Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.8, No.12, 2018

Study the Effect of Medicinal Plant Extracts on the Growth of Bacterial and Fungal

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Abstract
Study antimicrobial and antifungal activity of three selected plants medicinal ***Rosemary*** (*Rosmarinus officinalis*), ***Ginger (Zingiber officinale)*** and **Anise (*Pimpinella anisum* L.)**. The plants were extracted and dissolved with ethanol, and study the effects of them against growth of some bacterial pathogens isolates like (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus roseus and streptococcus pygenes*) also study the effects of this plants extracts against the growth of some fungal like *Candida tropicalis, Candida albicanus Penicillium sp. Fusarium sp. Rhizopus sp. Aspergillus niger* . The results showed that all plants extracts exhibited antibacterial activity and antifungal activity against the tested isolates in tow concentration (10^{-1} and 10^{-3}). *S. aureus* was the most bacterial species effected inhibition zone (18 mm) with *Rosemary* and the lowest species of bacteria effected and show minimum inhibition zone (8 mm) was Anise against *Pseudomonas aeruginosa*, on the other hand the highs inhibition zone against fungi was recorded with Anise so (18 mm) which inhabit growth of *Aspergillus niger* and the minimum inhibition zone (8 mm) show with *Ginger* against *C. albicanus*.
Keywords: Plant extracts, *Rosemary* , *Ginger* , *Anise*

1.0 Introduction

Medicinal plants have the ability to inhibit the growth of wide range of pathogenic microorganisms due to presence of essential oils. The antimicrobial impact of essential oils and its various components extracted from medicinal plants has been well documented. Medicinal plants contain many phytochemicals components such as flavonoids, alkaloids, tannins and essential oils. Among these plants, *Rosmarinus officinalis*, *Ocimum basilicum* and *Laurus nobilis*(Ouibrahim *et al.,* 2013; Akthar *et al.,* 2014). Plants are good source of a more kind of economically important compounds such as phenolic compounds, nitrogen containing compounds, vitamins and minerals which have anti-oxidant, anti-tumor, anti- mutagenic, anti- carcinogenic and diuretic activities. Many of the plant materials used in traditional medicine are readily available in rural areas. Medicinal plants have been a valuable source of natural active constituents that products for maintain human health and treatment of many human disease.(Malini *et al.,* 2013). Extracts of medicinal plants are effective against fungal and bacterial pathogens; meanwhile they are biodegradable compounds which have high potential for using in integrated pest management programs. The use of biological compounds extracted from plants may be an alternative to conventionally used fungicides to control phytopathogenic fungi, due to their being bioactive chemicals such as flavonoids, phenols, tannins, alkaloids, quinones, saponins and sterols(Manoorkar and Gachande, 2014). The antioxidant and antimicrobial properties of various extracts from medicinal plants have been of great interest because of their potential use as natural additives for the prevention of oxidation, controlling pathogens and/or toxin-producing microorganisms in foods . Medicinal plants have been used as traditional medicines all over the world for thousands of years . Bioactive compounds of medicinal herbs especially polyphenolics have possible health benefits with antioxidative, anticarcinogenic, antihypertensive, antimutagenic and antimicrobial activities(Haghju and Almasi, 2015).

Rosemary (Rosmarinus officinalis) plant with the scientific name of *Rosmarinus officinalis* L. is of Lamiaceae (Labiatae) family. This type has an evergreen bush which is a local plant of Mediterranean region with pharmacological and decorative value. It is a sustainable plant, so aromatic and has wooden stalks with 50 cm up to 2 m height growing in Mediterranean region and in particular in the littorals region through the minor Asia areas widely. Its leaves facing each other are turned down, narrow, long, thick, sharp-pointed and with a tough appearance (Moghtader and Farahmand ,2011). As medicinal plant rosemary belongs to the pool of herbs, which probably more than others, lies at the boundary between myth, superstition and traditional popular usages, but at the same time, its efficacy is largely acknowledged, being, in fact, listed in the official pharmacopoeia of several countries 3. Historically, rosemary has been used as a medicinal agent to treat renal colic and dysmenorrhea. It has also been used to relieve symptoms caused by respiratory disorders and to stimulate the growth of hair. Extracts of rosemary are used in aromatherapy to treat anxiety-related conditions and to increase alertness(Ceylan *et al.,* 2014). Its herb and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent and Rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram negative bacteria. High percent of the antimicrobial(Seleman *et al.,* 2016).

Ginger (Zingiber officinale) family, Zingiberaceae is widely used around the world as a spice or food additive

and medicine. This plant is a perennial herb consisting of an underground stem or rhizome, bearing erect leafy shoots. The pungent element of ginger is the oleoresin-gingeroles, shogaols and zingerone, which are credited with anti-nausea or antiemetic, abortifacient, antimicrobial, anti-inflammatory. antioxidant5 anticoagulant, antihypercholesterolemic, antihypertensive, antihyperglycaemic, and anti-spasmodic, aperient. alexeteric, circulatory stimulant, counter irritant, sialagogue and vasodilator effects, They contain chemical substances such as polyphenols, quinines, flavonols/flavonoids, alkaloids, polypeptides or their oxygen-substituted derivatives (Pokhrel *et al.,* 2012: Christaki *et al.,* 2012). Ginger which are routinely used for gastrointestinal problems as grandmother's remedies were tried on the pathogen in the present study A variety of volatile molecules present in essential oils like terpenes, terpenoids, phenol- derived aromatic and aliphatic compounds could be responsible for their bactericidal, virucidal and fungicidal action(Justin and Antony, 2016).
Anise (*Pimpinella anisum* L) is an annual important spice and medicinal plant belonging to the family Apiaceae and is considered as a natural raw material and used for pharmaceutics, perfumery, food and cosmetic industries. In recent times, this that is native to the Mediterranean region. Today, anise spice plant has attracted consumer attention due to its antimicrobial, antifungal, insecticidal, and antioxidative effect on human health.annual herb and a grassy plant with white flowers and a small green to yellow seed. The plant is self fertile; prefer light sandy and medium loamy and well drained soil. When threshed out, the fruit or the so- called seed (part used) may be easily dried in trays, in a current of air in half-shade, out-of-doors, or by moderated heat. The taste is sweet and spicy, and the odor aromatic and agreeable, The use of biological compounds extracted from plants may be an alternative to conventionally used fungicides to control phytopathogenic fungi, due to their being, bioactive chemicals such as flavonoids, phenols, tannins, alkaloids, quinons, saponins and sterols (Amin *et al.,* i,2012;Ullah *et al.,* 2013; Mohamed *et al.,* 2015). Anise (*P. anisum* L.). For medical purposes, they are used to treat dyspeptic complaints and catarrh of the respiratory tract, and as mild expectorants. It was also reported that extracts from anise fruits have therapeutic effects on several conditions, such as gynaecological and neurological disorders. Ethanolic extract of anise-fruits contains *trans*-anethole, methylchavicol (estragole), eugenol, psedoisoeugenol, anisaldehyde, coumarins (umbelliferon, scopoletin), caffeic acid derivatives (chlorogenic acid), flavonoids, fatty oil, proteins, minerals, polyenes and polyacetylenes as its major compounds(Kosalec *et al.,* 2005).

2.0 MATERIALS AND METHODS

2.1Collection of medicinal plant**:** *Rosemary (Rosmarinus officinalis), Ginger (Zingiber officinale) and Anise (Pimpinella anisum L.).* were purchased from an well known market in Amarha city, Misan Governorate ,lraq

2.2Microorganisms: uses In Studying Uses in this study isolations Microorganisms different from isolations bacterial diseases *(Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus roseus and streptococcus pygenes)* and use organisms testing Microorganisms detection activity inhibition of Extraction plants medicinal and source this isolations from Department Sciences Biology/College Sciences /University Misan. Uses in this study isolations Microorganisms different from isolations fungals and use organisms testing Microorganisms detection activity inhibition of Extraction plants medicinal and source this isolations *Candida tropicalis, Candida albicanus Penicillium sp. Fusarium sp. Rhizopus sp. Aspergillus niger* from Department Sciences Biology/College Sciences /University Misan.

2.3Ethanol Extraction
Plant extraction:100 g of the dried *Anise (Pimpinella anisum L.).* seeds were milled to a course powder using an electrical grinder and plants Fresh *Rosemary (Rosmarinus officinalis), Ginger (Zingiber officinale)* Two dried so were successively were extracted with 99% ethanol by stored at room temperature (25°C) over period of two week. 500 g of plant material and one liter of ethanol were used in the extraction. Ethanol containing the extract was then filtered through filter paper and the solvent was vacuum–distilled at 65°C in a rotary evaporator to ensure the removal of any residual solvent. Final extract was a dark green liquid. This ethanol extract was kept in deep freeze at -20°C until use (Alnamer *et al.,* 2012; Attitalla *et al.,* 2015).
2.4Antibacterial Activity: Isolates of Bacteria strains obtained from Department Sciences Biology/College Sciences /University Misan.. The antibacterial activity of the Extraction plants medicinal against Gram positive Bacteria like *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus roseus and streptococcus pygenes* were determined with the disc diffusion method National Committee for Clinical Laboratory Standards(Nccl,2002)Bacterial suspension was diluted with sterile normal saline up to 10^3 and this was used as inoculums. Muller-Hinton agar plates were inoculated by dipping a sterile cotton swap into inoculums and streaked all over the surface of Muller-Hinton agar plat three times, rotating the plates through an angle of 60° and finally the swap passed round the edge of the agar surface, then plates were left to dry for few minutes at room temperature. Filter paper discs (diameter, 5 mm) were saturated with different

concentrations (10^{-1}, 10^{-3}) of Ethanol plant extraction and then placed on the inoculated plates using sterile forceps. Discs were readily placed at 30-36 mm distance to avoid overlapping of inhibition zones. The plates were incubated (o/n) at 37°C. 5. After incubation, the diameter of each inhibition zone was measured (Alnamer *et al.,* 2012).

Fungal strains used Fungi were obtained and an examination of its sensitivity was carried out in Mycology Laboratory in from Department Sciences Biology/College Sciences /University Misan. and the fungal were as follows: *Candida tropicalis, Candida albicanus Penicillium sp. Fusarium sp. Rhizopus sp. Aspergillus niger* .

2.5Preparation of Inoculums :The suspension of fungus was prepared as per Mac-Farland Nephelometer Standard. A 24 h old culture was used for the preparation of fungus suspension. A suspension of fungus was made in a sterile isotonic solution of sodium chloride and the turbidity was adjusted such that it contained approximately 1.5 × 106 cells / ml. It was obtained by adjusting the optical density (650 nm) equal to 1.175% barium chloride in 100 ml of 1% sulphuric acid.

2.6Antifungal Susceptibility Test: Stock fungi were maintained at room temperature on Potato Dextrose Agar. Active fungi for experiments were prepared by seeding a loopful of fungi into Potato dextrose broth and incubated without agitation for 48 h at 25°C. The broth was diluted with Potato dextrose broth to achieve optical densities corresponding to 2.0 × 105 spore/ml for the fungal strains. The disc diffusion method was also used to screen for antifungal properties. In vitro antifungal activity was screened by using Potato Dextrose Agar (PDA). The PDA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 10 min and 1 ml of the test culture was introduced into agar and allowed to spread while the excess was drained off. The plate was incubated at room temperature for 10 min. A sterile cork borer of 5 mm diameter was used to make two ditches (wells) on each plate and filled with 1 ml (200 mg) of the plants extract. The same was repeated for each fungus strain using the extract. These were carried out in triplicate for each fungus. The plates were incubated at 25°C for 96 h and the resulting zone of inhibition around the ditches were measured to the nearest millimeter along two axes and the mean of the two measurements was calculated.

2.7Statistical Analysis: Data regarding two parameters (concentration and medicinal plants) were analyzed statistically using SAS program with completely randomized design (CRD).

3.0RESULTS

The antibacterial activity of ethanol plant extracts were tested against Gram positive and gram negative pathogen isolates species including isolations bacterial diseases *(Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus roseus and streptococcus pygenes)* The activities of the extracts were evaluated by measuring the diameter of inhibition zone around the respective discs in the concentrations (con. 10^{-1}, 10^{-3} ml). The results are presented in Tables 1 , 2,3 and 4. All plant extracts have potent activity against Gram positive than Gram negative bacteria. In this study, these extracts were for their antimicrobial activity against various microbes in the hope of finding a new antimicrobial agent. Although antimicrobial activity was highly dependent on different extracts structure, concentration and type of microbe, all synthesized extracts showed significance antimicrobial activity. were found significant ($p<0.05$) between concentration for extract**s**

Table 1. Antibacterial activity (inhibition zone/mm) of ethanol plant extracts (concentration 10^{-1}) against Gram positive and Gram negative pathogen isolates.

Ethanol plant extracts	***Escherichia Coli***(−)	***Pseudomonas aeruginosa***(−)	***Klebsiella pneumonia***(−)	***Staphylococcus aureus***(+)	***Micrococcus roseus***(+)	***streptococcus pygenes***(+)
Rosemary	15mm	13mm	12mm	18mm	17mm	17mm
Ginger	16 mm	14 mm	14 mm	16 mm	15 mm	16 mm
Anise	14 mm	12 mm	15 mm	17 mm	16 mm	17 mm

* Significant differences as a result of extracts plant bay treatments (P< 0.05)

Table 2. Antibacterial activity (inhibition zone/mm) of ethanolic plant extracts (concentration 10^{-3}) against Gram positive and Gram negative pathogen isolates.

Ethanol plant extracts	***Escherichia Coli***(−)	***Pseudomonas aeruginosa***(−)	***Klebsiella pneumonia***(−)	***Staphylococcus aureus***(+)	***Micrococcus roseus***(+)	***streptococcus pygenes***(+)
Rosemary	11mm	12mm	13mm	15mm	16mm	13mm
Ginger	10 mm	9 mm	11 mm	14 mm	15 mm	16 mm
Anise	9 mm	8 mm	10 mm	13 mm	16 mm	15 mm

* Significant differences as a result of extracts plant bay treatments (P< 0.05)

Table 3. Antifungal activity (inhibition zone/mm) of ethanol plant extracts (concentration 10^{-1}) against funals.

Ethanol plant extracts	***C. tropicalis***	***C. albicanus***	***Aspergillus niger***	***Rhizopus sp.***	***Fusarium sp.***	***Penicillium sp.***
Rosemary	15mm	14mm	16mm	17mm	13mm	14mm
Ginger	14 mm	13 mm	17 mm	15 mm	14 mm	16 mm
Anise	13 mm	13 mm	18 mm	16 mm	15 mm	17 mm

* Significant differences as a result of extracts plant bay treatments (P< 0.05)

Table 4. Antifungal activity (inhibition zone/mm) of ethanol plant extracts (concentration 10^{-3}) against funals.

Ethanol plant extracts	***C. tropicalis***	***C. albicanus***	***Aspergillus niger***	***Rhizopus sp.***	***Fusarium sp.***	***Penicillium sp.***
Rosemary	11mm	12mm	13mm	13mm	10mm	12mm
Ginger	10 mm	8 mm	10 mm	12 mm	12 mm	11 mm
Anise	12 mm	10 mm	11mm	14 mm	10 mm	14 mm

Significant differences as a result of extracts plant bay treatments (P< 0.05)

4.0 DISCUSSION

Medicinal plants contain many phytochemicals components such as flavonoids, alkaloids, tannins and essential oils. Among these plants, *Rosmarinus officinalis*, and it has three chemo types: cineol, camphor and verbenone , it contains also triterpene deri-vatives (2-4%), flavonoids, tannins and saponins, All organisms are sensitive to the three ethanol plant extracts except (Gram negative) which is more and more responsible for nosocomial infections. It has an intrinsic resistance to a wide range of antibiotics and also to ethanol plant extracts. This resistance is due to the impermeability of the wall of this bacterium(Ouibrahim *et al.,* 2013). showed that its

main constituents are a-pinene, borneol, 1,8-cineole and camphor , Several compounds found in the extracts to be inhibitory to several micro-organisms. It was confirmed that its extracts had shown antioxidant, antibacterial and antifungal activities.(Tzima *et al.,* 2015). The gram positive bacteria were more sensitive to spices than gram negative bacteria because of their cell wall structure. But in the present work the spices were found to be effective against both gram positive as well as gram negative bacteria.(Ceylan and Fung,2004; Shihabudeen *et al* ,.2010).

shows antimicrobial activity of the alcoholic extracts on nutrient agar and Muller Hinton agar medium against selected microbes for Alcoholic extracts of *Rosemary, Ginger* and *Anise* were effective and showed inhibitory zone in the range of 12-18mm at concentrations (10^{-1}) while concentrations (10^{-3}) inhibitory zone in the range of (8-16 mm inhibitory zone) of *Rosemary, Ginger* and *Anise.*

The plant extracts were found to be prominently active against the tested microorganisms *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus roseus and streptococcus pygenes*)) at the concentration of 10^{-1} and 10^{-3}, Generally, Gram-positive bacteria were more sensitive than Gram-negative bacteria Among the tested plant extracts, *Rosemary* ethanol extracts showed the highest activity (18 mm) of inhibition zone against *S. aureus* where was *Anise* so ethanol extracts showed the minimum inhibition zone against *P. aeruginosa* (8 mm) on the other hand all species of bacterial pathogens showed inhibition zone with all ethanol plant extracts in both concentration, Antibacterial properties of plants extracts depend not only on its chemical characteristics, but also on type of bacteria. Gram negative bacteria are less susceptible because their membrane contains hydrophilic lipopolysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds (Hyldgaard *et al.,* 2012). The results in table 3 and 4 show the activity of ethanol plant extracts against fungal of : *Candida tropicalis, Candida albicanus Penicillium sp. Fusarium sp. Rhizopus sp. Aspergillus niger* , generally show all fungal inhibited the growth with this plant extracts in different degree according to the kinds of extracts and the concentrations, the highest activity showed with *Anise* so (18 mm) of inhibition zone against *Aspergillus niger where was the* lowest inhibition zone (8 mm) with *Ginger* against *C. albicanus*. Other fungal of *Candida tropicalis, Penicillium sp. Fusarium sp. Rhizopus sp.* also show inhibition growth in different degree with the ethanol plant extracts. In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many different purposes due to decrease in natural richness and drawbacks. Like in many other countries, the plants known by people with health benefits are picked up and used for the treatment of various diseases in Iraq. In this study, the antimicrobial activity of the extracts from 3 spices and herbs against bacteria and fungi were determined The two test concentration have been utilized (10^{-1}, 10^{-3}) for the determination of antimicrobial activity of various samples, including plant extracts, The antimicrobial activity of the extracts of these spices and herbs was similar effective against bacteria and fungi. They contain chemical substances such as polyphenols, quinines, flavonols/flavonoids,Balkaloids, polypeptides or their oxygen-substituted derivatives . Some of these substances can act synergistically, so their bioactivity is enhanced (Christaki *et al.,* 2012).

5.0 Conclusion

The results obtained from this work showed that plant extracts exhibit antibacterial and antifungal effects. In ethanol extracts of all plant extracts offer effective bioactive compounds for growth inhibition of the bacteria and fungi. Even at low concentrations, these species showed antibacterial and antifungal activity. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antibacterial and antifungal activity.

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