tannins. Emblicanin A and B. Indian J Chem. 1996; 35B:941-948.
5. Varghese LS, Alex N, Ninan MA, Soman S. and Jacob S. Evaluation of in vitro antibacterial activity whole plant (fruits, seeds, stem, leaves and roots) of *Emblica officinalis* Gaertn. International journal of ayurvedic & herbal medicine. 2013; 3(6):1420-1425.
6. Nair R, Sumitra V, Chanda C. Antibacterial activities of some medicinal plants of western regions of India. Turk. J Biol. 2007; 3(1):231-236. 6
7. Sharma SK, Perianayagam BJ, Joseph A, Christina AJM. Anti inflammatory activity of ethanol in aqueous extracts of *Emblica officinalis* Gaertn. fruit. Hamdard Medicines. 2003; 46:75-78.
8. Perianayagam JB, Narayana S, Ganasekar G, Pandurengan A, Rajagopal K, Vijayakumar SG. Evolution of antidiarrheal potential of *Emblica officinalis*. Pharmaceut. Biol. 2005; 43(4): 373-377
9. Saeed S, Tariq P. Antibacterial activities of Emblica officinalis and *Coriandrum sativum* against Gram negative urinary pathogens. Pak J Pharm Sci. 2007; 20(1):32-5.
10. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ. 2005; 83:661-9.
11. Petersen PE. 2003. The World Oral Health Report continuous improvement of oral health in the 21st century - The approach of the WHO Global Oral Health Programme. Commun Dentist Oral Epidemiol. 2003; 31:3-24.
12. Petersen PE. The burden of oral disease: challenges to improving oral health in the 21st century. Bull World Health Organ. 2005; 83:3.
13. Rautemaa R, Lauhio A, Cullinan MP, Seymour GJ. Oral infections and systemic disease-an emerging problem in medicine. Clin Microbiol Infect. 2007; 13:1041-7.
14. Yeo BK, Lim LP, Paquette DW, Williams RC. Periodontal disease - The emergence of a risk for systemic conditions: pre-term low birth weight. Ann Acad Med Singap. 2005; 34:111-6.
15. Loesche W. Dental caries and periodontitis: contrasting two infections that have medical implications. Infect Dis Clin Nth Am. 2007; 21:471-502.
16. Jenkinson HF, Lamont RJ. Oral microbial communities in sickness and in health. Trends Microbiol. 2005; 13:589-95.
17. Tichy J, Novak J. Extraction, assay, and analysis of antimicrobials from plants with activity against dental pathogens (*Streptococcus* sp.). J Altern Complement Med. 1998; 4:39-45.
18. Badria FA, Zidan OA. Natural products for dental caries prevention. J Med Food. 2004; 7:381-4.
19. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12:564-82.
20. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem. 2003; 10:813-29.
21. Dygerak M, Bagecy E, Alma MH. Pharmaceutical Biol. 2002; 40(6):425-428.
22. Ibrahim SA, Dharmavaram SR, Seo CW, Shahbazi G. Intern. J Food Safety. 2002; 2:6-8.
23. Bassam A, Ghaleb A, Naser J, Awni A, Kamel A. Turk J. Biol. 2006; 30:195-198.
24. Farnsworth NR, Loub WD. Information gathering and data bases that are pertinent to the development of plant-derived drugs in Plants: The potentials for extracting Protein, Medicines, and Other Useful Chemicals. Workshop Proceedings.OTA-BP-F-23.U.S. Congress, Office of Technology Assessment, Washington, D.C. 1983, 178-195.
25. Patil SG, Deshmukh AA, Padol AR, Kale DB. In vitro antimicrobial activity of Emblica officinalis fruit by tube dilution method. Int. J Toxicol. Appl. Pharmacol. 2012; 2(4):49-51.
26. Biswas SK, Chowdhury A, Das J, Karmakar UK, Shill MC, Raihan SZ. Assessment of cytotoxicity, antibacterial activity and phytochemical screening of ethanol extract of *Phyllanthus acidus* L. (Family: Euphorbiacceae) bark. Journal of Applied Pharmaceutical Science. 2011; 01 (06):112-114.

27. Li XC, Cai L, Wu CD. Antimicrobial compounds from *Ceanothus americans* against oral pathogens. Phytochemistry. 1997; 46:92-102.
28. Hormer KA, Manji F, Beighton D. Inhibition of protease activities of peri-odontopathic bacteria by extracts of plant used in Kenya as chewing sticks (mswaki). Archives of Oral Biology. 1990; 35:421-424.
29. Ndukwe KC, Okeke WA, Lamikanra SKA, Aboderin O. Antibacterial activity of aqueous extracts of selected chewing sticks. J Contemp. Dent. Pract. 2005; 6(3):086-094.
30. Reena T, Rohitha P, Deepthi MS, Ramachanran RB, Sujatha S. Comparative effect of natural commodities and commercial medicines against oral thrush causing fungal organism of *Candida albicans*. Science Journal of Clinical Medicine. 2013; 2(3):75-80.