

Study of in vitro antibacterial activity of the essential oils of Cloves (*Syzygium aromaticum*) and the effect of temperature on antibacterial activity

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Abstract:

Essential oil of Cloves (*Syzygium aromaticum*) was obtained by steam distillation using a Clevenger-type system. The oil extraction was obtained from 250 g of dried plants flowers during 3 h for each run (each run was contained 25 g of plant with 300 ml distilled water). The aqueous phase was separated by separation funnel using diethyl ether, with vigorously shaken. In separation phase each run repeated for 3 times, and in each time the separation funnel containing 50 ml clove extract and 50 ml diethyl ether and shake vigorously for 15 min. Then the oil was obtained by using vacuum rotary evaporator apparatus, until all of the diethyl ether was completely evaporated, leaving the absolute essential oil.

This oil was analyzed for determination of antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*.

The effect of temperature on the antibacterial activity of essential oils of cloves against *Staphylococcus aureus*, *Escherichia coli* was determined. The essential oils (E.O.) of cloves showed antibacterial activity after treatment at 100°C for 30 min suggesting that the high temperature does not affect the activity of E.O.

الخلاصة:

تم استخلاص الزيت من زهور نبات القرنفل الجافة بواسطة عملية التبخير و التقطير باستعمال جهاز كلافنجر. حيث تم اخذ 250 من النبات وتم تقسيم هذه الكمية الى دفعات، حيث كل دفعة تحوي على 25 g من النبات و 300ml من الماء المقطر ووضع في الجهاز لمدة ثلاث ساعات. بعدها تم استخلاص الزيت من الماء بمزجه مع الداى اثير ووضع في قمع الفصل مع الرج القوي. خلال مرحلة الفصل كل دفعة تكرر ثلاث مرات، وفي كل مرة يحوي قمع الفصل فيها على 50 ml من مستخلص القرنفل و 50 ml من الداى اثير ويرج بقوة لمدة 15 min. ثم بعد ذلك تم تبخير الداى اثير باستعمال جهاز التبخير الدوار للحصول على الزيت فقط.

تم فحص فعالية الزيت المضادة للبكتريا، وتم استخدام بكتريا *Staphylococcus aureus*, *Escherichia coli*. تم دراسة تأثير درجة الحرارة على الفعالية المضادة للبكتريا للزيت. وقد أظهر الزيت فعالية مضادة للبكتريا حتى بعد المعاملة الى 100°C و لمدة نصف ساعة، مما أظهر ان ارتفاع درجة الحرارة لا يؤثر على فعالية الزيت المضادة للبكتريا.

Introduction:

Higher and aromatics plants have traditionally been used in folk medicine, showing inhibition against bacteria, fungi and yeasts (9). Most of their properties are due to

essential oils produced by their secondary metabolism (1). Essential oils and extracts from several plant species are able to control microorganisms related to skin (1), dental caries (6), and food spoilage, including Gram-negative and Gram-positive bacteria (7). Many countries have maintained research programs to screen traditional medicines for antimicrobial activity, as is the case of India (2), Palestine (3), Africa (4), Honduras (11), Jordan (12), Cuba (13) and Italy (15). Aromatic plants and spices have great importance for food, cosmetics and pharmaceutical industries. Their use have taken place since ancient times, and despite many of them were substituted by synthetic ones, the demand for natural products is increasing (8).

Clove is an herb. People used its oils, dried flower buds, leaves, and stems to make medicine. Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae. It is used for upset stomach and as an expectorant. Expectorants make it easier to cough up phlegm. Clove oil is used for diarrhea, hernia, and bad breath. Clove and clove oil are used for intestinal gas, nausea, and vomiting. Clove is applied directly to the gums (used topically) for toothache, for pain control during dental work, and for a complication of tooth extraction called “dry socket.” It is also applied to the skin as a counterirritant for pain and for mouth and throat inflammation. In foods and beverages, clove is used as a flavoring. In manufacturing, clove is used in toothpaste, soaps, cosmetics, perfumes, and cigarettes. Clove cigarettes, also called kreteks, generally contain 60% to 80% tobacco and 40% to 20% ground clove. Eugenol, one of the chemicals in clove, acts like menthol to reduce the harshness of tobacco smoke (5) (17).

In the present study, essential oils of Cloves (*Syzygium aromaticum*) was obtained by steam distillation using a Clevenger-type system, and the in vitro antibacterial activity of the clove essential oils and the effect of temperature on antibacterial activity of it was investigated.

Materials and methods:

•Essential oil extraction:

The oil extraction was obtained from 250 g of dried plants flowers by steam distillation using Clevenger system; during 3 h for each run, and each run was contained 25 g of plant with 300 ml distilled water. The aqueous phase was separated by separation funnel using diethyl ether, with vigorously shaken .In separation phase each run repeated for 3 times, and in each time the separation funnel containing 50 ml clove extract and 50 ml diethyl ether and shake vigorously for 15 min . Then the oil was obtained by using vacuum rotary evaporator apparatus, until all of the diethyl ether was completely evaporated, leaving the absolute essential oil.

•Microorganisms:

Antibacterial activity test was carried out against *Staphylococcus aureus*, as gram positive bacteria and *Escherichia coli*, as gram negative bacteria.

Antibacterial activity test:

The antibacterial activity of clove extracts was determined by the methods as described by Lee *et al* (10).

It was using disc which impregnated with clove E.O. before being placed on the agar plates which inoculated with *Staphylococcus aureus*, *Escherichia coli*.

The inoculated plates containing the impregnated discs were incubated in an upright position at 37C° overnight. The results were expressed as the zone of inhibition around the paper disc.

• The effect of temperature on the antibacterial activity:

The effect of temperature on the antibacterial activity of clove extracts was determined by the methods as described by Lee *et al* (10). The extracted oil was incubated at 4, 25, 37, 50, 75, and 100 C°, respectively, in a water bath for 30 min. Then, the extract heated at the different temperatures were cooled down and stored at 4 C° until use. Then, the antibacterial activity test was carried out against the bacteria *Staphylococcus aureus*, *Escherichia coli*, using discs which impregnated with heated clove E.O. before being placed on the inoculated agar plates.

Results and discussion:**Antibacterial activity of clove oil:**

It was found that The E.O. from clove has antibacterial properties and shown to inhibit all tested organisms (Table 1). This extract was more active on *Staphylococcus aureus* than on *Escherichia coli*. This result was agreed with that of Md. *et,al* (2008) (14). The results of the disc diffusion test revealed that the crude extracts of clove showed different degrees of growth inhibition, depending upon the bacterial strains (Table1).

The E.O.of clove showed notable antibacterial activity against Gram positive and Gram negative bacteria. It is well known that most spices are more active against Gram positive bacteria than Gram negative bacteria (16). So this study showed that E.O. of clove was more effective against Gram positive bacteria than Gram negative bacteria in vitro.

Table (1): Antibacterial activity of clove oil (+: one active fraction, ++: two active fractions)

Tested organism	Inhibition of Clove E.O.
<i>Staphylococcus aureus</i>	++
<i>Escherichia coli</i>	+

The effect of different temperature on the antibacterial activity of clove:

The effect of temperature on the antibacterial activity of extracts of cloves against the bacteria of *E. coli* and *S. aureus* were determined. The antibacterial activity of the EO from cloves was found unchanged at all temperatures applied (Table 2), this result was agreed with that of Md. *et.al* (2008) (14), suggesting that the active components of EO were not destroyed at high temperatures even with the 30 min treatment at 100C°.

Table (2): The effect of different temperature on the antibacterial activity of clove

Bacteria	Zones of inhibition\Temperature (C°)					
	4 C°	25 C°	37 C°	50 C°	75 C°	100 C°
<i>S. aureus</i>	11± 0.0	15.4± 0.35	18.5± 2.4	12.4± 8.7	14±0.02	10.22± 0.0
<i>E. coli</i>	9±0.06	11± 2.4	11.3± 2.8	9.2± 0.20	8.8± 0.31	8± 0.44

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