ANTIBACTERIAL ACTIVITY OF MOUTHWASH CONTAINING *MIMUSOPS ELENGI* LINN FRUIT SAPONINS AGAINST DENTAL PATHOGENS

ABSTRACT

This research work deals with formulation of mouthwash containing *Mimusops elengi* fruit saponins and studying *in vitro* antimicrobial activity against dental pathogens isolated from patients suffering from dental problems. In this study, saponins were isolated from the crude unripe fruit powder and tested against Gram positive microorganisms- *S. aureus*, *B. subtilis*, *S. fecalis*, Gram negative microorganisms – *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. typhi*, fungus *C. albicans* and clinical dental pathogens (Streptococci and Streptobacilli). The isolated saponins (1 % w/V) were incorporated in a suitable aqueous base to formulate a pharmaceutically accepted mouthwash with no after effects. The mouthwash was inherently sweet in taste and did not leave any dry feeling in the mouth after its usage. The mouthwash exhibited a good antibacterial activity against all the mentioned microorganisms and comparable activity to the marketed Listerine® mouthwash. Hence, the formulated mouthwash can be considered as an adjunct treatment for maintaining oral health.

Keywords: *Mimusops elengi*, fruits, saponins, mouthwash, antimicrobial, dental pathogens

INTRODUCTION

There are reported evidences of poor oral health linked to various chronic conditions, such as diabetes, cardiovascular diseases, rheumatoid arthritis and osteoporosis[1]. The early stage of gum diseases is development of dental caries, further decalcification and eventual tooth decay[2]. Penicillins and cephalosporins, erythromycin, tetracycline and metronidazole are reported to develop bacterial resistance[3]. Cetylpyridinium chloride, chlorhexidine and amine fluorides exhibit toxicity, staining of teeth, vomiting and diarrhoea[4-6]. Hence, exploring traditional knowledge which is safe with lesser side effects can be considered as good substitutes to existing synthetics.

Mimusops elengi Linn. belonging to family of Sapotaceae is popularly called as Bakul in Hindi. The bark extract is known to cure odontopathy, inflammation and bleeding of gums[7]. The bark and seed coat are used for strengthening the gum[8]. It is used in combination with *Acacia catechu* and *Punica granatum* bark and others in various herbal tooth powders, such as "Vajradanti"[9]. The unripe fruits and dried seeds are used to fix loose teeth. The young twigs of *M. elengi* are used as tooth brush for cleaning teeth[10]. The marketed ayurvedic preparation of Mimusops 'Bakuladya Taila' is applied topically for strengthening of gum and teeth[10].

Therefore, the present paper illustrates the formulation of *M. elengi* L. mouthwash, as a single active constituent and tested against clinical pathogens isolated from patients suffering from dental disease.

MATERIALS AND METHODS

Materials

Glycerine, sorbitol, sodium saccharine, menthol, solvents (AR grade) used in the extraction of saponins were purchased from S.D. Fine Chemicals Ltd. Nutrient agar (NA) and Sabourad dextrose agar (SDA) were procured from HiMedia Lab. Pvt. Ltd., India. Listerine® mouthwash (Johnson & Johnson Pvt. Ltd.) was purchased from the local market.

Methods

Procurement and identification of *M. elengi* L. fruits

The unripe fruits of *M. elengi* L. were collected from trees growing in S.N.D.T. University Juhu campus, Mumbai and identified at Zandu Pharmaceuticals Works Ltd., Mumbai. The fruits were dried and powdered in an electric mixer, stored in airtight container and kept in desiccator for further study.

Isolation of saponins from *M. elengi* L. fruits

The powdered unripe fruits were subjected to direct isolation of saponins by following two methods. Initially, 1g of fruit powder was defatted, in first method the remaining residue was dissolved in a little amount of methanol (10 mL) and poured in excess of solvent ether (500 mL) and filtered. The ether layer was evaporated[11]. In second method the residue was refluxed with methanol for 1 h and then partitioned between *n*-butanol and water. The *n*-butanol layer containing crude saponins was evaporated[11]. The solvent was further concentrated to dryness under vacuum using rotary evaporator.

Table I: Observation of average zone of inhibition of *in vitro* antimicrobial studies

Sr. no.	Organisms	Zone of inhibition (mm)					
		Fruit saponins			Mouthwash 1 % saponins	Marketed mouthwash	Chloramphenicol (250 µg mL^{-1})
		250 mg mL^{-1}	500 mg mL^{-1}	750 mg mL^{-1}			
1	*S. aureus* (ATCC 25923)	6.00± 0.030	9.00± 0.015	11.00± 0.014	5.00± 0.01	7.00± 0.00	16.00± 0.022
2	*B. subtilis* (ATCC 6633)	5.00± 0.041	8.00± 0.023	10.00± 0.031	6.00± 0.11	7.00± 0.006	13.00± 0.021
3	*E. faecalis* (ATCC 29212)	8.00± 0.056	10.00± 0.064	12.00± 0.025	7.00± 0.05	7.00± 0.002	16.00± 0.011
4	*P. aeruginosa* (ATCC 27853)	4.00± 0.022	6.00± 0.026	9.00± 0.000	7.00± 0.15	7.00± 0.015	13.00± 0.010
5	*K. pneumoniae* (ATCC 13883)	5.00± 0.014	8.00± 0.018	11.00± 0.021	7.00± 0.00	7.00± 0.001	12.00± 0.006
6	*E. coli* (ATCC 29522)	4.00± 0.00	6.00± 0.011	9.00± 0.001	7.00± 0.12	7.00± 0.014	14.00± 0.015
7	*S. typhi* (ATCC 6539)	6.00± 0.15	9.00± 0.021	11.00± 0.041	7.00± 0.09	7.00± 0.06	12.00± 0.000
8	*Streptococci* (P1)	7.00± 0.05	10.00± 0.042	12.00± 0.023	6.00± 0.012	7.00± 0.016	12.00± 0.010
9	*Streptococci* (P2)	4.00± 0.07	8.00± 0.034	11.00± 0.021	6.00± 0.011	7.00± 0.012	14.00± 0.017
10	*Streptobacilli* (P3)	5.00± 0.021	7.00± 0.017	12.00± 0.050	7.66± 0.00	7.00± 0.01	13.00± 0.00
11	*Streptobacilli* (P4)	6.00± 0.065	8.00± 0.044	11.00± 0.002	4.00± 0.002	7.00± 0.00	14.00± 0.011
							Clotrimazole (62.5 µg mL^{-1})
12	*Candida albicans* (ATCC 10231)	-	-	-	-	-	26±0.00

HPTLC technique for identification of *M. elengi* L. fruit saponins

The saponins was dissolved in methanol (10 mg mL^{-1}) and 10 µL solution was spotted with the help of CAMAG Linomat applicator on precoated silica gel 60 F_{254} plate. The solvent system was chloroform: methanol: hexane (4:0.5:1 V/V/V). The developed plate was dried and spots were scanned using a CAMAG scanner 3 under UV light of wavelength 254 nm in absorption mode and 366 nm in fluorescence mode. The plates were sprayed with ASR for the identification of saponins.

In vitro antimicrobial studies of *M. elengi* L. fruit saponins

The study were carried out using agar cup diffusion method. The microorganisms used for testing the antimicrobial activity are listed in Table I. The plaque samples of patients were collected from Nair Hospital Dental College, Mumbai and identified as *Streptococci* and *Streptobacilli*[12].

The saponins and standards (concentration as mentioned in Table I) were added to different cups bored

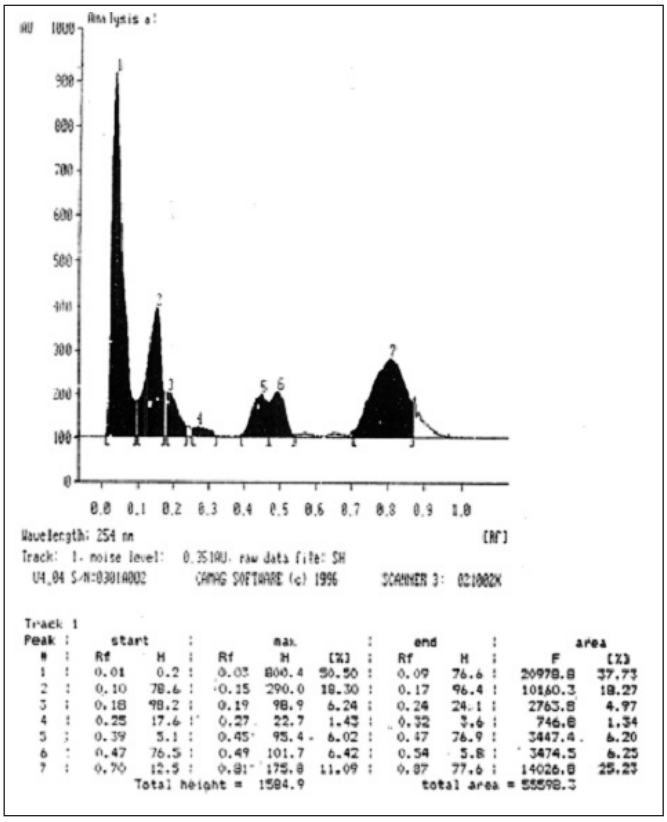

Fig. 1: HPTLC fingerprint of fruit saponins of *M. elengi* L. at 254nm

in agar plates containing bacterial and fungal culture. The prepared petriplates were incubated for 24 h at 37 ^{0}C for bacteria and 25 ^{0}C for fungi.

Formulation development of mouthwash containing *M. elengi* L. fruit saponins

1 % (w/V) saponins containing mouthwash was formulated. Further, glycerine, sorbitol, menthol and sodium saccharin previously dissolved in little amount of alcohol, were, added to the saponin solution. The pH of the mouthwash was adjusted with 10 % sodium hydroxide solution between 6.7-7.3. The prepared mouthwash was filled in clean transparent glass bottles and stored at RT.

Evaluation of mouthwash containing *M. elengi* L. fruit saponins

The prepared mouthwash was evaluated for the physicochemical parameters like appearance, colour, odour and the extract content. The efficacy of the prepared mouthwash was compared with the marketed mouthwash Listerine® by *in vitro* antimicrobial method.

RESULTS AND DISCUSSION

Isolation of saponins from *M. elengi* L. fruits

The percentage yield of saponins by method 1 (0.07 %w/w) was very less compared to method 2 (1.6 %w/w), hence procedure of method 2 was selected for isolation of saponins.

HPTLC technique for identification of *M. elengi* L. fruit saponins

The developed sprayed plate showed 4 purple coloured spots at Rf of 0.18, 0.36, 0.65, 0.73 indicating the presence of terpenoidal compounds and 3 green coloured spots at Rf of 0.02, 0.05, 0.27 showing the presence of steroidal compounds[13]. HPTLC fingerprints depict 7 and 6 components at 254 and 366 nm, respectively (Fig. 1).

In vitro antimicrobial studies of *M. elengi* L. fruit saponins

The saponins showed good activity at all prepared concentrations against the tested bacteria. Saponins at 750 mg mL^{-1} showed comparable activity to the standard chloramphenicol (250 µg mL^{-1}). The saponins were found to be very active against the bacteria isolated from dental patients. The average zones of inhibition measured are given in Table I.

Formulation and evaluation of mouthwash containing *M. elengi* L. fruit saponins

A clear yellowish green coloured mouthwash was prepared with a pH compatible to the oral cavity. The developed UV-Visible spectrophotometric method was found to be linear at λmax 276 nm with a regression coefficient 0.9994. The content of saponins in the mouthwash was found to be 99.09 % w/w. It was observed in incubated petriplates that the mouthwash penetrated more easily through the aqueous agar medium compared to the crude saponins, showing good antimicrobial activity at a low concentration (Table I).

CONCULSION

The saponins isolated from *M. elengi* L. unripe fruits and formulated mouthwash were found to be active against various gram positive and negative bacteria and the clinical isolates from dental patients. Mouthwash containing saponins was formulated with an acceptable colour and pleasant odour and good after effect. The results of the present study do confirm that the saponins containing mouthwash can be used as an adjunct to oral cleaning.

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