Study of the inhibitory effect of the ethanolic extract of a number of local medicinal plants on the growth of *proteus spp. in vitro*

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Summary

The present study was designed to evaluate the inhibitory effect of six local medicinal plants ethanolic extract (*Quercusrobur, Vitisvinifera, Cinamomumzeylanicum , Ginger officinale ,Thymus vulgaris and Coriandrumsativum)* on the growth of *Proteus spp.* in vitro. For this purpose graduate concentrates for each extract (50, 100, 200 and 400) mg/ml were prepared and tested. The result showed that all the studied medicinal plants have antibacterial activity against *Proteus spp*. with exception for *Coriandrumsativum* which didn't showed any inhibitory activity for the growing of the studied bacteria in Vitro. And the more active plant in inhibition the growth of the studied bacteria was *Quercusrobur* followed by *Vitisvinifera* and *Cinamomum zeylanicum , Ginger officinale ,Thymus vulgaris*. Also, there was no significant differences between the effect of the different concentrations of *Thymus vulgaris* and *Coriandrum sativum*, while there were a significant differences between effect of *Cinamomumzeylanicum* concentrations and the concentrations of (400 and 200 mg\ml) *Ginger officinale* and compared with 100 and 50 mg/ml of *Quercusrobur*, while, the ethanolic extract of the *Vitisvinifera* showed a significant differences between the effect of the concentration 400 mg\ml with the other studied concentration.

Keywords: Ethanolic extract, Medicinal plants, *Proteus spp.*, In-vitro.

Introduction

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (1). Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds .Further, scientific investigation and information of the therapeutic potential of the plant material is limited (2). The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases.

Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (3) World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug (4).The study aimed to evaluate the antimicrobial activity of six medicinal plants ethanolic extract on the growth of *Proteus spp.* In Vitro.

Materials and Methods

In this experiments six local medicinal plants were used that include: fruit of *Quercusrobur* , seeds of *Vitisvinifera*, stem of *Cinamomum zeylanicum*, root of *Ginger officinale*, leaves of *Thymus vulgaris and* leaves of *Coriandrum sativum*. All these plants were obtained from the local market and

identified by the national Iraqi institute for herbs, the fruits of the first plant, stem of the second plant and fruits of the third plant were taken, then all the chosen parts of the above plants were subjected to aerial drying for two weeks, after drying were grinded it very well until it became as a fine powder. The Ethanolic extractions of the three plants were done by Harborn method (5) by using of Ethanol at a concentration (96%).

Proteus spp. Isolates were identified by laboratory of Microbiology, College of Veterinary Medicine at Al-Qadissiyia University. For each of the tested medicinal plants a serial dilution were made to study the effect of the plants in inhibition the growth of *Proteus spp.* at a different concentrations and select the most effective concentrations of the plant extract depending on the zone of inhibition of growth that been given by each concentration , the studied concentrations include 400, 200, 100, 50 mg/ml. These serial dilutions were decided depending on clinical trials.

After preparation ethanolic extracts of the medicinal plants and activation of the pathogenic bacteria in the nutrient broth , the Mueller Hinton Agar (HIMEDIA –Mumbai-India) 18 Petri plates containing Mueller Hinton agar were used (18 plates for the medicinal plant extract study :3 plates for each extract). This study was done by taking swap from the test tube that contain the bacterial suspension and inoculated on the Petri plates that contain the Mueller Hinton agar, and then 0.1 ml was added from each concentration of plant extract on its own plates and we applied the chosen antibiotics discs after complete applying of all the medicinal plants extracts concentrations and the antibiotics were incubated all the Petri plates at 37C^{o} for 24 hours (6). The sensitivity of microorganisms towards the plants extracts was screened by following the agar well –diffusion method. The zone of inhibition (diameter in mm) in triplicates was measured and the mean value (μ) was tabulated (7).

Results and Discussion

In this study, *Quercusrobur*, *Vitisvinifera*, *Cinamomum zeylanicum* , *Ginger officinale* and *Thymus vulgaris* exhibited activity against the studied bacteria except for *Coriandrum sativum* that showed no activity against the studied bacteria. The antibacterial activity of Oak (*Quercusrobur*), Cinnamon (*Cinnamomum zeylanicum*) and Thyme (*Thymus vulgaris),* Grap seed *(Vitisvinifera),* Ginger *(Ginger officinale)* and Coriander (*Coriandrum sativum)* extracts were tested against *Protusspp.in vitro* and the results are listed in (Table,1 and Figure,1).

The results of the study showed that the ethanolic extract of Cinnamon produced antibacterial activity against the studied bacteria as follow for the studied concentrations (400, 200, 100 and 50) mg\ml (13.55±0.7, 20.55±0.55, 18.33±1.31 and 15.22±1.03) mm respectively, (Figure,2), the zones of inhibitions produced by the ethanolic extract of Ginger was as follow for the studied concentrations (17.55±0.44, 17.44±0.29, 15.44±1.06 and 14.55±0.7) mm respectively (Figure,3), the results of the ethanolic extract of Oak for the studied concentrations was as follow (24.22±0.22, 21.33±0.47, 24.88±0.93 and 20.11±0.53) mm respectively, (Figure,4) , the ethanolic extract of Grap seeds showed the followed result for the studied concentrations (20.88±0.35, 18.33±0.37, 19.77±0.54 and 20±0.76) mm respectively, (Figure,5), the results also showed that the ethanolic extract of Thyme have antibacterial activity against the studied bacteria appeared as a zones of inhibitions for the studied concentrations as follow (16.22±0.7, 17.66±0.16, 16.44±0.16 and 16.66±0.4) mm respectively, (Figure,6). While the ethanolic extract of Coriander showed no antibacterial activity against the studied bacteria (0±0) mm (Figure,7).

The antimicrobial effect of Cinnamon was identified in a laboratory experiment in which pure *Cinnamomum cassia* extract, mainly composed of the active ingredient cinnam aldehyde (8). The studies indicate to the fact that the antibacterial activity of Cinnamon may be due to this component (9).

Ginger contains phenol which is one of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative

toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (10). In addition, some authors have found that more highly oxidized phenols are inhibitorier (11 and 12). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (13).

*Vitisvinifera*L. (Vitaceae) is used in conditions like burning sensations, haemorrhages, anaemia, leprosy, skin diseases, syphilis, asthama, jaundice, bronchitis (14,15). The seeds contain polyphenol which is a proanthocyanidin the presence of this polyphenol may be responsible for it is antibacterial activity as we mentioned above in Ginger (16). The other main compounds are quercetin, catechin and epicatechin, *Vitisvinifera*has yet another molecule that has valuable potential as a detoxifying agent and that is the presence of a carotenoid called xanthophyll (17).

Oak contains Tannins, Polyphenols and Quercetin (16). Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. They also complex with polysaccharide (18). The antimicrobial significance of this particular activity has not been explored. Tannins in plants inhibit insect growth (19) and disrupt digestive events in ruminal animals (20). Scalbert (21) reviewed the antimicrobial properties of tannins in 1991. It listed 33 studies which had documented the inhibitory activities of tannins up to that point. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed (22) tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity.

The better solubility of the active components of plants in organic solvent may have an effect on its antibacterial activity (23). These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. Lin, *et. al.*, (24) reported that Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study.

The zone of inhibition produced by Cinnamon ethanolic extract in this study was ranged between (13.55-20.55) mm was disagree with the result of (25) found that Cinnamon produced no activity against *Proteus spp*. While for Ginger ethanolic extract the zone of inhibitions ranged from (14.55-17.55) mm and this was closed to the result of (26) who find that Ginger ethanolic extract had inhibitory activity against the growth of *Proteus vulgaris* ranged between (7-12) mm for its studied concentrations that ranged from (50-200) mg\ml which is closed to the concentrations used in our study, and also gave a zone of inhibitions ranged from (8-16) mm against *Proteus mirabilis* for the same concentrations.

The result of Oak ethanolic extract that were ranged from 20.11-24.88 mm was closed to the result of researcher (27) that reported that the ethanolic extract of Oak produced a minimal inhibitory concentrations (MIC) about 32 mm using a studied concentrations of the extract ranged from(0.1 -0.50)gm\ml.

The zone of inhibition for the Grape seeds ethanolic extract was ranged from (18.33-20.88) mm closed to the results described by authors (28) reported that the ethanolic extract of *Vitisvinifera* give a zone of inhibition against *P.mirabilis* (16) mm and against *P.vulgaris* (11) mm.

Thyme ethanolic extract gave azones of inhibitions ranged from (16.22-17.66) mm according to the studied concentrations was closed to the result other authors (29) showed that the zones of inhibition gave by alcoholic extract of Thyme against Proteus spp. With concentrations closed to what used in this study was (6-16)mm , while disagree with the result of workers (30), who found the at concentrations 20 mg\ml and 40 mg\ml of the ethanolic extract of Thyme there was no

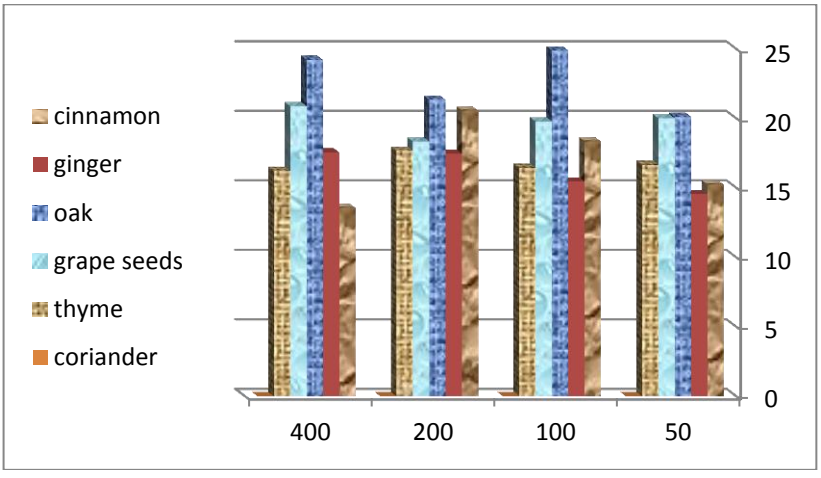
activity of this plant extract against *Proteus spp*.

In conclusion each of involved medicinal plants (ethanolic extract) have antibacterial activity against *Proteus spp*. In vitro except for Coriander that showed no antibacterial activity, there was a significant differences (P<0.05) between the effect of the studied plant and concentrations of each tested medicinal plants used in this study.

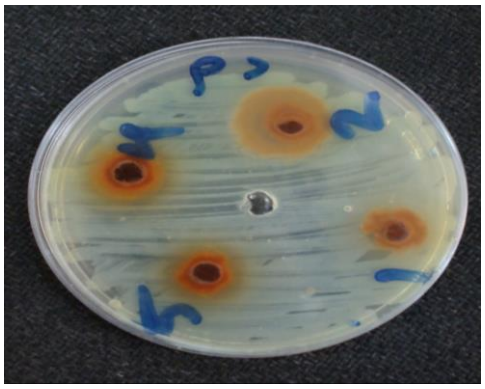
Table, 1: The antibacterial activity of the ethanolic extract of (*Quercusrobur, Vitisvinifera, Cinamomumzeylanicum , Ginger officinale ,Thymus vulgaris and Coriandrumsativum*) on *Proteus spp.*

Plants extract	Zone of inhibitions (mm)for each of the Concentrations (mg \ml)			
	400	200	100	50
Cinamomumzeylanicum	13.55±0.7 aA	20.55±0.55 bA	18.33±1.31 cA	15.22±1.03 dA
Ginger officinale	17.55±0.44 aB	17.44±0.29 aB	15.44±1.06 bB	14.55±0.7 cA
Quercusrobur	24.22±0.22 aC	21.33±0.47 aC	24.88±0.93 bC	20.11±0.53 cB
Vitisvinifera	20.88±0.35 aD	18.33±0.37 bD	19.77±0.54 bD	20±0.76 bB
Thymus vulgaris	16.22±0.7 aE	17.66±0.16 aD	16.44±0.16 aE	16.66±0.4 aC
Coriandrumsativum	0±0 aF	0±0 aE	0±0 aF	0±0 aD

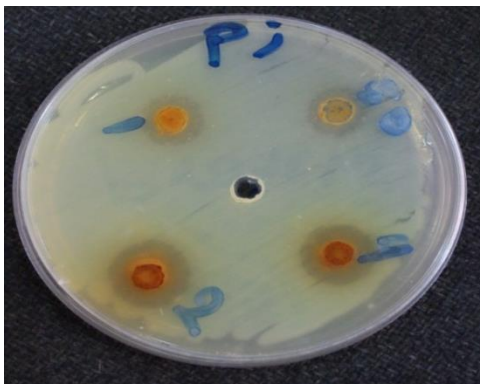
***Values were expressed as means ± standard error**
***Values with different capital letters are significant differences vertically at (P< 0.05).**
***Values with different small letters are significant differences horizontally at (P<0.05).**



Figure, 1: Zone of inhibition (mm) of *Proteus spp.* exhibited by the ethanolic extracts of the tested plants .



Figure, 2: Inhibition zones of *Proteus spp.*growth on Mueller-Hinton agar produced by ethanolic extract of *Cinnamomumzeylanicum*, the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol .



Figure, 3: Inhibition zones of *Proteus spp.* growth on Mueller-Hinton agar produced by ethanolic extract of *Ginger officinale*, the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol

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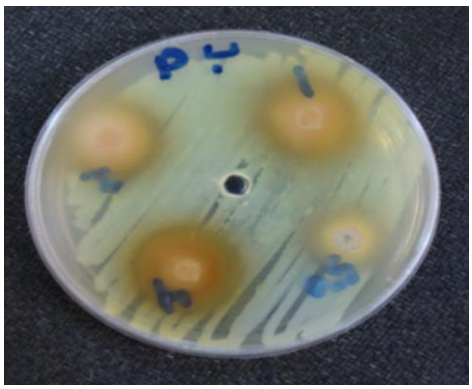
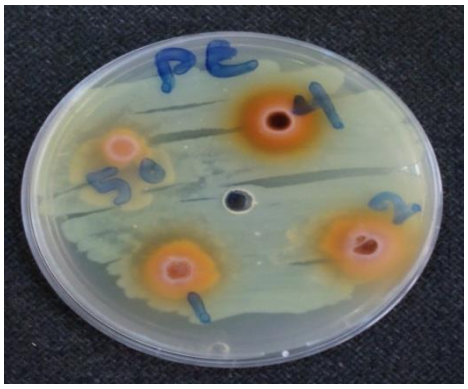
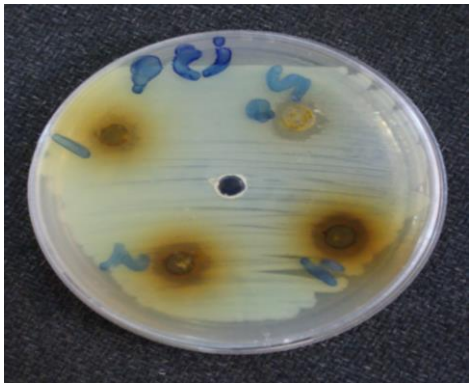


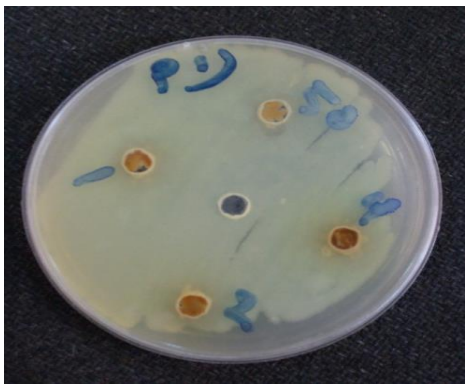
Figure, 4: Inhibition zones of *Proteus spp.*growth on Mueller-Hinton agar produced by ethanolic extract of *Quercusrobur*, the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol .



Figure, 5: Inhibition zones of *Proteusspp.* growth on Mueller-Hinton agar produced by ethanolic extract of*Vitisvinifera*, the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol



Figure, 6: Inhibition zones of *Proteus spp.*growth on Mueller-Hinton agar produced by ethanolic extract of *Thymus vulgaris* , the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol .



Figure, 7: Inhibition zones of *Proteus spp.* growth on Mueller-Hinton agar produced by ethanolic extract of *Coriandrumsativum*, the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol .

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دراسة التأثيرالمثبط للخلاصة الأيثانولية لعدد من النباتات الطبية المحلية على نمو جرثومة المتقلبات في الزجاج

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الخلاصة

صممت هذه الدراسة لتقييم التأثيرالمثبط للمستخلص الأيثانولي لستة من النباتات الطبية المحلية (البلوط، بذورالعنب، الدارسين، الزنجبيل، الزعتر و الكزبرة) في نمو جرثومة المتقلبات في الزجاج. ولهذا الغرض حضرت ودرست تراكيزمتدرجة لكل مستخلص (50، 100، 200 و 400) ملغم/مل. أظهرت النتائج ان لكل النباتات الطبية المدروسة فعالية مضادة للبكتريا ماعدا نبات الكزبرة الذي لم يظهر اي تأثير مثبط لنمو البكتريا المدروسة في المختبر. أن النبات الأكثرفاعلية في تثبيط نموالبكتريا المدروسة كان البلوط يليه بذورالعنب ثم الدارسين ثم الزنجبيل والزعتر. وايضا عدم وجود فرقا معنويا بين تأثيرالتراكيزالمدروسة لنباتي الزعترالكزبرة بينما كان هنالك فرقا معنويا بين تأثيرالتراكيزالمدروسة لنبات الدارسين وبين التركيزين (200 و 400) ملغم\مل لنباتي الزنجبيل والبلوط مع التركيزين (50 و 100) ملغم\مل، بينما لبذورالعنب فقد كان هنالك فرقا معنويا بين تأثيرالتركيز (400) ملغم\مل مع بقية التراكيزالمدروسة.

الكلمات المفتاحية : المستخلص الايثانولي, النباتات الطبية, الزوائف, في الزجاج .