



## Formulation and evaluation of slow releasing mouth dissolving films from *Emblica officinalis* fruit for prevention of dental caries

P. Shubha<sup>1</sup>, K. Namratha<sup>1</sup>, C. S. Vicas<sup>1</sup>, K. Byrappa<sup>1\*</sup>, B. M. Gurupadaiah<sup>2</sup>, N. G. Rashmi<sup>2</sup>, and C. G. Shinde<sup>2</sup>

<sup>1</sup>Centre for Materials Science and Technology, Vijnana Bhavan, Manasagangotri, Mysore, India

<sup>2</sup>Department of Pharmaceutical Chemistry, J. S. S. College of Pharmacy, SS Nagar, Mysore, India

### ABSTRACT

Intercepting dental caries with natural material based drug delivery systems is a novel method against chemical based formulations as the latter possess several disadvantages. The purpose of this study is to formulate antibacterial, slow releasing mouth dissolving films from *Emblica officinalis* aqueous extract which is active against *Streptococcus mutans*, the primary pathogen causing dental caries. *Emblica officinalis* aqueous extract was prepared by decoction method and gallic acid, a polyphenol responsible for antibacterial activity was qualified using ultra frequency liquid chromatography. Minimum inhibitory concentration of extract was found to be 5%. This dose was incorporated in hydroxypropyl methyl cellulose polymer with excipients using solvent casting method. Four different formulations were prepared by varying polymer-exciipient ratio along with placebo films. All four formulations were checked for mechanical properties out of which one of the formulations (F4) showed optimal properties which was used for further studies. The films were made slow releasing with 30 min dissolution time and 14.53 min disintegration time which is statistically significant ( $p < 0.05$ ). Aqueous suspension of films containing 5% extract showed antibacterial activity with 20 mm zone of inhibition and toxicologically were non-hematotoxic (less than 5% haemolysis) and non-teratogenic. Hence, this *in vitro* study concludes that *Emblica officinalis* aqueous extract is a natural antibacterial source which can be used in formulating slow releasing mouth dissolving films which are better over chemical formulations like mouth washes as stay-in-mouth time of these films are extended ensuring good antibacterial activity with extended local bioavailability with no drug like feel within oral cavity.

**Keywords:** *Emblica officinalis*, gallic acid, *Streptococcus mutans*, slow release, Antimicrobial.

### INTRODUCTION

Dental caries, the commonest of all human dental diseases is primarily caused by *Streptococcus mutans* a gram positive facultative anaerobe. The disease causes plaque-induced acid demineralization of enamel and dentin [1]. It is a multifunctional process initiated through bacteria, fermentable carbohydrate, saliva and fluoride. Pathologically, *S. mutans* breaks down sugar for energy and produces acidic environment causing demineralization of tooth. The resultant of this conversion disintegrates enamel of tooth and dissolves calcium molecule creating a hole [2].

Despite the presence of over 700 different species of indigenous oral flora, dental caries occurs due to overgrowth of handful of microorganisms, predominantly *S. mutans*. Considering this fact, control of dental caries in terms of reduction in number of *S. mutans* colonies in oral cavity is always an approach considered imperative [3].

Streptococcal colonies within oral cavity have been combated in different ways which include use of mouth washes, lozenges, chewing gums, topical applicants etc [4]. Mouth washes are the commonest of all chemical methods and uses chemicals like chlorhexidine gluconate, triclosan, sodium bicarbonate, sodium fluoride. It is a common observation that use of mouth washes made of chlorhexidine causes altered taste sensation, discoloration and reduced patient compliance [5]. Therefore, there has always been a search for novel alternative materials which gives better advantages over chemical formulations.

Recently, phytomedicines are largely being considered for therapeutic applications in dentistry. Most of phytocompounds are natural antimicrobial and antioxidants. Among them, *Emblica officinalis* is the most common herb used in Indian system of medicine. The plant is commonly available in India, especially in warmer and tropical areas. It is commonly known as Indian gooseberry or Amla and found to be richest source of vitamin C along with other active ingredients like gallic acid and tannic acid [6]. The gallic acid has been considered as an important active principle as it contributes to *E. officinalis* fruit's antimicrobial activity [7]. It is a natural polyphenol which acts as antioxidant and is reported to prevent microbial growth. In an earlier clinical study, 'Triphala' containing *E. officinalis* as one of its ingredient and a well-known ayurvedic formulation used in treating digestive system ailments, has been tested for its therapeutic actions considering gallic acid as marker compound [8]. Earlier reports have shown that 3, 4, 5,-trihydroxybenzoic acid or gallic acid has shown activity against pathogenic microorganisms [9-10]. Gallic acid causes irreversible changes in membrane properties of pathogenic bacteria through hydrophobicity changes, decrease of negative surface charge and occurrence of local rupture or pore formation in cell membranes with consequent leakage of essential intracellular components [11].

We have reported earlier that the extract showed antimicrobial activity against many oral and endodontic pathogens namely *Enterococcus fecalis*, *Lactobacillus acidophilus*, *Streptococcus mutans*, *Staphylococcus aureus* and *Escherichia coli*. The extracts were also found to be non -toxic to blood and chick embryos at different stages of their development [12]. The work was further continued to form slow releasing mouth dissolving films and to study their antimicrobial, toxicology and pharmaceutical properties.

Mouth dissolving films are recently upcoming drug delivery systems developed in lines with transdermal patches. The system consists of thin film which can be simply placed on patient's tongue or buccal mucosa which on coming in contact with saliva will get wet and release active pharmaceutical ingredient into oral cavity for quick therapeutic action [13-14]. There are a plenty of mouth dissolving formulations consisting of standard chemical pharmacological agents. Though there are herbal based formulations available for application in dentistry, incorporation of *E. officinalis* fruit in mouth dissolving film for dental caries interception has not been reported earlier. Therefore, incorporation of herbal medicines in mouth dissolving films for maintenance of oral care is a novel approach as the herb itself contains several phytoconstituents which might have complex interactions with polymer and other ingredients of the film [15]. Upon successful standardization of these films, they can be used as novel alternative to conventional mouth wash like formulation which needs dose adjustment and patient cooperation.

## EXPERIMENTAL SECTION

All chemicals and reagents used for RF-UFLC was HPLC grade and obtained from Merck Chemicals, Germany. HPMC E-50 was obtained from Sigma-Aldrich, PEG- 400 from Rolex Chemicals and glycerol from Ranbaxy Fine Chemicals Limited. The pure bacteriological cultures were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Culture media was obtained from HIMEDIA Laboratories. For synthesis methods and bacteriological activity studies ultra-pure water with resistivity of 18.2 M  $\Omega$  (Option Q7, ELGA, UK) was used.

### 2.1. Preparation of *Emblica officinalis* aqueous extract

About 200 g of fresh *E. officinalis* fruits were procured from local market. They were identified and authenticated from Department of Studies in Botany, University of Mysore. Fruits were washed, deseeded and sun-dried for 4-5 days, pulverized and sieved through cheese cloth and stored in air-tight container at 4° C.

About 5 g of powder was weighed and dispensed in 100 ml of ultrapure water of resistivity 18.2 M  $\Omega$  in a conical flask and kept at 100°C for 30 min with continuous stirring. After boiling cycle, the flask was cooled and contents were filtered through Whatmann filter paper #41 and filtrate was centrifuged for 10 min at a speed of 7450 rpm (Centrifuge 5430R, Eppendorf). The supernatant was collected in a large petri dish and dried at 50 °C for 48 hrs to obtain shiny black extract powder. The powder was scraped, weighed and stored at 4 °C until further use.

## 2.2. Formulation of mouth dissolving films of *E. officinalis* extract

Mouth dissolving films were formulated by solvent casting method using HPMC E-50, PEG 400, glycerol, sorbitol sodium and pine-apple flavor. Four different formulations of mouth dissolving films were prepared by varying the concentration of HPMC and other excipients [16-18] as mentioned in table 1.

Table 1 - Formulations showing different polymer excipient ratios used in film formation

Sl No	HPMC E-50	PEG-400	Glycerol	Sodium Sorbitol	Ultrapure Water	Amla extract (mg)	Pine apple flavor
F1	30 mg	0.8 ml	0.8 ml	15mg	10 ml	500	Q.S.
F2	60 mg	0.8 ml	0.8 ml	15mg	10 ml	500	Q.S.
F3	90 mg	0.4 ml	0.4 ml	15mg	10 ml	500	Q.S.
F4	120 mg	0.4 ml	0.4 ml	15mg	10 ml	500	Q.S.

Prewighted amount of amount HPMC E-50 was dispensed into a 100 ml capacity beaker. 8 ml of ultrapure water was slowly added to it and the mixture was kept for stirring. The temperature was slowly raised to 70°C and stabilized for 15 min. Further, temperature was slowly turned down to 40°C and maintained constant until the end of experiment.

The polymeric mixture was stirred continuously at the rate of 350 rpm/min for 1 hr. Later, predetermined amount of PEG -400 and glycerol were added. Finally 500 mg (50 mg/ml of H<sub>2</sub>O) of extract was dissolved in 2 ml of ultrapure water and slowly poured into polymer excipient mixture and was continuously stirred at 40<sup>o</sup> C for 1 hr. Quantity sufficient amount of pine apple flavor was added at the end. The polymer drug mixture which had attained considerable viscosity was sonicated for 20 min to remove entrapped air. The mixture was poured into 5 inch diameter petri-dishes carefully from sides and maintained at 45°C for 2 hrs. The placebo films were prepared in similar way without incorporating extract. The formed films were peeled from petri dishes and cut into 2 X 2 cm size using sterile Bard-Parker blade # 15. The formulations were named F1, F2, F3 and F4 of which F4 was used up for further studies due to its superior mechanical properties.

## 2.3. Characterization

### 2.3.1. Qualitative analysis of *E. officinalis* extract for gallic acid

Gallic acid identification was done using RF-UFLC with diode array detector at J .S. S. College of Pharmacy, S.S. Nagar, Mysuru.

Preparation of standard: 25 mg of standard gallic acid was weighed and dispensed into 50 ml volumetric flask and dissolved in methanol. The volume was made up to 50 ml by remaining methanol.

Preparation of sample: About 250 mg extract presumed to contain gallic acid was dispensed (equivalent to 25 mg gallic acid) into a 50 ml volumetric flask. Sample was dissolved to make up volume to 50 ml. Dilute 5 ml to 50 ml with mobile phase.

Preparation of mobile phase for UFLC: Ultra-pure water with 2% of acetic acid (solution A) acetonitrile (solution B) were mixed in 90:10 (A: B) ratio to be used as mobile phase. 5 ml of standard was diluted with 50 ml mobile phase.

UFLC Chromatographic conditions: Chromatographic separation was done using Phenomenex C Column (250 X 4.6 mm, 5μ ID). The mobile phase consisted of water with 2% acetic acid and acetonitrile (90:10, v/v). The flow rate was adjusted to 1.2 ml/min and run time was adjusted to 10 min. Gallic acid was detected at a wavelength of 272 nm using a PDA detector with retention time 2.5 min. 10μl of injection volume was used for injection.

### 2.3.2. FTIR spectroscopy of films

Interaction of drug and polymers was studied using Fourier transform infra-red spectroscopy. Spectrum was recorded between 400-4000 cm<sup>-1</sup> using the instrument JASCO-460 Plus, Japan, for *E. officinalis* extract, placebo film and extracts film.

### 2.3.3. Uniformity of film thickness

The film thickness was evaluated using Micrometer (Mitutoyo Co. Ltd., Japan) at 5 different locations and mean thickness was calculated. A total number of 30 samples were used for measurement.

### 2.3.4. Measurement of tensile strength

Tensile strength of films was measured using Universal Testing Analyzer (UTA-TX-XT2 texture analyzer) on sample dimension of 2×2 cm. The tensile strength was calculated by dividing applied load at rupture with cross-sectional area of film [20].

### 2.3.5. Folding endurance of film

Ten samples of size 2 X 2 cm were repeatedly folded at one place for several times. Number of times the film could be folded at same place without breaking gave value of folding endurance [21].

### 2.3.6. Surface pH

Surface pH was determined to keep the films close to salivary pH. A combined pH electrode was used to determine pH. Surface of films was slightly wetted with water and electrodes were touched to record pH [12].

## 2.4. Pharmacological studies

### 2.4.1. *In vitro* disintegration and dissolution studies using RF-UFLC method

The film size required for dose delivery (2×2 cm) was placed in a glass container containing 10 ml of ultrapure water. Time required for film to break was noted as *in vitro* disintegration time.

*In vitro* dissolution test was carried out in USP type II basket dissolution apparatus. Samples of films equivalently containing ~ 50 (2cm<sup>2</sup>) drug were cut and placed in 300 ml dissolution media, which consisted of freshly deionized simulated saliva (pH 6.8) maintained at 37 ± 1 °C at a stirring speed of 50 rpm. Samples of 5 ml quantity were withdrawn at predetermined time intervals and replaced with fresh medium. The solution was filtered using Whatman filter Paper # 41. The UFLC method was used to quantify gallic acid from films. A time versus extract release from film was constructed using data obtained.

### 2.4.2. Scanning Electron Microscopy

SEM studies were carried out to determine surface morphology of placebo films and films incorporated with drug [22] using Hitachi S3400N. Both back scattered electron and secondary electron imaging modes were used for scanning.

### 2.4.3. Bacteriological studies

Minimum inhibitory concentration of *E. officinalis* extract containing gallic acid was determined by agar well diffusion method using Brain-Heart-Infusion Agar. Serially diluted samples from 200 mg/ml to 50 mg/ml extract were diffused into wells and results observed after 24 hrs.

*In vitro* microbiological studies were carried out on *E. officinalis* films to confirm that the antimicrobial activity is retained after getting released from films [23]. A 2 x 2 cm *E. officinalis* film was dissolved in 25 ml ultrapure water. The well diffusion was performed using standard protocol [24] using BHI agar. The control and test well were bore and filled with aqueous solution of film containing MIC value of drug and other was left blank. The plates were incubated at 37° C.

## 2.5. Toxicological studies

### 2.5.1 Hemolytic assay

Hemolysis assay was done using fresh chicken blood to observe lysis of red blood corpuscles due to change in isotonicity and molecular interactions [25]. Hemolysis assay was carried out using Halfman group standard procedure on serially diluted samples from ~50mg/ml ~ 12.5 mg/ml and absorbance reading was recorded at 541 nm using UV-Vis spectrophotometer. The hemolytic activity was calculated using the following formula –

$$[(A_{bs} - A_{b_{-ve}}) / (A_{b_{+ve}} - A_{b_{-ve}})] \times 100$$

Where,

A<sub>bs</sub> -Absorbance of the sample

A<sub>b<sub>-ve</sub></sub> – Absorbance of negative control

A<sub>b<sub>+ve</sub></sub> – Absorbance of positive control

### 2.5.2 Genotoxicity and teratogenicity assay

Possible genotoxicity of films was assessed using hen's egg test [26-27] on 4-day-old fertilized hen eggs. 30 eggs were collected from National Hatcheries, Gundlupete.

Eggs were divided into 3 groups of 10 each and were named as control, blank and test group. 2 x 2 cm film containing 5% *E. officinalis* extract was cut in to small pieces using a sterile Bard-Parker blade. The chorioallantoic membrane (CAM) was located using high intensity candle light source. A small vent was made through middle of egg carefully using sterile needle. Shred pieces of films were inserted carefully through this small vent. The hole was carefully sealed using candle wax. The control group received 100 µl of ultra-pure water. All eggs were incubated at 37° C for 12 days.

### 2.6. Statistical analyses

The statistical analysis for drug release profile was carried out using Statistical Package for Social Sciences (SPSS) software using Pearson correlation ( $p < 0.05$ ). Correlation between extract released from films with respect to time was made keeping correlation significance level set at 0.05 [28].

## RESULTS AND DISCUSSION

Dental caries is one of the most common cost intensive dental diseases, although rarely life threatening, it is a major problem for dental health service providers [29]. This multifactorial disease is caused by presence of fermentable carbohydrates, microorganisms and biofilm adhering to tooth surface. *S. mutans* has been considered as a major pathogen causing dental caries. The primary habitat for *S. mutans* is mouth, pharynx, and intestine [30]. It is a facultative anaerobe; therefore, growth on sugar leads to formation of organic acids locally on tooth surface which reduces *pH* in bacterium's microenvironment. Since *S. mutans* is able to directly adhere to tooth's hydroxyapatite (HA) matrix, *pH* of tooth surface may be easily reduced to below critical *pH* of HA demineralization [31]. Therefore, establishing a control over *S. mutans* within oral environment is one of the critical factors in management of dental caries.

### 3.1. Quantitative analysis of gallic acid

Gallic acid which is a polyphenol is one of active ingredients of *E. officinalis* fruit extract and is considered to be responsible for its antimicrobial activity [32-33]. Therefore, we used this gallic acid containing *E. officinalis* extract in fabricating suitable film.

In the present technique using RF-UFLC, standard gallic acid showed a retention time of 2.5 min. Similar retention peak was seen in *E. officinalis* aqueous extract sample at 2.5 min as seen Fig 1 and Fig 2.

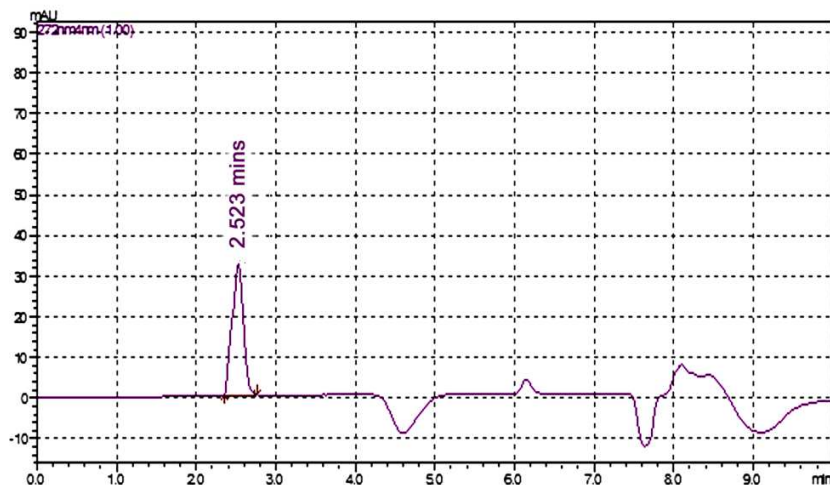


Fig 1. RF-UFLC Chromatogram of gallic acid standard showing a retention time of 2.52 min

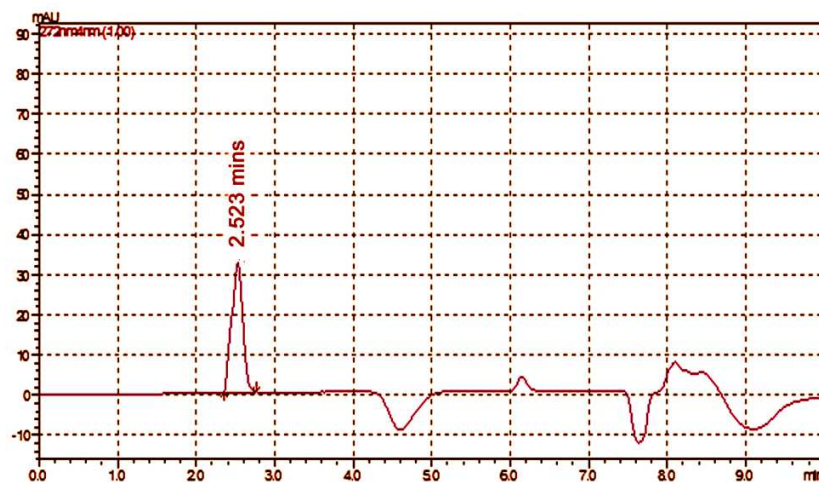


Fig 2. RF-UFLC Chromatogram showing a retention peak at 2.52 min confirming the presence of gallic acid in *E. officinalis* extract

### 3.2. FTIR Spectroscopy

FTIR analysis was performed to check the extract-material and polymer interaction. The spectrum obtained for *E. officinalis* extract shows peaks at  $3436\text{ cm}^{-1}$  (O-H) representing phenolic group,  $1632\text{ cm}^{-1}$  (C=C) alkenes,  $1044\text{ cm}^{-1}$  (C-N) stretching due to aliphatic amines as shown in Fig 3.

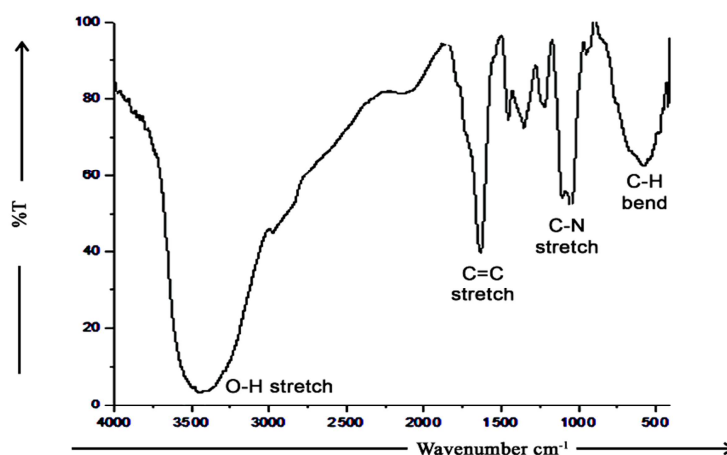


Fig 3. FTIR spectroscopy of *E. officinalis* extract with major functional groups

Similarly, spectra of placebo films shows  $2916\text{ cm}^{-1}$  (O-H), at  $2129\text{ cm}^{-1}$  (C≡C) alkynes,  $1948\text{ cm}^{-1}$  (C=O) aldehydes,  $1645\text{ cm}^{-1}$  (C=C) alkenes and that of *E. officinalis* film shows (O-H) group at  $3521\text{ cm}^{-1}$ , (C≡C) group at  $2125\text{ cm}^{-1}$  alkynes,  $1948\text{ cm}^{-1}$  (C=O) aldehydes assigned for unaltered polymeric material, as shown in Fig 4.

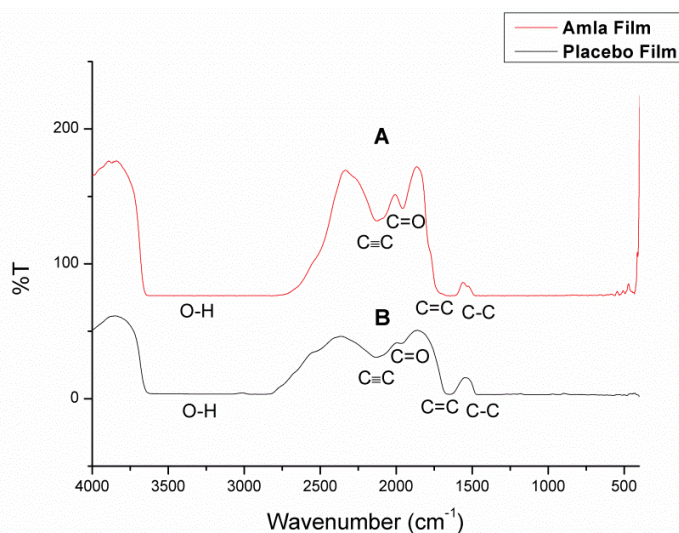


Fig 4. a. FTIR spectroscopy major functional groups of *E. officinalis* film; b. FTIR spectroscopy of placebo film demonstrating unaltered polymeric material.

### 3.3. Mechanical properties

Assaying mechanical properties of mouth dissolving films are essential for proper handling and dispensing of the films. Table 2 shows the mechanical properties of all the four formulations [34]. Out of the four formulations checked for mechanical properties, the F4 showed favorable mechanical properties with *pH* compatible with oral cavity and was used for further studies.

Table 2 -Mechanical properties of different film formulations

SL No.	Avg. thickness (in mm)	Tensile Strength (N/Cm <sup>2</sup> )	Folding Endurance	Weight Variation (mg)	Surface <i>pH</i>	Tackiness
F1	0.068±0.015	87±0.2	30	40.08	6.2	tacky
F2	0.091±0.017	98.57±0.2	34	40.44	6.3	tacky
F3	0.18±0.017	101.24±0.1	37	41.27	6.8	Slightly tacky
F4	0.21±0.017	107.65±0.1	45	42.39	6.7	Non tacky

### 3.4. Disintegration and dissolution studies

The term disintegration with respect to pharmaceutical formulations is defined as a state in which any residue of formulation except fragments of insoluble fractions remaining in media used for performance of test [35]. The disintegration time of formulations was 14.53 min.

One of the objectives of this study was to extend dissolution time of films so as to make the extract available for a longer duration within oral cavity for a better extract-bacteria interaction. The dissolution study was carried out using USP basket type apparatus [36]. More than 80% of *E. officinalis* extract with gallic acid from film was released by 10 min and from then a constant increase in release took place till 30<sup>th</sup> min. Continuous release for nearly 30 min within oral cavity ensures that sufficient extract is available for local action. RF-UFLC chromatogram of film layer shows release of gallic acid marker compound from film as demonstrated in Fig 5. The time versus extract release (in percentage) is demonstrated in Fig 6.

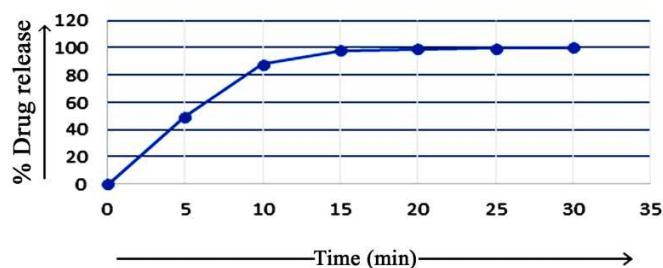


Fig 5. Release of gallic acid from *E. officinalis* film confirmed by UFLC method. In the present method, gallic acid of *E. officinalis* extract showed a retention time 2.5 min. The same was observed for the compound released from film confirming the presence of gallic acid



Fig 6. Time versus extract release plot of a 2x2 cm *E. officinalis* film. The graph demonstrates the availability of extract containing gallic acid in media (saliva) up to 30 min

### 3.5. Scanning electron microscopy

The scanning electron microscopy was done to assess the surface properties of film. Scanning electron micrograph of *E. officinalis* films showed considerable surface roughness morphologically. At 1800 X resolution the image showed flakes of extract material incorporated in polymeric matrix. Fig 7a and 7b shows the SEM images of *E. officinalis* film.

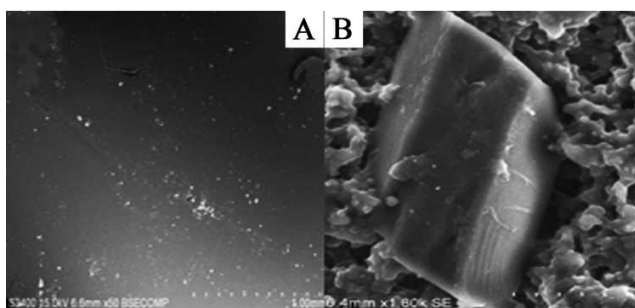


Fig 7. a. SEM micrographs of Amla film showing short range order polymer matrix incorporated with randomly dispersed extract material; b. SEM micrographs showing a single extract particle in the vicinity of polymer matrix

### 3.6. Antibacterial activity

The antibacterial activity of extract is attributed to the presence of gallic acid which was confirmed by RF-UFLC method. When the extract containing gallic acid was tested for antimicrobial activity against *S. mutans* it showed a MIC value of 50mg/ml (5%) with a zone of inhibition of 32 mm. Similarly, retention of antibacterial activity of formed films were confirmed using well diffusion method which showed inhibition of *S. mutans* around the test well filled with aqueous suspension of film sample containing extract. The zone of inhibition measured around 20 mm. Fig 8 shows antibacterial activity of *E. officinalis* extract and Fig 9 shows that of film.



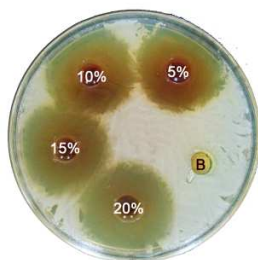


Fig 8. Agar plate showing minimum inhibitory concentration of *E. officinalis* extract. The MIC value was determined to be at 50 mg/ml

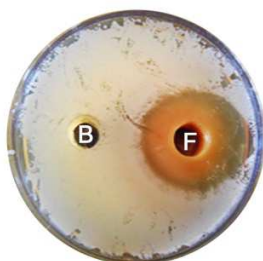


Fig 9. Antibacterial activity of mouth dissolving film containing *E. officinalis* extract. The test well (F) shows a zone of inhibition of 20mm

### 3.7. Toxicological studies

The preliminary toxicological screening tests were performed to assess possible biocompatibility of the extract and the film in *ex vivo* conditions so that further animal and human trials can be considered.

#### 3.7.1. Hemolysis assay

Hemolysis of fresh chicken blood was assessed to estimate lysis of RBC caused by films. Films containing 5% extracts showed a lysis activity of ~2.9% which falls within safe range of 5% lysis. This ensures that films containing extracts can be considered as haematologically safe. Fig10 shows percentage haemolysis activity by different concentrations.

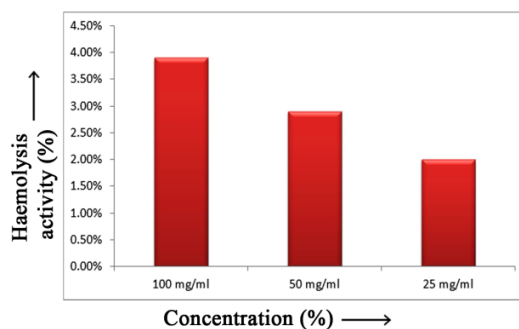


Fig 10. Bar graph representing the hemolytic activity of *E. officinalis* film. The film containing MIC value (50 mg/ml) extract showed less than 5% hemolytic activity indicating that the films containing extract are hematologically safe

#### 3.7.2. Genotoxicity assay

On 12<sup>th</sup> day of incubation, the eggs were dissected and embryos were observed for morphological changes. Both test and control group embryos showed normal growth of wings, limbs and beak, also there was no evidence of hepatomegaly and cardiomegaly. Breast plates were observed to be normal. This indicates that the films do not show any teratogenicity in embryos.

### 3.8. Statistical analysis

The statistical significance of gallic acid release pattern was calculated using UFLC data and Pearson correlation was applied and results showed that dissolutions studies on films were statistically significant with  $p < 0.05$  [37] and study can be applied for larger population.

In summary, a natural material having potent antibacterial activity has been used in formation of mouth dissolving films which were also suitably modified to release extract containing gallic acid within oral cavity for a longer duration against the conventional flash release type films. Therefore the films offer better compliance over other traditional formulations like mouth washes, gels and topical fluoride applicants whose stay-in-mouth time is 5-10 min and their effectiveness is based completely on the patient's ability to hold them inside mouth [38-39]. Further the polymers, HPMC E-50 and PEG-400 are completely biocompatible [40] and initial toxicology screening using chick embryo test and hemolysis test confirmed the same with the extract loaded films.

### CONCLUSION

From the present *ex vivo* study, the authors conclude that gallic acid containing *E. officinalis* fruit aqueous extract is a potential antimicrobial source which is successfully targeted against *S. mutans*. The same extract when loaded as drug inside biocompatible polymers used in the study did not alter which was confirmed by FTIR studies. The extract loaded films showed antimicrobial activity against target microbe with inhibitory zone of 20 mm. Further the films met the objective of extending release time up to 30 min which ensured that sufficient interaction time between pathogen and extract. Hence they can be considered as novel alternative to conventional mouth wash like formulations and can be applied into commercial usage after appropriate clinical trials.

### Acknowledgements

This work was supported by University Grant Commission, India, under University with Potential for excellence Programme (UPE), University of Mysore. We thank Prof. M. S. Thakur, Visiting Professor, Center of Material Science, University of Mysore, for extending his valuable support in preparing this manuscript. We extend our acknowledgements to Prof. H. G. Shivakumar, Principal, JSS College of Pharmacy, for his complete support towards UFLC identification and quantification of gallic acid isolate; Prof. Lancy De Souza, Maharaja College for his support towards statistical analysis; Prof. M. S. Sudarshana, Chairman, Department of Studies in Botany, for identification of *Embllica officinalis* plant and fruits used in the present study.

### REFERENCES

- [1] N Ramazani; HR Poureslami; R. Ahmadi; M. Ramazani. *J. Compr. Ped.*, **2010**, 2(2), 47-52.
- [2] SA Steinberg. *Dent. Today.*, **2007**, 26(1), 136-139.
- [3] FC Volpato; F. Jeremias; DM Spolidorio; SR Silva; A Valsecki Jr; FL Rosell. *J. Braz. Dent.*, **2011**, 22 (4), 280-284.
- [4] RM Murata; LB Almeida; EM Franco; R Yatsuda; MH Santos; SM De'alencar; H Koo; PL Rosalen. *Biofouling.*, **2010**, 26 (7), 865-872.
- [5] A Sathpathy; S Ravindra; A Porwal; M Kumar; I Mukhyopadhyaya. *J Oral Sci.*, **2013**, 55(2), 99-105.
- [6] P Javale; S Sabnis. *Asian J. Exp. Biol. Sci.*, **2010**, 1(Spl), 91-95.
- [7] P Mayachiew; S Devahastin. *LWT Food Sci. Technol.*, **2008**, 41(7), 1153-1159.
- [8] P Mukhargee; S Rai; S Bhattacharya; PK Debanath; T Biswas; U Jana; S pandit; B Saha; PK Paul. *IJPR.*, **2006**, 5(1), 51-64.
- [9] PR Bhandari; MA Kamdod. *Int. J. Green Pharm.*, **2012**, 6(4), 257-269.
- [10] A Borges; C Ferreira; MJ Saavedra; S Manuel. *J. Microb. Drug Resist.*, **2013**, 19 (4), 256-265.
- [11] H Akiyama; K Fujii; O Yamasaki; T Oono; K Iwatsuki. *J. Antimicrob. Chemother.*, **2001**, 48(4), 487-291.
- [12] CS Vicas; K Namratha; P Shubha; K Byrappa. *J. Green Sci. Techol.*, **2013**, 1(2):91-97.
- [13] N Thakur; M Bansal; N Sharma; G Yadav; P Khare. *Adv. Biol. Res.*, **2013**, 7(2), 50-58.
- [14] RP Dixit; SP Puthali. *J Cont. Release.*, **2009**, 139(2), 94-97.
- [15] C Jittinan; S Krisana; V Pakpachong. *IJIMT.*, **2013**, 4 (2), 265-268.
- [16] UC Galgatte; SS Khanchandani; GY Jadhav; PD Chaudhari. *AAPS Pharm. Sci. Tech.*, **2013**, 5(2), 1465-1472.
- [17] KB Liew; YT Tan; KK Peh. *Drug Dev. Ind. Pharm.*, **2014**, 40 (1), 110-119.
- [18] S Honary; M Golkar. *Iran J. Pharm. Res.*, **2003**, 2(2), 125-127.
- [19] K Vijayasri; P Rohini; P Reddy. *Asian J. Pharm. Clin. Res.*, **2012**, 5(4), 266-270.
- [20] P Gunjan; M Darshan. *J. Pharm. Bioallied. Sci.*, **2012**, 4(1), 35-46.
- [21] BK Satishbabu; BP Srinivasan. *Ind. J. Pharm. Sci.*, **2008**, 70(2), 175-179.
- [22] F Cilurzo; IE Cupone; P Minghetti; F Selmin; L Montanari. *Euro J. Pharm. Bio. Pharm.*, **2008**, 70 (3), 895-900.
- [23] KB Liew; YT Tan; KK Peh. *AAPS PharmSciTech.*, **2012**, 13(1), 134-142.

- [24] S Senthil Kumar; M Kamaraj. *Bot. Res. Intl.*, **2011**, 4(2), 41-52.  
[25] C Chen; YC Cheng; CH Yu; SW Chan; MK Chenug; PH Yu. *J. Biomed. Mater. Res. A.*, **2008**, 87(2), 290-298.  
[26] M Conceição; B Protti. *J. Ecotox. Env. Cont.*, **2012**, 7(1), 43-47.  
[27] V Hamburger; H Hamilton. *Reprinted J. Morph.*, **1992**, 88 (2), 231-272.  
[28] C Ball; KA Woodrow. *Antimicrob. Agents Chemother.*, **2014**, 58(8), 4855-4865.  
[29] PD Marsh. *Microbiol. Sgm.*, **2003**, 149(3), 279-284.  
[30] WJ Loesche. *Microbiol. Mol. Biol. Rev.*, **1986**, 50(4), 353-380.  
[31] SD Forssten; M Bjorklund; AC Ouwehand. *Nutrients.*, **2010**, 2(3), 290-298.  
[32] L Jagadish; VK Anand kumar; V Kaviyarasan. *Ind. J. Sci. Technol.*, **2009**, 2(1), 30-33.  
[33] SG Patil; AA Deshmukh; AR Padol; DB Kale. *Int. J. Toxic. App. Pharmacol.*, **2012**, 2(4), 49-51.  
[34] MDN Siddiqui; G Garg; PK Sharma. *Adv. Bio. Res.*, **2011**, 6(1), 291-293.  
[35] U Radhakisan; N Chauhan; N Tribhuvan. *Int. Res. J. Pharm.*, **2012**, 21(1), 169-178.  
[36] S Kalyan; M Bansal. *Int. J. Pharm. Tech. Res.*, **2012**, 5(4), 725-733.  
[37] EA Naumova; P Gaengler; S Zimmer; WH Arnold. *Open Dent. J.*, **2010**, 4, 185-190.  
[38] A Dinge; M Nagarsekar. *AAPS PharmSciTech.*, **2008**, 2(9), 349-356.  
[39] S Vijay Kumar; B Gavaskar; G Sharan; M Rao. *Int. J. Pharm. Pharm. Sci.*, **2010**, 2(3), 29-33.  
[40] K Ghosal; S Chakrabarty; A Nanda. *Der. Pharm. Sinica.*, **2011**, 2(2), 152-158.

Cover Art:

