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Study Effects of Plants Extracts on the Growth of Clinical Spices of Bactria and Fungi

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Abstract

Study antimicrobial and antifungal activity of three selected plants (*Rosmarinus officinalis, Anchusa so* and *Eminium spiculatum*). The plants were extracted and dissolved with ethanol, and study the effects of them against growth of some clinical bacterial pathogens isolates like (*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Staphylococcus aureus* and *streptococcus pygenes*) also study the efects of this plants extracts against the growth of some species of Candida like *C. tropicalis, C. albicanus, C. dublicans* and *C. krusei.* The results showed that all plants extracts exhibited prominent 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 Rosmarinus officinalisand the lowest species of bacteria effected and show minimum inhibition zone (9 mm) was Eminium spiculatum against *K. pneumonia*, on the other hand the highs inhabtion zone aginst fungi was recorded with Anchusa so (19 mm) which inhabit growth of *C. krusei* and the minimum inhibition zone (8 mm) show with *Eminium spiculatum* aginst *C. albicanus*

Keywords

Antimicrobial, Plant Extracts, *Rosmarinus officinalis*, *Anchusa so*, *Eminium spiculatum*

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1. Introductions

It is clear that plants extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food [1, 2]. Rosemary (Rosmarinus officinalis L.) originally grows in southern Europe. 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 [3] reported that 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. Studies on the morphology of this species are limited [4, 5]. A taxonomic revision of the genus Anchusa in Greece was performed by [6]. The great diversity of forms exhibited by this heterogeneous genus has generated rather variable interpretations. However, due to the lack of revisions, the identity and taxonomic status of several species were still uncertain [7].

Anchusa L., (Boraginaceae) is one of the major genera of flowering plants, consisting of about 170 taxa native to temperate and subtropical areas of the Old World. The major diversity centre of Anchusa is the southern part of the Balkan Peninsula [7]. The present great form diversity in this heterogeneous genus has generated variable interpretations at both species and generic level, Additionally, some species in

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this genus have been used as folk medicine, Anchusa officinalls L. has been used in folk medicine as a stimulant and as a drug against a number of illnesses, such as heart and lung disease [8].

The plant (Eminium spiculatum) is fairly common in the Mediterranean coastal region [9]. Many plants in this family are poisonous raw and if eaten raw, this toxin gives you sensation as if hundreds of tiny needles are sticking into the mouth tongue etc. However, it is easily destroyed by thoroughly cooking The toxic principles in this plant are glycosides, calcium oxalate crystals packaged into bundles called raphides and proteolytic enzymes recorded the plant contains a nicotinic like substances and triterpenoid. Some studies showed the plant can be used as insecticides antilipoperoxidative activity, antimicrobial activity and anticancer agent [10]. Blood coagulation is another example of a process that must happen quickly when needed, but which has catastrophic effects if it occurs at an inappropriate time or location. Blood coagulation, itself is a complex set of physical, cellular and biochemical events leading to thrombus formation [11]. Thrombus plays an important role in the pathogenesis and progression of atherosclerosis and cardiovascular disease [12, 13].

2. Materials and Methods

2.1. Plant Material

The leaves of Rosmarinus officinalis, Anchusa so and Eminium spiculatum were collected from Alkosh area in Kurdistan region, Iraq 2015,

2.2. Ethanol Extraction

Fresh leaves Achusa so were dried for 10 days at room temperature (25°C). Two plants Fresh Rosmarien officinial and dried Anchus so were successively 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 [14, 15].

2.3. Antibacterial Activity

Clinical isolates of Bacteria strains obtained from Biological Department. The antibacterial activity of the essential oils against Gram positive Bacteria like *Streptococcus pyogenes* and *Staphylococcus aureus* and Gram negative Bacteria like *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were determined with the disc diffusion method National Committee for Clinical Laboratory Standards [16]. A small portion of isolated single colony of each isolates was inoculated into 5 ml of brain heart infusion broth and incubated (o/n) at 37°C. 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 [17]. Fungal strains used Fungi were obtained and an examination of its sensitivity was carried out in Mycology Laboratory in the Biology Department, collage of Sciences, University of Dohuk, Kurdistan region of Iraq and the species were as follows: *C. tropicalis, C. albicanus, C. dublicans* and *C. krusei*

2.4. Preparation 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.

2.5. Antifungal 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. Each set of seeded plates were compared for confirmation. Control test was carried out using 10 mg/ml of fluconazole.

3. Results and Discussion

The antibacterial activity of ethanol plant extracts were tested against Gram positive and gram negative pathogen isolates species including *E. coli, P. aeruginosa, K. pneumonia S. aureus* and *S. pyogenes.* 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).

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

Ethanol plant extracts	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*S. aureus*	*S. pyogenes*
Rosmarinus officinalis	16 mm	12 mm	12 mm	18 mm	15 mm
Anchusa so	14 mm	11 mm	14 mm	16 mm	15 mm
Eminium spiculatum	15 mm	14 mm	13 mm	16 mm	13 mm
amoxicillin clavulanate	5	5	5	5	5
cefazolin sodium	5	5	5	5	5
Ethanol	------	----------	----------	---------	----------

The results are presented in Tables 1 and 2. All plant extracts have potent activity against Gram positive than Gram negative bacteria. In this study, these extracts were screened 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.

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

Ethanol plant extracts	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*S. aureus*	*S. pyogenes*
Rosmarinus officinalis	10 mm	10 mm	11 mm	14 mm	13 mm
Anchusa so	11 mm	8 mm	10 mm	13 mm	12 mm
Eminium spiculatum	13 mm	9 mm	9 mm	11 mm	10 mm
amoxicillin clavulanate	5	5	5	5	5
cefazolin sodium	5	5	5	5	5
Ethanol	------	----------	----------	---------	----------

The plant extracts were found to be prominently active against the tested microorganisms (*S. aureus*, *S. pyogenes, E. coli*, *P. aeruginosa* and *K. pneumonia*) at the concentration of 10^{-1} and 10^{-3}, Generally, Gram-positive bacteria were more susceptible than Gram-negative bacteria Among the tested plant extracts, Rosmarinus officinalis ethanol extracts showed the highest activity (18 mm) of inhibition zone against *S. aureus* where was Anchusa so ethanol extracts showed the minimum inhibition zone against *P. aeruginosa* (8 mm) on the other hand all species of clinical 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 [18].

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

Ethanol plant extracts	*C. tropicalis*	*C. albicanus*	*C. dublicans*	*C. krusei*
Rosmarinus officinalis	15 mm	16 mm	12 mm	14 mm
Anchusa so	13 mm	12 mm	17 mm	19 mm
Eminium spiculatum	12 mm	16 mm	14 mm	15 mm
Nystatin	----------	----------	5 mm	5 mm
70% Ethanol	----------	----------	----------	----------

The results in table 3 and 4 show the activity of ethanol plant extracts against species of candida, generally show all species of candida inhibited the growth with this plant extracts' in different degree according to the kinds of extracts

and the concentrations, the highest activity showed with Anchusa so (19 mm) of inhibition zone aginst *C. krusei* where was the lowest inhibition zone (8 mm) with *Eminium spiculatum* against *C. albicanus*. Other species of candida like *C. tropicalis* and *C. albicanus* 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, The use of some antibiotics is no longer recommended because of the potency of the widespread resistance to them [19]. Thus, these spices and herbs, like many other plants, can be used instead of antibiotics. The activity of some crude extracts used in the study against *P. aeruginosa, K. pneumonia S. aureus, S. pyogenes* and *E. coli* was more to that of amoxicillin clavulanate and cefazolin sodium. Furthermore, the antifungal activity of some of the crude extracts tested was more potent than the standard antifungal nystatin (100 units) against C. albicans, *C. tropicalis*, *C. dublicans,* and *C. krusei*

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

Ethanol plant extracts	*C. tropicalis*	*C. albicanus*	*C. dublicans*	*C. krusei*
Rosmarinus officinalis	11 mm	12 mm	10 mm	11 mm
Anchusa so	11 mm	12 mm	14 mm	12 mm
Eminium spiculatum	12 mm	8 mm	11 mm	13 mm
Nystatin	----------	----------	5 mm	5 mm
70% Ethanol	----------	----------	----------	----------

The plants investigated are known with healing powers, and used for the treatment of various diseases among people. The continuance of this study should include the isolation of the compounds responsible for the antimicrobial activity present in Rosmarinus officinalis, Anchusa so and Eminium spiculatum the plants showing the largest inhibitory activity over the growth of the microorganisms tested.

4. Conclusion

The results obtained from this work showed that plant extracts screened exhibit antibacterial and antifungal effects. In particular, 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 more to that of the commercial antibiotics used as a positive control. 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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