



Evaluation of antibacterial and antifungal efficacy and Taste Compliance of novel herbal mouth spray- Vedshakti Among Dental students: A Randomized Clinical Trial

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Citation this Article: Dr. Snehal Vilas Thamke, Dr. Prashanth Yachrappa Vishwakarma, Dr. Arun Suresh Dodamani, Dr. Shruti Rajendra Pundkar, Dr. Trupti Vasant Takle, Dr. Mitali Vilas Thamke, Dr. Snehal Sakharam Patil, Dr. Swapnali Satish Patil, “Evaluation of antibacterial and antifungal efficacy and Taste Compliance of novel herbal mouth spray- Vedshakti Among Dental students: A Randomized Clinical Trial”, IJMSIR- December - 2023, Vol – 8, Issue - 6, P. No. 168 – 178.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Numerous herbal formulations have been commercialized claiming antibacterial and antifungal properties, alike other formulation Vedshakti have been commercialized as an antimicrobial spray containing

clove, star anise, fennel and mint which have very well valued in Ayurveda.

Aim & objective: The aim of this study was to investigate the antibacterial and antifungal efficacy and also to observe the compliance of commercially available

herbal mouth spray against specific oral bacteria and fungi.

Settings and Design: In vivo invitro study in Private Medical Institute, Dhule

Materials and methods: 40 participants were selected, oral prophylaxis was carried out. Randomly divide 20 each in two groups: Group A- Vedshakti mouth spray and Group B- control. Gingival Index (GI) were assessed at baseline, 7, 14 and 21 days for both group in in-vivo study followed by questionnaire filled on taste compliance. The efficacy of herbal mouth spray was tested by zone of inhibition (ZOI) through agar diffusion method, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Streptococcus mutans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Candida albicans* in invitro study.

Result: GI was reduced in Group A compared to Group B and participant were compliant with the product. The efficacy of Vedshakti have shown resistance and sensitivity for zone of inhibition(ZOI), minimum inhibitory concentration(MIC) and minimum bactericidal concentration(MBC) against *Streptococcus mutans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Candida albicans*.

Conclusion: These commercially available spray provides the potential candidate for developing an oral antimicrobial and antifungal agent to control or prevent dental diseases associated with oral pathogenic bacteria and fungus.

Keywords: Gingival Index (GI), minimum bactericidal concentration (MBC), minimum inhibitory concentration (MIC), oral microorganism, Vedshakti, zone of inhibition.

Introduction

Oral health is important because it is part of overall public health. Teeth is a unity with the other parts of body^[1]. Vandalism the dental health can affect other parts of health, so that it can imping with daily activity.

Dental caries is an infectious disease correlated with numerous microorganisms, mainly *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus fermentum* and *Actinomyces* spp. Dental plaque is the key Aetiological factor of oral diseases, such as caries and gingivitis which is composed of bacterial biofilms that attach to tooth surfaces. Effects of dental plaque, however, are not merely limited to oral cavity, number of studies have shown associations with systemic health: aspiration pneumonia, bacteraemia and other infections.^[2] For maintenance of periodontal health mechanical plaque removal via toothbrushing and flossing serves as mainstay of daily oral hygiene. Some situations in which conventional oral hygiene measures may be compromised supplementary measures such as chemotherapeutic agents and assisted brushing, may be warranted. For example, such measures may be advocate following periodontal surgery and intermaxillary fixation, as well as for specific patient groups: (e.g. mentally or physically challenged individuals, institutionalized elders preschool children) ^[2], been established by numerous studies in recent decades. While another commonly used option is gel administration, its efficacy as a vehicle for such agents lacks concord due to differences in agents and concentrations investigated, along with wide disparity in application method and frequency (e.g. toothbrushes, trays). For physically or mentally challenged patients, many of whom may be unable to follow instructions for mouth rinsing, even dysphagic subjects at risk for aspiration of mouth rinses and gels, oral sprays may have potential as an alternative

delivery method for chemical plaque control agents in these individuals. A limited number of studies have shown that oral sprays are well accepted by patients, and there are fewer side effects than with mouth rinses.^[2] Even so, there remains a need to dictate effectiveness of oral sprays in relation to ameliorate clinical oral health.

Numerous herbal formulation have been commercialized claiming antibacterial & antifungal properties alike other formulation, Vedshakti have been commercialized as an antimicrobial spray, which contain the first Fennel (*Foeniculum vulgare* Miller), with a sweet, earthy flavour which has been used for many years as herbal remedy. Which have bactericidal, bacteriostatic effects & strong antifungal activity.

Second is Mint(Menthol) used as a raw material in toothpaste, toothpowder, chewing tobacco, confectionary, mouth fresheners, analgesic balms, cough drops, perfumes, chewing gums, candies and tobacco industry.^[3] The fresh or dried leaves are culinary source and used in drinks, antiseptic mouth rinses, mint, chocolate, teas, beverages, syrups, ice creams and also used in Touareg tea, a popular tea in northern African and Arab countries.^[3] It has shown antibacterial and antifungal activity.

Third is Star anise which has shown antioxidant activity (Singh et al.,2006), antibacterial effects (Iauk et al.,2003), antiviral activity(Song et al.,2007), antifungal properties(Mugnaini et al.,2012), insecticidal activity(Park and Shin,2005), and anticancer activity(Yadav & Bhatnagar,2007).^[4]

Fourth is Clove (*Eugenia caryophyllata*), is widely used in medicine as antiseptic against infectious diseases like periodontal disease due to antimicrobial activities against oral bacteria^[5] and potential antibacterial agent against periodontal pathogen. To see beneficial effect of this herbal spray, aim of present study was to investigate the

antibacterial and antifungal efficacy and also to observe the compliance of commercially available herbal mouth spray against specific oral bacteria and fungi.

Materials and methods

The present study is an in-vivo invitro randomized clinical trial conducted at Private Dental and Medical Institute, over a period of 1 months from February to March 2022. The purpose of study was to evaluate antibacterial and antifungal efficacy of commercially available herbal mouth spray, Vedshakti against specific oral bacteria and fungi and taste compliance. Ethical clearance for present study was obtained from the institutional ethical review committee and registered in clinical trial (CTRI/2023/02/049960).

Materials

Commercially available Vedshakti mouth spray (Colgate Palmolive Ltd.) (10g) was obtained from local pharmaceutical store for present study.

Method

40 participants were randomly selected aged 18-22 years who fulfilled the eligible criteria. Inclusion criteria: individuals who give consent to participate, participants having severe gingivitis; exclusion criteria: oral prophylaxis within the past three-month, participant using other mouth spray, participant undergoing orthodontic procedure, subjects suffering from any systemic diseases.

Sample size estimation: The 40-sample size was determined by using G Power 3.1.9.4 version software.

t tests - Means: Difference between two independent means (two groups)

Analysis: A priori: Compute required sample size

Input: Tail(s)	=	One
Effect size d	=	0.82
α err prob	=	0.05
Power (1- β err prob)	=	0.80

Output:

Allocation ratio N2/N1	=	1
Noncentrality parameter δ	=	2.5930677
Critical t	=	1.6859545
Df	=	38
Sample size group 1	=	20
Sample size group 2	=	20
Total sample size	=	40
Actual power	=	0.8163646

Assessment of gingival index

40 participants were selected and informed consent was obtained. GI was assessed at baseline followed by oral prophylaxis were carried out. Then participant was divided into 20 each in two groups; Group A- Vedshakti mouth spray and Group B- Control (without mouth spray) and GI was assessed for 7, 14, 21 days. All participant were instructed for proper oral hygiene technique (twice a day with toothbrush and toothpaste) Figure-1.

Assessment of taste compliance questionnaire

On 21st day the Vedshakti group were provide with questionnaire. It included a set of 12 question about knowledge, taste, sensation, its duration of particular sensation, effect on oral odour of mouth spray and overall effect of product. Validity and reliability of questionnaire was done before commencement of study. All assessment was done by investigator.

Test microorganisms

For present study Vedshakti spray was send to microbiological laboratory. This spray was tested against Gram positive bacteria Streptococcus mutans, two Gram negative organisms, Porphyromonas gingivalis,

Prevotella intermedia and one fungi Candida albicans for study. To assess the invitro antibacterial and antifungal efficacy, the present study was divided in 3 parts: Assessment of Zone Of Inhibition(ZOI) by disc diffusion method, Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).^[6]

Statistical Analysis

Statistical analysis was done using a computer with aid of a statistical package for social sciences (SPSS), version 23. Data were analysed using descriptive statistics. Frequency and percentages were calculated using descriptive statistics. GI was analysed by Friedman Test, Wilcoxon Signed Ranks Test, Mann-Whitney Test. ZOI was analysed by One-way ANOVA test, Post hoc tukey test. Level of significance was kept at $p \leq 0.05$.

Results

In the present study results for GI (Mean Rank) and comparison of GI within groups showed statistically significant in Group A compared to Group B as shown in Table 1, 2. Comparison of GI between groups showed statistically significant at 14 and 21 days compare to baseline and 7 days as shown in Table-3. Responses of participant on compliance of Vedshakti mouth spray shown in Table-4, figure-2,3,4,5. The Vedshakti spray was tested for its antimicrobial activity against three bacterial and one fungal strains by means of ZOI (statistically significant), MICs and MBCs at different concertation Table-5(I, II,III) and Image-1.

Table 1: Mean Rank of Group A and B

Group A(With mouth spray)	GI	Score 1	Score 2	Score 3	Mean Rank	P value
	Baseline	00	00	20(100)	3.53	0.000*
	7days	00	01(5)	19(95)	3.48	
	14 days	20(100)	00	00	1.50	
	21 days	20(100)	00	00	1.50	
Group B (Without mouth spray)	GI	Score 1	Score 2	Score 3	Mean Rank	P value
	Baseline	00	00	20(100)	2.63	0.061
	7days	00	00	20(100)	2.63	
	14 days	02(10)	00	18(90)	2.43	
	21 days	02(10)	01(5)	17(85)	2.33	

Friedman Test; * indicates significant difference at $p \leq 0.05$, GI- Gingival Index

Table 2: Comparison of GI within groups

Groups	GI	Mean Rank	P value
Group A	7 days - Baseline	1.0	0.000*
	14 days - Baseline	10.50	0.000*
	21 days - Baseline	10.50	0.000*
	14 days - 7 days	10.50	0.000*
	21 days - 7 days	10.50	0.000*
	21 days - 14 days	0.00	1.000
Group B	7 days - Baseline	0.00	1.000
	14 days - Baseline	1.50	0.157
	21 days - Baseline	2.00	0.102
	14 days - 7 days	1.50	0.157
	21 days - 7 days	2.00	0.102
	21 days - 14 days	1.00	0.317

Wilcoxon Signed Ranks Test; * indicates significant difference at $p \leq 0.05$, GI Gingival Index

Table 3: Comparison of GI between groups.

GI	Groups	Mean Rank	P value
Baseline	With mouth spray	20.50	1.000
	Without mouth spray	20.50	
7 days	With mouth spray	20.00	0.317
	Without mouth spray	21.00	
14 days	With mouth spray	11.50	0.000*
	Without mouth spray	29.50	
21 days	With mouth spray	11.50	0.000*
	Without mouth spray	29.50	

Mann-Whitney Test; * indicates significant difference at $p \leq 0.05$, GI- Gingival Index

Table 4: Responses of participant on compliance of Vedshakti

Question(Q)	Response	Frequency /Percentage
1.Have you heard of Vedshakti mouth spray?	Yes	20(100)
2.Have you heard about the ingredient present in Vedshakti?	No	4(20)
	Maybe	16(80)
3.Did you like the taste of the spray?	Very good	1(5)
	Good	19(95)
4.How does it taste?	Sweet	20(100)
5.Did you experience any of the following sensation in the mouth & how strong was the sensation?	Soothing	1(5)
	Refreshing	19(95)
6.How long you experience this mouth spray in your oral cavity?	10 minutes	17(85)
	30 minutes	2(10)
	1 hour	1(5)
7.Before using this product do you had bad breath?	Yes	20(100)
8.How did you feel whether it will help you to reduce in bad breath?	Yes	17(85)
	Don't know	3(25)
9.After using whether do you recommended this to your colleague, family or friends?	Yes	15(75)
	Maybe	5(25)
10.Was it helpful in reducing bad breath and refreshing the oral cavity?	Yes	20(100)
11.What are the other changes you like to inform?	No changes	20(100)
12.How much you liked the product overall?	Very much	19(95)
	Some what	1(5)

Table 5: (I, II,III) I. Comparison of zone of inhibition among different concentrations of mouth spray for each micro-organism

Concentration	Candida albicans		S. Mutans		Pg		Pi	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
75µl/ml	25.04	0.18	30.02	0.15	15.1	0.10	18.04	0.11
50µl/ml	23.04	0.11	25.06	0.11	13.04	0.09	13.08	0.0
25µl/ml	22.04	0.11	19.98	0.15	12.08	0.08	12.02	0.08
10µl/ml	15.14	0.13	15.04	0.11	10.04	0.11	10.08	0.08
5µl/ml	12.98	0.13	10.06	0.11	10.08	0.08	8.02	0.08
p value	<0.001*		0.001*		<0.001*		<0.001*	

One-way ANOVA test; * indicates significant difference at $p \leq 0.05$, S. Mutans- Streptococcus mutans, Pg- Porphyromonas gingivalis, Pi- Prevotella intermedia, SD- Standard deviation.

II. Pairwise comparison of zone of inhibition among different concentrations of mouth spray for each micro-organism

Pair	Candida albicans	S. Mutans	Pg	Pi
75µl/ml vs 50µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
75µl/ml vs 25µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
75µl/ml vs 10µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
75µl/ml vs 5µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
50µl/ml vs 25µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
50µl/ml vs 10µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
50µl/ml vs 5µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
25µl/ml vs 10µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
25µl/ml vs 5µl/ml	<0.001*	<0.001*	<0.001*	<0.001*
10µl/ml vs 5µl/ml	<0.001*	<0.001*	0.961	<0.001*

Post hoc tukey test; * indicates significant difference at $p \leq 0.05$, S. Mutans- Streptococcus mutans, Pg- Porphyromonas gingivalis, Pi- Prevotella intermedia

III. Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Results

MIC Results									
Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml 0.2 µg/ml
Pg	S	S	S	S	S	S	S	S	R R
Pi	S	S	S	S	S	S	S	S	R R
S.mutans	S	S	S	S	S	S	R	R	R R
C.albicans	S	S	S	S	S	S	R	R	R R
MBC Results									
Samples	100 µg/ml 50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Pg	NG NG	NG	NG	NG	NG	NG	NG	64	82
Pi	NG NG	NG	NG	NG	NG	NG	NG	84	98
S.mutans	NG NG	NG	NG	NG	NG	18	36	84	112
C.albicans	NG NG	NG	NG	NG	NG	56	64	89	92

S-Sensitive, R-Resistant, NG-no growth, S. Mutans- Streptococcus mutans, Pg- Porphyromonas gingivalis, Pi- Prevotella intermedia, C.albicans- Candida albicans
 MIC-Minimal Inhibitory Concentration, MBC-Minimum Bactericidal Concentration, SD- Standard deviation.

Figure 1: Sensation in the mouth

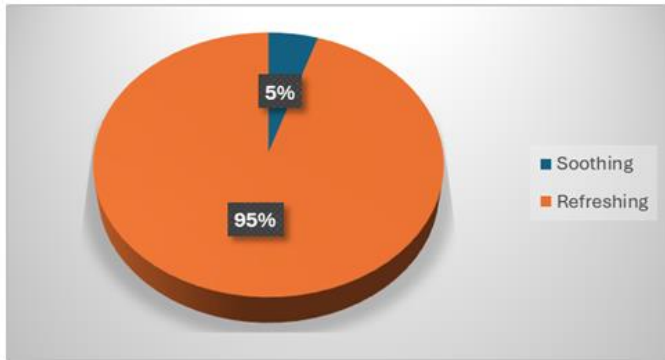


Figure 2: Long experience of mouth spray in your oral cavity

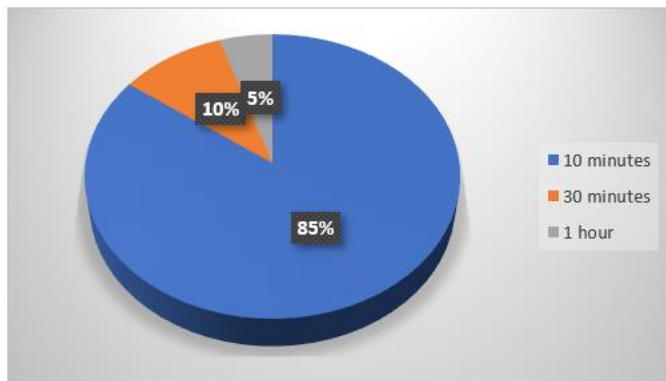


Figure 3: Help to reduce in bad breath.

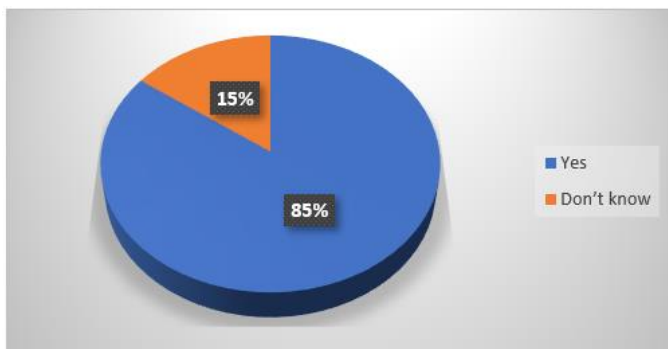
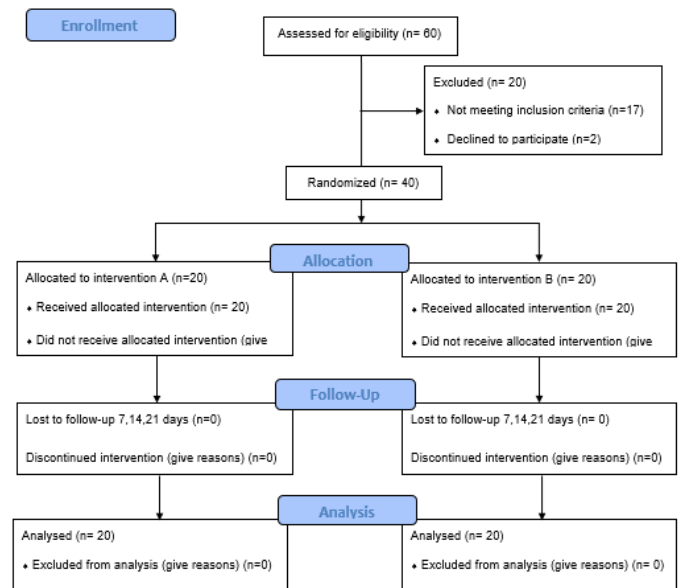
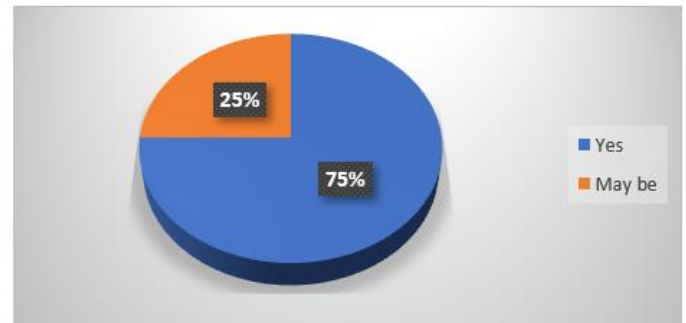


Figure 4: Recommended this mouth spray to your colleague, family or friends.



Discussion

In present study, GI, taste compliance, antimicrobial activity and effect of combining selected herbal extracts present in Vedshakti mouth protect spray were investigated.

In the in-vivo part of present study, Gingival index was assessed which showed that there was decrease in GI of Vedshakti group after 7,14 and 21 days, this may be due to star anise which is one of the ingredient in mouth spray, which correspond with the study by Assiery AA et al. which showed reduction of mean GI, papillary bleeding index and microbial count.^[7]

The taste compliance of mouth spray was assessed through questionnaire, participant observed soothing and

refreshing effect in oral cavity, this may be due to mint similar finding was observed by Diomedea L. study stated that the products containing menthol or eucalyptol are inhaled, chewed, consumed or applied to the skin, they produce a cooling effect or sensation.^[8]

Participant experience mouth spray in oral cavity for 30 minutes and taste of product was sweet this could be due to the fennel seed content in the mouth spray and was proven in the study conducted by Rajkumar et al., fennel seeds significantly increased the pH following from baseline and over a period of 30 min.^[9]

Nivetha et al.^[10] review showed clove oil can be a short remedy for halitosis which support to finding of present study which showed that participant observed reduction of bad odour as clove is present in mouth spray.

The invitro part of present study showed ZOI, MICs and MBC against 4 oral pathogens at different concentrations of spray, values indicated that Vedshakti spray was active against microbial strains tested in present study and exhibited strong antibacterial and antifungal activity. This effect observed is mainly due to 4 main ingredients present in mouth spray.

First ingredient is Fennel, one of the most popular medicinal plants.^[11] Dinesh et al.(2014),^[11] reported that ZOI caused by fennel EO against E. coli was 28mm and Ruberto et al.(2000)^[11] shown E. coli and S. aureus exhibited ZOI of 7.25mm and 16.5mm. Ghouati et al.(2014)^[11] mentioned that MIC of seed oil against S.aureus was 1/1000(v/v). Abdurahim et al.(2017)^[11] also reported that minimum concentration of fennel EO that inhibits the growth of S.aureus and E.coli was ≤ 0.025 . Similarly, results of Bakkali et al.(2008), Kalembe et al.(2003),^[11] Cantore et al (2004),^[12] Jazani et al.(2009),^[11] Akrayi(2012),^[11] Dadalioglu and Evrendilek(2004),^[11] Ağaoğlu et al.(2007).^[11] Gulfranz et al.(2008)^[11] and Abdurahman et al.(2010),^[11] Lo Cantore

et al.(2004),^[12] Dashti et al.^[13] showed antibacterial activity. Some study have shown antioxidant activity [Ruberto et al.(2000);^[11] Singh, et al.(2006)^[4]; Faudale et al^[11]], anti-inflammatory activity (Choi & Hwang,2004)^[14] and antifungal activity [Singh et al.(2006); Soyulu, et al.(2007);^[14] Ozcan et al.(2006)^[15]]. Antibacterial effect was seen through MIC and MBC against S.mutans, the maximum MBC was observed. MIC was sensitive from 3.12µg/ml to 100µg/ml concentration. So this strongly antifungal and antibacterial property of fennel have reflected in spray.

The oral microorganisms most likely that cause oral malodour are Gram-negative bacteria species including Treponema denticola, Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, Bacteriodes loescheii, Enterobacteriace-ae, Tannerella forsythensis, Centipeda periodontii, Eikenella corrodens, Fusobacterium nucleatum.^[16] The principle bacteria that are implicated in creation of oral malodour include F.nucleatum, P.intermedia and T.forsythensis.^[16] Various herbal product have shown beneficial effect to reduce malodour. One of them is mint which have been used since ancient times. Most study has shown antibacterial activity Para et al.; Golestan et al; Zaidi et al.^[17] Presence of this component in mouth spray have beneficial effect to reduce oral malodour. In present study minimum ZOI was observed against P.intermedia. This ingredient in product may have shown productive effect to suppress oral malodour.

Third content is star anise, which have therapeutic potential. It has received significant attention after shikimic acid extracted from star anise and it is used to manufacture Tamifu®, which is a drug used to treat influenzas (Wang et al.,2011).^[4] Most study shown antimicrobial activity Singh et al.(2006); Khesorn Nanthachit(2002); Yang et al.(2010); Mimica Dukic et

al.,(2003),^[4] antiseptic activity Verghese et al(1988) and antifungal activity De et al.,(2002);Singh et al.,(2006); Huang et al.,(2010).^[4] In present study it has shown strong antimicrobial effect of spray which have one of component in spray.

Last content is clove which had shown properties that could destroy cell walls and membranes of microorganisms, and permeate cytoplasmic membranes, then inhibit normal synthesis of DNA and proteins.^[5] Most of the study has shown antimicrobial activity Kuang et al; Badei et al.; Angienda et al; Lomarat et al; Shan et al; Bayoub et al.; Cui et al.; Badhe et al.; Liu et al.; Nassan et al; Keskin et al; Liang et al. and antifungal activity Schmidt, E. et al.^[5] So in present study antibacterial and antifungal effect of spray may be due to clove. This crucial four ingredient are present in Vedshakti mouth spray which has shown favourable effect like antibacterial and antifungal effect. No literature was found in relation between GI, taste compliance and microorganism with relation to Vedshakti mouth protect spray so strengths of present study is that it is the first study conducted.

Conclusion

Based on present research Vedshakti spray possessed good antimicrobial and antifungal activity against selected organism. The results of this study incite interest in evaluating various component present in this mouth spray which have shown beneficial effect on Gingival status and participant were compliant with the taste of mouth spray.

Furthermore clinical studies of the safety and efficacy of these spray will be important to establish whether they offer therapeutic benefits, either alone or in combination with conventional therapies that can help to reduce the overall burden of oral diseases.

Acknowledgement: The author appreciates all those who participated in the study and helped to facilitate the research process.

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