

Comparative study of the inhibitory efficacy of some medicinal plant oils on the growth of pure isolates from a group of pathogenic microorganisms *in vitro*

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Abstract:

The present *in vitro* study was undertaken to evaluate the antimicrobial efficacy of essential oils of four medicinal plants including: *Eugenia caryophyllus*, *Sesamum indicum*, *Linum usitatissimum*, and *Mentha piperita* against the growth of seven pathogenic microorganisms: two gram-positive bacteria; *Staphylococcus cohnii cohnii*, *Micrococcus spp.*, and five gram-negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*. The method employed to test the antimicrobial efficacy was the Agar Well Diffusion test. The antimicrobial activity of *Eugenia caryophyllus* oil against the growth of all the test microorganisms except *Proteus mirabilis* was statically superior to the rest of the test oils with mean diameter of zone of inhibition; 28.5 ± 0.87 mm, 12.25 ± 0.25 mm, 22.25 ± 0.66 mm, 23.08 ± 1.16 mm, 27.83 ± 1.39 mm, and 10.91 ± 0.39 mm against the growth of *Staphylococcus cohnii cohnii*, *Micrococcus spp.*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* respectively. *Sesamum indicum* oil produce significant effect against *Pseudomonas aeruginosa* and *Proteus mirabilis* with mean diameter of zone of inhibition; 21.75 ± 1.29 mm and 14.25 ± 1.03 mm respectively. *Linum usitatissimum* revealed positive results against *Micrococcus spp.* Only with mean diameter of zone of inhibition; 30.41 ± 0.46 mm. while the oil of *Mentha piperita* did not show any inhibitory activity against each of the test microorganisms.

دراسة مقارنة للتأثير التثبيطي لزيت عدد من النباتات الطبية على نمو عزلات نقية لمجموعة من الجراثيم المرضية المنمأة مختبرياً

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الخلاصة:

لقد أنجزت الدراسة الحالية في المختبر لتقييم الكفاءة المضادة للميكروبات ولأربع زيوت نباتية أساسية شملت زيت القرنفل، زيت السمسم، زيت بذور أكتان، وزيت النعناع ضد نمو اثنين من الجراثيم المرضية الموجبة لصبغة غرام هي: *Staphylococcus cohnii cohnii*، *Micrococcus sp.* وخمسة جراثيم

سالبة لصبغة غرام هي: *Klebsiella*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *pneumoniae*، *Enterobacter cloacae*. وقد استخدمت طريقة الأنتشار في حفر الأكار من أجل اختبار هذه الكفاءة.

ان أفعال المضادة للميكروبات لزيت القرنفل ضد نمو جميع الجراثيم المختبرة أعلاه فيما عدا جرثومة *Proteus mirabilis* كان الأعلى من بين باقي الزيوت النباتية المستخدمة، وقد كانت أقطار التثبيط المنتجة بواسطة زيت القرنفل هي: 28.5 ± 0.87 ، 12.25 ± 0.25 ، 22.25 ± 0.66 ، 23.08 ± 1.16 ، 27.83 ± 1.39 ، 10.91 ± 0.39 ملم ضد نمو جراثيم: *Micrococcus spp.*، *cohnii* *Staphylococcus cohnii*. *Micrococcus spp.*، *Enterobacter*، *Klebsiella pneumoniae*، *Escherichia coli*، *Pseudomonas aeruginosa*، *Pseudomonas aeruginosa* على التوالي. كما أظهر زيت السمسم تأثيراً معنوياً ضد جراثيم *Proteus mirabilis* و *Enterobacter cloacae* وبمعدل نطاق تثبيط: 21.75 ± 1.29 و 14.25 ± 1.03 ملم على التوالي. بينما أعطى زيت بذور أكتان نتيجة إيجابية فقط ضد نمو جرثومة *Micrococcus spp.* بمعدل نطاق تثبيط 30.41 ± 0.46 ملم في حين لم يظهر زيت النعناع أي تأثير تثبيطي ضد أي من الجراثيم المختبرة.

Introduction:

The research for components with antimicrobial activity has gained increasing importance in recent times due to growing world wide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (1).

Bacteria are very adaptable organisms because of their very short generation time (as little as 15 to 20 minutes for some species under ideal conditions) and their propensity for sharing genetic information even among different species of bacteria. The presence of an antibiotic may kill most of the bacteria in an environment but the resistant survivors can eventually re-establish themselves and pass their resistance genes on to their offspring and often to other species of bacteria. Both medical and veterinary uses of antibiotics have resulted in the appearance of resistant strains of bacteria which may cause disease that are difficult to treat (2, 3).

Also the problem posed by the high cost, adulteration and increasing toxic side effects of antibiotic coupled with their inadequacy in diseases treatment found more especially in the developing countries can not be over emphasized (4). However, there has also been a rising interest in the research for natural products from plants for the discovery of new antimicrobial agents in the last three decades and in recent times (5, 6).

More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compounds found in essential oils (7, 8, 9) with established potent antimicrobial activities which indeed was formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (10, 11). Santos et al., 1995 (12) remarked the World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. No doubts, some studies have identified and isolated the main active

ingredients in the plants responsible for this antimicrobial activity.

The purpose of this study was to investigate the antimicrobial efficacy of essential oils of four known medicinal plants; *Eugenia caryophyllus*, *Sesamum indicum*, *Linum usitatissimum*, and *Mentha piperita* against the growth of seven pathogenic microorganisms in order to prove the folkloric claims.

Materials and methods:

Selection of medicinal plant materials:

Essential oils of four medicinal plants including: *Eugenia caryophyllus* (Clove oil, HEMANI-IRP), *Sesamum indicum* (Sesame oil, HEMANI-IRP), *Linum usitatissimum* (Flax seed oil, Ashams for oil-ROI), and *Mentha piperita* (Mint oil, El-Captain Company-Egypt) were utilized in this study. All these plants oils were purchased from the local market and identified at the National Iraqi Institute for Herbs, Baghdad, Iraq.

Antibiotics:

Five standard antibiotics had been chosen according to their broad-spectrum activity used as positive control against each of the test microorganisms, they include: CIP 5 (Ciprofloxacin), C30 (Chloramphenicol-30 mcg), CN 10 (Gentamicin-10 mcg), T30 (Oxytetracycline-30 mcg), CE 30 (Cephadrine-30 mcg). All these antibiotics carried the same trade name that was (Bioanalyse)[®].

Microorganisms:

Microorganisms used were standard strains of gram-positive bacteria including: *Staphylococcus cohnii cohnii* and *Micrococcus spp.* Besides five strains of gram-negative bacteria including: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*. These strains were obtained from the Laboratory of Microbiology at the College of Veterinary Medicine, Al-Qadisiya University and all were identified and confirmed at the Central Laboratory of Health, Baghdad, Iraq.

Sensitivity test:

Inhibition of microbial growth was tested by using the agar well diffusion method (13). All the test microorganisms were subcultured in nutrient broth media (HIMEDIA Laboratories, Mumbai-India) which was prepared by dissolving 13 gm of nutrient broth in 1000 ml of distilled water, shaken well and heated for several minutes using water bath at a temperature of 80°C to ensure complete dissolving, then sterilized for 15 minutes at 15 lb pressure in an autoclave. Nutrient broth media was later poured into seven sterile test tubes at average of 10 ml of media for each tube, after that several colonies of each bacteria were suspended with the help of sterile cotton swab in one test tube containing 10 ml of nutrient broth, after mixing well, all the tubes were incubated at 37°C for 24 hours to produce bacterial suspensions revealed by the presence of turbidity. On the

other hand Mueller Hinton Agar (HIMEDIA Laboratories, Mumbai-India) which is a growth media used for testing antibiotics and the chosen plant oils susceptibility of the test microorganisms was prepared by dissolving 38 gm of Mueller Hinton agar in 1000 ml distilled water, shaken, heated, and sterilized by autoclave in a similar way to the preparation of nutrient broth. This media was poured aseptically at 45°C into sterilized Petri plates (two plates were used for each plant oil on each of the test microorganism besides seven plates for the antibiotic discs corresponding the seven test bacteria, so that the final number of Petri plates used in this study was sixty three plates). After complete solidification, four wells were made aseptically on the surface of each agar plate with a diameter of 5 mm (with exception of plates that were used for antibiotic study). A sterile cotton swab was dipped into the bacterial suspension produced by *Staphylococcus cohnii cohnii* to be inoculated on the Mueller Hinton agar surface by streaking of the swab over its. This step was repeated with other bacterial suspensions each on its own plates. Finally and after the inoculums were dried, 0.1 ml of each plant oil at a concentration of 100% was poured into the wells of its inoculated plates. As well as one disc of each antibiotic control was placed with the aid of sterile forceps over the surface of each plate (so that five different antibiotic

discs were placed for each of the seven bacterial inoculums).

All these plates were incubated at 37°C for 24 hours followed by measuring of the diameter of zone of inhibition with the aid of ruler. The values were given as mean \pm standard deviation and $P < 0.05$ was considered statistically significant. The data were analyzed by student's *t*-test using SPSS (Version 10).

Results:

Two gram-positive bacteria; *Staphylococcus cohnii cohnii* and *Micrococcus spp.*, and five gram-negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae* were used in the present study. The results of *in vitro* antimicrobial activity of essential oils of *Eugenia caryophyllus*, *Sesamum indicum*, *Linum usitatissimum*, and *Mentha piperita* are presented in Table 1.

The essential oil of *Eugenia caryophyllus* exhibited maximum activity against *Staphylococcus cohnii cohnii* with mean diameter of zone of inhibition; 28.5 ± 0.87 mm, followed by 27.08 ± 1.39 mm for *Klebsiella pneumoniae*, 23.08 ± 1.16 mm for *Escherichia coli*, 22.25 ± 0.66 mm for *Pseudomonas aeruginosa*, 12.25 ± 0.25 mm for *Micrococcus spp.*, and 10.91 ± 0.39 mm for *Enterobacter cloacae* which represented the lowest value among the positive results of essential oil of *Eugenia caryophyllus* when they were arranged in

descending pattern, where as *Proteus mirabilis* was found to resistant to *Eugenia caryophyllus* oil.

On the other hand, the essential oil of *Sesamum indicum* showed strong antimicrobial activity against *Pseudomonas aeruginosa* and *Proteus mirabilis* only with mean diameter of zone of inhibition; 21.75 ± 1.29 mm and 14.25 ± 1.04 mm respectively while the essential oil of *Linum usitatissimum* revealed inhibitory effect against *Micrococcus spp.* only with mean diameter of zone of inhibition; 30.41 ± 0.46 mm making its the strongest oil in comparison to *Eugenia caryophyllus* oil against *Micrococcus spp.* Essential oil of *Mentha piperita* showed negative results against all of the test microorganisms.

The present study had also been depended on the use of five standard antibiotics as a positive control for each of the test microorganism (Table 2).

CIP5 (ciprofloxacin) was the strongest among the used antibiotics, it produced significant inhibitory effect against the growth of *Staphylococcus cohnii cohnii*, *Micrococcus spp.*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae* with mean diameter of zone of inhibition; 28.66 ± 1.20 mm, 30 ± 1.00 mm, 32 ± 0.57 mm, 24.33 ± 0.88 mm,

34.33 ± 0.66 mm, 34 ± 0.57 mm, and 31 ± 0.0 mm respectively. C30 (chloramphenicol) produced positive results against all the above bacteria with mean diameter of zone of inhibition; 30 ± 0.57 mm, 24.66 ± 0.33 mm, 9.33 ± 0.33 mm, 21.66 ± 0.33 mm, 29.66 ± 0.88 mm, 9 ± 0.57 mm, and 19 ± 0.57 mm respectively. CN 10 (gentamicin) also showed significant inhibitory efficacy against all of the test microorganisms with values of; 24.33 ± 0.33 mm, 18 ± 0.57 mm, 15.33 ± 0.33 mm, 20.33 ± 0.33 mm, 25.33 ± 1.20 mm, 13.33 ± 0.33 mm, and 19 ± 0.0 mm respectively. T30 (oxytetracycline) exhibited positive results against the growth of *Staphylococcus cohnii cohnii*, *Micrococcus spp.*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, and *Enterobacter cloacae* with mean diameter of zone of inhibition; 32.66 ± 0.33 mm, 29 ± 1.00 mm, 12 ± 0.57 mm, 20.33 ± 0.33 mm, 10.33 ± 0.33 mm, and 20.66 ± 0.33 mm respectively. Finally CE 30 (cephradine) also it had been used, but it exhibited antimicrobial activity against gram-positive bacteria only (*Staphylococcus cohnii cohnii* and *Micrococcus spp.*) with mean diameter of zone of inhibition; 24.66 ± 0.33 mm, and 16 ± 0.0 mm respectively.

Table: Inhibition zones (mm) of the used essential oils obtained for each of the test microorganism.

Essential oils Microorganisms	<i>Eugenia caryophyllus</i>	<i>Sesamum indicum</i>	<i>Linum usitatissimum</i>	<i>Mentha piperita</i>
<i>Staphylococcus cohnii cohnii</i>	28.5±0.87 aA	0±0 cB	0±0 bB	0±0 aB
<i>Micrococcus sp.</i>	12.25±0.25 bB	0±0 cC	30.41±0.46 aA	0±0 aC
<i>Pseudomonas aeruginosa</i>	22.25±0.66 cA	21.75±1.29 aA	0±0 bB	0±0 aB
<i>Escherichia coli</i>	23.08±1.16 cA	0±0 cB	0±0 bB	0±0 aB
<i>Klebsiella pneumoniae</i>	27.83±1.39 aA	0±0 cB	0±0 bB	0±0 aB
<i>Proteus mirabilis</i>	0±0 dB	14.25±1.03 bA	0±0 bB	0±0 aB
<i>Enterobacter cloacae</i>	10.91±0.39 bA	0±0 cB	0±0 bB	0±0 aB

❖ Different small letters mean significant changes for vertical values at level ($p < 0.05$).

❖ Different capital letters mean significant changes for horizontal values at level ($p < 0.05$).

Table 2: Zones of inhibition (mm) produced by antibiotics on the test microorganisms.

Antibiotics Microorganisms	CIP 5	C 30	CN 10	T 30	CE 30
<i>Staphylococcus cohnii cohnii</i>	28.66±1.20	30±0.57	24.33±0.33	32.66±0.33	24.66±0.33
<i>Micrococcus spp</i>	30±1.00	24.66±0.33	18±0.57	29±1.00	16±0.0
<i>Pseudomonas aeruginosa</i>	32±0.57	9.33±0.33	15.33±0.33	12±0.57	0±0
<i>Escherichia coli</i>	24.33±0.88	21.66±0.33	20.33±0.33	20.33±0.33	0±0
<i>Klebsiella pneumoniae</i>	34.33±0.66	29.66±0.88	25.33±1.20	0±0	0±0
<i>Proteus mirabilis</i>	34±0.57	9±0.57	13.33±0.33	10.33±0.33	0±0
<i>Enterobacter cloacae</i>	31±0.0	19±0.57	19±0.0	20.66±0.33	0±0



Figure 1: Inhibition zones produced by *Eugenia caryophyllus* oil against *Klebsiella pneumoniae*.

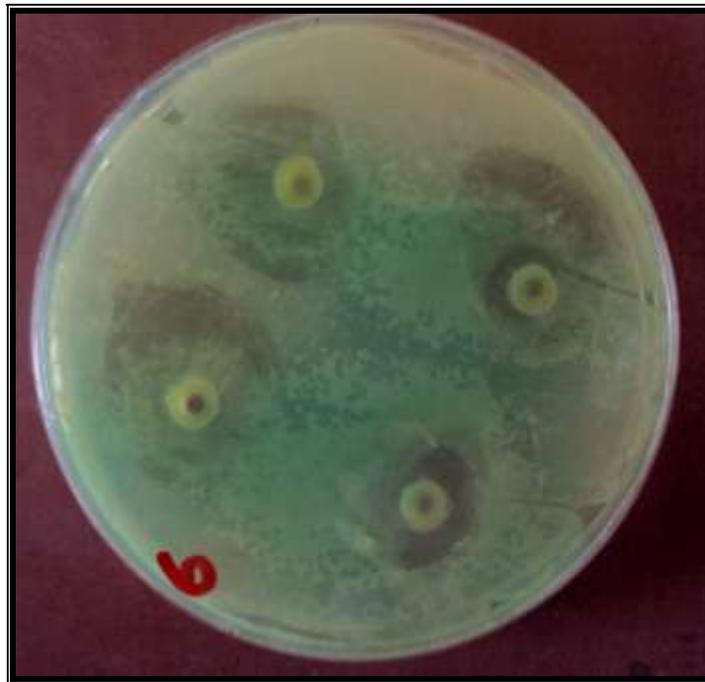


Figure 2: Inhibition zones produced by *Sesamum indicum* against *Pseudomonas aeruginosa*.



Figure 3: Inhibition zones produced by , *Linum usitatissimum* oil against *Micrococcus* spp.

Discussion:

Eugenia caryophyllus is the aromatic dried flower buds of a tree in the family Myrtaceae (14, 15). It is used as a carminative to increase hydrochloric acid in the stomach and to improve peristalsis (16). It is also used in dentistry where the essential oil of its was used as anodyne for dental emergencies (17). In addition it is antimutagenic (18), anti-inflammatory (19), antithrombotic (14), and antiparasitic (20). The essential oil extracted from the dried flower buds of *Eugenia caryophyllus* is used as a topical application to relieve pain and to promote healing (15). Several constituents of *Eugenia caryophyllus* had been identified mainly eugenol, eugenyl acetate, β -caryophyllene, 2-heptanone (21), acetyl eugenol, α -humulene, methyl salicylate, isoeugenol, methyl eugenol (20),

phenyl propranoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid (22). The antimicrobial activity of *Eugenia caryophyllus* had been studied by several authors (23). The spectrum of activity is fairly broad, with action against gram-positive and gram-negative rods and cocci, yeast and fungi (24). The present study had shown strong antimicrobial activity of *Eugenia caryophyllus* essential oil against *Staphylococcus cohnii cohnii*, *Micrococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* (Figure 1), and *Enterobacter cloacae* ; the highest effect was against *Staphylococcus cohnii cohnii* among the other test pathogens where as *Proteus mirabilis*

showed resistant to its. Similar results were achieved by Saeed and Tariq, 2008 (25), that reported antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*, but differ from our study by producing positive results against *Proteus mirabilis*. Our results also agreed with Ali et al., 2009 (26) in exhibiting antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Different results were achieved by Rams, 1999 (27) which found antimicrobial activity against *Staphylococcus aureus*, but no action against *Pseudomonas aeruginosa*, and *Escherichia coli*. A possible explanation for these diverse results is the fact that *Eugenia caryophyllus* oil composition is variable depending on the region and season that it is collected (28). Consequently, the active compounds may not present in sufficient quantities or quality. The mechanism of antimicrobial action of *Eugenia caryophyllus* oil, though not completely understood, seems to be complex and may vary according to its composition. The compounds known to have antimicrobial action are mainly the flavonoids and cinamic acids(29).

The results of our study had also shown the antimicrobial of *Sesamum indicum* oil. *Pseudomonas aeruginosa* (Figure 2), and *Proteus mirabilis* growth were sensitive and significantly inhibited by *Sesamum*

indicum oil. The strongest effect was against *Pseudomonas aeruginosa*, in compared with its efficacy on *Proteus mirabilis* growth; where as all of the other test microorganisms were resistant to *Sesamum indicum* oil. *Sesamum indicum* contained mainly essential oils such as aromatic phenolic compounds-sesamol, sesaminol, sesamin, carboxylic acids and other classes of compounds including fatty acids like palmitic acids, arachidonic acid, arachidic acid, stearic acid, myristic acid, oleic acid, linoleic acid, thiazole, pyrroles, disulphide and aldehyde (30). Many studies had proven the antimicrobial effect of *Sesamum indicum* oil and leaves extract as general. Annussek, 2001 (31) had found that *Sesamum indicum* seeds oil contain natural antibacterial agents that are effective against common skin pathogens; *Staphylococcus spp.* which showed resistance in our study. Anand et al., 2008 (32) had also proven the *in vitro* antimicrobial activity *Sesamum indicum* oil against dental caries causing bacteria, but there is no any available study about the antibacterial efficacy of *Sesamum indicum* oil against the growth of *Pseudomonas aeruginosa*, and *Proteus mirabilis*. The mechanism by which *Sesamum indicum* oil cause bacterial growth inhibition is not well known. *Sesamum indicum* oil has three lignans; sesamin, sesaminol, and sesaminol that have antioxidant properties, it has increased polyunsaturated fatty acids and reduced the lipid peroxidation (33).

The other possible mechanism might be the saponification or the soap making process that occurs as a result of the alkali hydrolysis of fat (34). The unsaponifiable fraction, a class of substances not found in the fats (sesamin or sesamolin) can probably protect against infection by its antioxidant property. These mechanisms could have been the reason for the reduction of microorganism growth, but more studies have to be done to check and prove the antimicrobial effect of compounds of *Sesamum indicum* oil (35).

Essential oil of *Linum usitatissimum* had produced significant inhibitory effect against the growth of gram-positive bacteria; *Micrococcus* spp. (Figure 3) only which was more sensitive to this oil whose its benefits are still being studied. There was no previous research that could support this positive result so it may be considered as a study of first kind. *Linum usitatissimum* oil is a rich source of essential fatty acids and is the richest natural source of α -linolenic acid (omega-3 fatty acid) that is important in the treatment of inflammatory disease and linoleic acid (omega-6 fatty acid) (36). It also contains high levels of lignans-active polysaccharides which have antibacterial, antiviral, and antifungal properties (37).

Mentha piperita oil also used in this study, but it had produced negative activity against all of the test microorganisms. All the test bacteria were highly sensitive to ciprofloxacin

that was the strongest among the other used antibiotics. The highest value was to *Klebsiella pneumoniae* and the lowest value was to *Escherichia coli*. Chloramphenicol also produced positive results against all of the test bacteria with highest effect on *Staphylococcus cohnii cohnii* and lowest effect on *Proteus mirabilis*. Furthermore, all the test bacteria were also sensitive to Gentamicin with highest effect on *Klebsiella pneumoniae* and lowest activity on *Proteus mirabilis*. Oxytetracycline was resisted by *Klebsiella pneumoniae*, but it was significantly effective on growth of the rest of test bacteria, the highest value was of *Staphylococcus cohnii cohnii* and lowest value was of *Proteus mirabilis*. Finally, Cephadrine was resisted by all the test bacteria with exception of gram-positive cocci producing high effect on *Staphylococcus cohnii cohnii*. In conclusion, findings showed that *Eugenia caryophyllus* oil presented "in vitro" broad antimicrobial activity against all the tested gram-positive and gram-negative organisms except *Proteus mirabilis*. *Sesamum indicum* oil was significantly possessed antimicrobial effect against *Pseudomonas aeruginosa*, and *Proteus mirabilis* (gram-negative), where as *Linum usitatissimum* oil inhibits the growth of *Micrococcus* spp. only. Further study must be done to elucidate the active ingredients which responsible for the antimicrobial properties of the tested plant oils.

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