

INVESTIGATION OF ACTIVE COMPOUND IN CLOVE (*Syzygium aromaticum*) EXTRACT AND COMPARED WITH INHIBITORS OF GROWTH OF SOME TYPES OF BACTERIA CAUSING FOOD POISONING

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ABSTRACT

Antimicrobial properties of *Syzygium aromaticum* were investigated and phytochemical active groups were done, clove oil contain a unique active compound known as eugenol was extracted and isolated to be tested. Four different pathogenic bacterial strains (*Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*) were obtained and isolated from contaminated food, three concentrations for the plant (eugenol extract) were prepared to be a treatments, T1, T2 and T3 with percentage of 100%, 50%, 25% respectively. Antimicrobial activity was measured by using disc diffusion method and the best treatment was T1, which tested for eugenol qualitative and quantitative activity that was determined by using (HPLC). The results showed that the inhibition zones were increased with the increasing of concentrations of eugenol extract thus it was very clearly that the results encourage the use of natural Sources like some plants or some parts of plants to solve some problems done by bacteria activities that cause food poisoning.

key words: volatile oil, eugenol, hplc, clevenger.

كرم

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التحري عن المركب الفعال في مستخلص القرنفل ومقارنته مع مثبطات نمو بعض انواع البكتريا المسببة للتسمم الغذائي

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المستخلص

اختبرت الخصائص المضادة للميكروبات في القرنفل *Syzygium aromaticum* و تم عمل الاختبارات الكيميائية للمجاميع الفعالة. زيت القرنفل يحتوي مركب فعال فريد يدعى اليوجينول تم استخلاصه وعزله لاختباره أربع سلالات بكتيريا مرضية مختلفة (*Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*) عزلت من الأغذية الملوثة و حضرت ثلاثة تركيزات لمستخلص اليوجينول لتكون معالجات ، T1 و T2 و T3 و بنسب تراكيز 100 % و 50 % و 25 % على التوالي. تم قياس الفعالية المضادة للميكروبات باستخدام طريقة الانشار بالقرص وافضل معالجة كانت T1 والتي اختبرت فعاليتها الكمية والنوعية لليوجينول والتي حددت باستعمال تقنية كروماتوجرافيا السائل عالي الأداء(HPLC). أظهرت النتائج أن مناطق التثبيط تزداد مع زيادة تركيز مستخلص اليوجينول لذا كان واضحا جدا أن النتائج تشجع استخدام المصادر الطبيعية مثل بعض النباتات أو أجزاءها لحل بعض المشاكل التي تحدثها أنشطة البكتيريا التي تسبب التسمم الغذائي.

الكلمات المفتاحية: زيت طيار, يوجينول, hplc, الكلافنجر.

INTRODUCTION

Food spoilage is a process in which foods to be undesirable, unacceptable and unuseful for human consumption due to changes in almost many characteristics, also it is clear that spoiled foods may be unsafe to eat specially when they contain pathogens or a toxin present (18). Many problems were reported that we cannot use chemical preservatives products safely any more due to carcinogenic effects of these chemicals, thus the requirement has been increased for natural preservatives (27, 28). Bacteria are an expected component of decomposing animals and plants (7). A group of Gram-positive bacteria, including *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus*, under low oxygen, low temperature, and acidic conditions, turn to be spoilage organisms on foods. *Listeria* spp., *Staph* sp. diseases that can be serious and cause damages to human and animals. (3, 4). Many pathogenic microbial organisms such as Enterobacteriaceae they are gram-negative, facultative anaerobic bacteria that include a number of human pathogens (*Salmonella* sp., *E. coli*, *Shigella*, *Yersinia*). *Bacillus* and *Geobacillus* spp. *Clostridium* spp. cause spoilage of canned foods. These bacteria are widespread in nature in soil, on plant surfaces and in digestive tracts of animals and are therefore present in many foods. (10, 26). In many parts of the world clove used for malaria, cholera, tuberculosis and parasitic that cause illnesses for human. Cloves also may use to alleviate muscle spasms, skin ulcers and sties in the eyes. Clove oil is also a potent insecticide, repelling disease-causing mosquitoes and other insects. Clove oil is an essential oil extracted from clove plants especially from its flowers, stems and leaves. The quality of clove oil is normally indicated by its eugenol and caryophyllene contents (25, 31). Methyl eugenol (ME), as a constituent in leaves, fruits, stems, and/or roots, may be released when that part of a plant is damaged. Methyl eugenol is a component of several essential oils that are sold for use in aromatherapy, oils that used for massage treatment and alternative medicines (25). Many pathogenic organisms had become resistant nowadays for many manufactured antibiotics (9). So various researches have

been done to improve and focus on the pharmaceutical characteristics of plants and their parts as alternative sources for many synthetic medicinal drugs, for example clove *Syzygium aromaticum*. (29).

MATERIALS AND METHODS

Sample preparation and extraction

Dried clove samples were collected from the local markets of Baghdad\ Iraq. Grinding was done for clove bud with grinder in the laboratory of Market Researches and Consumer Protection Center. The samples were kept in closed containers after being chopped into small pieces (1 mm).

Preparation extracts (oil Extract)

Clevenger is used for oil extraction to extract cloves volatile oil from *S. aromaticum* plant. 10g of ground cloves and 150 mL of distilled water. The cloves were allowed to be wetted in the water for about 15 min, then the mixture was distilled after that transferring the distillate quantitatively to the separator funnel, the distillate was extracted twice with 2.0 mL of CH₂Cl₂ Dichloromethane (DCM), the DCM extracts were combined, adding Na₂SO₄ to be dried, gently evaporated to get eugenol as a pale yellow oil (15, 20). Three different concentrations were prepared to be tested as treatments (T1 = 25% extract +75% DW), (T2 = 50% extract + 50% DW), (T3 = 100% extract) Then keep the oil in the refrigerator until use.

Determination of active compounds

The determination of some active groups, like {Flavonoids, Phenols, Tannins, Terpenes, Alkaloids, Coumarins} (13). HPLC (Shimadzu\Japan) used for purification and quantification of phenolic compounds in volatile oil of clove buds plant, the mobile phase solution consists of (Acetonitrile: Deionized distilled water with the ratio of 40:60) respectively, then the column C18 was used as stationary phase, flow rate 1.25 ml / min and the wavelength 210 nm, and injection was 25 µl of sample (15).

Bacterial isolation

Four types of bacteria were isolated from contaminated food: *Salmonella* sp. bacteria was isolated from several samples of meat and meat products, *Escherichia coli* bacteria was isolated from several vegetables samples, *Lesteria* sp. and *Staph* sp. were the gram

positive bacteria that were isolated from ice cream and vegetable fresh foods, These bacteria were identified according to several tests were depended first the Gram stain, second microscopic examination for morphology exam and some biochemical tests (5, 11), all these tests were done in Center of Market Researches and Consumer Protection.

Antimicrobial activity assay

Antibacterial activity was confirmed by use of disc diffusion method (27). The clove extract and the antibiotic drug Oflaxacin, stocks were also made for it at 30 µg \ml the Oflaxacin served as positive controls. The nutrient agar plates were spread with 100 µL of respective culture of pathogenic bacteria and the loaded

discs were placed onto the surface of agar. The plates were left for 5 min for drying then plates were incubating at 37°C for 24 h. The results shows the inhibition zone which were measured in millimeters was determined (19). All tests in this study were done with 5 replications.

RESULTS AND DISCUSSION

Determination of active compound

All treatments of oil extraction from powder samples of *S. aromaticum* plant were detected for their quality of active groups, so some active substances such as Flavonoids, Phenols, Tannins, Terpenes and Alkaloids were optioned as it found in table (1).

Table 1. Active compounds of studied treatments in oil extraction for *Syzygium aromaticum*

<u>Extractions</u>	<u>Flavonoids</u>	<u>Phenols</u>	<u>Tannins</u>	<u>Terpenes</u>	<u>Alkaloids</u>
T1(25%)	+	+	+	-	-
T2(50%)	++	++	+	-	+
T3(100%)	++	+++	++	+	++

It was clearly that the active compounds were in positive relation due to the rising of extract concentration and that results is compatible with the results of a study by Abo El- Maati, *et al* 2016, that concluded cloves extracts may be an effective antioxidant and antimicrobial and bioactive agents to promote health(1) and the these quality test shows that flavonoid and phenols were the highest results. The most suitable explanation were applied in a results study by Adaramola and Onigbinde (2), they found that clove bud has more flavonoids and phenols than Tannins and Terpenes or the extraction solvents were able to extract more flavonoids and phenols than other compounds. Positive correlation among all active compounds of clove bud and their antimicrobial and antioxidant capacity which may be due the presence of many phytochemicals and synergistic effect of all the phytochemicals that may be present. (6, 14) as shown in table (1).

Antimicrobial activity

Four pathogenic bacteria were collected, isolated and identified according to their morphological and chemical tests and gram stain test. Tow were positive gram, *Lesteria sp.* and *Staph sp.*, others were gram negative,

Escherichia coli and *Salmonella typhimurium*. These bacteria were chosen for their high resisted towards many chemical antibiotics also they had a high ferociousness. Disc diffusion method was confirmed and inhibition zones were measured in millimeters. The inhibition zones for tow positive tested bacteria *Lesteria sp.* and *Staph sp.* increased with the increasing of the concentration of the extract, these results were in agreement with results of many researches (8, 23, 27). It is found that the negative gram tested bacteria, *Escherichia coli* and *Salmonella typhimurium* were proportional in their response toward the treatments and shows a gradual increasing in inhibition zones with the rising of extract concentrations, these facts were in accord with some studies (21, 32, 33) as it shown in table (2). All tests in this study were done with 5 replications. All bacterial strains were sensitive to the activity of clove extract which damages the cells and inhibits the growth of both gram positive and gram negative bacteria this was explained and found that the primary mechanism of action of clove extract is membrane damage, which leads to cell death. (12, 17, 30).

Table2. Growth inhibition zone (mm) by clove extract T1, T2 and T3 against pathogenic bacteria and standard antibiotic (control treatment).

Pathogenic bacteria	T1(100%)of extraction	T2(50%) of extraction	T3(25%) of extraction	Oflaxacin Control
<i>Lesteria sp.</i>	8	6.5	5	10
<i>Staph sp.</i>	11	9.5	7	12
<i>Escherichia coli</i>	12	10	7	14
<i>Salmonella typhi</i>	12	8	6	15

Each sample result is a mean for five replications.

It was clearly that there is a positive relation between the concentration of the extract and the inhibition zones, after using a method to extract and isolate the eugenol so it is clear that the major phytochemical in clove is eugenol, that was in agreement with a study by Mittal, *et al* 2014, they recorded that major phytochemicals found in clove oil is mainly eugenol that is about (70-85%) followed by eugenyl acetate (15%) and finely β -caryophyllene which is less than 5% (22). It was obvious the best result is at the treatment T3 so the HPLC test was done and the results shows that the most important active compound which was detected is the aromatic oil called eugenol, the retention time (RT) of the extract compared with retention time (RT) of standard compound (15). RT of eugenol

stander was 12.20 as showed in figer (1), and the RT for T3 was tested and it was 12.19 as showed in figer (2) and this result shows the major area for eugenol which is a prove that this active component was responsible for the greet activity of this plant and that was coinciding with a study by some researchers that identified and analyzed the chemicals of clove extract, (16, 24). The results of this study encourage the use of natural sours like some plants or some parts of plants and the present tests improve the bioactivity including antibacterial action thus related to active compounds in the clover (*Syzygium aromaticum*) especially in the buds, actually this could be one of many promised treat to solve some series problems done by bacteria activities that cause food poisoning and harmful for humans health.

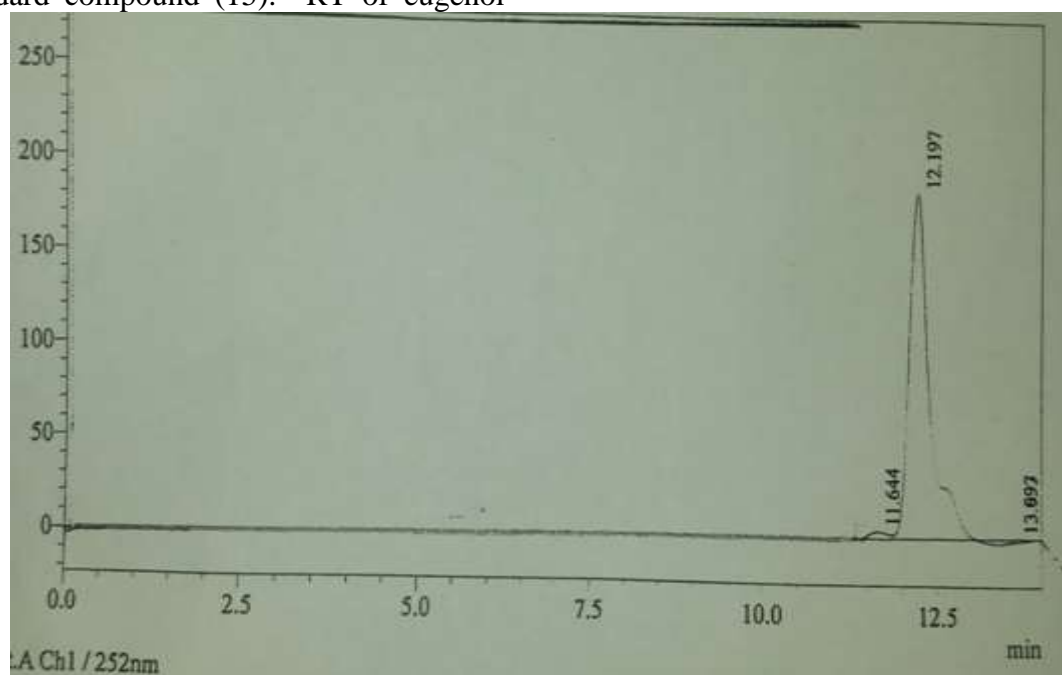
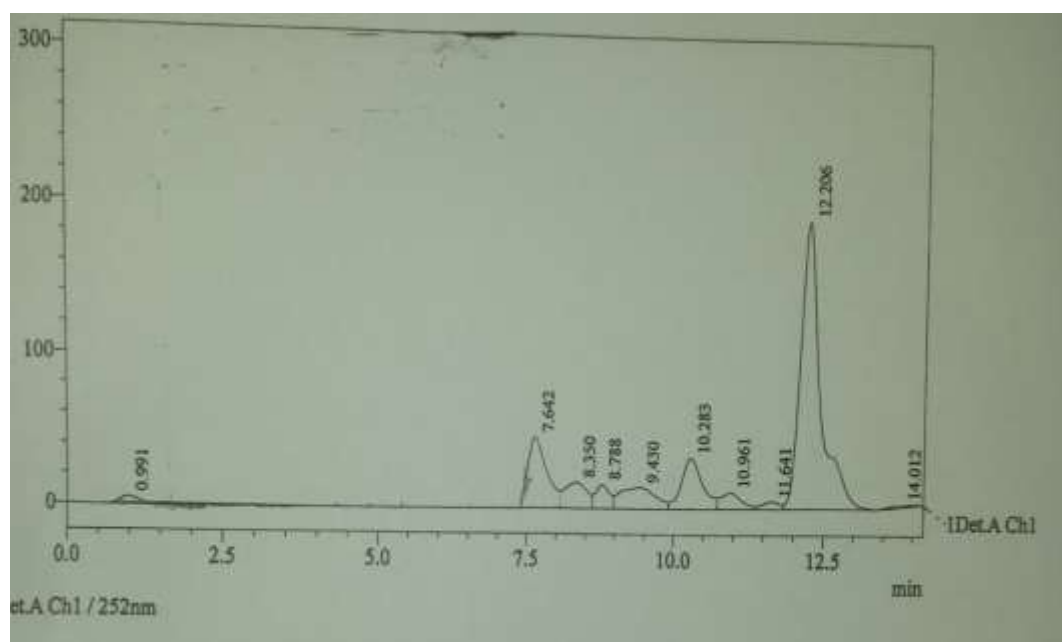


Figure 1. Stander of Eugenol analyzed by HPLC. Retention Time is in 12.19 min



Figur. 2 Eugenol analyzed by HPLC in tested treatment (T3). Retention Time is in 12. 20 min

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