In vitro Comparison of the Antimicrobial Activity of Five Herbal Extracts, and Selected Mouthwashes Marketed in Egypt against Cariogenic *Streptococcus Mutans*

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ABSTRACT

Dental caries is a multifactorial human disease that has widely affected many populations all over the world. Streptococcus mutans plays a major role in dental caries development. Few of mouthwashes, however, have undergone rigorous testing and lack the quality of an ideal agent as evidenced by the limited amount of information on their safety and efficacy in the literature. Thus presently the antibacterial activity of herbal agents is being extensively studied. The present study aims to compare the antimicrobial effects of 70% aqueous ethanol extract obtained from traditional Egyptian plants with the most common Egyptian mouthwashes brands against S.mutans. S.mutans isolated on Mitis Salivarius Agar (MSA) and confirmed by ***API 20 Strep C****. 70% aqueous ethanol extracts of Achillea fragrantissima Sch.Bip (No 5), Euphorbia hirta L. (No 2), Nymphaea alba L. (No 1), Thymus vulgaris L.(No 4) and Plectranthus amboinicus L (No 3) were prepared.The susceptibility pattern of 25 S. mutans bacterial isolates obtained from 82 dental plaque samples of patients having dental caries to the selected mouthwashes and plant extracts was determined using the agar well diffusion method. The zones of inhibition produced by the mouthwashes and herbal extracts against the bacterial isolates were measured compared with chlorhexidine positive control. Chlorhexidine formulations listermix and jase were the most effective mouthwash preparations, while chlorhexidine free formulation betadine was the least effective preparation against S.mutans. Extracts No 1 and 2 exhibited the highest antimicrobial effect, while extract No 4 showed the least one. Chlorhexidine formulations Jase and listermix are considered to be the most effective mouthwash anticaris. N. alba, E. hirta plant extracts have the greatest antibacterial activity against S.mutans. If similar results are confirmed in clinical trials, these plant extracts can be used alone or in combination to produce new, useful and economic antimicrobial mouthwashes alternative to commonly known mouthwashes with less side effects.*

Key words: *Streptococcus mutans, mouthwashes, plant extracts, antimicrobial activity*

INTRODUCTION

Dental caries is a microbial disease that continues to pose a significant public health problem in several countries[1]. Caries are a multifactorial infectious disease caused by accumulation of biofilm on tooth surface [2]. Despite the implementation of measures to control and treat dental caries with fluoride, they remain the most prevalent dental disease[3]. Manifestations of the disease occur when there is an imbalance between the biofilm and the host due to changes in biofilm matrix pH caused by diet, microorganisms, or salivary flow and their components[4].Colonization of teeth by cariogenic bacteria is one of the most important risk factor in the development of dental diseases[5]. *Streptococcus mutans (S.mutans)* has been implicated as the principal etiological agent in the development of dental caries in humans, and is frequently isolated from human dental plaque[1]. Insoluble glucans synthesized by *S.mutans* increase the pathogenicity of oral biofilm by promoting the adherence and accumulation of cariogenic bacteria on tooth surface[6]. This microorganism is also highly acidogenic and aciduric, meaning that they produce acids which can dissolve the tooth substance - calcium phosphate in the form of hydroxyapatite crystals - and that they can survive and produce acids in a low pH environment [7].

Although classical antibiotics (amoxicillin, penicillin, ampicillin, erythromycin) can prevent dental caries; however, there is a problem of multidrug resistant strains of bacteria toward the antibiotic and undesirable side effects will happen[8]. In addition there are several products have been used to control dental caries, such as fluoride, chlorhexidine, and their associations[9].

Chlorhexidine, polyvalent cations and non-ionic surfactants were observed to possess the capacity of plaque inhibition, suppression of oral microorganisms, and promising of being potential candidates as preventive for dental caries.[10,11] Supplementation of mechanical brushing with effective antimicrobial mouthwash has proven beneficial in the control of plaque. The greatest success has been found with chlorhexidine, which is incorporated into mouth rinse solutions and now considered the gold standard against which the potential antiplaque agents are measured.[12]

There are many brands of mouthwashes marketed in Egypt each with its own deodorant, antiseptic, disinfectant, analgesic and/or astringent property. The roles of mouthwash in the prevention and treatment of dental caries cannot be overemphasized as a result of the pains and/or mouth-odour that have been linked to bacterial activity in the pulp of a carious tooth and the antibacterial activity of some mouthwashes. Despite good plaque control and an antimicrobial effect, mouthwashes have several adverse effects such as burning sensation, vomiting, diarrhea, tooth and oral tissue staining.[13, 14] Natural substances obtained from medicinal plants and used in the alternative medicine were reported to possess antibacterial action. This action is mainly due to the flavonoids contents, which act on bacterial cells disrupting the cytoplasmic membrane and inhibiting the enzymatic activity.[15] Researchers are trying to pay more attention to these natural products aiming to find an effective antimicrobial mouthwash having the advantage of decreasing the side effects of synthetic one.

The antiseptic properties of aromatic and medicinal plants as well as their extracts have been recognized since antiquity while attempts to characterize these properties in the laboratory date back to the early 1900s.[16]

Thymus vulgaris L (F. Lamiaceae), is a widely distributed perennial plant for its aromatic use, it was reported for its wide antiseptic and antimicrobial effects.[17, 18] The evaluation of its constituents revealed the presence of phenolic monoterpenes, thymol and carvacrol monoterpene glucoside as (R)-p-cymen-9-yl beta-D-glucopyranoside 2- and 5-beta-D- glucopyranosyl thymoquinols, (-)-angelicoidenol-beta-D-glucopyranoside.[19,20] *Plectranthus amboinicus* L (F. Lamiaceae) has been used traditionally for treatments of burns, insect bites and malaria fever.[21,22] Moreover its antimicrobial activity was evaluated.[23,24] Phenolic compounds represent the main constituents of *P. amboinicus*. 3-methoxygenkwanin, (crisimaritin), p- coumaric acid, (taxifolin), rosmarinic acid, apigenin and 5-O-methyl-luteolin are the main identified phenolics.[25] *Achillea Fragrantissima* Sch.Bip (F. Asteraceae) has been widely used in the Egyptian folk medicine in the treatment of gastrointestinal disorders. In addition, it was reported that the plant exhibits antidiabetic, antinflammatory and antimicrobial effects.[26,27,28] Moreover the phytochemical evaluation of *A. Fragrantissima* revealed the presence of sesquiterpene and flavonoid as the main active constituents.[29,30] *Nymphaea alba* Linn (F. Nymphaceae) is commonly named as European white waterlily and white Lotus. It has been used traditionally as aphrodisiac, anodyne, antiscrophulatic, astringent, cardiotonic, demulcent, sedative and anti-inflammatory.[31,32,33] It was reported that *N. alba* showed anxiolytic, antioxidant and antimicrobial activities.[34,35,36,37] Chemical evaluation of *N. alba* revealed the presence of alkaloids and phenolics as the major active constituents.[38, 39]

Euphorbia hirta L. (Euphorbiaceae) was traditionally used in respiratory system disorders, as laryngeal spasms, emphysema, asthma, bronchitis, hay fever, cough, common cold[40], menstrual cycle disorders, kidney stones, sterility and sexually transmitted diseases.[41]. It was reported that *E. hirta* exhibited antibacterial effect[42,43] antioxidant and antiproliferative effect on Hep- 2 cells.[44,45,46] Chemical evaluation of *E. hirta* revealed the presence of triterpene, sterols in addition to phenolic acids and flavonoid as gallic acid, scopoletin, scoparone, isoscopoletin, quercetin, isorhamnetin, pinocembrin, kaempferol, luteolin.[47,48]

This study aims to compare the antimicrobial effects of 70% aqueous ethanol extract obtained from traditional Egyptian plants with the most common Egyptian mouthwashes brands against *S.mutans*.

MATERIALS & METHODS

A total of 82 dental plaque samples were collected using sterile cotton swabs from patients who are admitted to October University for Modern Sciences and Arts (MSA) Dental Clinics, 6th October City, Egypt. All the patients signed a written consent form before participating in the study.

The aerial parts of *P. amboinicus* (No 3), *T. vulgaris* (No 4) and leaves of *N. alba* (No 1) were collected from El-Orman garden, Giza, Egypt. Achillea ((No 5) aerial parts are collected from Saint Catherine, Sinai, Egypt.

Moreover the leaves of *E. hirta* (No 2) were collected from Ismailia road, Egypt. All plant samples were collected in November 2011 and they were identified by Dr. Therse Labib Youssef, Orman Botanic garden, Giza, Egypt. Voucher specimens are kept in the department of pharmacognosy, MSA University. 6 October City, Egypt

Isolation and characterization of bacteria

Dental examinations were performed under natural light, using a plane dental mirror and explorer. One plaque sample was collected from each carious teeth patients (those had received antibiotics within the previous 3 months or with systemic disease were excluded) along the cervical margin of the teeth by excavator and then put on the sterile cotton swabs and immediately swabbed on the surface of prepared sterile Mitis Salivarius Agar (MSA) (Difco Lab., USA) supplemented with 0. 1% potassium tellurite, 0.2 units (2.8 µg/ml) of bacitracin (Sigma Chemical Co., USA). The bacitracin was freshly prepared immediately before use. The Plates were incubated at 37 ^{o}C for 48 h in an anaerobic jar. Bacterial isolates were identified morphologically (small blue colony on MSA & greenish discoloration on blood agar), Gram stain (Gram positive coccci in chain) and biochemical tests (catalase & optochin) [49].

API 20 Strep C (bioMerieux, Inc France) was used as a confirmatory identification method for all isolates. The procedures were done according to the manufacturer's instructions. The reactions are read according to the reading table and the identification is obtained by using the identification software.

Preparation of plant extracts

Air dried powdered (100g) plant samples under investigation were extracted by 70% aqueous ethanol on cold. The residue left after evaporation of solvents under reduced pressure is kept at 20°C till used.

Estimation of the phenolic content

Total phenolic content was estimated by the Folin- Ciocalteu method[50]. Concentration of phenolic content was expressed as gallic acid equivalent (GAE).

Screening for antimicrobial activity

Antimicrobial effectiveness of various mouthwashes and plant extracts was assessed by using agar well diffusion method[51]. Mouthwashes were purchased from a pharmacy outlet, Cairo, Egypt and their compositions are listed in Table (1).The inoculums of the test strains were adjusted to 0.5 McFarland standard by adding sterile saline. A lawn of the test pathogen was prepared by spreading 100 µl inoculums, on the entire surface of sterile brain heart infusion agar plate and allowed to set. Six wells were bored into each agar plate using a sterile Wassermann tube and 100 µl of both mouthwash and the extract (dissolved in DMSO) were dropped into separate wells. Chlorhexidine solution 0.12 % (Sigma-Aldrich, St. Louis.MO. USA) was used as positive control while saline and DMSO were used as negative control respectively. The plates were left for 1 h to allow for diffusion of the samples into the agar medium. All the plates were then incubated at 37°C for 24 h and the zones of inhibition measured using an accurately calibrated transparent ruler. The mean diameter of the zones of inhibition was calculated. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922(obtained from Dar El Fouad hospital) were used as Gram positive and Gram negative control, respectively. All solvents used are of analytical grade.

Table (1): Composition of mouthwashes brands marketed in Egypt

Mouthwashes	Composition
Listermix plus	Chlorhexidine gluconate (0.100 %), thymol (0.064%), eucalyptol (0.092%), menthol (0.042%), clove oil (0.06%)
JASE	Chlorhexidine gluconate (0.3%), thymol (0.05%), menthol (0.2 %), clove oil (1%), glycerine (5%)
Tantum verde	Benzydamine hydrochloride (0.15g)
Betadine	povidone iodine (1% W/V), glycin, saccharine sodium, ethyl alcohol, sodium hydroxide, methyl salicylate, methanol, purified water

Statistical analysis

The inhibition zones produced by both mouthwashes and extracts were statistically analyzed by using one-way ANOVA test followed by Tukey's Kramer Multiple Comparison Test to determine if there was significant difference in their susceptibility patterns at 95 % confidence level. p value <0.05 was considered as significant. Statistical results are represented in (Fig. 1)

RESULTS

Phenolic content of different plant extracts under investigation revealed that *T. vulgaris* has the highest phenolic content (63.87 ± 1.2 mg/g GAE) followed by *E. hirta* (47.4 ± 0.95 mg/g GAE) and *A. fragrantissima* (40.97 ± 0.76 mg/g GAE) while *P. amboinicus* and *N. alba* possess nearly the same amount being (32.4 ± 0.84 mg/g GAE) and (32.8±0.75 mg/g GAE) respectively.

S. mutans were identified in 25 (30.5%) samples out of the total 82 dental swabs used in the study. The results of susceptibility pattern of both mouthwashes and extracts against *S.mutans* and control organisms are presented in Tables (2 and 3).

All mouthwashes showed significant antibacterial activity with variable degrees against the tested isolates compared with positive control. Listermix plus, jase and tantam verde showed the same antibacterial activity as that of chlorhexidine. Moreover betadine exhibited a significantly lower antibacterial activity than that of chlorhexidine. Listermix, jase showed significantly higher antibacterial activity than betadin.

All 70% aqueous ethanol extracts under investigation showed antibacterial activity against all isolates with variable degrees compared with that of chlorhexidine. Extracts No 1, 2, 3, and 5 showed the same antibacterial activity as that of chlorhexidine. On the other hand extract 4 exhibits a significantly lower antibacterial activity than that of chlorhexidine. Comparing antibacterial effect of mouthwashes with that of plant extracts it was noticed that, extracts No 1, 2 and 3 exhibited a significantly greater antibacterial activity than that of betadin, moreover the extracts 1, 2 and 3 showed non-significant antibacterial activity compared to listermix , Jase and tantum verde .On the other hand extract No 4 showed lower significant antibacterial activity than that of listermix and Jase.

Table (2): Mouthwashes sensitivity results (zone of inhibition in mm)

Sample No.	Saline (-ve control)	Chlorohexidine (+ve control)	Betadine	JASE	LISTERMIX	Tantum Verdae
Staph aureus	R	27	21	29	26	12
E.coli	R	27	R	20	25	15
S002	R	40	13	43	44	25
S003	R	26	R	12	25	R
S006	R	32	15	38	37	19
S007	R	32	15	28	27	16
S010	R	22	R	24	24	13
S012	R	20	12	22	22	R
S018	R	10	R	30	23	19
S022	R	20	R	20	22	R
S025	R	25	13	35	32	20
S026	R	27	14	26	30	16
S029	R	32	10	35	33	20
S031	R	24	R	23	25	18
S034	R	25	13	25	29	23
S038	R	10	R	28	24	15
S039	R	8	R	26	25	17
S041	R	40	11	42	43	24
S049	R	27	R	30	31	16
S052	R	8	R	40	40	30
S058	R	16	13	25	27	18
S066	R	26	13	12	25	13
S069	R	33	18	35	37	22
S079	R	23	13	25	25	15
S100	R	35	R	37	39	24
S103	R	36	R	30	26	27
S105	R	18	R	28	25	26

Saline: 100% resistant (negative control). Chlorohexidine: 100% sensitive (positive control). Betadine: 52% sensitive. Jase: & Listermix: 100 % sensitive. Tantum verdae: 88 % sensitive.

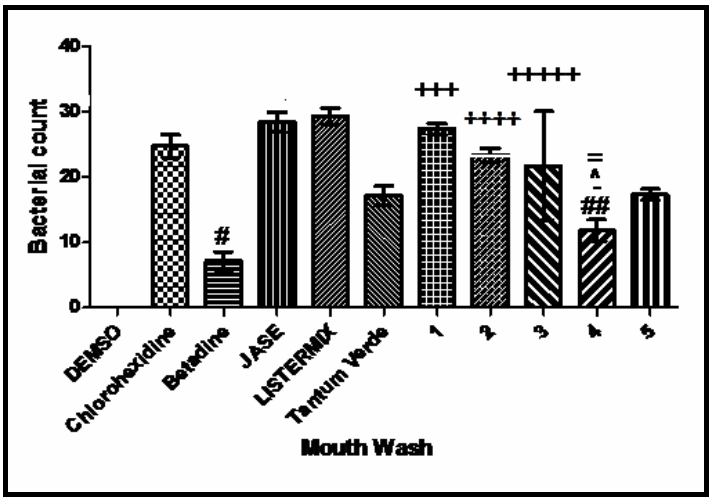

Fig (1): statistical results

Significant difference between chlorohexidine and betadine at P<0.05
Significant difference between chlorohexidine and extract 4 at P<0.05
+++ Significant difference between betadine and extract 1 at P<0.05
+ Significant difference between betadine and JASE at P<0.05
++Significant difference between betadine and LISTERMIX at P<0.05
++++ Significant difference between betadine and extract 2 at P<0.05
+++++Significant difference between betadine and extract 3 at P<0.05
_ Significant difference between JASE and extract 4 at P<0.05
^ Significant difference between LISTERMIX and extract 4 at P<0.05

Table (3): Plant extracts sensitivity results (zone of inhibition in mm)

Sample No	**DMSO (-ve control)**	**Chlorohexidine (+ve control)**	**1**	**2**	**3**	**4**	**5**
Staph aureus	R	27	23	19	25	**15**	R
E.coli	R	27	30	25	19	**16**	R
S002	R	40	29	27	R	**18**	١٧
S003	R	26	24	27	14	**21**	١٠
S006	R	32	28	29	11	**21**	١٠
S007	R	32	30	29	R	**20**	٣٠
S010	R	22	37	22	234	**25**	٣٠
S012	R	20	30	20	21	**23**	١٢
S018	R	10	29	26	20	**11**	١٣
S022	R	20	30	27	16	**16**	١٥
S025	R	25	25	23	17	**17**	١٦
S026	R	27	26	24	19	**14**	١٠
S029	R	32	33	21	12	**14**	R
S031	R	24	28	29	19	**20**	١١
S034	R	25	25	27	23	**21**	١٨
S038	R	10	23	29	R	**20**	٢٠
S039	R	8	31	24	16	**25**	٨
S041	R	40	25	23	16	**17**	٨
S049	R	27	29	19	18	**16**	١٤
S052	R	8	30	21	17	**14**	١٠
S058	R	16	29	29	19	**17**	١٩
S066	R	26	24	28	20	**16**	٢٠
S069	R	33	23	25	21	**17**	١٤
S079	R	23	31	20	10	**21**	١٥
S100	R	35	32	21	R	**11**	R
S103	R	36	17	10	R	**8**	R
S105	R	18	18	8	R	**10**	R

1= *N. alb,* 2= *E. hirta*,3= *P. amboinicus*,4= *T.vulgari*s and 5= *A. frangrantissima*

DISCUSSION

Despite great improvements in the global oral health states, dental caries are still remains of the most prevented diseases.[52] Chlorhexidine as a gold standard chemical agent appears to be the most effective antimicrobial agent for reduction of both plaque and gingivitis. The concentration selected (0.12%) is the most commonly indicated because this concentration provides antimicrobial efficacy with less sever adverse effects.[53] Also this concentration corresponds to that used clinically for substitutive plaque control.[54]

Antimicrobial susceptibility profiles reveal that mouthwashes Jase and listermix plus displayed the most effective antibacterial activity against all tested isolates including control organisms (100%). They contain chlorhexidine gluconate as the main active ingredient in their composition. Chlorhexidine has been extensively marketed as an anti-plaque agent and as a component of topical, slow-release vehicles for treatment of periodontal diseases.[55] Chlorhexidine gluconate is a cationic biguandide with broad spectrum antimicrobial action. The mode of action of Chlorhexidine gluconate in dental cares is the inhibition of plaque formation via an immediate bactericidal effect, followed by prolonged bacteriostatic action resulting from its adsorption into the biofilm-coated enemal surface.[56] Chlorhexidine formulations like Jase and listermix are considered to be the gold standard antiplaque mouthwashes due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential.[57] The obtained results have been supported by Satoshi *et al*.[58], who reported that chlorhexidine as an adjunct to mechanical tooth cleaning markedly reduced the number of microorganisms that could be detected in saliva. The number of salivary bacteria may influence the amount of plaque that formed during the early phase of poor oral hygiene. Also agreed with, Shaker[59] who reported that formulations that contain Chlorhexidine gluconate as the main active constituent were the most effective mouth wash preparations. Moreover Aneja *et al.*[52] and Aldhaher[60] reported that chlorhexidine formulations showed excellent antimicrobial activities. In addition Jase and listermix plus contain thymol, eucalyptol and menthol which cause bacterial cell destruction and bacterial enzymes inhibition. Moreover, they have anti-inflammatory action and have been proved efficacious for reduction of dental plaque and gingivitis.[61] In the current study, tantum verde and betadine exhibited 12% and 48 % resistance to isolated strains respectively. This may be due to that they are chlorohexidin free. This is in accordance with Shaker[59]; Kocak *et al*.[62] and Aneja *et al.*[52] who found that chlorhexidine free formulations displayed very little or totally lacked antimicrobial activity. Moreover Da Silva *et al*.[63] found that listerine (mouthrinse similar in its composition to Jase &listermix except chlorohexidin free) did not exert inhibitory effect against any of their tested strains.

In spite of the wide use of mouthwashes they have a number of drawbacks even chlorhexidine which is considered as a gold standard mouth rinse since it is not free of adverse effects, as extrinsic tooth staining, restorations, altered taste sensation, and occasionally associated with supragingival calculus build up, moreover limited information on their safety and efficacy in the literature magnify these drawbacks.[64]

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries for primary health care because of better cultural acceptability, better compatibility with the human body, and fewer side effects. Hence efforts have been made for development of alternate mouthwash from natural products to be safer, easily available and substitute standard pharmaceutical remedies.[65] Antimicrobial activity of plant phenolics has been intensively studied. Their activity against human pathogens has been investigated to characterize and develop new healthy food ingredients, medical compounds, and pharmaceuticals.[66, 67]

The observed significant antimicrobial activity of *N. alba*, *E. hirta* and *P. amboinicus* is strongly correlated with previous reports. Where the strong antimicrobial activity of *N. alba* [37] is correlated to the previously isolated phenolic acid, hydrolysable tannins and the high phenolic content expressed in this study. The strong biological activity of *P. amboinicus* widely used in the Indian system of medicine is due to the previously identified caffeic acid, rosmarinic acid, coumaric acid in addition to the flavonoid contents.[25] On the other hand, *E. hirta* high activity is highly attributed to its phenolic constituents as afzelin, quercitrin, myricitrin, rutin, quercitin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D, 2,4,6-tri-O-galloyl-β-D-glucose, 1,3,4,6-tetra-O-galloyl-β-D-glucose, kaempferol, gallic acid, and protocatechuic acid.[45] Despite the high phenolic content of *T. vulgaris*, it showed lower activity compared with the previous medicinal plants. Sengul *et al.*[68] revealed that total phenolic

content determined according to the Folin-Ciocalteu method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic compounds have different antioxidant activities, depending on their structure and consequently different biological activity. Previous studies revealed that *T. vulgaris* extract had a significant antimicrobial activity against *Streptococci mutans* with significant reduction in the counts of salivary *Streptococci mutans* after one hour.[69] This activity is mainly contributed to its high phenolic content.

CONCLUSION

This study demonstrates that Chlorhexidine formulations Jase and listermix are considered to be the most effective anticaris mouthwashes. *N. alba*, *E. hirta* plant extracts have the greatest antibacterial activity against *S.mutans*. If similar results are confirmed in clinical trials, these plant extracts can be used alone or in combination to produce new, useful and economic antimicrobial mouthwashes alternative to commonly known mouthwashes with less side effects.

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مقارنة التأثير المضاد للجراثيم لخمس مستخلصات عشبية مع بعض المستحضرات التجارية المستخدمة كغسول للفم فى مصر على الجرثومة المكورة السبحية الطفرية المسببة لتسوس الاسنان

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يهدف هذا البحث الى مقارنة التأثير المضاد للجراثيم لخمس مستخلصات عشبية باستخدام الايثانول المائى ٧٠%مع بعض المستحضرات التجارية المستخدمة كغسول للفم فى مصر على الجرثومة المكورة السبحية الطفرية المسببة لتسويس الاسنان وذلك على سلالات مرجعية استافيلوكوكاس اوريوسATCC25923 وايشريشا كولاى ATCC25922 باستخدام طريقة الانتشار من الحفرة الى الاجار.

تم تحديد نمط ٢٥ عترة من المكورة السبحية الطفرية من ٨٢ مريض باعراض تسوس الاسنان بعد زراعة اللطخات البكنيرية على وسط غذائى خاص لنمو المكورة السبحية الطفرية وهو الميتس ساليفريس اجار(Mitis Salivarius Agar (MSA فى ظروف لاهوائيه لمدة ٤٨ ساعة عند ٣٧°م والتاكد باستخدام API 20 Strep C. تم تحضير مستخلصات ٧٠% مائى كحولى لكل من زنبق الماء (*Nymphaea alba* L)، اللبينه(*Euphorbia hirta* L) ، الزعتر الجبلى (*Plectranthus amboinicus* L)، القيصوم (*Achillea fragrantissima* Sch.Bip L) و الزعتر البلدى (*Thymus vulgaris* L).

وقد اظهرت النتائج الاوليه ان المستحضرات المحتوية على الكلورهكسيدين(Listermix و Jase) هى الاكثر فاعيلية على المكورة السبحية الطفرية عن بقية المستحضرات التى لا تحتوى على الكلورهكسيدين مثل(بيتادين).ووجد ان المستخلصات المستخرجة من زنبق الماء(*Nymphaea alba* L) ، اللبينه(*Euphorbia hirta* L) ، الزعتر الجبلى (*Plectranthus amboinicus* L) من اكثر المستخلصات النباتية الاكثر فاعلية مضادة للبكتيريا وهى مماثلة للمستحضرات المحتوية على الكلورهكسيدين ، يليها الزعتر البلدى (*Thymus vulgaris* L). فى حين ان مستخلص القيصوم (*Achillea fragrantissima* Sch.Bip L) كانت الاقل فاعليه مضادة للبكتيريا وهى مماثلة للمستحضرات التى لا تحتوى على الكلورهكسيدين.

طبقا لهذه النتائج ، المستحضرات التى تحتوى على الكلورهكسيدين هى الاكثر فاعلية للتحكم فى تسوس الاسنان وايضا يمكن استخدام زنبق الماء و اللبينه،بعد التاكد من نتائجهما فى التجارب السريرية كبدائل لغسولات الفم المعروفه بآثار جانبية أقل.