

## Antibacterial activity of ethanolic extract of *Myrtus communis*. L leaves against salivary *Mutans streptococci*

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هدفت الدراسة الى تقييم فعالية المستخلص الكحولي لأوراق الياس في تقليل أعداد العقديات الطافرة في اللعاب بالمقارنة مع كلورهكسيدين في تجارب مختبرية وفي الأشخاص الأصحاء. أجريت الدراسة في فرع الأدوية ، كلية الطب -الجامعة المستنصرية بالتعاون مع فرع وقاية الأسنان في كلية طب الأسنان في جامعة بغداد (بغداد- العراق) خلال عام ٢٠٠٦. حضر مستحضر الكحول الأثيلي لأوراق الياس (٢٪) وأخضع لمسح مقياس الطيف الضوئي للأشعة فوق البنفسجية والمرئية سعياً للبحث عن ذروات المركبات الفعالة. تم احتساب تراكيز التثبيط الأدنى لنمو العقديات الطافرة المعزولة من اللعاب والمشخصة بكتريولوجياً لكل من مستخلص الياس الأثيلي وكلورهكسيدين. وتم التقصي عن تأثير جرعة واحدة من شطف فموي ، عند الأشخاص الأصحاء، لمستخلص الياس الأثيلي أو كلورهكسيدين على اعداد وحدة تكوين مستعمرة العقديات الطافرة في اللعاب عقب تناول الشطف الفموي لفترة ساعتين. أظهرت النتائج وجود ذروة كبيرة عند طول موجي ٢٦٦,٥ نانومتر وأخرى صغيرة عند طول موجي ٦٧٢ نانومتر في رسم مقياس الطيف الضوئي للأشعة فوق البنفسجية والمرئية دالة على وجود مركبات فلافونود. بلغ تركيز التثبيط الأدنى لنمو العقديات الطافرة المعزولة من اللعاب لمستخلص الياس الأثيلي ١٠٦,٦ ميكروغرام / ميليلتر مقابل ٣,٣ ميكروغرام / ميليلتر لكلورهكسيدين. تناول جرعة واحدة بهيئة شطف فموي من مستخلص الياس الأثيلي تسبب في تدني اعداد وحدة تكوين مستعمرة العقديات الطافرة في اللعاب بدلالة نوعية متميزة إلا ان تأثيره اقل من كلورهكسيدين بدلالة نوعية متميزة. نستنتج من هذه الدراسة ان تأثير المضاد الميكروبي لأوراق الياس يمتد الى العقديات الطافرة بسبب احتوائه على مركبات فلافونود ، وبهذا التأثير يمكن لمستخلص الياس الأثيلي ان يمنع استعمار العقديات الطافرة وما يترتب عليها من حصول تسوس الأسنان.

**OBJECTIVE:** The aim of this study was to evaluate the efficacy of ethanolic extract of *Myrtus communis* (myrtle) leaves in the selective reduction of *Mutans streptococci* count in saliva compared to chlorhexidine through *in vitro* and *in vivo* experiments. **MATERIALS and METHODS:** Ethanolic extract (2%) was prepared and screened by UV-visible spectrophotometer to detect peaks of active compounds. The minimum inhibitory concentrations (MICs) of myrtle extract as well as chlorhexidine (0.12%) were determined against *Mutans streptococci* isolated from human saliva and identified bacteriologically *in vivo* experiments, the effect of single mouth rinse of myrtle extract or chlorhexidine was investigated against the number of colony forming unit (CFU) of *Mutans streptococci* for two hours after rinse. **RESULTS:** The results showed the presence of one large peak at  $\lambda_{266.5}$  nm and a small one at  $\lambda_{672}$  of the extract in UV-visible spectra suggesting the presence of flavanoid. The MIC of myrtle was 106.6  $\mu\text{g/mL}$  compared with 3.3  $\mu\text{g/mL}$  of chlorhexidine. Single mouth rinse of myrtle extract significantly reduced the number of CFU of salivary *Mutans streptococci* but its effect was significantly less than that of chlorhexidine. **CONCLUSION:** It was concluded that the antibacterial effect of myrtle on *Mutans streptococci* was due to its flavanoid content. Therefore, ethanolic extract of myrtle could be a potential remedy for the prevention of colonization by *Mutans streptococci* thereby preventing or retarding development of dental caries.

## INTRODUCTION

The *Mutans streptococci* comprise a group of seven species isolated from human saliva and dental plaques.<sup>1</sup> *Mutans streptococci* infection has been implicated in dental caries and plaque formation.<sup>2,3</sup> Its growth is inhibited by topical chlorhexidine,<sup>4</sup> a cationic bis-biguanide biocide with low

mammalian toxicity and broad spectrum antibacterial activity.<sup>5</sup> Several reports have demonstrated the efficacy of certain herbs against *Mutans streptococci* in both *in vitro* and *in vivo* studies.<sup>6-10</sup>

Myrtle (*Myrtus communis*, Myrtaceae) was originally from North Africa but it is now widely cultivated around the Mediterranean region. It was used for medicinal purposes by the ancient Greeks and Romans for a variety of ailments and as an aphrodisiac.<sup>11</sup> Myrtle inhibits a number of health benefits which include antibacterial,<sup>12-14</sup> antiprotozoal,<sup>15</sup> anti-inflammatory,<sup>16</sup> antioxidant,<sup>17,18</sup> anti-mutagenic<sup>19</sup> and hypoglycemic activities.<sup>20</sup> Methanolic extract of myrtle seeds inhibited the growth of *Staphylococcus aureus*, *B. cereus* and *B. bronchoseptica*<sup>12</sup>

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and the aqueous extract of myrtle leaves showed an excellent inhibitory effect on *Pseudomonas aeruginosa* growth.<sup>21</sup> In 1974, Rotestein *et al.*<sup>13</sup> isolated acylphloroglucinols from myrtle leaves that inhibited the growth of eight positive microorganisms including *Streptococcus faecalis*.

The aim of this study was to test the hypothesis that the known antibacterial effect of myrtle against gram positive bacteria could apply to cariogenic bacteria, *Mutans streptococci*. Therefore, we studied the effect of ethanolic extract of myrtle leaves in both *in vitro* and *in vivo* experiments, using chlorhexidine as reference medicament.

## MATERIALS AND METHODS

This study was done in the Department of Pharmacology, College of Medicine, Al-Mustansiriya University in cooperation with the Department of Preventive Dentistry and Paedodontics, College of Dentistry, Baghdad University in Baghdad, Iraq during 2006.

### Collection of Plant Materials

Fresh leaves of *Myrtus communis* were collected from the bush in the gardens of Al-Mustansiriya University in Baghdad during the spring of 2006. The plant was identified and authenticated at the Department of Biology, College of Science, Al-Mustansiriya University.

### Preparation of Myrtle Extract

The fresh leaves were dried in a dark place at room temperature for several days and then were reduced to fine powder with an electric grinder. Two grams of fine powder were blended with 100 mL of ethanol (100%) in closed dark container and left for 24 hours at room temperature (25-30°C). The obtained supernatant

was filtered using Whatmann filter paper (0.45 $\mu$ ). The ethanolic extract was diluted 1:32 in distilled water and scanned by UV-visible spectrophotometer (Aquarius, Cecil Series, France). The absorption spectra ranged from 200 nm to 900 nm in 4 mL quartz cuvette.

## *In Vitro* Experiments

### Isolation of Mutans Streptococci

Samples of paraffin-stimulated saliva were obtained from adults with dental caries by collecting saliva into receptacles over 5 minutes. Saliva samples were immediately brought to the laboratory, centrifuged for 30 seconds and diluted 1:100 in physiological normal saline. Fifty microliters aliquots were spread onto mitis-salivarius agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) supplemented with 0.2 unit bacitracin and sucrose (20% final concentration). After 48 hours of anaerobic incubation at 37°C in candle jar, the isolates were identified microbiologically and biochemically (using Epi-20 strep) according to the standards of the National Committee for Clinical Laboratory (NCCLs). The isolates were maintained by culturing on brain heart infusion (BHI) agar with 5% (v/v) defibrinated blood, and incubated in an anaerobic atmosphere at 37°C.

### Determination of Minimum Inhibitory Concentration (MIC)

Suspensions of *Mutans streptococci* were prepared at the time of MIC experiments. The colonies were inoculated in normal saline to give  $\lambda_{620}$  of optic density equal to 0.08 (using visible spectrophotometer, Spectronic 20). This optic density represented 10<sup>8</sup> bacteria/mL on McFarland turbidity standards. The MICs of chlorhexidine gluconate (Perio Rx 0.12%, Discus Dental, Culver City, USA)

as reference drug and myrtle ethanolic extract were determined by broth dilution method with concentration ranging from 0.125 to 1280 µg/mL. The MIC was defined as the lowest concentration at which no visible growth, and no changes in transmission percent (%T) at  $\lambda_{620}$  in comparison with pretreatment data. The mean of triplicate experiments of each treatment was determined.

### **In Vivo Experiments**

#### **Subjects**

The study was double blind clinical trial approved by the Scientific Postgraduate Committee of the College of Dentistry, Baghdad University. All subjects signed written informed consent forms to participate in the study. The criteria of inclusion include healthy young adults of both sexes. The criteria of exclusion included systemic illness (e.g. diabetes mellitus), missing teeth, dental prosthesis, orthodontic appliances, and antimicrobials remedy up to two weeks before experiments. A total number of 18 young adults (9 males and 9 females) with median age of 22 years participated. They were distributed randomly into three groups of six each.

#### **Treatment Groups**

The subjects received 15 milliliters mouth rinse as follows:

Group I: Distilled water (as control)

Group II: Ethanolic extract of myrtle (2%)

Group III: Chlorhexidine (0.12%)

The subjects were asked to report any adverse reaction.

#### **Determination of Colony Forming Unit (CFU)**

Samples of paraffin stimulated saliva were obtained for determination of baseline

*Mutans streptococci* count (CFU/mL) and after treatments, at three intervals of 30, 60 and 120 minutes, respectively. Saliva samples were immediately brought to the laboratory, centrifuged and diluted 1 :10<sup>2</sup> to 1:10<sup>4</sup> in normal saline, and then 100 µL of each dilutes saliva was spread on to selective agar media (mitis-salivarius agar) supplemented with 0.2 unit bacitracin and sucrose (20% final concentration) and incubated anaerobically at 37°C for 48 hours. Colonies were counted and the number of bacteria per milliliter of saliva was calculated based on colony forming units.

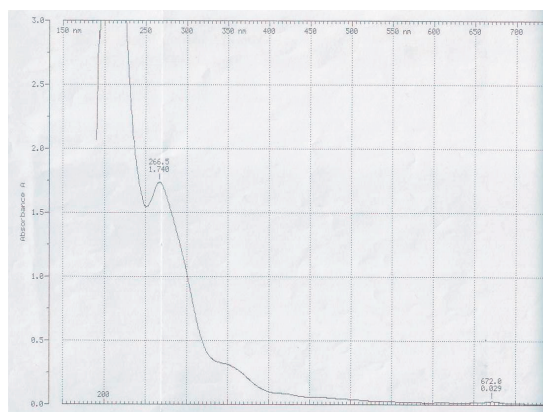
#### **Statistical Analysis**

All values were expressed as median and range. The treatment effect in each group compared with baseline as well as between treatment groups was analyzed with Wilcoxon signed rank test. Differences at probability (*P*) values of ≤ 0.05 were considered statistically significant.

## **RESULTS**

Scanning of UV-visible spectra of myrtle ethanolic extract revealed the presence of large peak at  $\lambda_{266.5}$  and a small peak at  $\lambda_{672}$  (Fig. 1).

The mean MIC of myrtle ethanolic extract was 106.6 µg/mL in comparison with 3.3 µg/mL of chlorhexidine. Table 1 shows that ethanolic extract of myrtle leaves significantly reduced the count of CFUs in comparison with control group over three time intervals. Chlorhexidine significantly reduced the CFUs count of *Mutans streptococci* in comparison with Groups I and II over all time intervals of the study (Tables 1 and 2). Other than bitter taste and slight burning sensation, there were no adverse reactions reported by the volunteer subjects.



**Fig. 1** UV-visible spectra of ethanolic extract of *Myrtus communis* L. leaves.

**Table 1.** Changes in colony counts (CFU) of *Mutans streptococci* before and after treatments using mouth rinse of distilled water (Group I), 2% ethanolic myrtle extract (Group II) and 0.12% chlorhexidine gluconate (Group III)

Treated groups	Baseline	Time (minutes) after mouth rinse treatment		
		30	60	120
Group I	410000 (255000-650000)	380000 (236000-690000)	425000 (266000-630000)	445000 (270000-730000)
Group II	585000 (420000-880000)	100500 (67000-144000)*	148500 (123000-182000)*	256000 (176000-287000)*
Group III	470000 (290000-650000)	63000 (18300-78000) *†	58000 (34000-98000)†	75000 (36000-133000) *†

Values are median (range) of CFU/mL saliva (n=6 for each treatment). CFU were determined by conventional culture method.

\* Significant differences versus baseline ( $P < 0.05$ ).

† Significant differences versus 2% ethanolic myrtle extract ( $P < 0.05$ ).

## DISCUSSION

The data presented in this study support the hypothesis that alcoholic extract of myrtle affect gram positive microorganisms like *Mutans streptococci*. In the study, significant immediate reduction in salivary *Mutans streptococci* count was observed. There were studies on the antimicrobial action of myrtle but so far no data are available on salivary *Mutans streptococci* count reduction. The

**Table 2.** The calculated smaller total ranks by Wilcoxon signed rank test for colony counts (CFU) of *Mutans streptococci* before and after treatments using mouth rinse of distilled water (Group I), 2% ethanolic myrtle extract (Group II) and 0.12% chlorhexidine gluconate (Group III).

Treatment	Time (min)	Group II	Group III
Group I	0 (baseline)	31	38.5
	30	21*	21*
	60	21*	21*
	120	21*	21*
Group II	0 (baseline)		32
	30		22.5*
	60		21*
	120		21*

The smaller total rank is used for determination the level of significance.

\*  $P < 0.05$ .

antimicrobial effect of myrtle mouth rinse may be due to the release of biological active chemicals that was demonstrated by UV-spectra at  $\lambda_{266.5}$ . The presence of such peak indicates the presence of flavanoids,<sup>22,23</sup> but the possibility of antibacterial effects of trepenoides<sup>24</sup> or polyphenols<sup>25</sup> could not be excluded. Extraction of myrtle in absolute alcohol achieves high yield of polyphenols. Eloff<sup>26</sup> found that about fifteen active compounds could be extracted by ethanol solvent. In comparing the degree of reduction of salivary count of *Mutans streptococci* between chlorhexidine and myrtle extract, care should be taken with the interpretation of the data since the experiments (*in vitro* and *in vivo*) were performed on extract and not on isolated pure compounds. This could explain the higher MIC of myrtle extract as well as significantly reduced effect on *Mutans streptococci* count in comparison with chlorhexidine. Also the presence of absolute ethanol in myrtle extract (100% vs 11.6% in chlorhexidine pharmaceutical preparation) enhances the antimicrobial effect of myrtle due to the reduction in hydrophobicity of *Mutans streptococci*.<sup>27</sup> Although the residual effect of myrtle extract was not investigated, it seems

that single mouth rinse resulted in marked reduction of salivary *Mutans streptococci*.<sup>28, 29</sup> It is important to observe that myrtle extract reduced the salivary *Mutans streptococci*  $\leq 10^5$  / mL within one hour after mouth rinse. Such effect may reduce the probability of colonization by *Mutans streptococci* occurring when the microbial count is at least  $10^5$  / mL saliva.<sup>30, 31</sup>

In conclusion, it appears ethanolic myrtle extract could be a potential remedy for preventing the colonization of teeth by *Mutans streptococci* and retarding development of dental caries through its antibacterial effect upon *Mutans streptococci*.

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