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In Vitro Antimicrobial Activity of *Thymus Vulgaris,Origanum Vulgare* and *Rosmarinus Officinalis* Against Dental Caries Pathogens

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Abstract

The *in vitro* antimicrobial activity of *Thymus vulgaris,Origanum vulgare* and *Rosmarinus officinalis* water extracts were studied against *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans* isolates two of each isolate. All cold water extracts exhibited antimicrobial activity against all tested microorganisms. The largest zone of inhibition was obtained against *Candida albicans* isolates. *Streptococcus mutans* isolates and one isolate of *Lactobacillus acidophilus* showed no effect in 300mg\ml concentration of *Thymus vulgaris* extract,while the same concentration of *Rosmarinus officinalis* extract had no effect on all *Streptococcus mutans* isolates and *Lactobacillus acidophilus* isolates .*Origanum vulgare* extract had inhibitory effect in 200mg\ml concentration with (10mm) diameter against all isolates .This study depicts that the extract of *Thymus vulgaris,Origanum vulgare* and *Rosmarinus officinalis* extracts possess very good antifungal and antibacterial activities respectively and can be used as a potential source of novel antimicrobial agents used to cure dental caries.

Key words: *In-vitro*, antimicrobial activity, herbal extracts, dental pathogens.

Introduction

Dental caries are one of the public health concerns for several reasons. Teeth affected with dental caries are sources of infection, which can cause an inflammation of dental pulp, periodonteum and gums. If left untreated, this disease gradually leads to teeth loss, which causes chewing difficulties and aesthetic problems [1]. It remains one of the most widespread diseases of the mankind. In developing countries, dental caries is often at epidemic proportions, especially among the poor. Since the 19th century, when sucrose became a daily used sweetener by many people worldwide, the increasing prevalence of dental caries had also been noticed[2],therefore clear that dental diseases have detrimental effect on quality of life both in childhood and older age [3].Medicinal plants since ancient times have been employed for prophylactic and curative purposes[4].

Many studies have investigated the antimicrobial activity of different plant species in various geographical regions in search for new antibiotics. The use of plant derivatives as antimicrobials has not been extensively addressed until recently since most antibiotics were derived from bacterial or fungal origin. With the increase in resistance and the realization that the effective life span of any antibiotic is limited, new sources especially plant sources are currently being heavily investigated. Thousands of phytochemicals with antimicrobial activity have already been identified but they should be subjected to animal and human studies to study their toxicity and their effectiveness in whole organism systems. Several phytochemicals are already being studied in humans [5].

Thymus vulgaris (garden thyme) is a small shrubby plant with a strong, spicy taste, and odor. This plant is indigenous to the Mediterranean region of Europe and is extensively cultured in

the United States. Known primary constituents of the thyme include essential oil (borneol, carvacrol, cymol, linalool, and thymol), tannin, flavonoids (apigenin and luteolin), saponins, and triterpenic acids. Essential oil and extracts from fresh leaves and flowers can be used as aromatic additives in foods, pharmaceuticals, and cosmetics. *Thymus vulgaris* has antispasmodic, carminative, diaphoretic, expectorant, and sedative properties. Thyme is frequently used in throat and bronchial problems, including acute bronchitis, laryngitis, and whooping cough, and also for diarrhea, chronic gastritis, and loss of appetite [6].An aqueous extract of 1:1 thyme dose-dependently inhibited *Helicobacter pylori* in a growth inhibition test and two urease activity assays *in vitro* [7]. The thyme extracts have direct antimicrobial actions and also potentiate the effectiveness of tetracycline against drug-resistant *Staphylococcus aureus* [8].*Rosmarinus officinalis (*Rosemary) have been known to have antimicrobial, antioxidant, anti-diabetic, and anti-tumorigenic activities [9-12].In addition, it is known to affect the cell's adhesive properties, self-aggregation, and protein secretion; and this might help in the treatment of cardiovascular diseases and thrombosis [9] .*Rosmarinus officinalis* have anticancer properties [13]. *Rosmarinus officinalis (*Rosemary) was traditionally used for the treatment of several illnesses such as urinary tract infections, rheumatoid cholecystitis, diarrhea, and hypertension.*Origanum vulgare*(oregano) has antioxidant properties and exhibits antimicrobial activity against bacteria and fungi[14-15].The objective of this study was to see *in vitro* antibacterial and antifungal properties of *Thymus vulgaris* , *Rosmarinus officinalis* and *Origanum majorana* water extract against common dental caries causing microorganisms that can be assigned as a novel remedy for dental caries.

Materials and Methods

Test microorganisms

Six isolates of *Streptococcus mutans*,*Lactobacillus acidophilus* and *Candida albicans* two of each were collected from students in Biology department of College of Science, the organisms were identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics[16].

Collection of the plant samples

The herbal sample consisted of three different plants: leaves of *Rosmarinus officinalis* (Rosemary), leaves of *Origanum vulgare* (oregano) and *Thymus vulgaris* (thymus) .They were collected from local market, identified, and characterized by a taxonomist. Collected plants were washed thoroughly, dried and grinded into powdered form for preparation of cold water extract and stored in air-tight bottles at 4 C°.

Preparation of the cold water extract

50 grams of plant powder was dissolved in 500 ml of distilled water, put in shaking incubator for 24hrs at 28 C° then filtered by filter paper. The mixture were then centrifuged at 3000 rpm for 10 minute, the supernatant filtered by Whattman No.1 filter paper, then evaporated in the incubator at 37C °for 48hrs to obtain the crude extract, kept in clean vial at 4C°. The procedure was applied for all three plants [17].

The antimicrobial activity

The antimicrobial activity of *Rosmarinus officinalis* (Rosemary),*Origanum vulgare*(oregano) and *Thymus vulgaris* extract was determined by agar well diffusion method against two isolates of each *Streptococcus mutans*,*Lactobacillus acidophilus* and *Candida albicans* isolate as described by[18]. In this method, pure isolate of 24hrs growth was cultured

in Muller-Hinton Agar plate (Hi Media, Mumbai, India) by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 8.0mm was used to bore five wells in each agar plates. Five concentration of the crude extract were made by dissolving 1 gram of the crude extract in 2ml distilled water to obtain 500mg\ml filtered in Millipore filter paper and used as stock to prepare the other concentrations(100,200,300,400)mg\ml .A 10µL volume of each concentration was applied by micropipette in the wells into Muller-Hinton Agar plate. Distilled water served as control [19]. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37ºC for 24hrs. The zone of inhibition was recorded. Each experiment was performed in duplicate.

Result and Discussion

All aqueous extracts exerted antimicrobial activity against all isolates with different diameters of inhibition in different concentrations. The inhibitory activity of water extract of *Thymus vulgaris* lasted up to 300mg\ml concentration in case of one isolate of *Lactobacillus acidophilus* and *Candida albicans* isolates, while *Streptococcus mutans* showed no effect at the same concentration as shown in Table **1**.

In our study *Thymus vulgaris* extract inhibited growth of *Candida albicans* at low concentration and that agree with [20], another study revealed that exposure of *S. mutans* to thyme extract showed a time and concentration-dependent decrease in bacterial viability. The greatest effect was observed when *S. mutans* had been exposed to 20% thyme extract for a period of 48 h which resulted in 96% inhibition of bacterial growth. Furthermore, the adhesion of *S. mutans* to buccal epithelial cells was also reduced when either buccal epithelial cells or *S. mutans* had been pre-incubated with different concentrations of aqueous thyme extracts (83-98% and 75-89% inhibition respectively). There was also greater reduction in the adherence of bacterial cells to buccal epithelial cells after mouth rinsing with 20% aqueous thyme extract compared to rinsing with chlorhexidine digluconate (45% and 89% inhibition of bacterial adhesion respectively). [21].In general, the mechanisms by which micro-organisms survive the action of anti-microbial agents are poorly understood and remain de-batable [22].The reason why yeast is more susceptible to the extracts than bacteria is unclear .In this study, the incubation time and temperature were the same for bacteria and fungi.

In Table **2** the results indicate inhibitory activity against all isolates used in this study up to 200mg\ml concentration of *Origanum vulgare* water extract. The inhibition zones obtained from aqueous *Origanum vulgare* extract varied from 9-16 mm in diameter. scientists studied the antifungal activity of thymol, a component in oregano oil against fluconazole-sensitive and –resistant strains of *Candida albicans* and found it effective against 24 fluconazole-resistant and 12 fluconazole-susceptible clinical isolates of *Candida albicans*[23]. But water extract studies are very little. That may due to the extraction of secondary metabolites which highly depends on using extractor techniques that depend on the chemical properties of these compounds. Water-soluble compounds and proteins can be extracted in water or polar solvents whereas water insoluble compounds can be extracted with organic solvents [24].Another study [25]studied 82 Indian medicinal plants traditionally used in medicines, by using well diffusion method and determined that alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts[26].While *Candida albicans* isolates were inhibited by *Rosmarinus officinalis* extract and the diameter of inhibition ranged (10-20) mm,the inhibitory effect stopped at 300mg/ml concentration against *Lactobacillus acidophilus* isolates as shown in Table **3**.On the other hand *Rosmarinus officinalis* water extract showed inhibitory activity against *Streptococcus mutans* this come along with [27] which demonstrated that rosemary has antibacterial activity against *Streptococcus mutans*.. The antimicrobial activity of plant extracts is due to the presence of secondary metabolites,

such as alkaloids, flavonoids, phenolics, polyphenols, quinines, flavones, flavonols, tannins, terpenoids, lectins, polypeptides, proanthocynidins, tannins, and quercetin[28].Plant extracts provide protection by immune stimulation and do not have any known side effects[29].To the best of our knowledge, as few studies have been done on antimicrobial effects of medical plants against oral pathogens, it is better that the effect of herbal extracts on other oral bacteria that have cariogenic activity be studied because of the antimicrobial effects of some medical plants, which have minimal side effects in comparison with chemical drugs, more in vivo and in vitro investigations about oral cavity flora should be recommended and it is suggested that more research should be carried out to find plants with antimicrobial activity for producing herbal mouthwashes,if a similar results are confirmed in clinical trial, this plant extracts can be used to produce new, useful and economic antimicrobial agent to cure from dental caries caused by different dental pathogens.

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Table 1: Antimicrobial activity of *Thymus vulgaris* extract against test organisms.

Test organism	***Thymus vulgaris* extract concetratiom mg\ml**				
	500mg\ml	**400mg\ml**	**300mg\ml**	**200mg\ml**	**100mg\ml**
	Inhibition zone (mm)				
Streptococcus mutans 1	15	10	-	-	-
Streptococcus mutans 2	15	11	-	-	-
Lactobacillus acidophilus 1	12	10	-	-	-
Lactobacillus acidophilus 2	15	13	9	-	-
Candida albicans 1	20	18	14	-	-
Candida albicans 2	20	17	13	-	-

*Inhibition zone (mm)

Table 2: Antimicrobial activity of *Origanum vulgare* extract against test organisms.

Test organism	***Origanum vulgare* extract concetratiom mg\ml**				
	500mg\ml	**400mg\ml**	**300mg\ml**	**200mg\ml**	**100mg\ml**
	Inhibition zone (mm)				
Streptococcus mutans 1	14	12	10	9	-
Streptococcus mutans 2	13	12	11	9	-
Lactobacillus acidophilus 1	15	14	13	10	-
Lactobacillus acidophilus 2	15	14	12	10	-
Candida albicans 1	16	13	12	10	-
Candida albicans 2	16	14	14	10	-

*Inhibition zone (mm)

Table 3: Antimicrobial activity of ***Rosmarinus officinalis*** extract against test organisms.

Test organism	***Rosmarinus officinalis* extract concetratiom mg\ml**				
	500mg\ml	**400mg\ml**	**300mg\ml**	**200mg\ml**	**100mg\ml**
	Inhibition zone (mm)				
Streptococcus mutans 1	13	10	-	-	-
Streptococcus mutans 2	13	10	-	-	-
Lactobacillus acidophilus 1	12	10	-	-	-
Lactobacillus acidophilus 2	13	11	-	-	-
Candida albicans 1	15	13	10	-	-
Candida albicans 2	20	14	10	-	-

*****Inhibition zone (mm)

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دراسة الفعالية ضد ميكروبية للمستخلص المائي لنبات الزعتر،اوريكانو و اكليل الجبل ضد بعض الممرضات المسببة لتسوس الاسنان

بان عدي عبد الستار ، علي مرتضى حسن ، اشرف سامي حسن
قسم علوم الحياة ، كلية العلوم ، الجامعة المستنصرية
استلم البحث في :3 تشرين الاول 2011 قبل البحث في : 11 كانون الثاني 2012

الخلاصة

تم في هذه الدراسة استعمال المستخلص المائي لنبات الزعتر،اوريكانو و اكليل الجبل ضد بعض الممرضات المسببة لتسوس الاسنان وذلك بأستعمال طريقة الانتشار بالحفرضد ست عزلات من *Streptococcus mutans* ، *Lactobacillus acidophilus* و *Candida albicans* عزلتين من كل نوع.اظهرت كل المستخلصات فعالية عالية ضد كل العزلات واكبر منطقة تثبيط ظهرت على عزلات*Candida albicans*. لم تتأثرعزلات *Streptococcus mutans* وعزلة واحدة من *Lactobacillus acidophilus* بمستخلص الزعتر بتركيز 300ملغم\مل.بينما كل عزلات *Streptococcus mutans* و *Lactobacillus acidophilus* قيد الدراسة لم تتاثر بالتركيز نفسه من مستخلص اكليل الجبل في حين مستخلص الاوريكانو اظهر تاثيراً تثبيطياً بتركيز 200 ملغم\مل وبقطر 10 ملم ضد كل العزلات قيد الدراسة.يشير البحث الى ان المستخلص المائي لنبات لنبات الزعتر،اوريكانو و اكليل الجبل يحوي مواد فعالة ضد الفطريات والبكتريا المسببة لتسوس الاسنان وبالامكان استعمال هذه المستخلصات في علاج تسوس الاسنان.
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