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Scientific validation of a traditional toothpaste formulation to treat halitosis

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Abstract

Since the beginning of human history, *Cuminum cyminum* seeds are habitually used in several cuisines of different food cultures. In India it is used in both whole and ground form as a traditional ingredient to make innumerable dishes without knowing their medicinal uses and properties. In Kerala, the decoction of the cumin seeds made from drinking water used for drinking purpose instead of ordinary water and in Tamilnadu cumin seeds used in daily to make food items called Rasam(soup) to eat with rice to increase the digestion. The fruits of *Solanum lycopersicum* are one of the important savoury vegetables used to increase the flavour of cooked foods. The oil and solid albumen ripe of *Cocos nucifera (L.)* fruits are commonly used in cooking and frying. Hence in the present study, the composition (paste) prepared by mixing of 2 drops of *Solanum lycopersicum* fruit juice, 2 drops of *Cocos nucifera (L.)* and 1gram of *Cuminum cyminum* seed powder were tested on the 100 subjects who are felt bad breath. The result of the present study showed effectively controlled bad breath in 91 patients even after on weak period of clinical trial and except 09 diabetic patients.

Keywords: Oral hygiene, Bad breath, Halitosis, *Cuminum cyminum*, *Solanum lycopersicum*, *Cocos nucifera* (L.).

INTRODUCTION

Halitosis is a common unpleasant or offensive oral odor related health condition and it affects the majority of human population at all age groups. Throughout the world, nearly 30% to 50% of the population has experienced the problem of halitosis [1, 2, 3]. Halitosis, oral malodour, or bad mouth breath is a universal medico social problem in all communities and refers to the unpleasant odour that originates from the mouth or elsewhere in the oral cavity [4]. Almost all the people have unpleasant oral odour at some time [5]. When the halitosis occurs severe or longstanding, it may psychologically decrease the self-confidence and social interactions of affected person [4]. Formation of halitosis may be due many reasons such as lack of proper oral cleansing and xerostomia (dryness of the mouth) [6], oral cavity contains volatile sulphur compounds (VSCs) such as hydrogen sulphide, methyl mercaptan, dimethyl-sulphide, and organic acids [7, 8, 9, 10] and the volatile sulphur compound producing bacteria associated with gingivitis and periodontitis such as *Porphyromonas gingivalis, Prevotella intermedias*, *Actinobacillus actinomycetemcomitans*, *Campylobacter rectus*, *Fusobacterium nucleatum, Peptostreptococcus micros, Bacteroides forsythus*, *Eubacterium* species, and spirochetes [11], medical problems such as renal failure, cirrhosis of the liver, and diabetes mellitus [1, 12, 13], result of the microbial degradation of proteins [11], the presence of decay or retention of food between the teeth. Till date no any effective tooth paste or oral mouth wash to clear this halitosis. Hence, in the present investigation, the composition "CUSOCU" were prepared in the form of paste from usual food items such as cumin (*Cuminum cyminum*) seeds, fruits of tomato (*Solanum lycopersicum)* and oil of coconut (*Cocos nucifera (L.)* and were clinically tested among the peoples who are having halitosis condition to validate this medicinal preparation.

MATERIALS AND METHOD

Study sample selection

For this study, samples of more than 100 peoples between the ages of 18 and 45 years were randomly selected as a study subjects from Katpadi Town, near Vellore District. A simple and short question contained questionnaire was given to each person to know what he felt about his oral hygiene and halitosis. Based on

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their answer, the 100 subjects who strongly believe to existence of halitosis conditions were underwent to halitosis detection test to ensure the halitosis condition. The halitosis detection test was conducted in all study subjects by standard method called organoleptic method. In all 100 samples 22 were females and 78 were males.

Halitosis detection

The organoleptic method is a simple, easy and frequently used method to detect the halitosis. In this method, a simple plastic tube is placed in the mouth of the persons being examined for this study to prevent the dilution of the breath by the surrounding air and other end of the tube is very near to the nose of the assessor. While the study subjects slowly exhales, the "judges" assesses the odour at the other end of the tube. In this test the embarrassing situation between both study subjects and the assessor were avoided by keeping an opaque privacy screen between them to ensure no visual contact. All the study subjects were tested for the halitosis by this organoleptic method. The individuals with halitosis were identified and experimented with present composition to validate their potentiality in prevention of halitosis and maintenance of oral hygiene.

Experiment with halitosis affected human

The persons who are having the halitosis were subjected to validation experiment of present medicinal preparations. For all patients, the paste containing herbal compositions and the procedure to prepare the tooth paste were clearly explained (1gm *Cuminum cyminum*+ 5drops of *Solanum lycopersicum* fruit extract+2 drops of pure *Cocos nucifera* oil) and also recommended instead of chemical paste already in the usage of everyday practice. The paste obtained by mixing of three food ingredients are named as CUSOCU for our convenience based on the joining of first two letters of all three food ingredients. All the patients were checked for their halitosis level in the 0 day (just before the first day of brushing) 5th day, 10th day, 15 and 30th day after the experiment (used after the new herbal composition paste). The halitosis level of all patients of from 0 to 30th days results were compared to know if any improvement exists in the halitosis reduction. All the patients are requested to brush their teeth at least twice a day to achieve a healing effect and use the dental floss after eating because normal brushing cannot eliminate bacterial plaque in between teeth. Addition to these, they are all asked to do tongue cleaning because many people have a habit of brushing their teeth but they do not know that the tongue is also a part to be cleaned.

Phytochemical analysis of medicinal preparation

For the present study, the medicinal preparation "CUSOCU" have been analysed by FTIR to know the functional group present in that composition. In addition to this, the preparation "CUSOCU" comprising individual materials such as *Cuminum cyminum, Solanum lycopersicum* and *Cocos nucifera* oil were also analysed separately for their functional groups.

Collection and preparation of "CUSOCU" for FTIR analysis

Dried *Cuminum cyminum* seeds, fresh *Solanum lycopersicum* and pure *Cocos nucifera* oil were obtained from nearby grocery shop. The methanol extraction obtained from the powder of *Cuminum cyminum* seeds, fresh juice of *Solanum lycopersicum* oil of *Cocos nucifera* were analysed individually and in mixed form in FTIR to know their functional group. The extract for dried seed powder of *Cuminum cyminum* were prepared by maceration process and filtered by No.1 Whatman filter paper. The juice of *Solanum lycopersicum* obtained by squeezing the fruits and dried and the oil were filtered inNo.1 Whatman filter paper and taken to FTIR analysis.

Pellet preparation and FTIR analysis

The 10 mg dried powder samples and trace amount of KBr (30mg) have been taken in a mortar and ground well with pistol up to it becomessomewhat "pasty" and sticks to the mortar (3 to 5 minutes).The pelletpress were fixed together and die was inserted into the cavity. The sample mix is transferred and evenly spread into the cavity by using a metal spatula. The bolt press was inserted and rotated into the cavity to distribute the particles. The whole die set transferred to the hydraulic pellet press andthe wheel was rotated to secure it tightly. The valve of the hydraulic press was closed and moves the handle up and down until the handle becomes tight. Five minutes after, move up the wheel of the press to release the pressure and the die was taken out. A transparent pellet obtained from pellet press was transferred to pellet holder and analysed by JASCO, FT/IR-4600, Serial No DO86061786,

RESULTS AND DISCUSSION

Halitosis is a common non-risk health problem among humans and can be caused by oral or non-oral. The term Halitosis derived from halitus (breathed air) and the osis (pathologic alteration) [14]. It means disagreeable bad or unpleasant odor emanating from the mouth air and breath. More than 50% of the general population have halitosis [15]. Most of the bad breath comes mainly due to inadequate hygiene or due to hygiene deficient in contact points, the formation of periodontal pockets due to the presence of supra-gingival or sub-gingival plaque or calculus and the surface of the tongue, whose anatomy favors the accumulation of bacteria [16, 17, 18]. However, they are not form any health risk, the social anxiety disorder among these halitosis patients is high and it tends to precede depression and alcoholic dependence. Worldwide, one-third of social anxiety disorder patients have concomitant psychological disorders such as depression or alcohol dependence [19]. Sometimes, the social anxiety disorder amalgamated with other psychological diseases leads to increase the risk of suicide [20]. Excessive anxiety in social situations causes a considerable distress and impairs in functional ability in daily lives [21]. Most of the halitosis patients turn to "soft" medicine that offers a wide range of disciplines to treat bad breath; homeopathy, herbal medicine and aromatherapy as an alternative treatments, or complementary to conventional medicine. In India, particularly in Tamilnadu, majority of the people used the traditional mode of treatment to treat the halitosis. Hence, in the present investigation, the herbal treatments which were used generation by generation to treat halitosis were experimented on halitosis affected persons to validate and evaluate their potential in curing halitosis. For this study, totally 100 individuals who felt the bad mouth smell was randomly selected and they were re-checked and confirmed byorganoleptic method. According to the obtained results of the present study, five individual out of 100 has pseudo-halitosis effect. Even though, these five individuals are not having any bad smell in their mouth they are psychologically worried about the halitosis. Few people in whom symptoms of halitosis are not present, and yet they complain about having them, psuedohalitosis or halitophobia [22]. Five (5) individuals who were having pseudo halitosis were identified at organoleptic test who were all counselled in the 1st day of experiment and then they were released from the experimental study and another 12 individuals are not having any halitosis and even not felt the halitosis are identified. The validation of present herbal composition was tested only in 83 subjects excluding these 12 +5 individuals. The results of organoleptic test showed30 individuals with discontinuous halitosis, 29 individuals with slight halitosis and the remaining 24 individuals with worst halitosis conditionamong these 83. The present herbal composition CUSOCU was recommended to all (88) subjects out of 100 except the sampling individuals (12) who are not having any halitosis and even not felt the halitosis. The halitosis conditionsof all 88 individuals were checked by organoleptic method 0, 7, 15 and 30th days after the use of this herbal tooth paste to brush the

tooth. The obtained results showed 67 individuals are not having any bad smell in their mouth have some concordance with the findings that in-vivo studies of chlorhexidine mouth rinse, containing pericarp extracts of *Garcinia mangostana L*. showed a significant reduction of VSCs level in gingivitis patients [23], 14 individuals have a moderate level of smell and remaining 7 are not having any improvement out of 88 individuals. The results of 15th day observation showed 75 individuals without any halitosis, 6 individuals with moderate halitosis and 7 individuals without any improvement. The 30th observation exhibited 81 individuals are not having any halitosis showed a similarity with that report of toothpaste mixed with Chinese herb extract showed better in vitro inhibition effect on VSCs genesis anaerobic bacteria than most other toothpaste on market [24]. Even after30 days treatment, 7 individuals are not having any improvement in their halitosis condition were reanalysed to find weather it may be due to oral and dental problems or some other problems. The results of the present study observed at 0 days and 7th day, 15th day and 30 day of experiment interprets that both worst halitosis (24) and moderate halitosis (59) of 0 days drastically reduced to 07 and 14 at 7th day observation. The halitosis reduction in worst and moderate patients gives clear cut evidence for the ability of present herbal composition in curing halitosis. The worst halitosis observed at 7th day were not observed further change up to 30thday. But the moderate conditions of 14 individuals were further reduced to 06 number and 0 level at 15th and 30th day observation. Already many plants such as *Elettaria cardamomum, Salvadora persica, Illicium verum,* and *Origanum vulgare* were widely used by 76% traditional healers to treat halitosis and 64.9% patients themselves used to treat halitosis without any scientific validation. Salvadorapersica, (Miswak) was widely used to treat halitosis because of anti-inflammatory effect [25] and contains vitamin C that helps in healing gingival edema and bleeding [26]. The alcoholic extract of *Curcuma zedoaria* was used auxiliaries component in the mouthwash to treat dental plaque and gingivitis [27]. Hence, in the present investigation, the traditional preparation CUSOCU was tested to validate scientifically its efficacy on halitosis cure and the phytocompounds responsible for it.

Table 1: Validation of a new herbal composition CUSOCU on halitosis patients

Halitosis condition	Number of Individuals			
	0 day	7th day	15th day	30th day
Worst	24	07	07	07
Moderate	59	14	06	0
Pseudo halitosis	0	0	0	0
Without halitosis	0	62	71	76
Total	83	83	83	83

Cumins are common spices used in foods and are necessary commodity of kitchens [28]. Its extract contained a large amount of cuminaldehyde (61.65%) and other active chemical compounds such as cumene; p-cymene, β-pinene, acetic acid, p-cymen-7-ol, and γterpinene has a significant antibacterial effect [29, 30] against E. faecalis persistent endodontic infections [31, 32, 33]. Cuminaldehyde is an active compound of cumin extract, which is present in the highest concentration and with potential antibacterial effects [34, 35, 36]. The cumin extract had better antibacterial effects on E. faecalis than 2% CHX gel at a concentration of 0.7 mg/mL [29]. The seed powder of *Cuminum cyminum*was taken as one of the ingredients in medicinal preparation *CUSOCU* for halitosis. In the present investigation, the functional groups exhibited in the methanol extract of *Cuminum cyminum* was estimated by FTIR analysis. The FTIR absorptionfrequency for methanol extract of *Cuminum cyminum* showed twenty seven peaks. For each peak, the functional group and its corresponding intensity is further detailed in Table 2. The peak at 3543.36 corresponding to reference ranges 3500-3700 showed functional group alcohols and phenols by the presence of O-H group (H-bonded) (usually broad) exhibited resemblance with the FTIR peak at 3404 and 3533 for *Sennaitalica* [37]. The peaks at 2991.05, 2921.63, 2873.42 and 2856.06 between the reference ranges 2850-3000 indicated the presence of functional group alkanes by the presence of N-H stretching and CH3, CH2 & CH2 or 3 bands showed a concrete similarities with the spectral peaks at 2873.94and 2929.87 (CH antisym and Sym stretching) for $–CH_3$ and CH_2 aliphatic compound in the methanolic extract of leaf of *Indigofera tinctoria* [37]**.** Further**,** presence of phyto-compounds alkanes were identified due to peak at 2873.42 and 2856.06 [38], peak at 2770.24 indicates aldehydes & ketones, peak at 2141.56 showed alkynes [40], carboxylic acid, imine/oxime, nitro compound, aromatic, carboxylic acid, sulfonyl chloride, phenol, alkyl aryl ether, sulfoxide, carboxylic acids, aromatics, alkyl halides, alkynes and alkyl halides confirmed by the FTIR peak contains functional groups C-H (aldehyde C-H), S-C≡N Stretching, C=O stretching, dimer, C=N, Stretching, N-O Stretching, C-C Stretch, O-H bend, S=O strtching, C-H "oop", C-Cl Stretch, -C=C-H; C-H bend and C-Br stretching.These results have seen a concordance with the FTIR results of [41]. Alkynes are unsaturated hydrocarbons. They are hydrophobic, more reactive and contain at least one carbon-carbon triple bond. Alkynes used in lot of medicines including the noretynodrel pill, antiretroviral efavirenz and the antifungal terbinafine [42], Nitro compounds are used in the treatment of many diseases, including hypertension, coronary artery disease, and heart failure, and in the prevention of stroke in atrial fibrillation and thromboembolism. Drugs that are used in cardiovascular diseases include organic nitrates that are characterized by C-ONO_2, β-blockers, calcium channel blockers, anticoagulants, fibrinolytics, omega 3-fatty acids, and free radical scavengers [43, 44].

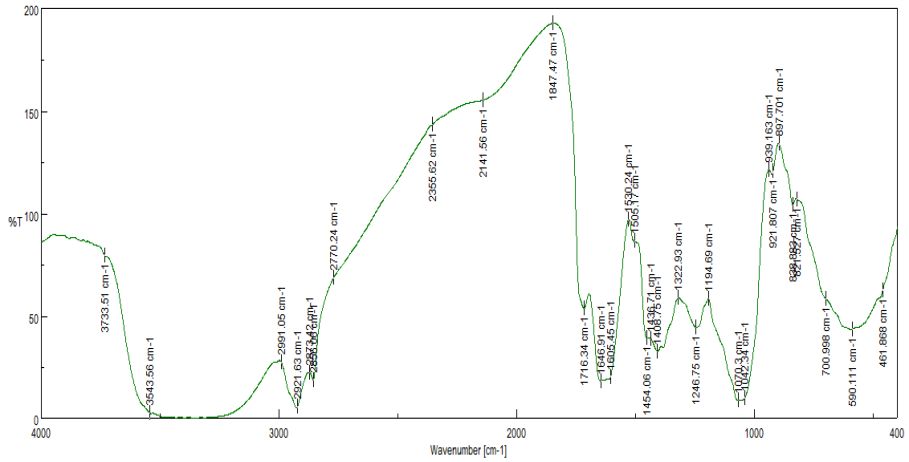

Figure 1: FTIR spectrum of methanolic extract of *Cumin cyminum* seeds

Table 2: IR spectral frequencies related functional groups for methanol extract of *Cumin cyminum* seeds

S.No	Peak Value absorption (cm^{-1})	Reference ranges	Functional group (Bonds)	Intensity	Compound
1.	3543.56	3500-3700	O-H (H-bonded), usually broad	Strong	Alcohols & Phenols
2.	2991.05	2850-3000	N-H stretching	Strong broad	Alkanes
3.	2921.63		N-H stretching	Strong broad	Alkanes
4.	2873.42		CH3, CH2 & CH 2 or 3 bands	Strong	Alkanes
5.	2856.06		CH3, CH2 & CH 2 or 3 bands	Strong	Alkanes
6.	2770.24	2695-2830	C-H (aldehyde C-H)	Medium	Aldehydes & Ketones
7.	2141.56	2100-2260	C-Ctriple bond	Strong	Alkynes
8.	1716.34	1706-1720	C=O stretching, dimer	Strong	Carboxylic acid
9.	1646.91	1640-1690	C=N, Stretching	Strong	Imine/Oxime
10.	1665.45				
11.	1530.24	1500-1550	N-O Stretching	Strong	Nitrocompound
12.	1505.17				
13.	1454.06	1400-1500	C-C Stretch	Stromg	Aromatic
14.	1436.71	1395-1440	O-H bend	Strong	Carboxylic acid
15.	1408.75	1380-1410	S=O strtching	Strong	Sulfonyl chloride
16.	1322.93	1210-1390	O-H bending	Medium	Phenol
17.	1246.75	1020-1250	C-N Stretching	Medium	Alkyl aryl ether
18.	1194.69				
19.	1070.3	1030-1070	S=O	Strong	Sulfoxide
20.	1042.34				
21.	939.163	910-959	O-H bend	Medium	Carboxylic acids
22.	921.807				
23.	897.701	675-900	C-H “oop”	Strong	Aromatics
24.	828.882	550-850	C-Cl Stretch	Medium	Alkyl halides
25.	821.527				
26.	700.998	610-700	-C=C-H; C-H bend	Broad strong	Alkynes
27.	590.111	515-690	C-Br stretching	Medium	Alkyl halides

The tomato species *Solanum lycopersicum* one of the component in CUSOCU medicinal preparation were underwent to FTIR analysis exhibited 22 peaks.The fruit juice of *Solanum lycopersicum* have seen *FTIR* peaks at 2991.05, 2921.63, (N-H stretching) 2873.42, 2856.06, (CH3, CH2 & CH 2 or 3 bands) between the reference ranges 2850-3000 and 2695-2830 indicated the presence of alkanes. The peaks at 2770.24 (2695-2830), 2141.56 (2140-2175), 1716.34 (1706-1720), 1646.91, 1665.45 ((1640-1690), 1530.24, 1505.17 (1500-1550), 1454.06 (1400-1500), 1436.71 (1395-1440), 1408.75 (1380-1410), 1322.93 (1210-1390), 1246.75, 1194.69 ((1020-1250), 1070.3, 1042.34 (1030-1070), 939.163, 921.807 (910-959) and 897.701 (675-900) specifies the presence of aldehydes & ketones, thiocyanate, carboxylicacid, imine/oxime, nitrocompound, aromatic, carboxylicacid, sulfonylchloride, phenol, alkyl aryl ether, sulfoxide, carboxylic acids and aromatics respectively. The peaks at 828.882, 821.527 (550-850), 700.998 (610-700) and 590.111 (515-690) represents the existence of alkyl halides and alkynes. These are all functional groups signifies the presence of phytocompounds and nutrients such as potassium, folic acids, neoxanthin, lutein, α-cryptoxanthin, α-carotene, β-carotene, cyclolycopene, and β-carotene 5, 6-epoxide [45], flavonoids, vitamin C, hydroxycinnamic acid derivatives and anti-oxidative and anti-cancer phytocompund lycopene’s [46, 47]. The lycopene is an important and major phytocompounds in tomato plays a multifunctional role as a nonsurgical aid in the treatment of oral diseases like leukoplakia, oral submucous fibrosis, lichen planus, oral squamous cell carcinoma, and also prevents the destruction of periodontal tissues [48]. Among these, the peak for functional group alcohol, nitro compound, sulfonyl chloride, carboxylic acids, aromatic, alkyl aryl ether, alkyl halides and alkynes were observed as similar as present in *Cuminum cyminum.* Addition to these, presence of some new compounds such as aliphatic primary amine, amines, allens, α, β –unsaturated ketone, tertiary alcohol, primary alcohol and alkenes were also observed in *Solanum lycopersicum* which was evidenced by the existence of related functional groups such as N-H Stretching (medium), C-H Stretching (medium), C=C=C Stretching, C=C Stretching, C-O stretching, Strong, C-O stretching, strong and C=C, bending (medium) respectively.Lycopene is a fat-soluble carotenoid discovered by Ernest *et al*. in 1959 [49] Tomatoes and tomato-based products are the major sources of lycopene in the human diet [50]. Lycopene has some beneficial effects in the treatment of certain diseases of oral cavity including oral cancer and precancerous lesions [51]. There is a positive relationship between lycopene consumption and a reduction in the risk of development of degenerative diseases caused by free radicals, such as cancer, cardiovascular diseases, asthma, arthritis, stroke, cataractogenesis, hepatitis and also periodontitis [52, 53, 54]. Tomato *Solanum lycopersicum* fruit paste is listed as one of the home remedy material which is taken as mouth wash to clear the oral diseases [55].

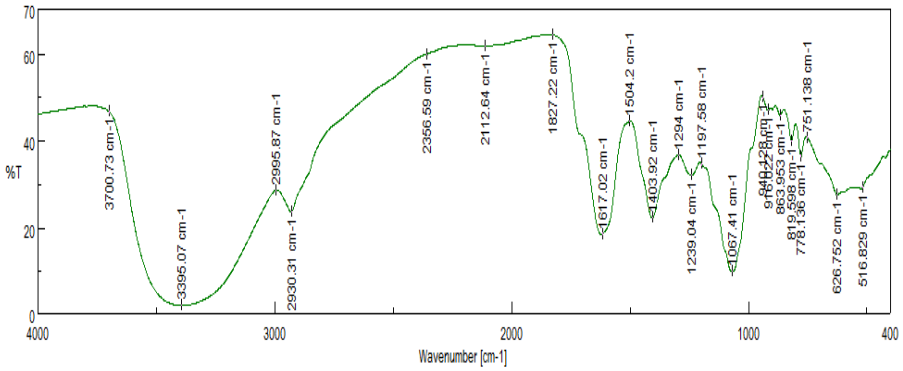

Figure 2: FTIR spectrum of fruit juice of *Solanum lycopersicum*

Table 3: IR spectral frequencies related functional groups for fruit juice of *Solanum lycopersicum*

S.No	Peak Value absorption (cm^{-1})	Reference ranges	Bonds	Functional group
1.	3700.73	3584-3700	O-H stretching, Medium, Sharp	Alcohol
2.	3395.07	3200-3550	N-H Stretching (medium)	Aliphatic primary amine
3.	2995.87	2800-3000	C-H Stretching (medium) C-H Stretching (medium)	Amines
4.	2930.31			
5.	2356.59	-	-	Unknown
6.	2112.64	2100-2140	-C≡C- Stretching (weak)	Alkynes
7.	1827.22	1650-2000	C=C=C Stretching	Allene
8.	1617.02	1610-1620	C=C Stretching,	α, β –unsaturated ketone
9.	1504.2	1500-1550	N-O stretching, Strong	Nitro compound
10.	1403.92	1380-1410	S=O stretching, Strong	Sulfonyl chloride
11.	1294	1266-1342	C-N stretching, Strong	Aromatic
12.	1239.04	1200-1275	C-O stretching, Strong	Alkyl aryl ether
13.	1197.58	1124-1205	C-O stretching, Strong	Tertiary alcohol
14.	1067.41	1050-1085	C-O stretching, Strong	Primary lcohol
15.	940.128	910-950	O-H bend (medium)	Carboxylic acid
16.	916.022			
17.	863.953	675-900	C-H 'opp'' (strong)	Aromatics
18.	819.598	790-840	C=C, Bending Medium	Alkenes
19.	778.136	550-850	C-Cl Stretching, Medium	Alkyl halides
20.	751.138			
21.	626.752	610-700	-C≡C-H:C-H bend (broad, strong)	Alkynes
22.	516.829	515-690	C-Br Stretching (medium)	Alkyl halides

Oral infectious diseases gives painful conditions as well as cause teeth decay, oral malodor, bleeding gums, halitosis, reduce the strengthening of teeth, gums, and jaw. But the peoples in rural India is not give an importance to this oral infectious diseases because of expensive to treat specially in a country like India where oral health care is still beyond the reach of rural population, further they depend the economically chief traditional Siddha and Ayurveda medicinal system to treat oral infectious diseases. Among these treatments the practice of oil pulling therapy is very popular to treat all oral related infectious diseases [56]. Pure oils acts as antibacterial agents so, oil pulling inhibit the harmful bacteria, fungus, and other organisms of the mouth, teeth, gums, and throat [57]. This oil pulling concept is not a new concept. it is an ancient ayurvedic therapy for maintaining oral hygiene [58] (Oakley, 2016).It has been described in the ancient Ayurvedic text "CharakaSamhita" as "Kavalagraha" or "Kavala Gandoosha." It is a powerful detoxifying folk technique traditionally followed in India that has recently become popular as a complementary and alternative remedy to prevent decay, oral malodor, bleeding gums, and for strengthening teeth, gums, and jaws [59, 60]. In coconut oil contain lauric acid one of the fatty acids is a proven antimicrobial agents it can kill bacteria, viruses, and fungi that make it especially well-suited for oral health [61]. In coconut oil, medium-chain fatty acids called lauricacid are present at 50% in total phytochemical contents it has a significant antimicrobial and anti-inflammatory benefits [62]. In addition to these, different researchers suggested various concepts about how the oil pulling reduce and prevent oral infectious diseases. Oil pulling generates antioxidants which damage the cell wall of microorganisms and kill them [63]. This oil will coat the teeth and gingiva and inhibits bacterial co-aggregation and plaque formation [62]. Thus plaque building bacteria responsible for dental caries, gingivitis, periodontitis and bad breath are removed from the oral cavity. Oil pulling prevents dental caries, gingivitis, oral candidiasis and periodontitis from occurring, helps to reduce tooth pain, fixes mobile teeth and achieves vigorous oral hygiene [58, 64, 60]. It contains lauric acid which can react with alkalis present in saliva such as

sodium hydroxide and bicarbonates to form sodium laureate-soap like substance, which reduces plaque adhesion and accumulation, and possesses cleansing action [65, 62, 66]. Hence in the present CUSOCU medicinal preparations containoil of coconut (*Cocos nucifera*)is taken to FTIR analysis. The FTIR made for the oil of *Cocos nucifera* showed 28 peaks, among these functional groups and phytocompounds for13 spectral peaks as similar as to what would be observed in the seeds of *Cuminum cyminum* and fruits of *Solanum lycopersicum* except three functional group such as C-H stretching (medium), -C=N-H stretching, C=O stretching (strong), C=C stretching (medium), O-H bending (medium) and C-O stretching (strong) related phytocompounds alkane, nitriles, ǫ-Lactone, conjugated alkene, phenol and secondary alcohol respectively.

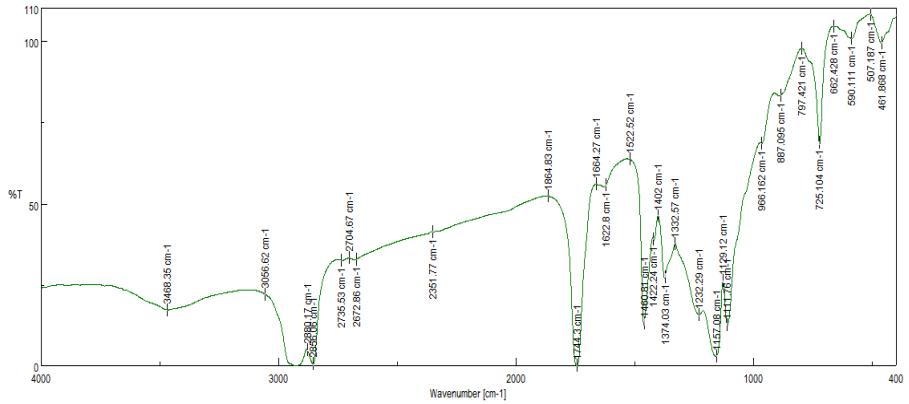

Figure 3: FTIR spectrum of oil of *Cocos nucifera*

Table 4: IR spectral frequencies related functional groups for *Cocos nucifera* oil

S.No	Peak Value absorption (cm^{-1}) of test sample	Reference ranges	Bonds	Functional group
1.	3468.35	3200-3550	O-H Stretching (strong, broad)	Alcohol
2.	3056.62	3000-3100	C-H Stretching, medium	Alkenes
3.	2886.17	2840-3000	C-H stretching (medium)	Alkane
4.	2856.06			
5.	2735.53	2695-2830	C-H stretching (medium)	Aldehyde
6.	2704.67			
7.	2672.86	2500-3600	O-H stretch (strong)	Carboxylic acid
8.	2351.77	2000-2400	-C=N-H stretching	Nitriles
9.	1864.83	1650-2200	C-H bending (weak)	Aromatic compound
10.	1744.3	1735-1750	C=O stretching (strong)	ǫ-Lactone
11.	1644.27	1640-1690	C=N Stretching (medium)	Imine/oxime
12.	1622.8	1600-1650	C=C stretching (medium)	Conjugated alkene
13.	1522.52	1500-1550	N-O stretching (strong)	Nitro compound
14.	1460.81	1300-1550	N-O asymmetric stretch (strong)	Nitro compound
15.	1422.24	1395-1440	O-H Stretch (Strong)	Carboxylic acid
16.	1402	1380-1410	S=O Stretching (strong)	Sulfonyl chloride
17.	1374.03	1310-1390	O-H bending (medium)	Phenol
18.	1332.57			
19.	1232.29	1020-1250	C-O stretching (strong)	Amine
20.	1157.08			
21.	1111.76	1087-1124	C-O stretching (strong)	Secondary alcohol
22.	966.162	960-980	C=C bending (strong)	Alkene
23.	887.095	885-895	C=C bending (strong)	Alkene
24.	797.421	790-840	C=C bending (medium)	Alkene
25.	725.104	665-730	C=C bending (medium)	Alkenes
26.	662.428	515-690	C-Br bending	Halo copound
27.	590.111	500-660	C-l stretching (strong)	Halo compound
28.	507.187			

The whole traditional herbal composition recommended for halitosis treatment were tested in combined form in FTIR analysis showed certain new functional groups which may be due to the reaction between the phytochemicals of all these three components. Present herbal composition contains seed powder of *Cuminum cyminum,* fruits of *Solanum lycopersicum* and oil of fruit of *Cocos nucifera* form two layers when kept in mixed form. These three components were kept in mixed paste format, they form liquid layer in the bottom, oil layers on the top. So, both the liquid and oil layer were taken to FTIR analysis showed different spectral peaks, functional groups and phytocompounds.The results of FTIR analysis of this mixture showed some functional groups and phytocompounds as similar as observed in seed powder of *Cuminum cyminum,* fruits of *Solanum lycopersicum* and oil of fruit of *Cocosnucifera* in separate analysis and addition to these few new compounds also exhibited which are not observed in individual analysis. The bottom layer of the mixture showed 49 peaks at different places in between the ranges of 679.785^{cm-1} to3816.44 $^{cm-1.}$ The peaks at 3388.32, 2807.85, 2140-1990, 1329.68, 1225.54, 1039.44 showed new phytocompound saliphatic primary amine, amine salt, isothiocyanate, sulfone, vinyl ether and sulfoxideforrelated functional groups of N-H stretching (narrow, strong), N-H stretching (strong, broad), N=C=S stretching (strong), S=O stretching (strong), C-O stretching (strong) and S=O stretching (strong) respectively.

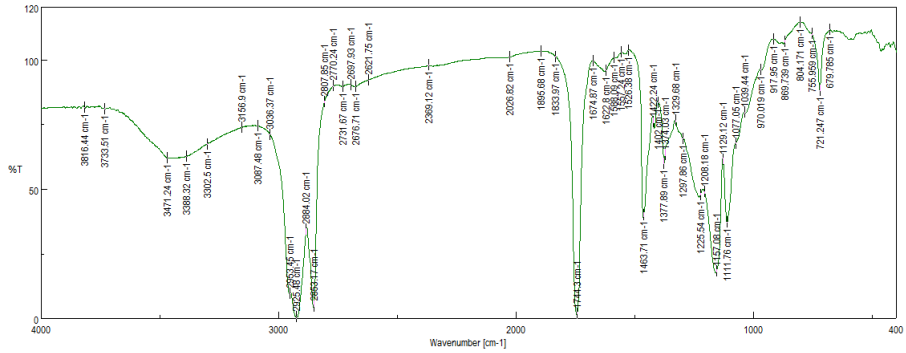

Figure 4: FTIR spectrum of bottom layer of *Cumin cymium* seed powder, *Solanum lycoperisicum* juice and *Cocus nusifera* oil composition.

Table 5: IR spectral frequencies related functional groups for bottom layer of *Cumin cymium* seed powder, *Solanum lycoperisicum* juice and *Cocus nusifera* oil composition.

S.No	Peak Value absorption (cm^{-1})	Reference ranges	Bonds	Functional group
1.	3816.44	-	-	Unknown
2.	3733.51	-	-	
3.	3471.24	3500-3200	O-H Stretching, H-bonded (Strong, Sharp)	Alcohols, phenols
4.	3388.32	3400-3300	N-H stretching (narrow, strong)	Aliphatic primary amine
5.	3302.5	3330-3270	-C≡C-H: C-H stretching (medium)	Alkynes (terminal)
6.	3156.9	3200-2700	O-H stretching (weak, broad)	Alcohol
7.	3087.48	3100-3000	C-H stretching (medium)	Alkenes
8.	3036.37			Alkenes
9.	2953.45	3000-2840	C-H stretching(medium)	Alkane
10.	2925.48			
11.	2884.02			
12.	2853.17			
13.	2807.85	3000-2800	N-H stretching (strong, broad)	Amine salt
14.	2770.24	2830–2695	H-C=O: C-H stretching (medium)	Aldehydes
15.	2731.67			
16.	2676.71	3300-2500	O-H stretching (strong, broad)	Carboxylic acid
17.	2697.93	2830-2695	C-H stretching (medium)	Aldehyde
18.	2621.75	3300-2500	O-H stretching (strong, broad)	Carboxylic acid
19.	2369.12	-	-	Unknown
20.	2026.82	2140-1990	N=C=S stretching (strong)	Isothiocyanate
21.	1895.68	2000-1650	C-H bending (weak)	Aromatic compound
22.	1833.97			
23.	1744.3	1750-1735	C=O stretching (strong)	δ-lactone
24.	1674.87	1675-1665	C=C stretching (weak)	Alkene
25.	1622.8	1650-1600	C=C stretching (medium)	Conjugated alkene
26.	1588.09	1650–1580	N-H bend (medium)	1°amines

27.	1557.24	1560-1500	N-H (2ȷ-amide) II band (medium)	Carboxylic Acids & Derivatives
28.	1526.38	1550-1500	N-O stretching (strong)	Nitro compound
29.	1463.71	1470–1450	C-H bend (medium)	Alkanes
30.	1422.24	1440-1395	O-H bending (medium)	Carboxylic acid
31.	1402	1410-1380	S=O stretching(strong)	Sulfonyl chloride
32.	1377.89	1390-1310	O-H bending (medium)	Phenol
33.	1374.03			
34.	1329.68	1350-1300	S=O stretching (strong)	Sulfone
35.	1297.86	1310-1250	C-O stretching (strong)	Aromatic ester
36.	1225.54	1225-1200	C-O stretching (strong)	Vinyl ether
37.	1208.18			
38.	1157.08	1205-1124	C-O stretching (strong)	Tertiary alcohol
39.	1129.12			
40.	1111.76	1124-1087	C-O stretching (strong)	Secondary alcohol
41.	1077.05	1085-1050	C-O stretching (strong)	primary alcohol
42.	1039.44	1070-1030	S=O stretching (strong)	Sulfoxide
43.	970.019	980-960	C=C bending (strong)	Alkene
44.	917.95	950–910	O-H bend(medium)	Carboxylic acids
45.	869.739	900–675	C-H ‘ oop’’ (strong)	Aromatic
46.	804.171	840-790	C=C bending (medium)	Alkene
47.	755.96	850-550	C-Cl stretching (strong)	Halo compound
48.	721.247	730-665	C=C bending (strong)	Alkene
49.	679.785	700–610	-C≡C-H: C-H bend (broad, strong)	Alkynes

The FTIR analysis of top layer of the mixture has totally 42 peaks, among these, the peaks appeared at 2569.68, 2414.44 and 2358.52, 1910.15, 1747.19, 1616.06, 1405.85, 1132.01 and 1042.34 indicate the presence of some new functional groups such as thiol, allene, Ester and saturated aliphatic, α, β-unsaturated ketone, sulphate and aliphatic ether and anhydride.Among these, the phytocompounds sulfoneandthiol are having potential antibiotic and free radical scavenging activities. sulfoneantibiotics are used for infectious diseases like leprosy and are effective in inflammatory diseases. The mechanism of action may involve inhibiting free radical formation by neutrophils. In most case reports, these medications are effective only in purely cutaneous forms of urticarial vasculitis [67]. Sulfones are one of the members of organosulfur compounds have various biological activities primarily as antimicrobial [68, 69, 70, 71, 72, 73, 74, 75]. The thiol (-SH) functional group is found in a number of drug compounds and serving as radical scavengers [76]. Hence, in the present investigation, the phytocompounds present in CUSOCU medicinal preparation act as a strong antibacterial and antifungal activities which may eradicate the microbes responsible for halitosis present on biofilms of oral cavity and tooth and also prevent their growth at significant levels.

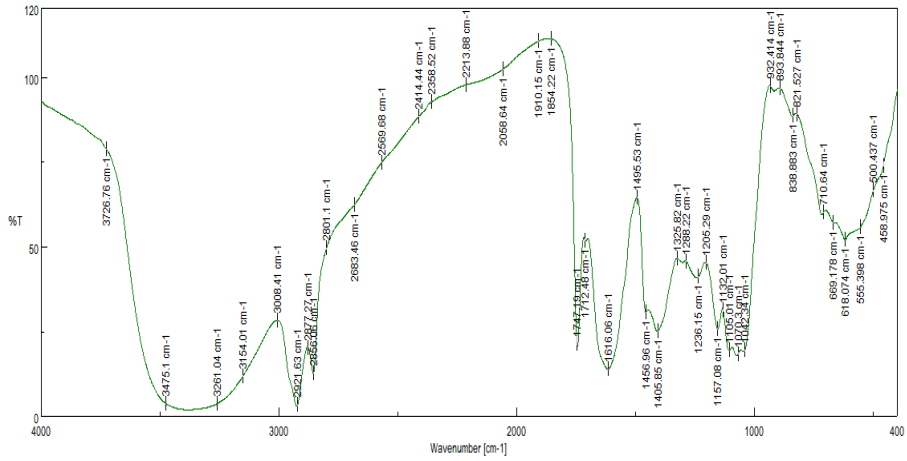

Figure 5: FTIR spectrum of top layer of *Cumin cymium* seed powder, *Solanum lycoperisicum* juice and *Cocus nusifera* oil composition

Table 6: IR spectral frequencies related functional groups for top layer of *Cumin cymium* seed powder*, Solanum lycoperisicum* juice and *Cocus nusifera* oil composition.

S.No	Peak Value absorption (cm^{-1})	Reference ranges	Bonds	Functional group
1.	3726.76	-	-	Unknown
2.	3475.1	3500–3200	O-H Stretching, H-bonded (Strong, Sharp)	Alcohols, phenols
3.	3261.04	3400–3250	N-H stretching (medium)	1°2°amines, amides
4.	3154.01	3300–2500	O-H stretching (medium)	Carboxylic acid
5.	3008.41	3100-3000	C-H stretching (medium)	Alkene
6.	2921.63	3000-2840	C-H stretching (medium)	alkane
7.	2877.27			
8.	2856.06			
9.	2801.1	2830-2695	C-H stretching (medium)	Aldehyde
10.	2683.46	3300-2500	O-H stretching (strong)	carboxylic acid
11.	2569.68	2600-2550	S-H stretching (weak)	Thiol
12.	2414.44	-	-	Unknown
13.	2358.52	-	-	
14.	2213.88	2260-2190	C≡C stretching (weak)	Alkyne
15.	2058.64	2140-1990	N=C=S stretching (strong)	Isothiocyanate
16.	1910.15	2000-1900	C=C=C stretching (medium)	Allene
17.	1854.22	2000-1650	C-H bending (weak)	aromatic compound
18.	1747.19	1750–1735	C=O stretching (strong)	Ester, saturated aliphatic
19.	1712.48	1720-1706	C=O stretching (strong)	carboxylic acid
20.	1616.06	1620-1610	C=C stretching (strong)	α, β-unsaturated ketone
21.	1495.53	1500–1400	C-C stretching (in-ring) medium	Aromatics
22.	1456.96	1470–1450	C-H bend (medium)	Alkanes
23.	1405.85	1415-1380	S=O stretching (strong)	Sulfate
24.	1325.82	1390-1310	O-H bending(medium)	Phenol
25.	1288.22	1310-1250	C-O stretching (strong)	Aromatic ester
26.	1236.15	1250–1020	C-N stretching (medium)	Aliphatic amine
27.	1205.29	1225-1200	C-O stretching (strong)	Vinyl ether
28.	1157.08	1205-1124	C-O stretching (strong)	Tertiary alcohol
29.	1132.01	1150-1085	C-O stretching (strong)	Aliphatic ether
30.	1105.01	1124-1087	C-O stretching (strong)	secondary alcohol
31.	1070.3	1085-1050	C-O stretching (strong)	Primary alcohol
32.	1042.34	1050-1040	CO-O-CO stretching (strong, broad)	Anhydride
33.	932.414	950–910	O-H bend (medium)	Carboxylic acids
34.	893.844	1000–650	=C-H bend (strong)	Alkenes
35.	838.883	840-790	C=C bending (medium)	Alkene
36.	821.527	850–550	C–Cl stretch (medium)	alkyl halides
37.	710.64	730-665	C=C bending (strong)	Alkene
38.	669.178			
39.	618.074	690-515	C-Br stretching (strong)	Halo compound
40.	555.398	850–550	C-Cl stretching (medium)	Alkyl halides
41.	500.437	600-500	C-I stretching (strong)	halo compound
42.	458.975	-	-	Unknown

CONCLUSION

The Siddha medicinal system is one of the most important and frequently used healthcare practices in the rural area of Tamilnadu. The people use this method to cure various diseases even without the prescription of Siddha Doctors and proper advice because of zero side effects. Halitosis is a very common problem among majority of the people. The peoples are using variety of paste formulation and mouthwashes to escape from this problem but no any permanent solution. Hence, in the present investigation a simple siddha formulation containing *Cuminum cyminum* seeds*, Solanum lycopersicum* fruits and

Cocos nucifera oil which are frequently used as a food additive in south Indian food tradition were used to cure the Halitosis. The validation study for this mixture was conducted in the random samples selected from Katpadi, Vellore District, Tamilnadu, India. From the observed results, it was clearly indicated that the Halitosis curing potential of this mixture is very high when compared with any other paste and mouthwashes available in the medical shop. These results may be due to the presence of various bioactive phytocompounds present in this mixture.

Conflict of interest

The auther reports no conflicts of interest.

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