In vitro investigation of antimicrobial activities of ethnomedicinal plants against dental caries pathogens

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Abstract

The study aimed to evaluate the antimicrobial activity of medicinal plant extracts against the bacterial pathogens prominent in dental caries. A total of 20 plant species (herbs, shrubs and trees) belonging to 18 genera and 15 families were documented for dental caries. Antimicrobial activity of solvent extracts and essential oil from plants were determined by zone of inhibition on the growth of *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) using the agar well diffusion method. The results of in vitro antimicrobial assay prove that methanol is more successful in the extraction of phytochemicals from plant samples than aqueous solvent, as methanol extracts show higher antimicrobial activity than aqueous extracts against both the test pathogens. Methanol extracts of *Nigella sativa*, *Psidium guajava* and *Syzygium aromaticum* were the most effective among all 20 plant samples and have potent inhibitory activity against both dental caries pathogens with minimum inhibitory concentration of 0.2 mg mL⁻¹. *N. sativa* seed methanol extract was more effective with 22.3 mm zone of inhibition at 0.2 mg mL⁻¹ against *S. mutans* (MTCC 497), while *L. acidophilus* (MTCC 10307) was more sensitive to *S. aromaticum* bud methanol extract at 11.3 mm zone of inhibition at concentration 0.1 mg mL⁻¹. Essential oil extracted from plants also possesses strong antimicrobial activity for both test pathogens, with a minimum inhibitory concentration range of 0.05–0.16 mg mL⁻¹. *Syzygium aromaticum* bud essential oil at 0.05 mg mL⁻¹ was most active against *S. mutans* (MTCC 497). Plant extracts viewing antimicrobial activity with minimum inhibitory concentration show the efficacy of the plant products that could be considered as a good indicator of prospective plants for discovering new antimicrobial agents against dental caries pathogens. The findings of this study provide a lead to further polyherbal formulations for the treatment of dental caries malaise.

Keywords Dental caries · *Streptococcus mutans* · *Lactobacillus acidophilus* · Antimicrobial activity · Minimum inhibitory concentration

Introduction

Dental caries is one of the widespread oral diseases in humans which causes irreversible damage to the tooth enamel, with difficulties in food intake and hence causes great distress. The occurrence of dental caries is mainly

associated with oral microbial pathogens, especially cariogenic bacteria (Kouidhi et al. 2010; Besra and Kumar 2016). The biological nature of the disease is a microbial infection caused primarily by the bacterium *Streptococcus mutans* (Clarke 1924; Loesche et al. 1975; Loesche 1986; Tanzer et al. 2001). The leading cariogenic pathogen *S. mutans* carries an enzyme glucosyltransferase (Gtf) that synthesizes water-insoluble glucan from sucrose and forms a layer on the surface of tooth enamel, which serves as an adhesive material for *S. mutans* itself and other *Streptococci* sp. (Besra and Kumar 2016). In the second stage, by the accumulation of acidogenic bacteria like *Lactobacillus* sp. produce acid from sucrose fermentation, create an acidic environment. The colonization and co-aggregation of bacteria able to produce acid (acidogenic) and tolerate the acidic environment (aciduric), final form dental plaque

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and thus cause local destruction of the tooth because of the acid accumulation and mineral decalcification (Losche 1986; Dhruwet al. 2012). Left untreated, dental caries will gradually lead to tooth loss. Dental caries prevention is preferable to treatment, due to high cost and lack of resources at primary levels. Wide-ranging antimicrobial agents like penicillin, cephalosporin, erythromycin and tetracycline are prescribed by the dental practitioners for dental infections. Exposure to wide range of antibiotics results in evolution of antibiotic resistance in oral pathogens. In addition, allergic reaction of all degrees of severity can occur with most antibiotics (Montgomery and Kroeger 1984). Dental caries is frequent in town residents in comparison to rural inhabitants, as they are less exposed to synthetic antibiotics; they also rely on natural remedies for dental care. In recent years, plant-derived products are gaining popularity in preventing oral illness, due to adaptation of antibiotic resistance in oral microbial pathogens against synthetic antibiotics (Lynch and Robertson 2008). Natural plant products ensure the absence of secondary effects and possible long-term usage in the oral cavity.

Since time immemorial, plants have been used for food, fodder and medicine. The World Health Organization (WHO) suggested that as much as 80% of the world's people depend on traditional medicine for their primary health care needs (Khan 2002). The primary inhabitants of natural ecosystem or the community people possess a vast amount of traditional knowledge using local biodiversity (Pradhan and Badola 2008). Herbal plant extracts have been used as antimicrobial agents in traditional medicines and have attracted considerable interest in the prevention of dental caries (Lee et al. 2003; Lim et al. 2003; Limsong et al. 2004; Song et al. 2007; Sampaio et al. 2009). In this study, an ethnobotanical survey was conducted in some rural region of Dhanbad district of Jharkhand, India (Supplementary Fig. 1). Although Dhanbad has an abundance of coal and minerals, there is an affluent ethnic tradition in terms of medicinal practices also, and only few dedicated ethnomedicinal studies have been published so far. The present study was undertaken to scientifically enumerate medicinal plants used by the rural communities of Dhanbad to treat severe oral ailment dental caries. Moreover, in vitro study of plant extracts was also performed to confine to qualitative and quantitative analysis in small extent.

Materials and methods

Plant sample collection

Frequent field visits were made to different rural regions of Dhanbad district, Jharkhand, India (latitude 23°37'3"N and 24°4'N and longitude 86°50'E). Information about the

traditional medicinal plants used to treat dental caries was obtained from experienced traditional healers and local individuals. Total number of plant samples collected for this study, local names of the plants, habit, plant part(s) used and mode of application were recorded. Plants were identified using regional floristic literature.

Preparation of plant-solvent extracts and essential oil

The plant samples were shed-dried at room temperature and ground into powder using a grinder. The powdered plant sample (10 g) was extracted using a Soxhlet apparatus with 200 mL of solvent for 10 h. The solvents used were methanol and distilled water. The solvent from extracts was evaporated under reduced pressure at 40 °C using a rotary evaporator. Stock solutions were prepared for each plant extract in 10% aqueous dimethylsulfoxide (DMSO) and preserved at 4 °C for further antimicrobial test.

An essential oil from plant samples was extracted by hydro-distillation technique using Clevenger's apparatus. 100 g fresh plant sample was homogenized and extracted with 500 mL of distilled water for 12 h. Collected essential oils were dried over anhydrous sodium sulphate and kept at 4 °C for antimicrobial test.

Bacterial strain and culture condition

The bacterial strains, *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) were requested from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. The growth media employed were, brain heart infusion broth (Himedia, India) for *S. mutans* and Lactobacilli MRS broth (Himedia, India) for *L. acidophilus* for the revival of strains. Further strains were preserved on agar plates of their respective growth media.

Antimicrobial assay

In vitro antimicrobial activity of plant extracts and essential oil against *S. mutans* and *L. acidophilus* was determined using the agar well diffusion method with modifications. In this method, 0.5 McFarland standard culture (1.5×10^8 cfu mL⁻¹) for each test microbe was prepared in Muller Hinton broth (Himedia, India). 100 µL inoculum of each test microbe was spread onto Muller Hinton agar (Himedia, India) plates using a sterile cotton swab and allowed to dry and wells were made with a sterile borer in the inoculated agar plates. Different test concentrations, 5.0–0.02 mg mL⁻¹, of plant extracts and essential oil were prepared in 10% aqueous DMSO. 100 µL volumes of each test concentration were propelled into the wells for each test organism.

Then the plates were incubated at 37 °C for 24 h. 10% aqueous DMSO was used as the negative control; ampicillin and tetracycline served as the positive control. Antimicrobial activity of plant extracts and essential oils indicated by a zone of inhibition around well was recorded. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with standard deviation were calculated (Aneja and Joshi 2009).

The minimum inhibitory concentration (MIC) of plant extracts and essential oil was determined using 96-well microtiter plates (CLSI 2012). An 80 µL aliquot of test concentration of each extract, was released into a 96-well microtiter plate. Then each well was inoculated with 100 µL microbial inoculums (0.5 McFarland standard culture, 1.5×10^8 cfu mL^{-1}) prepared in MHB medium. The microtiter plates were incubated for 18 h at 37 °C. 10% aqueous DMSO was used as the negative control; ampicillin and tetracycline served as the positive control.

Results and discussion

A total 20 plant species reported by the informants primarily used for dental caries treatment are summarized in supplementary Table 1. These plant species belong to 18 genera of 15 different families in which plants from the families *Amaranthaceae*, *Dipterocarpaceae*, *Euphorbiaceae*, *Lamiaceae*, *Meliaceae*, *Myrtaceae* and *Sapotaceae* were majorly preferred for dental caries. The gathered information includes scientific name, local name, botanical family, plant part used and usage. Usually, the plants were used when fresh or dry, essentially in the form of toothbrush (*Azadirachta indica*, *Jatropha curcas*, *Jatropha gossypifolia*, *Shorea robusta*, *Achyranthes aspera*), also as decoction and mouthwash (*Allium cepa*, *Ocimum sanctum*, *Ocimum basilicum*, *Psidium guajava*, *Syzygium aromaticum*, *Terminalia arjuna*, *Vitex negundo*), sometimes a powder form of seed from *Solanum surratense* and *Mimusop elengi* is applied on the caries infected tooth. Essential oil from *S. aromaticum* dried bud is used to relieve the pain caused by caries. When analyzing the number of citations for the plant

Fig. 1 Antimicrobial activity (zone of inhibition) of all 20 plant methanol extracts **a–d** with 10% DMSO negative control **c** and positive controls ampicillin and tetracycline **e** on *Streptococcus mutans* (MTCC 497)

Fig. 2 Antimicrobial activity (zone of inhibition) of all 20 plant methanol extracts **a–d** with 10% DMSO negative control **c** and positive controls ampicillin and tetracycline **e** on *Lactobacillus acidophilus* (MTCC 10307)

Fig. 3 Zone of inhibition of plant methanol extracts *A. aspera* (MIC 0.5 mg mL⁻¹), *A. indica* (MIC 0.25 mg mL⁻¹), *N. sativa* (MIC 0.2 mg mL⁻¹), *P. guajava* (MIC 0.2 mg mL⁻¹), *S. aromaticum* (MIC 0.2 mg mL⁻¹) and positive control ampicillin (MIC 0.05 mg mL⁻¹)

and tetracycline (MIC 0.02 mg mL⁻¹) and 10% DMSO negative control C on *A. Streptococcus mutans* (MTCC 497) and B *Lactobacillus acidophilus* (MTCC 10307)

parts used to prepare local remedies, a preference for the use of stem becomes noticeable. The inhabitants use the tender stem mostly as toothbrush on a daily routine, because of accessibility and the simplest mode of application. Most of the reported preparations in the area are drawn from a single plant, mixtures are used very rarely. In the current study, the medicinal plants reported were from each habit, 25% of the reported species were herbs, shrub (45%) and the tree (30%).

The most commonly used plant species was *A. indica* leaf used for malaria, leprosy, skin ulcers, cardiovascular diseases, gingivitis, liver problems and diabetes. *P. guajava* leaves are medicinally useful and its fruit is a nutritional powerhouse. The leaves are full of antioxidants, antimicrobial and anti-inflammatory agents. Informants present least use reports of *Lantana camara*, *S. surratense* and *V. negundo*, this might be because they are wild plants and are less prominent in human use. Four plants were also reported to have highest fidelity for use in dental caries treatment were *P. guajava*, *A. aspera*, *S. surratense* and *M. elengi*. Some plants like *A. aspera*, *A. indica*, *J. gossypifolia*, *M. elengi*, *P. guajava*, *S. surratense* and *V. negundo* were also

cited for use against dental pathogens by different workers (Singh et al. 2012).

Antimicrobial activity of plant extracts and essential oil

All plant extracts (methanol and aqueous) show significant antimicrobial activity against both test bacteria *S. Mutans* (MTCC 497) (Fig. 1) and *L. acidophilus* (MTCC 10307) (Fig. 2) with MIC value range from 0.2 to 5.0 mg mL⁻¹ (Tables 1, 2). Methanol extracts of all plants were more active than aqueous extracts. Methanol extracts of *A. aspera*, *A. indica*, *N. sativa*, *P. guajava* and *S. aromaticum* show significant inhibitory activity for both pathogens (Fig. 3). *S. mutans* were more sensitive to *N. sativa* with zone of inhibition 22.3 mm and MIC 0.2 mg mL⁻¹ (Tables 3, 1) while, *L. acidophilus* was more susceptible to *S. aromaticum* with zone of inhibition 11.3 mm and MIC 0.1 mg mL⁻¹ (Tables 4, 2). Essential oil from *O. basilicum*, *O. sanctum*, *N. sativa*, *S. aromaticum* and *V. negundo* were also active against *S. mutans* and *L. acidophilus* (Table 5). It was found

Table 1 Minimum inhibitory concentration value of plant extract against *Streptococcus mutans* (MTCC 497)

S. no.	Plants (parts) ↓	MIC (mg mL ⁻¹)	
		Methanol	Aqueous
1	<i>S. aromaticum</i> (bud)	0.2	0.25
2	<i>N. sativa</i> (seeds)	0.2	0.3
3	<i>P. guajava</i> (leaves)	0.2	0.5
4	<i>A. indica</i> (leaves)	0.25	0.75
5	<i>A. cepa</i> (bulb)	0.4	1.5
6	<i>A. aspera</i> (root)	0.5	1.0
7	<i>O. basilicum</i> (leaves)	0.7	1.0
8	<i>O. sanctum</i> (leaves)	0.75	1.0
9	<i>C. longa</i> (rhizome)	0.75	1.5
10	<i>M. elengi</i> (seeds)	1.0	3.0
11	<i>P. nigrum</i> (fruit)	1.5	2.5
12	<i>M. arvensis</i> (aerial parts)	2.0	2.5
13	<i>S. robusta</i> (stem)	2.5	3.5
14	<i>J. gossypifolia</i> (stem)	2.5	5.0
15	<i>M. indica</i> (leaves)	3.0	3.5
16	<i>J. curcas</i> (stem)	3.0	4.5
17	<i>T. arjuna</i> (bark)	3.5	4.5
18	<i>L. camara</i> (leaves)	4.0	0
19	<i>S. surratense</i> (seeds)	4.0	5.0
20	<i>V. negundo</i> (leaves)	4.0	6.0
	Ampicillin [#]	0.05	
	Tertracycline [#]	0.02	

MIC minimum inhibitory concentration

[#]Positive control (ampicillin and tetracycline)

Table 2 Minimum inhibitory concentration value of plant extract against *Lactobacillus acidophilus* (MTCC 10307)

S. no.	Plants (parts) ↓	MIC (mg mL ⁻¹)	
		Methanol	Aqueous
1	<i>L. camara</i> (leaves)	0	0
2	<i>S. aromaticum</i> (bud)	0.1	0.5
3	<i>P. guajava</i> (leaves)	0.2	0.3
4	<i>A. cepa</i> (bulb)	0.2	1.0
5	<i>A. indica</i> (leaves)	0.25	0.5
6	<i>N. sativa</i> (seeds)	0.5	0.3
7	<i>A. aspera</i> (root)	0.5	1.5
8	<i>C. longa</i> (rhizome)	0.5	1.5
9	<i>P. nigrum</i> (fruit)	1.5	2.5
10	<i>M. arvensis</i> (aerial parts)	2.0	2.2
11	<i>M. elengi</i> (seeds)	2.0	3.0
12	<i>O. basilicum</i> (leaves)	2.0	4.0
13	<i>O. sanctum</i> (leaves)	2.5	4.5
14	<i>S. robusta</i> (stem)	3.0	3.3
15	<i>M. indica</i> (leaves)	3.0	4.0
16	<i>J. gossypifolia</i> (stem)	3.0	5.0
17	<i>T. arjuna</i> (bark)	3.5	4.2
18	<i>J. curcas</i> (stem)	3.5	4.6
19	<i>S. surratense</i> (seeds)	4.0	0
20	<i>V. negundo</i> (leaves)	4.0	4.5
	Ampicillin [#]	0.05	
	Tertracycline [#]	0.02	

MIC minimum inhibitory concentration

[#]Positive control (ampicillin and tetracycline)

Table 3 Zone of inhibition of plant extract against *Streptococcus mutans* (MTCC 497)

S. no.	Plants (parts) ↓ Solvents →	ZI (mm) ^a	
		Methanol	Aqueous
1	<i>N. sativa</i> (seeds)	22.3±0.57	15.3±0.57
2	<i>A. indica</i> (leaves)	18.3±0.57	11.3±0.57
3	<i>P. guajava</i> (leaves)	18.3±0.57	9.6±0.57
4	<i>S. aromaticum</i> (bud)	16.3±0.57	10.1±0.57
5	<i>A. cepa</i> (bulb)	16.3±0.57	8.3±1.52
6	<i>M. arvensis</i> (aerial parts)	15.6±1.15	7.3±0.57
7	<i>P. nigrum</i> (fruit)	14.6±0.57	4.6±0.57
8	<i>A. aspera</i> (root)	12.3±0.57	5.6±0.57
9	<i>S. surratense</i> (seeds)	11.3±1.15	9.6±0.57
10	<i>C. longa</i> (rhizome)	12.0±0.00	4.6±1.52
11	<i>M. elengi</i> (seeds)	11.3±0.57	7.3±0.57
12	<i>O. basilicum</i> (leaves)	11.0±0.0	9.3±0.57
13	<i>T. arjuna</i> (bark)	9.6±0.57	1.3±1.15
14	<i>O. sanctum</i> (leaves)	8.3±1.52	7.3±0.57
15	<i>S. robusta</i> (stem)	8.9±0.57	2.3±0.57
16	<i>M. indica</i> (leaves)	7.6±0.57	3.3±0.57
17	<i>J. gossypifolia</i> (stem)	5.3±0.57	2.6±1.15
18	<i>J. curcas</i> (stem)	4.6±0.57	2.6±0.57
19	<i>V. negundo</i> (leaves)	3.6±0.57	7.7±0.57
20	<i>L. camara</i> (leaves)	1.7±1.15	0
	Ampicillin [#]	27.3±0.57	
	Tetracycline [#]	29.3±0.57	

ZI zone of inhibition

[#]Positive control (ampicillin and tetracycline)^aResults are mean ± SD values for three replicates

that *S. mutans* was more susceptible to essential oil than *L. acidophilus*. *S. aromaticum* bud essential oil shows strong inhibitory activity with MIC 0.05 and 0.08 mg mL⁻¹ for *S. mutans* and *L. acidophilus*, respectively.

This study represents the significant in vitro antimicrobial activity of solvent extracts of collected plant samples against dental caries pathogens and also suggest their efficacy for the treatment of dental caries. The results of our study revealed that the standard strains *S. mutans* (MTCC 497) and *L. acidophilus* (MTCC 10307) were more sensitive to plant extracts studied. Our result was also supported by previous workers (Nair and Chanda 2006). It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells (Walsh et al. 2003).

Plant species used in this study have previously been tested against both caries pathogens. *P. guajava* have shown better results against caries pathogens as reported in earlier studies (Palombo 2011; Garode and Waghode 2014; Gurnani et al. 2016). Antimicrobial activity of *O. sanctum*

Table 4 Zone of inhibition of plant extract against *Lactobacillus acidophilus* (MTCC 10307)

S. no.	Plants (parts) ↓ Solvents →	ZI (mm) ^a	
		Methanol	Aqueous
1	<i>P. guajava</i> (leaves)	22.6±0.57	9.6±0.57
2	<i>N. sativa</i> (seeds)	18.6±0.57	12.3±0.57
3	<i>O. basilicum</i> (leaves)	14.3±0.57	6.3±0.57
4	<i>P. nigrum</i> (fruit)	14.3±0.57	4.6±0.57
5	<i>O. sanctum</i> (leaves)	11.3±1.52	5.6±0.57
6	<i>M. arvensis</i> (aerial parts)	11.6±1.15	7.3±0.57
7	<i>M. elengi</i> (seeds)	11.6±0.57	7.6±0.57
8	<i>J. curcas</i> (stem)	11.6±0.57	2.6±0.57
9	<i>S. aromaticum</i> (bud)	11.3±0.57	9.6±1.15
10	<i>A. indica</i> (leaves)	11.3±0.57	9.3±1.0
11	<i>T. arjuna</i> (bark)	11.3±0.57	1.3±0.57
12	<i>A. cepa</i> (bulb)	10.3±0.57	8.3±0.0
13	<i>A. aspera</i> (root)	8.6±1.15	5.6±0.23
14	<i>V. negundo</i> (leaves)	8.3±0.57	5.6±0.57
15	<i>M. indica</i> (leaves)	8.3±0.57	5.3±0.57
16	<i>C. longa</i> (rhizome)	7.3±0.57	9.6±1.15
17	<i>S. robusta</i> (stem)	6.6±0.57	2.3±0.57
18	<i>J. gossypifolia</i> (stem)	5.6±0.57	2.6±0.57
19	<i>S. surratense</i> (seeds)	4.3±0.57	0
20	<i>L. camara</i> (leaves)	0	0
	Ampicillin [#]	30.3±0.57	
	Tetracycline [#]	29.3±0.57	

ZI zone of inhibition

[#]Positive control (ampicillin and tetracycline)^aResults are mean ± SD values for three replicates

against *S. mutans* and *L. acidophilus* has been published recently by Gadiyar et al. (2017). Susceptibility of dental caries pathogens to *S. surratense* and *M. arvensis* were also reported (Raja and Devi 2010). Solvent extracts of *A. indica* have strong antimicrobial activity against dental caries pathogens (Lekshmi et al. 2012; Lakshmi et al. 2015; Kaushik et al. 2016). Strong anti-*Streptococcus mutans* activity of *P. guajava*, *P. nigrum* and *S. aromaticum* were reported by Rosas-Pinon et al. (2012). Methanolic extract of *A. indica*, *O. sanctum*, *M. elengi* has considerable antimicrobial activity against *S. mutans* (Mistry et al. 2014). Freires et al. (2015) also reported *S. aromaticum* bud essential as most promising species against cariogenic bacteria. Most active antimicrobial plant species with strongest anti-caries activity were *A. indica*, *P. guajava*, *N. sativa* and *S. aromaticum*. The rest of the plant species show concentration dependent activity against both tested bacterial strains with MIC ranges from 0.3 to 6.0 mg mL⁻¹. This study also reports diverse ethnobotanical taxa as a source of natural alternatives to synthetic antimicrobial agents for the treatment of dental caries.

Table 5 Antimicrobial activity of essential oil against dental caries pathogens

S. no.	Plants (parts)	<i>Streptococcus mutans</i> (MTCC 497)		<i>Lactobacillus acidophilus</i> (MTCC 10307)	
		ZI (mm) ^a	MIC (mg mL ⁻¹)	ZI (mm) ^a	MIC (mg mL ⁻¹)
1	<i>S. aromaticum</i> (bud)	16.3 ± 0.57	0.05	12.2 ± 0.57	0.08
3	<i>N. sativa</i> (seeds)	11.6 ± 0.57	0.06	9.5 ± 0.57	0.08
2	<i>O. basilicum</i> (leaves)	9.4 ± 0.57	0.06	9.3 ± 0.57	0.08
4	<i>O. sanctum</i> (leaves)	13.3 ± 0.57	0.08	11.6 ± 0.57	0.1
5	<i>V. negundo</i> (leaves)	3.3 ± 0.57	0.15	5.4 ± 0.57	0.16
	Ampicillin [#]	27.3 ± 0.57		30.3 ± 0.57	
	Tetracycline [#]	29.3 ± 0.57		29.3 ± 0.57	

ZI zone of inhibition, MIC minimum inhibitory concentration

^aResults are mean ± SD values for three replicates

[#]Positive control (ampicillin and tetracycline)

Conclusion

Dental caries is a pathological condition of the teeth resulting in decalcification of the tooth enamel and disintegration of the remaining organic material. Nature provides diverse plant taxa as a source for antimicrobial agents, which can be a substitute for synthetic antibiotics that generate so many allergic reactions to humans. The present study has revealed significant results on antimicrobial activity of selected medicinal plants against cariogenic pathogens *S. mutans* and *L. acidophilus*. The most active plant was *P. guajava* (leaves), *N. sativa* (seeds), *A. indica* (leaves), *A. aspera* (root), *S. aromaticum* (bud). These in vitro antimicrobial results suggest the efficacy of these plants in dental caries. Use of more than one plant part, even a combination of plants for the formulation in the treatment of a single ailment can come as a success to complete cure of dental caries.

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Compliance with ethical standards

Conflict of interest The authors have none to declare.

References

- Aneja KR, Joshi R (2009) Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens. *Jundishapur J Microbiol* 2:105–111
- Besra M, Kumar V (2016) Antimicrobial activity of essential oils and herbal extracts against etiological agent of dental caries. *J Essent Oil Bear Pl* 19:1807–1815
- Clarke JK (1924) On the bacterial factor in the etiology of dental caries. *Br J Exp Pathol* 5:141–147
- CLSI, (2012) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard, 9th edn. In: CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA
- Dhruw C, Rajmani H, Bhatt R, Verma P (2012) Isolation of dental caries bacteria from dental plaque and effect of tooth pastes on acidogenic bacteria. *Open J Med Microbio* 2:65–69
- Freires IA, Denny C, Benso B, de Alencar SM, Rosalen PL (2015) Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review. *Molecules* 20:7329–7358
- Gadiyar A, Ankola AV, Rajpurohit L (2017) Evaluation of antimicrobial activity of *Ocimum sanctum* L. (Tulsi) extract against *Streptococcus mutans* and *Lactobacillus acidophilus*—an in vitro study. *Int J Health Res* 7:224–228
- Garode AM, Waghode SM (2014) Antibacterial activity of guava leaves extracts against *S. mutans*. *Int J Bioassays* 3:3370–3372
- Gurnani P, Krishnan CGA, Gurnai R, Ghosh A, Shah A (2016) Antibacterial activity of Guava leaves extract against *Lactobacillus acidophilus*: an in-vitro study. *Int J Oral Health Med Res* 2:37–40
- Kaushik A, Tanwar R, Kaushik M (2016) Ethnomedicine: application of neem (*Azadirachta indica*) in dentistry. *Dent Hypotheses* 3:112–114
- Khan US (2002) History of decline and present status of natural tropical thorn forest in Punjab. *Pak Biol Conserv* 63:210–250
- Kouidhi B, Zmantar T, Bakhrouf AV (2010) Anticariogenic and cytotoxic activity of clove (*Eugenia caryophyllata*) essential oil against a large number of oral pathogens. *Ann Microbiol* 60:599–604
- Lakshmi T, Krishnan V, Rajendran R, Madhusudan N (2015) *Azadirachta indica*: a herbal panacea in dentistry—an update. *Pharmacogn Rev* 9:41–44
- Lee ES, Ahn TY, Yoon JJ, Kook JK, Lee BR, Kim DK (2003) Restraint effect of leaf extract from *Camellia sinensis* and seed extract from *Casia tora* against period onto pathogens. *J Korean Acad Dent Health* 27:569–579
- Lekshmi NCJP, Sowmia N, Viveka S, Brindha JR, Jeeva S (2012) The inhibiting activity of *Azadirachta indica* against dental pathogens. *Asian J Plant Sci* 2:6–10
- Lim SH, Seo JS, Yoon YJ, Kim KW, Yoo SO, Kim SH, Kook JK, Lee BR, Cha JH, Park JY (2003) Effect of leaf extract from *Camellia sinensis* and seed extracts from *Casia tora* on viability of mutans streptococci isolated from the interface between

- orthodontic brackets and tooth surfaces. *Korean J Orthod* 33:381–389
- Limsong J, Benjavongkulchai E, Kuvatanasuchati J (2004) Inhibitory effect of some herbal extracts on adherence of *Streptococcus mutans*. *J Ethnopharmacol* 92:281–289
- Loesche WJ (1986) Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 50:353–380
- Loesche WJ, Rowan J, Straffon LH, Lops PJ (1975) Association of *Streptococcus mutans* with human dental decay. *Infect Immun* 11:1252–1260
- Lynch AS, Robertson GT (2008) Bacterial and fungal biofilm infections. *Annu Rev Med* 59:415–428
- Mistry K, Sanghvi Z, Parmar G, Shah S (2014) The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. *Eur J Dent* 8:172–177
- Montgomery EH, Kroeger DC (1984) Use of antibiotics in dental practices. *Dent Clin North Am* 28:433–53
- Nair R, Chanda S (2006) Activity of some medicinal plants against certain pathogenic bacterial strains. *Indian J Pharmacol* 38:142–144
- Palombo EA (2011) Traditional plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. *Evid Based Complement Alternat Med* 2011:1–15
- Pradhan BK, Badola H (2008) Ethnomedicinal plants used by Lepcha tribe of Dzongu valley, bordering Khangchendzonga Biosphere Reserve, in North Sikkim, India. *J Ethnobiol Ethnomed* 4:22
- Raja RR, Devi BP (2010) Phytochemical and antimicrobial screening of *Gymnema sylvestre*, *Mentha arvensis*, *Solanum surratense*, extracts in dental caries. *Int J Pharm Res* 3:21–23
- Rosas-Pinon Y, Mejia A, Diaz-Ruiz G, Aguilar MI, Sanchez-Niteo S, Rivero-Cruz JF (2012) Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections. *J Ethnopharmacol* 141:860–865
- Sampaio FC, Pereira MS, Dias CS, Costa VC, Conde NC, Buzalaf MA (2009) *In vitro* antimicrobial activity of *Caesalpinia ferrea* Martius fruits against oral pathogens. *J Ethnopharmacol* 124:289–294
- Singh H, Krishna G, Baske PK (2012) Ethnomedicinal plants used for dental care in Sundargarh, Mayurbhanj, Angul and Balangir districts of Odisha, India. *Ind J Nat Prod Resour* 4:419–424
- Song JH, Yang TC, Chang KW, Han SK, Yi HK, Jen JG (2007) *In vitro* effects of a fraction separated from *Polygonum cuspidatum* root on the viability, in suspension and biofilms and biofilm formation of mutans streptococci. *J Ethnopharmacol* 112:419–425
- Tanzer JM, Livingstone J, Thompson AM (2001) The microbiology of primary dental caries in humans. *J Dent Edu* 65:1028–1037
- Walsh SE, Maillard JY, Russel AD, Catrenich CE, Charbonneau AL, Bartolo RG (2003) Activity and mechanism of action of selected biocidal agents on Gram -positive and -negative bacteria. *J Appl Microbiol* 94:240–247