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In vitro Antibacterial Activity of *Manilkara hexandra*(Sapotaceae) Seed Extracts and Violacein against Multidrug Resistant *Streptococcus mutans*

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Abstract

Extracts of the *Pongamia pinnata*, *Manilkara hexandra*, and *Pyrus pyrifolia* seeds prepared by microwave assisted extraction method, and the violet pigment- violacein extracted from *Chromobacterium violaceoum* were screened for their antibacterial activity against *Streptococcus mutans*. *M. hexandra* extracts were able to inhibit both the test strains of *S. mutans* used in this study with minimum inhibitory concentration (MIC) ranging from 600-800 µg/mL. These extracts exerted bactericidal action against *S. mutans* with minimum bactericidal concentration (MBC) of 600-900 µg/mL. Acetone extract of *M. hexandra* seeds registered highest average total activity of 231.20 mL/g. Extraction efficiency was found to have a moderately good correlation with antibacterial activity. Violacein exerted bactericidal action with MIC and MBC of less than 2µg/mL against both the strains.

Keywords: Bactericidal, biofilm, microwave assisted extraction

1. Introduction

Dental caries and periodontal diseases are two of the most common infections of humans. In India, the prevalence of dental caries varies from 33.7% to 90% in the child population, and is increasing at an alarming rate [1]. In the United States, dental caries affects 59% of children between the ages of 5 and 17. Plaque bacteria are among one of the major etiological elements involved. *Streptococcus mutans* and *Streptococcus sobrinus* are considered to be the most important species in the initiation of enamel caries. In cariogenic communities, *S. mutans* comprises approximately 10% of the total cultivable microbiota. Oral Streptococci are among the dominant members of the oral microbiota, and are important primary colonisers of mucosal and tooth surfaces. Many are able to synthesize polysaccharides, which constitute the major component of the matrix of dental plaque and can also serve as carbohydrate sources for other oral species. *Streptococcus* is among the predominant genera detected in the oral cavity. Members of the mutans group of oral Streptococci (*S. mutans* and *S. sobrinus*) are found mainly in dental plaque and are associated with dental caries. Some oralStreptococci are associated with endocarditis and abscess formation [2].

Organisms present in the oral cavity such as *Streptococcus mutans* are capable of substantial biofilm formation on the surfaces of teeth [3]. Biofilm formation is very important and characteristic feature of the organisms inhabiting the oral cavity. An important consideration in the use of antibiotics to treat plaque-related diseases is the well-documented recalcitrance of biofilms to antimicrobial agents. Antibiotics such as

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ciprofloxacin have little effect on the oral microbiota. There is increasing evidence that the resistance of oral bacteria to many antibiotics (such as Tetracycline, Penicillin, Amoxycillin, Erythromycin, and Clindamycin) is increasing. A wide range of antimicrobial agents have been incorporated into toothpastes and other dentifrice products in order to improve the levels of achievable oral hygiene. Certain plant extracts are also being used as ingredients of few oral care products.

Finding novel products (including those of plant origin) active against cariogenic microbes like *S. mutans* is important as even some commercially available dentifrice products are also reported not to be effective against their biofilms. Listerine® mouth wash, *Cymbopongon citrates* (lemon grass), *Plectrantus amboinicus* (mexican mint), and *Conyzabonariensis*(hairy fleabane) tinctures were reported to possess no inhibitory action against the dental biofilm-forming bacteria [4]. Not so appreciable antimicrobial activity in Listerine®, while complete lack of antimicrobial activity in Toss-K and Senquel-AD against four different dental caries pathogens (including *S. mutans* strain employed in our work) was reported by Aneja et al. [5]. Although several antiplaque agents are available in the market, the search for more effective agent(s) still continues [6]. Plant products effective against oral pathogens like *S. mutans* can find use as ingredients in chewing sticks, toothpastes or other dentifrice products.

The present study was aimed at investigation of antibacterial potential of three different plant seeds i.e. *Pongamia pinnata* Lam. (Papilionaceae), *Manilkara hexandra* Roxb. (Sapotaceae), and *Pyrus pyrifolia* Burm. (Rosaceae) against *Streptococcus mutans*. Common Indian names for these plants are Karanj, Rayan, and Nashpati respectively. Mahida and Mohan reported *M. hexandra* (leaves or tubers) extracts to possess antibacterial activity [7]. Chanda and Parekh [8] reported antimicrobial activity of *M. hexandra* leaf extracts against few gram- positive and gram-negative bacteria, as well as fungi. *P. pinnata* seed oil has been reported for antibacterial as well as antifungal property [9]. *P. pinnata* bark, leaves, and seed extracts were reported for their antioxidant and antimicrobial properties by Sajid et al [10].

Additionally, we also tested Violacein for its activity against *S. mutans*. Violacein is a violet colored pigment produced mainly by bacteria of the genus *Chromobacterium* e.g., *Chromobacterium violaceum*. Violacein has been reported for its antitumor, antimicrobial, and antiparasitary properties [11].

2. Materials and Methods

2.1 Plant Materials

Seeds of *P. pinnata* were collected from its tree in the Nirma University campus (Ahmedabad, Gujarat, India). Seeds of *M. hexandra*, and *P. pyrifolia* were procured during October, 2013 to February, 2014, from the fruits purchased from local market in the city of Ahmedabad. They were authenticated for their unambiguous identity by Dr. Himnashu Pandya, Department of Botany, Gujarat University, Ahmedabad.

2.2 Bacterial Cultures

Three bacteria used in this study (Table 1) were procured from Microbial Type Culture Collection (MTCC), Chandigarh.

2.3 Extraction of Plant Materials

Seeds were extracted in three different solvents (Merck, Mumbai, India) - acetone, methanol, and ethanol (50%) by microwave assisted extraction (MAE) method [3, 12]. One gram of dry seed powder was soaked into 50 mL of solvent, and subjected to microwave heating (Electrolux EM30EC90SS) at 720 W. Total heating time was kept 120, 90 and 70 second for acetone, methanol and ethanol, respectively, with intermittent cooling. This was followed by centrifugation (at 7,500 rpm for 20 min), and filtration with Whatman paper # 1 (Whatman International Ltd., Maidstone, England). Solvent was evaporated from the filtered extract and then the dried extracts were reconstituted in dimethyl sulfoxide (DMSO) for antimicrobial assay. Reconstituted extracts were stored under refrigeration for further use. Extraction efficiency was calculated as percentage weight of the starting dried plant material.

2.4 Extraction of Violacein

C. violaceum was grown in nutrient broth (HiMedia) for 72 h at 35˚C. After measuring growth by recording OD at 625 nm (Elico SL210), Violacein extraction from

Table 1: Test organisms

No.	Organism	MTCC code	[a]Remarks (with input from MTCC manual)
1	Streptococcus mutans	497	Isolated from carious dentine, Streptomycin resistant up to 30 µg/ml,Cefaclor and Cefotaxime resistant (30 µg/ml).
2	Streptococcus mutans	890	Opportunistic pathogen, causative agent of dental caries, production of Streptokinase in small amount, Streptomycin resistant till 30 µg/ml
3	Chromobacterium Violaceum	2656	Producer of violet pigment-Violacein

[a]Antibiotic susceptibility determined by microbroth dilution assay and disc diffusion assay in our lab.

C. violaceum culture was carried out as described in Choo et al. [13]. Briefly, the culture broth was centrifuged (Nüve NF 800 R) at 12,000 rpm for 15 min, and the resulting supernatant was discarded. The remaining cell pellet was resuspended in DMSO (Merck, Mumbai) , and incubated at room temperature for 30 min, followed by centrifugation at 12,000 rpm for 15 min. TheViolacein extracted in the supernatant was estimated by measuring OD at 585 nm [14]. Concentration of Violacein was calculated using its molar extinction coefficient at 585 nm ($\in$ = 0.05601 mL μg^{-1} cm^{-1}) [15]. Spectrum of the extracted Violacein was also generated to confirm purity (Fig. 1).

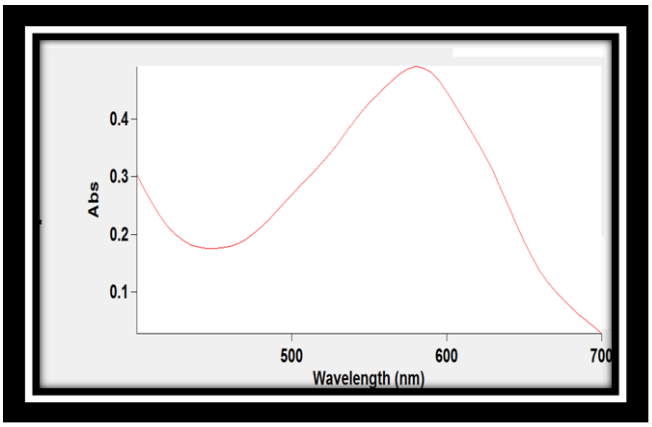


Fig. 1. Visible spectrum of violacein showing maximum absorbance (λ_{max}) at 585 nm.

2.5 Broth Dilution Assay against Planktonic Cells

Both the test organisms were challenged with different concentrations of all the three seed extracts and Violacein. MIC (minimum inhibitory concentration) was determined using microbroth dilution method as described in [3], in accordance with NCCLS guidelines [16]. Assay was performed in 96-well microtitre plates (HiMedia TPG96). Total volume of the assay system in each well was kept 200 µL. Brain heart infusion broth (BHI; HiMedia) was used as growth medium. All the extracts of *P. pinnata,* and methanolic extract of *P. pyrifolia* were getting precipitated in BHI, and hence could not be tested. Inoculum density of the test organisms was adjusted to that of 0.5 McFarland standard. Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. Extracts (reconstituted in DMSO) or Violacein were serially diluted into each of the wells. A DMSO control was included in all assays [17]. Gentamicin and Streptomycin (HiMedia) served as positive control. Appropriate abiotic controls (containing media and extract, but no inoculum) were also set. Plates were incubated at 35°C for 48 h, before being read at 655 nm in a plate reader (BIORAD 680). MIC was recorded as the lowest concentration at which no growth was observed. All MICs were determined on three independent occasions. Concentration at which growth was inhibited by 50% was recorded as IC_{50}value. After reading the plates for MIC, subculturing was made on nutrient agar from the wells showing no growth, so as to determine whether the extract is bactericidal or bacteriostatic. Incubation was extended for 72 h to detect any possible post extract effect (PEE) [12], as the agents exhibiting a post antibacterial effect (PAE) require extended incubation following subculture in either time-kill or minimum lethal concentration (MLC) determinations

in order to ensure the detection of slow-growing but not dead organisms [18]. Growth on the plate indicated bacteriostatic action, absence of growth was interpreted as bactericidal action, and the concentration at which nearly 99.9% killing was observed was taken as minimum bactericidal concentration (MBC). Total activity (mL/g) was calculated as [19]: Amount extracted from 1 g (mg) / MIC (mg/mL). Activity index was calculated as ratio of MIC of antibiotic (positive control) to MIC of test extract against susceptible organisms [20].

Simultaneously, the test organisms were also challenged with Triclosan, Chlorhexidine, and two commercially available mouthwashes (Colgate Plax® and Listerine®) for comparison, using the method described above for extracts. Former two are commonly used ingredients of many dentifrice formulations [21, 22]. Test with Chlorhexidine was performed using solid media i.e. BHI agar.

2.6 Susceptibility Testing against Biofilm

Test substances which exerted antibacterial activity against planktonic *S. mutans* in broth dilution assay, were also evaluated against its biofilm. Standardized inoculum was added into the wells of a 96-well surface treated polystyrene microtiter plate (HiMedia, TPP96), using BHI supplemented with 2% sucrose [23, 24] as the growth medium. Uninoculated autoclaved medium was put in wells corresponding to sterility control. Total volume of the content filled in the wells was kept 300 µL. Incubation to allow biofilm formation was carried out at 35˚C for 48 h. Following incubation spent medium was removed from the wells under aseptic condition, and wells were filled with minimal media containing test substance (or antibiotic used as positive control). DMSO replaced the extract in the wells corresponding to negative control. Organism was incubated in this medium containing test substance for 24 h at 35˚C. Each concentration of the test substance was put in three replicate wells. Following incubation in media containing test extract, these three wells were used for assessment of biofilm viability. Viability of the biofilm was estimated by tube method (suspending the biofilm in liquid media). This method was found to be at par with viable count performed on agar plates in our previous study [3]. Biofilm was removed from the well surface by scraping with a sterile micropipette tip. This scrapped biofilm was added to sterile normal saline, and vortexed for 30 s; measured volumes of which were inoculated into defined quantity of sterile nutrient broth, and incubated for 24 h at 35°C. Following incubation growth was quantified in terms of OD at 625 nm (Spectronic 20D+, Thermo scientific). Percentage viability in the tubes corresponding to test wells was calculated in relation to OD of the negative control. Tubes from antibiotic treated wells were compared with the growth control.

2.7 High Performance Liquid Chromatography (HPLC)

Extracts dissolved in their respective solvent (methanol / ethanol / acetone) were filtered through a PVDF hydrophilic membrane syringe filter (0.22 µm; Himedia) and 10 µl aliquots of the filtrate were injected into HPLC system (Agilent 1260) with a ZORBAX Eclipse Plus C_{18} column (4.6 mm x 250 mm). Mobile phase consisted of Orthophosphoric acid (0.05%): Acetonitrile (Merck). A gradient elution was set at 1 ml/min. A gradient of mobile phase was applied by varying the relative proportion of both component solvents of the mobile phase. Ratio employed was- solvent A to solvent B (0–6 min: 5% B, 6–15 min: 15% B, 15–35 min: 20% B, 35–40 min: 40% B; A: Orthophosphoric acid, B: Acetonitrile). Detection was carried out at 220/270 nm.

3. Results

Results of MAE are reported in Table 2. Results of broth dilution assay of all the test substances against both the *S. mutans* strains are presented in Table 3. Results obtained while evaluating the test substances against biofilm are recorded in Table 4.

Table 2: Extraction and reconstitution efficiency for all the seed extracts

Seed	Solvent	Extraction Efficiency (%)	Reconstitution Efficiency (%)
Pongamia pinnata	Ethanol (50%)	21.58	19.28
	Methanol	17.08	29.86
	Acetone	17.42	26.41
Manilkara hexandra	Ethanol (50%)	13.24	83.23
	Methanol	13.78	74.31
	Acetone	17.92	5.80
Pyrus pyrifolia	Ethanol (50%)	10.40	78.85
	Methanol	9.78	77.09
	Acetone	9.32	17.59

4. Discussion

All the three extracts of *M. hexandra* were able to inhibit both the strains of *S. mutans,* with MIC values falling in the range 600-800 µg/mL (Table 3). All of these three *M. hexandra* extracts were bactericidal with MBC values falling in the range 600-900 µg/mL. For *M. hexandra* extracts the MBC/MIC ratio was found to be 1, except that of methanolic extract against MTCC 497. For the latter this ratio was found to be 1.12. A small MBC/

Table 3: Results of broth dilution assay

Seed/ Test substance	Solvent	*S. mutans strain*	IC_{50} (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	Total Activity (mL/g)	Average total activity (mL/g)	Activity Index		MBC/ MIC
								Gentamcin	Strepto-mycin	
M. hexandra	Ethanol (50%)	MTCC 497	500	600	600	220	211.53	0.016	0.066	1
		MTCC 890	550	650	650	203.07		0.015	0.061	1
	Methanol	MTCC 497	500	800	900	152.22	185.71	0.011	0.04	1.12
		MTCC 890	600	625	625	219.20		0.016	0.064	1
	Acetone	MTCC 497	ND	750	750	238.66	231.20	0.013	0.053	1
		MTCC 890	500	800	800	223.75		0.012	0.05	1
P. pyrifolia	Ethanol (50%)	MTCC 497	>1000							
	Acetone	MTCC 497	>1000				NA			
Violacein		MTCC 890		1.28	1.28		NA	7.812	31.25	1
		MTCC 497		1.66	1.66			6.024	24.098	1
Colgate Plax® (% v/v)		MTCC 497		1	2					2
		MTCC 890		1	1			NA		2
Listerine® (% v/v)		MTCC 497		>5						
		MTCC 890		>5				NA		
Chlor-hexidine		MTCC 497	ND	2.5	2.5			0.25	0.00625	1
		MTCC 890		1	1		NA	0.1	0.025	1
Triclosan	Dissolved in sterile distilled water	MTCC 497		>50	NA					
		MTCC 890		>50	NA			NA		
Gentamicin		MTCC 497		10	10					1
Streptopmycin		MTCC 890		40	40			NA		1

NA: Not applicable; ND: not determined

Table 4: Colgate Plax®against S. mutans biofilm

Organism	Concentration (% v/v)	Loss of viability (%)
S. mutans (MTCC 497)	1	No viability loss
	2	33.63±9.00**
	3	No viability loss
	4	100**
	5	100**
S. mutans (MTCC 890)	1	6.52±0.33
	2	5.74±1.44
	3	No viability loss
	4	100**
	5	100**

**p<0.01

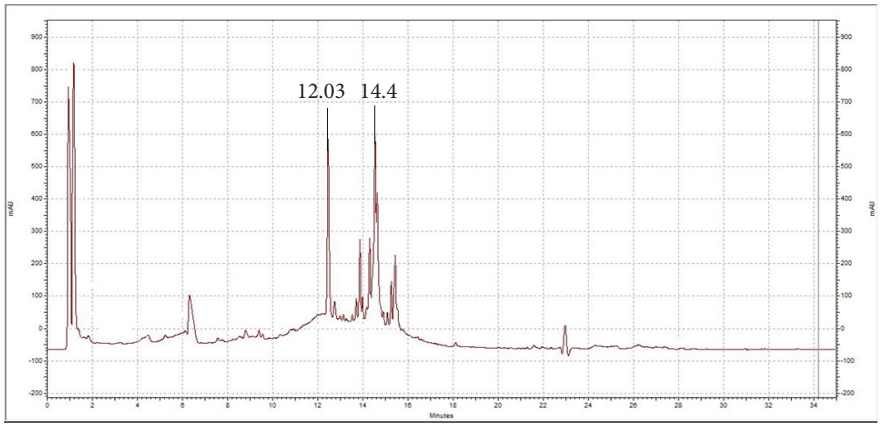
MIC ratio (<4 to 6) is generally expected for bactericidal agents [25]. This ratio between 1-2 is considered as an indication of bactericidal mode of action [26-28]. Maximum average total activity was registered by the acetone extract of *M. hexandra*. Total activity is a measure of the amount of material extracted from a plant in relation to the MIC of the extract, fraction or isolated compound. It indicates the degree to which the active fractions or compounds present in 1 g can be diluted and still inhibit growth of the test organism [19]. For example a total activity of 238.66 mL/g found for the acetone extract of *M. hexandra* against *S.mutans* (MTCC 497) means that if 1 g of this extract is diluted in 238.66 mL of the solvent, still it would be able to inhibit this organism. The *average total activity* was found to have a moderately strong linear correlation (r=0.76)with the *extraction efficiency*. These two quantities were found to have a strong correlation in our previous studies too [29, 30, 3]. This shows the importance of selecting a proper extraction method while screening for a desired bioactivity. The MAE extraction method employed in this study was previously also reported by us to be an effective method for extraction of antibacterial compounds from plant seeds [31]. *P. pyrifolia* extracts prepared in Ethanol and Acetone could not inhibit either of the *S. mutans* strains notably till 1000 µg/mL.

Among the antimicrobial agents used as positive control in this study, Chlorhexidine, Gentamicin, and Streptomycin were able to inhibit *S. mutans* with MIC values falling in the range 1-40 µg/mL, whereas Triclosan failed to inhibit *S. mutans* till 50 µg/mL. Triclosan (0.07 mmol/L) was shown to kill nearly 40% of multi-species biofilm of oral bacteria [32]. Lower efficacy of Triclosan against *Streptococci* has earlier also been reported [33, 34]. Among all the positive controls, Chlorhexidine proved to be most effective with an MIC of 1-2.5 µg/mL. Chlorhexidine gluconate was reported to kill approximately 40% of multi-species bacterial biofilm at 2.23 mmol/L [35]. From the two commercial mouthwashes tested, CP was able to inhibit both the *S. mutans* strains at 1 % v/v, and the effect was found to be bactericidal. Listerine® was not able to notably inhibit any of the test strains upto 5% v/v. This variation in the efficacy of these commercial products may be attributed to the difference in their composition. Not so appreciable efficacy of Listerine® has been reported by other workers too [4, 5].

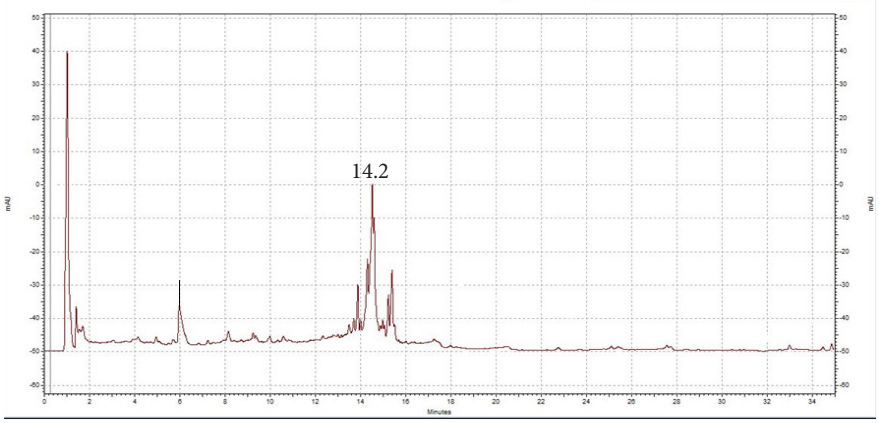
Violacein extracted from *C. violaceum* exerted bactericidal action against both the *S. mutans* strains, with an MIC of 1.28 and 1.66 µg/mL against MTCC 497 and MTCC 890 respectively. Owing to its lower MIC, it also registered appreciable activity index values (Table 3). Notably violacein had lower MIC than the commercial antibiotics (Chlorhexidine, Gentamicin, and Streptomycin) used as positive controls. Inhibitory action of Violacein on the growth of gram-positive bacteria has been reported earlier. Growth of *Bacillus* and *Micrococcus* was reported to be inhibited by Violacein at 2-20 µg/mL [36]. Nakamura et al. reported Violacein to have a cell killing effect above 15 µg/mL against *B. licheniformis*, *B. subtilis*, *B. megaterium*, and *Staphylococcus aureus*, whereas in the present study Violacein was found to be bactericidal against *S. mutans* at less than 2 µg/mL [37]. Violacein has also been reported to possess in vitro antimycobacterial activity with a MIC of 64 µg/mL and MBC of 128 µg/mL. MIC of Violacein against *S. aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Vibrio cholerae,* and *Salmonella typhi* was reported to be in the range of 5.7-20 µg/mL. Violacein has also been shown to work synergistically with most commercial antibiotics, and hence may be used as drug in combination with other antimicrobial agents [38–40]. Violacein was also reported to have an inhibitory effect on gram-negative *Meningococcus*, and the mold *Blastomyces dermatitidis* [41]. A large number of organisms have been demonstrated to be susceptible to Violacein, including *P. aeruginosa*, herpes simplex virus, polio virus, *Leishmania* species [42], and *Trypanosoma cruzi* [43]. Many of the biological effects of Violaceinare related to its ability to interfere with enzyme activities

such as phosphatases [44]. Antimicrobial potential of Violacein may also come from its ability to act as a potent inhibitor of DNA topoisomerase [45] and protein kinases [46]. Based on the active ingredient Aztreonam, originally isolated from *C. violaceum*, a synthetic bactericidal antibiotic has been marketed under the brand name AZACTAM®. Aztreonam acts by inhibition of bacterial cell wall synthesis, and its MIC against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) is in the range 0.06-0.25 µg/mL and 2-8 µg/mL respectively [47].

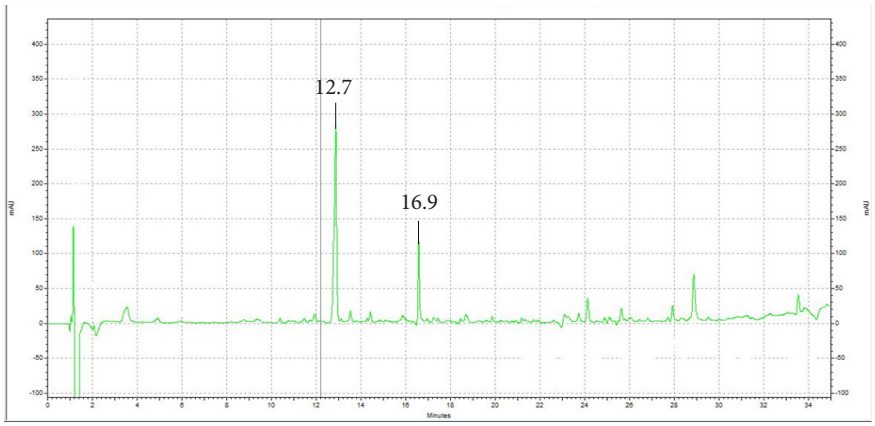
The three extracts of *M. hexandra* found effective against planktonic form of *S. mutans* were subjected to HPLC for generating their chromatographic fingerprint. Fig. 2 shows these chromatograms with retention time



(A)



(B)



(C)

Fig. 2. HPLC profile of M. hexandra seed extracts. (A): Ethanolic extract; (B): Methanolic extract; (C): Acetone extract. Retention time (in min) is indicated on top of the peaks.

of the major peaks. Since crude extracts can show considerable lot-to-lot variation with respect to their composition, chromatographic profiles of the bioactive extracts can be useful in their characterization [48]. These three extracts were also tested against biofilm form of *S. mutans*. But we could not obtain reproducible results for experiments with biofilm, except in case of CP. This product was able to kill 100% cells in *S. mutans* biofilm, at 4%v/v (Table 4). However this was higher than that required to inhibit planktonic cells of the same organism. Biofilms are well known to be much more resistant to antimicrobial agents than their planktonic counterparts [49, 50].

This study has identified *M. hexandra* extracts to be effective against *S. mutans* strains with MIC values in the range 600-800 µg/mL. All parts of *M. hexandra* plant are reported to contain Taraxerol, Cinnamates, alpha-sipnasterol, Ursolic acid, and Quercetin. Quercetin has been reported for its antimicrobial action against bacteria [51] as well as fungi [12].Mahida and Mohan found methanolic extract of *M. hexandra* leaves to be effective against *Staphylococcus epidermidis* and *S. aureus* at 2 mg/mL [7]. Chanda and Parekh reported *M. hexandra* leaf extracts to be effective against multiple gram-negative and gram-positive bacteria, and fungi with MIC values ranging from 250 to 32,000 µg/mL [8]. Such MIC values of *M. hexandra*leaves reported by other workers against different gram-positive bacteria are much higher than MIC values found in our study for *M. hexandra* seed extracts. It may be that seeds of this plant contain better antimicrobial potential than its leaves. Though simple comparisons among antimicrobial activity of different extracts are difficult because of differences in the method of extraction, microbial strains used, susceptibility assays employed, etc. Still a broad comparison of MIC values of different extracts against same pathogen may give some useful indication. *S. mutans* was found to be inhibited by *E.officinalis* fruit extracts at 12.5-50 µg/mL [5]. Smullen et al. reported MIC of different polyphenol containing plant extracts against *S. mutans* within the range 0.5-4 mg/mL [52]. Xanthorrhizol purified from the rhizome of *Curcuma xanthorrhiza* was reported to completely inhibit biofilm formation of *S. mutans* at a concentration of 5 µmol/L. This compound could kill 76 % of *S. mutans* biofilm at 50µmol/L [23]. *Rheedia brasilensis* fruit extracts could inhibit *S. mutans* at 12.5-25 µg/mL [53]. Bioactive compound (7-epiclusianone) isolated from the crude extract registered lesser MIC (1.25–2.5 µg/mL), which is comparable to the MIC of Violacein (1.28-1.66 µg/mL) reported in the present study. Islam *et al*. found *Morus alba* leaf extracts to be effective against *S. mutans* at 125 mg/L, the purified compound (1-deoxynojirimycin) from this extract had an MIC of 15.6 mg/L [54]. Islam *et al.* reported *T. indica* and *P. emblica* extracts to be effective against *S. mutans* at 6.25 mg/mL [55]. Larsen *et al*. reported Paprika and Rosemary leaves extracts to be inhibitory to *S. mutans* at MIQ (minimum inhibitory quantity) of 24–180 mg/mL [56]. Jebashree *et al.* found *Psidium guajava* and *Terminalia chebula* extracts to be effective against *S. mutans* at MIC values ranging from less than 0.076 mg/mL to more than 5 mg/mL [57]. Bacteriostatic activity of Guaijaverin, isolated from *Psidium guajava* Linn. leaves against *S. mutans* strains with MIC values of 2–4 mg/mL was reported by Prabu *et al.* [6]. Al-Sohaibani and Murugan showedthe MIC of methanol extracts of *Salvadora persica* (miswak) against cariogenic *S. mutans*to be 2.6 mg/mL [58].

5. Conclusion

This study has identified three *M. hexandra* extracts and violacein to be effective against two different *S. mutans* strains. To search for novel products effective against cariogenic bacteria such as *S. mutans* is important, as even some commercial oral care products are also reported not to possess enough efficacy against planktonic and/or biofilm forms of these organisms. Despite the availability of many dentifrice products in market, the search for better and more effective agents continues [6]. Natural products with high efficacy against oral pathogens including *S. mutans* can be screened for their suitability as ingredients of oral care formulations e.g. chewing sticks, toothpastes or mouthwashes. A crude natural product may contain relatively small amounts (<1 % w/v) of an active compound [59], and MIC of the crude extract may be 100-1000 times greater than the possible pure active compound obtained at the end of a successful fractionation and purification operation. Fractionation of the *M. hexandra* extracts may further yield the active principle(s) with still lesser MIC than the crude parent extracts. Structural studies of such active principle(s) may result in identification of new lead compound(s).

6. Acknowledgement

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