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EFFECT OF CLOVE OIL AND AQUEOUS INFUSION OF MISWAK ON GLUCAN PRODUCTION BY ORAL VIRIDANS GROUP STREPTOCOCCI

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ABSTRACT

The effect of clove oil (*Eugenia caryophyllata* L.) and aqueous infusion of miswak (root bark of *Salvador apersica* L.) on glucan production by oral viridans group streptococci (VGS) was performed by precipitation method in terms of reduced activity of GTFs (glucosyltransferases). The VGS isolates producing large amount of glucan were selected for the study. The different concentrations of clove oil (0.5%, 0.25%, 0.125%, 0.0625% and 0.0313%) and aqueous infusion of miswak (10%, 8%, 5%, 2.5%, 1.25% and 0.5%) exhibited varying degree of reduction in glucan production in terms of reduced activity of GTFs. Comparatively, clove oil was found more effective than aqueous infusion of miswak, and reduced the formation of glucan by approximately 80% in a dose dependent manner. Future research studies should be focused on mode of action and chemical nature of active constituents of clove as well as miswak.

Key words: glucosyltransferases (GTFs), viridans group streptococci (VGS), aqueous infusion, glucan, clove, miswak

INTRODUCTION

Medicinal plants have been utilized as conventional medicines for various human illnesses therapy since time immemorial. Pakistan has a great diversity of medicinal flora and people use these ethno-medicines to deal with oral problems (Megersa *et al*., 2019). There have been various reports of the utilization of medicinal plants and their products in dental practice for the treatment of toothaches and oral infections. The accessibility, cost effectiveness, and lower incidence of side effects of plant products offer considerable advantages in comparison to synthetic drugs. The knowledge of indigenous medicinal plants is a part of Pakistani culture and traditionally, mostly in the rural areas, is still being used safely and effectively by both the humans and livestock for various ailments. They are cheaper, especially for those living in the rural areas with lower income. However, living in the urban areas also believe in the traditional usage of these remedies (Ahmed *et al*., 2012; Khan *et al*., 2019). The use of traditional medicinal plants is practiced regularly in homes and transferred from generation to generation as a cultural virtue. Considering above views, the present study was aimed to determine the effects of aqueous infusion of miswake and clove oil on glucan producing potential of oral viridans group streptococci (VGS) in terms of reduced activity of GTFs (glucosyltransferases). GTFs are one of the crucial virulence factors of *Streptococcus mutans*, a major etiological pathogen of dental caries. All the available researches indicate that extracellular polysaccharide, particularly glucans produced by *S. mutans* GTFs; contribute to the cariogenicity of dental biofilms. Therefore, inhibition of GTFs activity and the consequential polysaccharide synthesis may impair the virulence of cariogenic biofilms, which could be an alternative strategy to prevent from dental caries. Up to now, many GTF inhibitors have been recognized in natural products, which remain the major and largely unexplored source of GTFs inhibitors. These include catechin-based polyphenols, flavonoids, proanthocyanidin oligomers, polymeric polyphenols, and some other plant-derived compounds. Metal ions, oxidizing agents, and some other synthetic compounds represent another source of GTFs inhibitors, with some novel molecules either discovered by structure-based virtual screening or synthesized based on key structures of known inhibitors as templates. Although many agents have been shown to possess potent inhibitory activity against glucan synthesis by GTFs, bacterial cell adherence, and caries development in animal models, much research remains to be performed to find out their mechanism of action, biological safety, cariostatic efficacies, and overall influence on the entire oral community. As a strategy to inhibit the virulence of cariogenic microbes rather than eradicate them from the microbial community, GTFs inhibition represents an approach of great potential to prevent dental caries (Ren *et al*. 2016). In this regards, clove oil was selected because it is not seasonal, easily available and traditionally being used as a home ready to treat different types of dental disorders such as toothache. Whereas, miswak is also commonly used as natural tooth brush for cleaning teeth all over the world. It is an inexpensive source, easily available and reported to have many pharmacological properties including antimicrobial, antifungal, antiprotozoal and anti-plaque activities.

MATERIALS AND METHODS

The effect of clove oil and aqueous infusion of miswak on the glucan producing potential of VGS was determined according to the method described by Masumoto *et al*. (1987) and Figueiredo *et al*. (2010) in terms of reduced activity of GTFs.

ISOLATES

Fifty three isolates belonging to 6 different species of *Streptococcus* viz., *S. anginosus* (08), *S. intermedius* (05), *S. sanguis* (05), *S. salivarius* (01), *S. oralis* (04) and *S. mutans* (26) were used for the study. These isolates were recovered from oral cavity of carious and non-carious subjects from different areas of Karachi, Pakistan and isolates were maintained on sodium azide blood agar medium (Baron *et al*. 1994).

SELECTION, COLLECTION AND AUTHENTICATION OF CLOVE OIL AND AQUEOUS INFUSION OF MISWAK

Clove oil (*Eugenia caryophyllata* L.) and aqueous infusion of miswak (root bark of *Salvadora persica* L.) were selected based on previous preliminary screening for their strong antibacterial activities on growth of VGS. Both were purchased from local market of Karachi, Pakistan and identified by taxonomist and the herbaria were made and deposited at Department of Botany, University of Karachi.

CONCENTRATIONS OF AQUEOUS INFUSION OF MISWAK AND CLOVE OIL

The different concentrations of clove oil (0.5%, 0.25%, 0.125%, 0.0625% and 0.0313%) and aqueous infusion of miswak (10%, 8%, 5%, 2.5%, 1.25% and 0.5%) was prepared to check their activity on glucan production (Mandava *et al*. 2019).

BASE MEDIUM

Brain Heart Infusion Broth (BHIB) (Merck) was used for preparation of inoculum and 5% Sucrose broth containing 0.04% Sodium azide was used for preparation of cell-free supernatant used as the crude GTFs (Masumoto *et al*. 1987).

PRECIPITATION OF GLUCAN

Precipitation of glucan was performed by precipitation method (Masumoto *et al*. 1987; Mandava *et al*. 2019). One milliliter standardized inoculum was transferred to 9 ml BHI broth and incubated at 35-37˚C for 18-24 hours. After incubation, the tube was centrifuged at 3000 × g for 10 minutes at 4˚C to obtain cell-free supernatant. This cell-free supernatant was used as the crude GTFs (crude glucosyltransferase). BHI broth containing 5% sucrose (8ml) was prepared and different concentrations of clove oil and aqueous infusion of miswak (1ml) were inoculated into the media. Furthermore crude GTFs (1ml) was incorporated into the media to increase the glucan production. All the sets of tubes containing different concentrations of clove oil and aqueous infusion of miswak including positive and negative controls were incubated at 35-37˚C for 24h. After incubation, the inoculated tubes of different concentrations of clove oil and aqueous infusion of miswak were centrifuged at 3000 x g for 10 minutes at 4˚C. The precipitates of insoluble glucan were deposited at the bottom of the tubes and supernatant was decanted carefully without disturbing the precipitates. The precipitate was then washed with sterile distilled water three times and centrifugation was performed at 3000 x g for 10 minutes at each step. The precipitate of insoluble glucan was dried and weighed.

INTERPRETATION

The difference in the amount of glucan produced by VGS in control and at various concentrations of aqueous infusion of miswak and clove oil was noted in terms of the dry weight in milligram (mg) (Figueiredo *et al*. 2010).

RESULTS AND DISCUSSION

In the present study, clove oil and aqueous infusion of miswak were selected to determine their effects on glucan production of VGS in terms to reduce GTFs. However, the effect of clove oil and aqueous infusion of miswak against glucan production by VGS have not been thoroughly studied. But the demands of natural plant based remedies like clove and miswak is increasing in developed and underdeveloped countries because these are natural products, having no side effects, not seasonal, easily available at affordable prices, being non narcotic and sometimes the only way of healthcare available to the poor. Besides, they have been reported for treatment of

different oral problems (Nunez and Aquino, 2012). As clove oil is being used for toothache since long time whereas miswak is commonly used as toothbrush for cleaning teeth (Chavan *et al*. 2014; Vahabi *et al*. 2011; Chaieb *et al*. 2007).

Table 1. Effect of clove oil in terms of reduced activity of crude glucosyltransferase.

Organisms	Subjects	Code No.	Control (mg)	Glucan production (mg)				
				0.5%	0.25%	0.0125%	0.0625%	0.0313%
S. anginosus	**Carious**	SAC1	73.4	24.3	27.1	28.3	29.2	29.7
		SAC2	81.9	ND	ND	0.7	1.1	1.9
		SAC3	131.1	9.0	10.7	12.1	12.9	13.2
		SAC4	186.2	9.3	9.9	10.6	11.1	14.3
		SAC5	244.6	54.2	103.5	154.1	201.2	208.5
		SAC6	272.5	152.3	173.2	181.4	190.3	198.8
		SAC7	388.5	108.4	130.1	175.5	215.7	304.8
	Non-carious	SAN1	12.3	ND	ND	ND	0.8	3.6
		SAN2	16.6	ND	ND	2.8	3.0	3.2
		SAN3	41.8	ND	6.0	7.9	8.0	9.1
		SAN4	97.8	ND	ND	6.0	9.6	10.2
		SAN5	160.7	131.3	151.6	151.8	152.3	157.1
S. intermedius	**Carious**	SIC1	62.5	3.0	7.4	8.3	10.1	13.4
		SIC2	97.4	ND	ND	ND	ND	5.2
		SIC3	101.9	ND	ND	5.7	7.9	9.3
	Non carious	SIN1	18.7	ND	ND	3.6	9.3	9.9
		SIN2	48.4	ND	ND	ND	4.8	8.3
S. sanguinis	**Carious**	SSNC1	206.2	ND	3.7	8.6	9.1	14.3
		SSNC2	297.7	ND	3.3	4.6	7.3	9.6
		SSNC3	324.6	ND	135.2	145.1	160.8	163.6
	Non-carious	SSNN1	89.4	2.5	3.4	3.9	5.2	7.9
		SSNN2	122.4	ND	ND	41.1	97.5	121.7
S. salivarius	**Carious**	SSLC1	77.3	0.9	1.1	1.8	1.9	2.1
	Non-carious			ND				
S. oralis	**Carious**	SOC1	34.3	ND*	5.4	6.9	8.1	8.7
		SOC2	132.2	ND	ND	ND	35.5	45.0
		SOC3	340.7	147.4	203.1	211.4	235.1	326.2
	Non-carious	SON1	18.3	ND	ND	9.1	9.9	10.1
S. mutans	**Carious**	SMUC1	12.2	1.2	2.6	3.4	8.1	10.4
		SMUC2	39.3	9.3	17.3	19.8	19.8	20.3
		SMUC3	39.9	ND	ND	14.3	18.7	19.0
		SMUC4	46.5	ND	ND	ND	0.9	1.7
		SMUC5	52.1	9.3	11.0	11.4	14.3	14.9
		SMUC6	54.3	ND	ND	ND	19.3	21.3
		SMUC7	54.4	ND	ND	0.6	9.0	9.0
		SMUC8	54.8	1.1	1.9	3.4	7.7	7.9
		SMUC9	59.4	11.3	12.4	12.9	12.9	14.1
		SMUC10	67.8	13.8	20.1	21.4	27.4	27.9
		SMUC11	88.1	ND	ND	ND	0.3	0.7
		SMUC12	99.3	ND	21.3	23.4	27.4	29.1
		SMUC13	111.3	ND	ND	5.8	7.7	8.1
		SMUC14	122.5	ND	13.0	39.1	48.1	99.1
		SMUC15	177.7	53.1	122.4	142.4	147.4	161.4
		SMUC16	221	144.3	147.2	171.9	184.4	184.8
		SMUC17	333.2	121.5	132.4	150.1	154.1	251.9
		SMUC18	353.6	88.8	134.7	164.3	168.5	168.5
		SMUC19	541	125.3	207.4	287.5	448.2	451.4
	Non-carious	SMUN1	43.5	ND	ND	ND	ND	1.3
		SMUN2	73.1	1.4	2.4	2.9	3.1	4.3
		SMUN3	78.8	7.1	8.4	8.8	10.4	19.1
		SMUN4	88	3.9	9.7	9.9	10.3	11.9
		SMUN5	98.3	4.7	12.1	14.4	16.1	18.8
		SMUN6	101.9	ND	ND	ND	ND	3.2
		SMUN7	521.1	ND	ND	3.1	12.2	44.6

*ND : Not done

Table 2. Effect of aqueous infusion of miswak on the reduced activity of crude glucosyltransferase

Organisms	Subjects	Code No.	Control (mg)	Glucan production (mg)					
				10%	8%	5%	2.5%	0.125%	0.5%
S. anginosus	Carious	SAC1	73.4	69.9	71.1	71.7	72.4	72.9	73.1
		SAC2	81.9	33.2	55.3	57.0	63.0	66.8	69.3
		SAC3	131.1	87.3	92.4	99.1	99.7	103	114
		SAC4	186.2	29.1	52.1	62.6	77.4	79.3	92.4
		SAC5	244.6	229.9	238.4	239.7	240.4	241.6	242.9
		SAC6	272.5	213.4	239.9	250.8	254.9	256.8	259.2
		SAC7	388.5	319.2	338.8	339.1	361.2	364.5	369.1
	Non-carious	SAN1	12.3	3.3	4.7	5.3	5.6	9.1	10.7
		SAN2	16.6	4.1	8.5	9.9	10.8	11.4	16.3
		SAN3	41.8	2.5	3.6	4.8	6.1	8.6	9.3
		SAN4	97.8	13.4	45.3	49.1	80.5	82.3	84.1
		SAN5	160.7	154.9	156.8	157.1	157.4	159.8	160.2
S. intermedius	Carious	SIC1	62.5	50.3	51.1	60.8	61.1	61.8	62.2
		SIC2	97.4	22.5	37.9	39.7	41.8	44.3	67.8
		SIC3	101.9	6.9	90.6	98.9	103	105	107
	Non-carious	SIN1	18.7	11.3	13.2	13.6	13.7	13.9	14.1
		SIN2	48.4	9.5	17.8	28.9	29.8	30.4	44.8
S. sanguinis	Carious	SSNC1	206.2	32.7	66.2	80.5	83.7	105.6	137.2
		SSNC2	297.7	32.2	51.3	57.8	60.3	67.8	69.1
		SSNC3	324.6	200.8	224.1	228.3	257.7	259.2	278.2
	Non-carious	SSNN1	89.4	4.7	6.2	6.4	6.4	7.8	9.9
		SSNN2	122.4	111.9	113.5	119.9	120.1	120.7	121.9
S. salivarius	Carious	SSLC1	77.3	11.3	12.0	12.3	13.9	14.0	14.4
	Non-carious			ND					
S. oralis	Carious	SOC1	34.3	30.2	31.5	31.9	32.1	32.6	33.1
		SOC2	132.2	90.5	91.4	96.2	98.7	109.9	119.3
		SOC3	340.7	298.1	300.4	311.9	318.5	333.1	337.4
	Non-carious	SON1	18.3	14.4	15.2	16.3	16.8	18.1	18.1
S. mutans	Carious	SMUC1	12.2	9.4	10.0	10.9	11.3	11.9	12.0
		SMUC2	39.3	13.4	31.1	37.3	37.9	38.1	38.9
		SMUC3	39.9	21.5	27.8	29.1	33.1	35.2	36.8
		SMUC4	46.5	13.6	35.8	41.3	41.8	44.8	45.3
		SMUC5	52.1	33.1	37.8	41.1	42.4	46.8	51.1
		SMUC6	54.3	14.4	30.5	31.4	51.8	53.1	53.7
		SMUC7	54.4	11.1	20.3	29.3	33.4	40.3	49.8
		SMUC8	54.8	22.1	44.3	44.9	46.3	51.0	52.1
		SMUC9	59.4	34.1	40.2	41.1	47.2	49.1	51.2
		SMUC10	67.8	33.6	45.8	49.1	57.4	58.2	59.1
		SMUC11	88.1	12.8	15.5	18.9	29.7	53.6	66.8
		SMUC12	99.3	57.1	57.4	62.1	63.4	68.7	81.5
		SMUC13	111.3	50.2	66.4	68.1	69.4	90.5	99.5
		SMUC14	122.5	99.2	99.8	100.8	103.0	107.8	121.9
		SMUC15	177.7	169.9	173.0	173.1	173.8	174.4	174.9
		SMUC16	221.0	209.8	211.4	213.9	217.7	219.3	220.4
		SMUC17	333.2	309.9	311.5	314.9	319.5	324.8	329.4
		SMUC18	353.6	330.4	331.8	332.2	332.9	333.8	339.3
		SMUC19	541.0	524.4	527.3	530.4	534.1	537.3	539.8
	Non-carious	SMUN1	43.5	19.7	20.5	22.1	22.9	27.4	29.1
		SMUN2	73.1	54.1	55.3	57.5	60.5	65.1	67.3
		SMUN3	78.8	42.3	52.1	54.6	61.1	69.4	69.9
		SMUN4	88.0	29.1	44.8	49.3	50.8	51.7	59.3
		SMUN5	98.3	29.7	60.3	81.1	83.7	86.6	89.4
		SMUN6	101.9	4.7	5.9	44.7	46.7	50.2	95.6
		SMUN7	521.1	5.1	61.2	73.1	99.4	120.7	148.0

ND : Not done

Table 3. Percentage reduction in Glucan production by viridans group streptococci in response to aqueous infusion of Miswak and Clove oil.

Organisms	Subjects	Code No.	Control (mg)	Percentage Reduction in glucan production								
				Aqueous infusion of Miswak (%)					Clove oil (%)			
				10	8	5	2.5	0.125	0.5	0.25	0.0125	0.0625	0.0313
S. anginosus	Carious	SAC1	73.4	4.8	3.2	2.4	1.4	0.7	0.5	66.9	63.1	61.5	60.3	59.6
		SAC2	81.9	59.5	32.5	30.5	23.1	18.5	15.4	0	0	99.1	98.7	97.7
		SAC3	131.1	33.5	29.6	24.5	24	21.5	13.1	93.2	91.9	90.8	90.2	90
		SAC4	186.2	84.4	72.1	66.4	58.5	57.5	50.4	95.1	94.7	94.4	94.1	92.4
		SAC5	244.6	6.1	2.6	2.1	1.8	1.3	0.7	77.9	57.7	37	17.8	14.8
		SAC6	272.5	44.2	36.5	33.5	30.2	27.1	21.7	12	8	6.5	5.8	4.9
		SAC7	388.5	17.9	12.8	12.7	7.1	6.2	5	72.1	66.6	54.9	44.5	21.6
	Non-carious	SAN1	12.3	73.2	61.8	57	54.5	26.1	13.1	0	0	0	93.5	70.8
		SAN2	16.6	75.4	48.8	40.4	35	31.4	1.9	0	0	83.2	82	80.8
		SAN3	41.8	94.1	91.4	88.6	85.5	79.5	77.8	0	85.7	81.2	80.9	78.3
		SAN4	97.8	86.3	53.7	49.8	17.7	15.9	14.1	0	0	93.9	90.2	89.6
		SAN5	160.7	3.7	2.5	2.3	2.1	0.6	0.4	5.7	5.6	5.6	5.3	2.3
S. intermedius	Carious	SIC1	62.5	19.6	18.3	2.8	2.3	1.2	0.5	95.2	88.2	86.8	83.9	78.6
		SIC2	97.4	76.9	61.1	59.3	57.1	54.6	30.4	0	0	0	0	94.7
		SIC3	181.9	52.3	50.2	45.7	43.4	42.3	41.2	0	0	96.9	95.7	94.9
	Non-carious	SIN1	18.7	39.6	29.5	27.3	26.8	25.7	24.6	0	0	80.8	94.2	47.1
		SIN2	48.4	80.4	63.3	40.3	38.5	37.2	7.5	0	0	0	91.1	82.9
S. sanguinis	Carious	SSNC1	206.2	84.2	67.9	61	59.6	48.8	33.5	0	98.3	95.9	95.6	93.1
		SSNC2	297.7	89.2	82.8	80.6	79.8	77.3	76.8	0	98.9	98.5	97.6	96.8
		SSNC3	324.6	38.2	31	29.7	20.7	20.2	14.3	0	58.4	55.3	50.3	30.3
	Non-carious	SSNN1	89.4	94.8	93.1	92.9	92.9	91.3	89	97.3	96.2	95.7	94.2	91.2
		SSNN2	122.4	8.6	7.3	2.1	1.9	1.4	0.5	0	0	66.5	20.4	0.6
S. salivarius	Carious	SSLC1	77.3	85.4	84.5	84.1	82.1	81.9	81.4	98.9	98.6	97.7	97.6	97.3
	Non-carious	ND												

Table 3 (Continued)

Organisms	Subjects	Code No.	Control (mg)	Reduction in glucan production (%)										
				Aqueous infusion of Miswak						Clove oil				
				10	8	5	2.5	0.125	0.5	0.5	0.25	0.0125	0.0625	0.0313
S. oralis	Carious	SOC1	34.3	12	8.2	7	6.5	5	3.5	0	84.3	79.9	76.4	74.7
		SOC2	132.2	31.6	30.9	27.3	25.4	16.9	9.8	0	0	0	73.2	66
		SOC3	340.7	12.6	11.9	8.5	6.6	2.3	1	56.8	40.4	38	31	4.3
	Non-carious	SON1	18.3	21.4	17	11	8.2	1.1	1.1	0	0	50.3	46	44.9
S. mutans	Carious	SMUC1	12.2	23	18.1	10.7	7.4	2.5	1.7	90.2	78.7	72.2	33.7	14.8
		SMUC2	39.3	66	20.9	5.1	3.6	3.1	1.1	76.4	56	49.7	49.7	48.4
		SMUC3	39.9	46.2	30.4	27.1	17.1	11.8	7.8	0	0	64.2	53.2	52.4
		SMUC4	46.5	70.8	23.1	11.2	10.2	3.7	2.6	0	0	0	98.1	96.4
		SMUC5	52.1	36.5	27.5	21.2	18.7	10.2	2	82.2	78.9	78.2	72.6	71.5
		SMUC6	54.3	73.5	43.9	42.2	4.7	2.3	1.2	0	0	0	64.5	60.8
		SMUC7	54.4	79.6	62.7	46.2	38.7	26	8.5	0	0	98.9	83.5	83.5
		SMUC8	54.8	59.7	19.2	18.1	15.6	7	5	98	96.6	93.8	86	85.6
		SMUC9	59.4	42.6	32.4	30.9	20.6	17.4	13.9	81	79.2	78.3	78.3	76.3
		SMUC10	67.8	50.5	32.5	27.6	15.4	14.2	12.9	79.7	70.4	68.5	59.6	58.9
		SMUC11	88.1	85.5	82.5	78.6	66.3	39.2	33	0	0	0	99.7	99.3
		SMUC12	99.3	42.5	42.2	37.5	36.2	30.9	18	0	78.6	76.5	72.5	70.7
		SMUC13	111.3	54.9	40.4	38.9	37.7	18.7	10.7	0	0	94.8	93.1	92.8
		SMUC14	122.5	19.1	18.6	17.8	16	12	0.5	0	89.4	68.1	60.8	19.2
		SMUC15	177.7	4.4	2.7	2.6	2.2	1.9	1.6	70.2	31.2	19.9	17.1	9.2
		SMUC16	221.0	5.1	4.4	3.3	1.5	0.8	0.3	34.8	33.4	22.3	16.6	16.4
		SMUC17	333.2	7	6.6	5.5	4.2	2.6	1.2	63.6	60.3	55	53.8	24.4
		SMUC18	353.6	6.6	6.2	6	5.9	5.6	4	74.9	62	53.6	52.4	52.4
		SMUC19	541.0	3.1	2.6	2	1.3	0.7	0.3	76.9	61.7	46.9	17.2	16.6
	Non-carious	SMUN1	43.5	54.8	52.9	49.2	47.4	37.1	33.2	0	0	0	0	97.1
		SMUN2	73.1	26	24.4	21.4	17.3	11	8	98.1	96.8	96.1	95.8	94.2
		SMUN3	78.8	46.4	33.9	30.8	22.5	12	11.3	91	89.4	88.9	86.9	75.8
		SMUN4	88.0	67	49.1	44	42.3	41.3	32.7	95.6	89	88.2	88.3	86.5
		SMUN5	98.3	70.4	38.7	17.5	14.9	12	9.1	95.3	87.7	85.4	83.7	80.9
		SMUN6	101.9	95.4	94.3	56.2	54.2	50.8	6.2	0	0	0	0	96.9
		SMUN7	521.1	90.3	88.3	86	81	76.9	71.6	0	0	99.5	97.7	91.5

Table 4.Incidence of isolates of VGS exhibiting ≥ 80% reduction in Glucan amount.

Organisms	No. of isolates exhibiting ≥ 80% reduction in glucan amount										
	Aqueous infusion of miswak (%)						Clove oil (%)				
	10	8	5	2.5	1.25	5	0.5	0.25	0.125	0.0625	0.0313
S. anginosus	02	01	01	01	0	0	03	03	06	07	05
S. intermedius	01	0	0	0	0	0	01	01	03	04	03
S. sanguinis	03	02	02	01	01	01	03	03	03	03	03
S. salivarius	01	01	01	01	01	01	01	01	01	01	01
S. oralis	0	0	0	0	0	0	0	01	0	0	0
S. mutans	03	03	01	0	0	0	07	07	08	09	11

The results are presented in Tables 1,2,3 and 4. Different codes were assigned for respective isolates used in the study i.e. SMUC and SMUN for *S. mutans* isolated from carious and non-carious subjects respectively followed by SAC and SAN for *S. anginosus*, SSNC and SSNN for *S. sanguinis*, SIC and SIN for *S. intermedius*, SOC and SON for *S. oralis* (04) and SSLC for *S. salivarius*. The effects of different concentrations of clove oil (5%, 2.5%, 1.25%, 0.625% and 0.313%) and aqueous infusion of miswak (100%, 80%, 50%, 25%, 12.5% and 5%) were observed on glucan production in terms of reduced activity of GTFs. The concentrations which did not inhibit the growth of VGS were selectedbased on previously reported MICs/MBCs against VGS (Ahmed *et al*. 2012).The GTFs are sucrose metabolizing enzymes that play an essential role in the formation of dental plaque as well as in the sucrose dependent cellular adhesion (Shemesh *et al*. 2006). The production of glucans is catalyzed by GTFs using dietary sucrose. There are three different types of GTFs such as GTFB, GTFC and GTFD. Among types of GTFs, GTFB, GTFC and GTFD are responsible to synthesize insoluble glucans, both soluble and insoluble glucans and only soluble glucans respectively. GTFB and GTFC are of great concern with respect to the virulence of cariogenic organisms and pathogenesis of dental caries (Mandava *et al*. 2019). GTFs are active in nature and found on the surface of cariogenic microorganisms. They are also present in the saliva and salivary pellicle which formed on the surface of tooth in human oral cavity (Koo *et al*. 2003). As shown in Table I, overall, the results of present study showed that the production of glucan by crude GTFs from all oral VGS obtained from carious and non-carious subjects was significantly suppressed in the presence of various concentrations of clove oil. It is might be due to presence of variety of constituents of clove oil. A major component of clove oil is eugenol (90-95%) and other important essential constituents are acetyl eugenol, beta-caryophyllene and vanillin, crategolic acid, tannins (bicornin), methyl salicylate, gallotannic acid, the flavonoidseugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids (oleanolic acid, campesterol and stigmasterol), and several sesquiterpenes(Uddin *et al*. 2017).

Few promising results are mentioned here. Like, in case of *S. anginous* obtained from carious subjects, glucan production of SAC2, SAC4 and SAC3 were strongly reduced by concentrations of clove oil as compared to the control used in the study. For *S. anginous* isolated from non-carious subjects, glucan production of SAN1, SAN2, SAN3 and SAN4 were significantly decreased. As far as *S. intermedius*, *S. sanguinis*, *S. salivarius* and *S. oralis* are concerned, a significant reduction in glucan synthesis by crude GTFs was found at all concentrations of clove oil. The results of present study suggest the clove oil is very effective for oral VGS. Except in few cases, glucan production was strongly reduced from different species of *S. mutans* obtained from carious (SMUC1 to SMUC15, SMUC19) and non-carious subjects (SMUN1 to SMUN7) (Table 1).

In case of aqueous infusion of miswak, the results of present study revealed that it did not show strong effects as compared to clove oil. Overall, all concentrations of aqueous infusion of miswak were gradually reduced the glucan synthesis and activity of GTFs but significant results were noted from 100% and 80% concentrations. Both concentrations promising results but their effectiveness varied specie to specie (Table 2). This might be due to bioactive contents of miswak. Constituents of miswak have potential to inhibit glucan production in terms to reduce GTFs activity. The effectiveness of miswak is related to the presence of benzyl isothiocyanate, sulfur, alkaloids (saladorine), tannis and chloride. The miswak also contains other few chemical components such as tri-methyamin, salvadrin, fluoride, silica, mustard, vitamin C, calcium, and phosphorous. Although effects of miswak was not much stronger than clove oil but present study could say that miswak containing mouth rinse could be considered as a suitable oral hygiene alternative for use in subjects of all ages, socioeconomic backgrounds, and health conditions

especially as a long-term measure due to its safety, efficacy, cost-effectiveness, availability, and ease of use (Jassoma *et al*. 2019).

In the present study, all the selected concentrations exhibited varying degree of reduction in glucan production (Table 3). However, the effect of clove oil was stronger than aqueous infusion of miswak for inhibiting glucan production as most of the concentrations of clove oil reduced ≥ 80% glucan production % in a dose dependent manner (Table 4).

CONCLUSION

The results of present study suggest that clove oil, compared to aqueous infusion of miswak strongly inhibit the GTFs and glucan producing properties of most of species of VGS. But still further research studies are necessary to be clarifying the active constituents of clove oil and other plants responsible for such biomolecular activities to suppress or inhibit GTFs and glucan production by VGS as well as to improve the quality of treatment for dental caries and many oral disorders.

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