



SYNERGISTIC EFFECT OF ALCOHOL EXTRACT OF *MENTHA SPECITA L* AND *CONOCARPUS LANCIFOLIUS* AGAINST BACTERIA CAUSING DENTAL CARIES AND SOME INFECTIONS OF GUMS AND MOUTH

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Abstract

This study included the isolation and diagnosis of bacteria causing tooth decay and infections of the gums and mouth and spread among 40 samples from the students in the Department of biology/ at the University of Samarra and tested the biological effectiveness of alcohol extract of *mint and conocarpus* and their mixture against bacteria, three concentrations were used (30, 40, and 50 mg/ml). the results showed a difference in the ability of each extract, since the highest inhibition diameter was shown when the extracts were used synergistically and significantly different from their use separately or alone. *Streptococcus pyogenes* and *Staphylococcus aureus* were affected with the highest inhibition diameter (2.3 and 1.9), respectively. When the concentration increased to 40 mg/ml, it inhibited growth for all species and the highest inhibition was *K. pneumoniae* which was (2.5), when the mixture was used, while increasing the concentration to 50 mg/ml inhibited the growth of all species and the highest inhibition diameter was for *S. Pyogenes* when the mixture was used.

Introduction

The dental caries is a huge challenge that affects health and a financial burden on both developed and developing countries. It affects public health and body weight (Sheiham, 2006). Dental caries levels have declined in recent years, but their rates now tend to increase due to increased consumption of sugars and insufficient exposure to fluoride. The decline in rates over the past 20 years has been observed in industrialized countries due to the adoption of several health measures to reduce the disease, including the effective use of fluoride as well as improving living conditions and lifestyles (Petersen *et al.*, 2005). Dental caries is the most common disease in the world. It affects 60-80% of schoolchildren and the vast majority of adults. Causes these injuries in all people, the infection is chronic and progress slowly over many years and the oral cavity microflora cause these infections in all people and the body's immune system cannot identify these infections and the formation of immunity against them with time (Cury *et al.*, 2001) and lead to decay resulting in a local crash to the installation of hard teeth caused by the secretions of bacteria in the oral cavity begins to form cavities with small areas free of minerals in the these areas, then widen through the ivory and the pulp causing pain as caries initially arise in the form of a white spot without pain, and then develop cavities appear in brownish tints; when caries reach the ivory the pain appears due to thermal stimulation or eating sweets food and drink the sour (Amoroso *et al.*, 2003).

Natural products have been used to cure form many diseases for centuries. Researchers in the field of chemistry have discovered many biologically active products of metabolism. Many individuals in developed countries prefer medicines that contain compounds derived from medicinal plants (Owolabi *et al.*, 2007) because they are safer, more accessible and cheaper. One of the most commonly used medicinal plants is peppermint. It is one of the most common annual plants in different parts of the world, it belongs to the oral family. *Conocarpus lancifolius* is one of the plants belonging to the *combretaceae* family. Based on the above, this study aimed to test the effectiveness of a mixture of mint

and damas plants against bacteria isolated from people with dental caries.

Materials and Methods

Samples collection

40 samples of different ages for both sexes were collected from the students of the biology department, those suffering from dental caries and prefer to eat sugars. The samples were taken in the form of cotton swap for the period from 1/11/2018 to 1/12/2018 and elected the most common types among the infected .

Preparation of plant extracts

The leaves of the mint and conocarpus plants were obtained from the gardens of the University of Samarra, and the mint was diagnosed according to the phenotypic and anatomical qualities according to (Al-Musawi, 1987). The leaves of the plants were collected separately and washed with water several times to remove impurities, then they were left to dry under laboratory conditions, stirring to prevent rotting, and the dry leaves were ground with an electric grinder for fine powder. The extraction was carried out using ethanol alcohol (99%) and soxholet apparatus. After extraction the plant was dried at 40 °C and then dissolved (1 g) of dry extract in 10 ml of distilled water to have an original stock solution of 10 mg/ml. 30, 40 and 50 mg/ml were then sterilized by filtration and tested for their biological effectiveness (Shareef, 1998).

Preparation of agricultural media

The media was prepared based on the information installed on the containers by the manufacturer and dissolved in distilled water, and then sterilized by an autoclave at a temperature of 121 °C and pressure of 15 lbs for 15 minutes, then the dishes were incubated aerobically at 37 °C for 24 hours to investigate the non-contamination, then kept in the refrigerator, the media used in this study are:

1. **Nutrient broth** : This medium was used to develop and isolate bacteria as well as to preserve samples with glycerol.

2. **Nutrient agar** : This medium was used for the purifying cultures and keeping for short times.
3. **Mannitol Salt agar** : This medium was then used as a selective medium for *Staphylococci spp.* .
4. **MacConkey agar** : This medium was used for the development of Gram negative bacilli and also used to distinguish between fermented and non-fermented intestinal species of lactose
5. **Blood Agar** : This medium was Prepared according to the manufacturer's instructions and after leaving to cool at a temperature of 50-45 °C 5% of human blood was adding, and then pouring in sterile dishes. This medium was used to detect the ability of the isolates to produce the blood enzymes.

Diagnosis of bacterial isolates

The morphological and biochemical characteristics of developing colonies were observed:

1. **Microscopic examination and culture characteristics:** Bacteria were initially diagnosed by observing the culture characteristics of the growing colonies on the media used in terms of size, height and color of the colony.
2. **Bacteriological tests:** IMVIC tests (including Indol test, methyl red, Vogt's proskauer, Cimmom Citrite), as well as Catalase and Coagulase tests were carried out to confirm the isolated bacterial species (Collee *et al.*, 1996).

Biochemical Tests

1. **Catalase test** : This test was conducted according to (Baron *et al.*, 1994).
2. **Coagulase test** : This test was performed according to (Collee *et al.*, 1996).
3. **Mannitol fermentation test** : This was done according to (Alfred, 2005).
4. **Hemolysin production test.** : The test was conducted according to (Baron *et al.*, 1994).
5. **Bacitracin Sensitivity test** : This test was performed according to (Koneman *et al.*, 1997).
6. **Optochin Sensitivity test** : This test was conducted according to (Cappuccino & Sherman, 2011).

Effectiveness Test of plant extracts

The agar well diffusion method was used to measure the biological efficacy of plant extracts, inoculating the nourishment of the bacterial feed containing 3×10^9 colony forming units. A sterile cork drill was applied and placed 200 microliters of plant extract in each hole with 3 replicates, and incubated for 24 hours at 37 ° C. Results were measured by

measuring the diameter of the inhibition zone in mm around each hole.

Results and Discussion

The results of the present study showed the different effects of alcoholic extracts of mint and conocarpus plants and their mixture. This study showed that the two types of extracts did not inhibit *Klebsiella* growth, while *S. Pyogenes* and *S. aureus* were affected. The highest inhibition diameter was observed when using the mixture of 2.3 and 1.9 respectively, significantly different from the two extracts used separately.

The results also showed that increasing the concentration of extracts to 40 mg / ml (Table 2) inhibited all studied species with the highest inhibition diameter of (2.5 and 2.4) for the using of the extract mixture against *Klebsiella* and *S. Pyogenes* respectively, and a significant difference from the inhibition diameter when used separately, the inhibition diameters when tested for the effect of the extracts on *S. aureus* species were convergent and showed no significant differences.

The results showed that the highest inhibition diameter was found on *S. Pyogenes* when the mixture was used, it was 2.7, significantly different from the use of extracts separately, then *Klebsiella*, the inhibition was 2.5, and there was a significant difference when used was Separately, *S. aureus* had a convergent diameter when conocarpus extract and mixture 2.3 and 2.4 were significantly different from the use of mint alone.

The results showed that both peppermint and conocarpus inhibited the growth of dental caries bacteria and that the inhibition diameters increased when used synergistically, and that this study was corresponding with (Shamkhi, 2013 and Basheer *et al.*, 2013) in the effectiveness of peppermint extracts against bacteria as it came similar to (Saddeq *et al.*, 2019 and Hayssam *et al.*, 2013) which they founded antibacterial activity the conocarpus extract.

This variance in the results depends on the plant content of the active substances and the inhibitory ability of the plant extract is affected by various factors such as temperature and pH (Mitschrs *et al.*, 1972) and the extraction method as some volatiles are less effective depending on the type of solvent used as well as different bacterial type. The type of soil planted, the climate, the drying process, storage conditions and the size of the bacterial vaccine have effects on the anti-plant activity (Shamkhi, 2013). The inhibition of bacteria is increasing with the increasing of concentrations, and synergies between mint and conocarps plants have yielded better results than using them separately. This is because mixing the two extracts together contributes to increasing the diversity of the active substances and increasing their concentration, thus increasing their biological activity.

Table 1 : The Effect of mint and conocarp extracts and their mixture at a concentration of 30 mg/ml

The type of bacteria	Control/distilled water	mint extracts	conocarp extracts	mixture
<i>K. pneumoniae</i>	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
<i>S. Pyogenes</i>	0.0 ±0.0	1.8 ±0.5 b	1.5 ±0.5 b	2.3 ±0.5 a
<i>S.aureus</i>	0.0 ±0.0	1.3 ±0.23 b	1.5 ±0.23 b	1.9 ±0.23 a

*Numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%

* Each number represents a rate of 3 replicates.

Table 2 : Effect of Mint and Conocarp extracts and their mixture at a concentration of 40 mg/ml.

The type of bacteria	Control/distilled water	mint extracts	conocarp extracts	mixture
<i>K. pneumoniae</i>	0.0 ±0.0	1.9 ±0.7 b	1.4 ±0.7 b	2.5 ±0.7 a
<i>S. Pyogenes</i>	0.0 ±0.0	1.9 ±0.2 a	1.8 ±0.2 b	2.4 ±0.2 a
<i>S.aureus</i>	0.0 ±0.0	2.1 ±0.2 a	2.2 ±0.2 a	2.1 ±0.2 a

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%

* Each number represents a rate of 3 replicates.

Table 3 : The Effect of mint and conocarp extracts and their mixture at a concentration of 50 mg/ml.

The type of bacteria	Control/distilled water	Mint extracts	Conocarp extracts	Mixture
<i>K. pneumoniae</i>	0.0 ±0.0	1.9 ±0.7 b	1.6 ±0.6 b	2.5± 0.6a
<i>S. Pyogenes</i>	0.0 ±0.0	2.4±0.3b	2.4±0.3b	2.7±0.3a
<i>S.aureus</i>	0.0 ±0.0	2.1±0.2b	2.3±0.2a	2.4±0.2a

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%

* Each number represents a rate of 3 replicates.

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