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ANTIMICROBIAL ACTIVITIES OF CLOVE BUDS AND MISWAK AGAINST VIRIDANS GROUP STREPTOCOCCI

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ABSTRACT

Viridans group streptococci (VGS), normal inhabitant of oral cavity, are involved in a number of oral and extra-oral diseases. Among oral diseases, dental caries is the most common public health problem throughout the world for all age groups. In the present study, the preliminary screening and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were conducted by well diffusion and tube dilution methods. Overall, clove buds (*Eugenia caryophyllata*) have potential to show strong antibacterial activities against tested VGS as compared miswak (root bark of *Salvadora persica*). The highest antibacterial activity was noted for clove oil as all the isolates were found susceptible. It exhibited strong antibacterial activity against *Streptococcus mutans* (20.2mm mean zone of inhibition±3.4SD) from carious subjects and *S. sanguinis* (17.7mm mean zone of inhibition ±1.1SD) from non-carious subjects. The aqueous decoction of clove buds was next to clove oil, exhibited the highest zone of inhibition against *S. mutans* obtained from carious subjects (16.8mm±3.4SD) followed by non-carious subjects (14.3mm±2.3SD). The aqueous infusion of clove buds showed the highest zone of inhibition against *S. mutans* isolated from carious (13.2mm±3.2SD) and non-carious (13.6mm±4.2SD) subjects. Aqueous infusion and aqueous decoction of miswak failed to inhibit the tested VGS. The MICs and MBCs of the clove oil, aqueous infusion and decoction of buds of clove against VGS was recorded as 5 – 0.625%, 5% and 5 – 2.5%, respectively.

Key-words: VGS, MIC, MBC, SD, aqueous infusion, well diffusion

INTRODUCTION

Due to increase in antibiotic resistance, there is a need of alternative, curative and preventive measures which should also be safe, economical and beneficial for mankind. Uncountable species of plants, herbs and spices have been used since long time (Lee, 2013). They have active potential to prevent from various diseases caused by oral bacteria such as dental caries.Dental caries is the most common prevalent health problem throughout the world (Ferrazzano *et al*., 2011; Mathur and Dhillon, 2018). Currently, there are many plant extracts, aqueous infusions and aqueous decoctions being used for prevention of dental caries, gingivitis, periodontal disorders and toothache (Jeevarathan *et al*., 2007). In this connection, different home remedies have been proposed as preventive measures for the control of dental caries such as clove buds and root bark of miswak are being used worldwide. In view of above, the aim of present study was to determine the antibacterial activities of clove bud and miswak against viridans group streptococci (VGS) isolated from carious and non-carious subjects.

MATERIALS AND METHODS

Collection of Clove Oil, Clove Buds and Miswak

Clove oil, clove buds (*Eugenia caryophyllata*) and miswak (root bark of *Salvadora persica*) were purchased from local market of Karachi, Pakistan.

Preparation of Aqueous Infusions and Aqueous Decoctions

The aqueous infusion and aqueous decoction of clove buds and miswak were prepared (Saeed and Tariq 2008). They were kept at 4°C until used.

ISOLATES

A total of 80 isolates belonging to 07 different species of viridans group streptococci (VGS) i.e. *S. anginosus* (19), *S. mutans* (10), *S. mitis* (14), *S. intermedius* (10), *S. sanguinis* (10), *S. oralis* (10) and *S. salivarius* (7) isolated from carious and non-carious individuals.

BASE MEDIA

Mueller Hinton Broth (MHB) (Merck) and Mueller Hinton Agar (MHA) (Merck) were used for the preparation of the inoculum and as a base medium, respectively, for the screening of the antibacterial activities of the selected plants.

Determination of Preliminary Screening

The preliminary screening to check antibacterial activities of aqueous infusion and decoction of clove buds and miswak and clove oil were determined by well diffusion method (Cheesbrough, 2000). The inoculated MHA plates were incubated at 37˚C for 18-24 hours. After incubation, diameter of the zone of inhibition was measured to nearest millimeter (mm). The values are reported as Mean ± Standard Deviation (mm±SD) of zone of inhibition (Essex-Sorlie, 1995).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous infusion, aqueous decoction and oil of clove buds exhibiting antibacterial activity during screening assay was determined by using tube dilution method (Baron *et al*., 1994, Mirpour *etal*., 2015). The different concentrations of infusion and decoction and oil of clove buds (5, 2.5, 1.25, 0.625 and 0.313%) were used. Negative and positive growth controls were also prepared of selected concentrations. All the tubes were incubated at 37˚C for 24 hours. After incubation, the tubes were examined for the presence of turbidity visually and compared with the respective negative growth controls. The lowest concentration showing absence of turbidity was recorded as the MIC value.To determine the MBC, the dilution representing the MIC and at least two of the more concentrated test product dilutions are plated and enumerated to determine viable CFU/mL. The MBC is the lowest concentration that demonstrates a pre-determined reduction (such as 99.9%) in CFU/mL when compared to the MIC dilution (Mirpour *etal*., 2015).

RESULTS AND DISCUSSIONS

In the present study, a total of 80 isolates of VGS were selected for antibacterial activities of oil, aqueous infusion and decoction of clove buds and aqueous infusion and decoction of miswak. These isolates were belonging to 07 different species of VGS i.e. *Streptococcus anginosus* (19), *S. mutans* (10), *S. mitis* (14), *S. intermedius* (10), *S. sanguinis* (10), *S. oralis* (10) and *S. salivarius* (7). The results for screening of antibacterial activities are presented in Table 1 to 3. Overall, clove oil exhibited strong antibacterial activities against tested VGS. Whereas, aqueous infusion and aqueous decoction of clove had moderate antibacterial effects but aqueous infusion and aqueous decoction of miswak completely failed to inhibit any of the tested VGS.

In the present study, clove oil was strongly active against all VGS isolates obtained from carious and non-carious subjects. In case of VGS isolated from carious subjects, the largest inhibitory zone was noted against *S. mutans* (carious 20.2 mm ± 3.4 SD vs. non-carious 12.2 mm ± 3.1 SD) followed by *S. anginosus* (carious15.4 mm ± 2.2 SD vs. non-carious 12.5 mm ± 3.1 SD) and *S. mitis* (carious 14.9 mm ± 4.2 SD vs. non-carious 11.8 mm ± 2.2 SD). Whereas, in case of VGS isolated from non-carious subjects, clove oil exhibited good inhibitory activity against few species with maximum inhibitory zone i.e. *S. sanguinis* (carious 14.2 mm ± 1.1 SD vs. non-carious 17.7 mm ± 1.1 SD), *S. oralis* (carious 11.9 mm ± 3.2 SD vs. non-carious 17.3 mm ± 2.2 SD), *S. intermedius* (carious 11.7 mm ± 1.1 SD vs. non-carious 14.8 mm ± 2.2 SD) and *S. salivarius* (carious 12.4 mm ± 2.6 SD vs. non-carious 14mm) (Table 3). The bioactive components of clove oil can disrupt different metabolic activities and permeability of bacterial cell membrane (Zhang *et al*., 2017). Substantial research has documented clove as a traditional plant for treatment of various ailments. In this respect, buds of clove and clove oil both have strong medicinal importance. Clove (*Eugenia caryophyllata*) is aromatic dried flower bud belonging to Myrtaceae family. Clove buds contain a sharp acrid taste with strong phenolic smell. It contains tanene, fixed and essential oil. The principle constituent of clove oil is the volatile oil. The volatile oil consists of eugenol, beta caryophyllene and eugenyl acetate. Eugenol has the broad spectrum antibacterial potential. It is also reported to have strong antifungal, antiviral and antiparastic properties. It is evident from available literature that the aqueous preparation of clove buds is responsible to stop the inflammation process either acute or chronic. It has potential to modulate a cascade of biochemical reactions that propagate and mature the inflammatory response. Besides, it is responsible to inhibit granuloma and formation of edema (Agrawal *et al*., 2014). It has also been reported for the treatment of diarrhea, arthritis, ulcers, asthma, bronchitis, athlete's foot, warts and wound. Furthermore, it is used to treat cough, sore throat, nausea, vomiting, fever and common cold. Clove oil is commonly used to prevent stomach upsets as well as hernia. Clove buds and its parts are used to make various herbal medicines for skin infections. Clove is also responsible to reduce bad breath

(Dominque, 2002; Mathur and Dhillon, 2018). It is reported as an expectorant. Generally, it is used for toothache and controlling pain during tooth extraction and other dental procedures. It is also famous as a counterirritant that can be applied inside of mouth and throat for reducing pain and inflammation. It acts as a flavoring agent in food and beverage industries. Besides, it is evident from previous literature that clove can be used as a food preservative (Dominque, 2002). It is reported as a carminative agent for improving peristalsis and increasing hydrochloric acid in the stomach. In traditional medicine, clove is also used to relief musculoskeletal pain and nasal obstructions. Clove and its oil have been reported to have strong antibacterial potential against oral bacteria such as VGS i.e. *S. mutans* and *S. salivarius*. It has also strong bacteriostatic and bacteriocidal potential against *E. coli*, *S. aureus* and *H. pylori* (Pandey and Singh, 2011).

Table 1. Antibacterial activities of aqueous infusions of clove buds and miswak against VGS.

Organisms	**Total No. of isolates**	**Subjects**	**No. of isolates**	**Mean zone of inhibition ± standard deviation (mm ± SD)**	
				Clove	**Miswak**
S. anginosus	19	Carious	07	12.2 ± 1.7	0
		Non-carious	12	10.4 ± 3.1	0
S. mutans	10	Carious	07	13.2 ± 3.2	0
		Non-carious	03	13.6 ± 4.2	0
S. mitis	14	Carious	09	10.7 ± 3.4	0
		Non-carious	05	11.2 ± 2.2	0
S. intermedius	10	Carious	03	10.7 ± 2.4	0
		Non-carious	07	11.2 ± 1.4	0
S. sanguinis	10	Carious	04	10.9 ± 2.3	0
		Non-carious	06	13.3 ± 3.2	0
S. oralis	10	Carious	03	12.3 ± 1.1	0
		Non-carious	07	11.3 ± 2.4	0
S. salivarius	07	Carious	06	10.8 ± 2.4	0
		Non-carious	01	0	0

*Zero shows no activity; Viridans Group Streptococci (VGS)

Table 2. Antibacterial activities of aqueous decoctions of clove buds and miswak against VGS.

Organisms	**Total No. of Isolates**	**Subjects**	**Number of isolates**	**Mean zone of inhibition ± standard deviation (mm ± SD)**	
				Clove	**Miswak**
S. anginosus	19	Carious	07	9.1 ± 2.6	0
		Non-carious	12	10.2 ± 1.3	0
S. mutans	10	Carious	07	16.8 ± 3.4	0
		Non-carious	03	14.3 ± 2.3	0
S. mitis	14	Carious	09	14.7 ± 2.4	0
		Non-carious	05	11.4 ± 2.1	0
S. intermedius	10	Carious	03	11.4 ± 3.3	0
		Non-carious	07	13.9 ± 3.7	0
S. sanguinis	10	Carious	04	10.8 ± 2.4	0
		Non-carious	06	14.2 ± 2.2	0
S. oralis	10	Carious	03	12.2 ± 4.3	0
		Non-carious	07	13.9 ± 2.8	0
S. salivarius	07	Carious	06	11.7 ± 3.4	0
		Non-carious	01	10.00	0

*Zero shows no activity

Table 3. Antibacterial activity of clove oil against VGS.

Organisms	**Total Number of Isolates**	**Subjects**	**Number of isolates**	**Mean zone of inhibition ± standard deviation (mm ± SD)**
				Clove oil
S. anginosus	19	Carious	07	15.4 ± 2.2
		Non-carious	12	12.5 ± 3.1
S. mutans	10	Carious	07	20.2 ± 3.4
		Non-carious	03	12.2 ± 3.1
S. mitis	14	Carious	09	14.9 ± 4.2
		Non-carious	05	11.8 ± 2.2
S. intermedius	10	Carious	03	11.7 ± 1.1
		Non-carious	07	14.8 ± 2.2
S. sanguinis	10	Carious	04	14.2 ± 1.1
		Non-carious	06	17.7 ± 1.1
S. oralis	10	Carious	03	11.9 ± 3.2
		Non-carious	07	17.3 ± 2.2
S. salivarius	07	Carious	06	12.4 ± 2.6
		Non-carious	01	14.00

Table 4. Overall comparison of MIC and MBC of aqueous infusion of clove buds againstvgs.

S. No.	**Organisms**	**No. of Isolates**	**Minimum Inhibitory Concentration**	**Minimum Bactericidal Concentration**
			%	
1	*S. anginosus*	19	5	5
2	*S. mutans*	10	5	5
3	*S. mitis*	14	5	5
4	*S. intermedius*	10	5	5
5	*S. sanguinis*	10	5	5
6	*S. oralis*	10	5	5
7	*S. salivarius*	07	5	5

Table 5. Overall comparison of MIC and MBC of aqueous decoction of clove buds against VGS.

S. No.	**Organisms**	**No. of Isolates**	**Minimum Inhibitory Concentration**	**Minimum Bactericidal Concentration**
			%	
1	*S. anginosus*	19	5	5
2	*S. mutans*	10	5 – 2.5	5 – 2.5
3	*S. mitis*	14	5 – 2.5	5 – 2.5
4	*S. intermedius*	10	5 – 2.5	5 – 2.5
5	*S. sanguinis*	10	5 – 2.5	5 – 2.5
6	*S. oralis*	10	5 – 2.5	5 – 2.5
7	*S. salivarius*	07	5	5

Table 6. Overall comparison of MIC and MBC of oil of clove buds against VGS.

S. No.	Organisms	No. of Isolates	Minimum Inhibitory Concentration	Minimum Bactericidal Concentration
			%	
1	*S. anginosus*	19	5 – 1.25	5 – 1.25
2	*S. mutans*	10	5 – 0.625	5 – 0.625
3	*S. mitis*	14	5 – 1.25	5 – 1.25
4	*S. intermedius*	10	5 – 0.625	5 – 0.625
5	*S. sanguinis*	10	5 – 2.5	5 – 2.5
6	*S. oralis*	10	5 – 1.25	5 – 1.25
7	*S. salivarius*	07	5 – 2.5	5 – 2.5

In case of aqueous decoction of clove buds, the screening results revealed the maximum inhibitory activity for *S. mutans* (carious 16.8mm ± 3.4SD vs. non-carious 14.3mm ± 2.3SD) isolated from carious subjects followed by *S. mitis* (carious 14.7mm ± 2.4SD vs. non-carious 11.4mm ± 2.1SD) and *S. salivarius* (carious 11.7mm ± 3.4SD vs. non-carious 10mm). While, in case of non-carious subjects, the highest antibacterial activity of aqueous decoction of clove buds was noted against *S. sanguinis* (carious 10mm ± 2.4SD vs. non-carious 14.2mm ± 2.1SD), *S. oralis* (carious 12.2mm ± 4.3SD vs. non-carious 13.9mm ± 2.8SD), *S. intermedius* (carious 11.4mm ± 3.3SD vs. non-carious 13.9mm ± 3.7SD), and *S. anginous* (carious 9.1mm ± 2.6SD vs. non-carious 10.2mm ± 1.3SD) (Table 2).

As far as aqueous infusion of clove buds is concerned, the antibacterial potential of aqueous infusion of clove buds was found next to aqueous decoction of clove buds. It was interesting to note that VGS isolates obtained from non-carious subjects were highly sensitive to aqueous infusion of clove buds as compared to carious subjects except in few cases (Table 1). The inhibitory zone was noted as 12.2mm ± 1.7SD (carious) and 10.4mm ± 3.1SD (non-carious) for *S. anginosus*, 13.2mm ± 3.2SD (carious) and 13.6mm ± 4.2SD (non-carious) for *S. mutans*, 10.7mm ± 3.4SD (carious) and 11.2mm ± 2.2SD (non-carious) for *S. mitis*, 10.7mm ± 2.4SD (carious) and 11.2mm ± 1.4SD (non-carious) for *S. intermedius*, 10.9mm ± 2.3SD (carious) and 13.3mm ± 3.2SD (non-carious) for *S. sanguinis*, 12.3mm ± 1.1SD (carious) and 11.3mm ± 2.4SD (non-carious) for *S. oralis* and 10.8mm ± 2.4SD (carious) for *S. salivarius*. While, the only isolate of VGS obtained from non-carious subject was not inhibit by aqueous infusion of clove buds (Table 1). The results of present study are in harmony with Natta *et al*. (2008) who reported strong antibacterial potential of aqueous infusion of clove buds against isolates of *S. mutans*. The reason of absence or limited antibacterial potential of aqueous infusion of clove buds might be polarity of antibacterial constituents of clove buds which make them more easily extracted by organic solvents compared to aqueous preparations.

MIC and MBC of aqueous infusion, aqueous decoction and oil of buds of clove against different species of VGS isolated from carious and non-carious subjects was also conducted. Miswak was not selected because it did not show any antibacterial activity in screening test. The results of MIC/MBC are shown in Table 4 to 6. In present study, MIC/MBC values were similar for tested isolates obtained from carious and non-carious subjects. Overall, different concentrations of oil of buds of clove showed strong inhibitory activity as compared to the aqueous decoction and aqueous infusion of buds of clove (Table 6).The comparison tables clearly showed that 5% concentration of oil, aqueous decoction and aqueous infusion of buds of clove was effective for most of the tested species of VGS.

CONCLUSION

It is concluded from the findings of present study that clove oil strongly affected the growth of VGS, followed by aqueous decoction and aqueous infusion of clove buds but would require more research work to check the activity of clove on acidogenic potential and glucan (extracellular polysaccharide) production as both factors attribute and play an important role in the cariogenicity of oral VGS and process of dental caries.

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