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Evaluation of Antifungal Activity of Plant Extracts of (*Thymus vulgaris*) and (*Cinnamomum)* against fungal

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Abstract
The present study impression concentrations different from extract alcohol some of the medicinal plants thyme (*Thymus vulgaris*) and cinnamon (*Cinnamomum zylanicum*) agent isolation the fungal and which included (*Aspergillus niger*, *Penicillium* ssp, *Fusarium* ssp, *Alternaria* ssp, *Cladosporium* ssp, *Mucor* ssp, *Rhizopus* ssp, *Botrytis* ssp *)* where showed the results if extract thyme give higher inhibition against fungal at concentration 1500 mg/ml *Aspergillus niger*, *Penicillium* ssp, *Fusarium* ssp, *Alternaria* ssp, *Cladosporium* ssp, *Mucor* ssp, *Rhizopus* ssp, *Botrytis* ssp. of 9 mm , 5 mm, 7 mm, 8 mm, 9 mm, 8 mm, 10mm,8 mm on respectively comparative other while was extract cinnamon effected low from extract thyme agents fungal at concentration 1500 mg/ml *Aspergillus niger*, *Penicillium* ssp, *Fusarium* ssp, *Alternaria* ssp, *Cladosporium* ssp, *Mucor* ssp, *Rhizopus* ssp, *Botrytis* ssp. of 11 mm , 8 mm, 9 mm, 9 mm, 10 mm, 9 mm, 11mm,10 mm on respectively . got decrease inhibition against fungal *Aspergillus niger*, *Penicillium* ssp, *Fusarium* ssp, *Alternaria* ssp, *Cladosporium* ssp, *Mucor* ssp, *Rhizopus* ssp, *Botrytis* ssp both extract (thyme and cinnamon) .
Key words. :Plant extracts, fungal, *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Mucor*, *Rhizopus*, *Botrytis*

1.0 IUNTRDUCTION

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 constitute major sources of number of primary and secondary metabolites which are the bioactive compounds of great therapeutic value(Akthar *et al.*, 2014). Herbs and spices have been used since ancient times, not only as antioxidants and flavoring agents, but also for their antimicrobial activity against degradation induced by foodborne pathogens and food spoilage bacteria. Many plants used in traditional medicine represent rich sources of natural bioactive. These plant are rich source of scents and used in food preservation and aromatherapy. These possess multiple antimicrobial i.e., antibacterial (Ozcan *et al*., 2006), antifungal anticancer, antiviral and antioxidant properties against viruses, bacteria and fungi. Some essential oils such as aniseed, calms, camphor,cedar-wood, cinnamon, eucalyptus, geranium, lavender (Upadhyay *et al.*,,2010;Nabavi *et al.*, 2015).

1.1.Thyme(*Thymus vulgaris)*Thymus vulgaris* L. (thyme), locally known"zaatar" or "zaitra', a member of the family Lamiaceae, is widely used in Morocco folk medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminativeand diuretic properties. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants all over the world (Imelouane *et al*., 2009). *Thymus* species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants The extracts of many Thymus species (Lamiaceae family) native to Mediterranean basin are widely used in pharmaceutical, cosmetic and perfume industry, and for flavouring and preservation of several food products .The essential oils of *Thymus* species are rich sources of phenolic monoterpenes such as thymol and carvacrol (El Ouariachi *et al*., 2011). Thymol and carvacrol constituted the main phenolic compound of Thyme oil. The major non-phenolic compounds were linalool and p-cymene been reported that its essential oils possesses numerous biological activities including anti-worm, antiseptic, antispasmodic, antimicrobial and antioxidant. The antifungal nature of thymol is caused by thymol's ability to alter the hyphal morphology and cause hyphal aggregates, resulting in reduced hyphal diameters and lyses of hyphal wall Additionally, thymol is lipophilic, enabling it to interact with the cell membrane of fungus cells, altering cell membrane permeability by permitting the loss of macromolecules(Numpaque *et al.*, 2011;Moghtader,2012; Agili,2014).

2.2.Cinnamon *(Cinnamomum zylanicum*): The genus Cinnamomum (family Lauraceae) contains more than 300 evergreen aromatic trees and shrubs . Four species have great economic importance for their multiple culinary uses as common spices worldwide: *Cinnamon zeylanicum*. Moreover, cinnamon is used in various savory dishes, pickles, soups, and Persian sweets. Cinnamon bark, leaves, flowers and fruits are used to prepare essential oils, which are destined for use in cosmetics or food products. Moreover, according to traditional Chinese medicine (dating roughly 4000 years), cinnamon has been used as aneuroprotective agent and for the treatment of diabetes(Khasnavis *et al.*, 2012; Nabavi *et al.*, 2015). Antimicrobial compounds are chemical or natural components, which have bactericidal effect or growth-inhibitory effect on microorganisms. The essential

oils of aromatic plants are commonly used in food preservation and flavoring ,such as cinnamon (*Cinnamomum zeylanycum*). It is well documented that compounds that have phenolic groups are the most effective; thus plants medicinal have been found to be most effective against foodborne microorganisms. The antimicrobial effect of cinnamon essential oil against various bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus fecalis*, *S. aureus*, *Salmonella* sp., and *Vibrio parahaemolyticus* (Rezaei *et al.*, 2010; Raeisi *et al.*, 2015).

2.0 Material and methods

2.1 Preparation of simple

Leaves plants were collected from local city markets in Amarah city /Iraq (thymus, cinnamon). The plant material was thoroughly washed with clean water to remove soil and other dirt and Mill the leaves in miller for powder and putting powder both plant in tins glassy of time the extraction.

2.2 Preparation of Plant Extracts

The air-dried plant materials were separately extracted twice at room temperature with ethanol 95% (500 ml/100 g of plant material each run). The final ethanol extract of each plant part was filtered using filter paper (Whatman) and was evaporated under vacuum at 40°C using rotary vacuum evaporator resultant residues from the different plants parts and were stored at -20 °C for further analysis (Mahasneh, 2002)..

2.3 Antifungal Test: Activation isolations the fungal include isolations *Aspergillus niger*, *Penicillium* ssp, *Fusarium* ssp, *Alternaria* ssp, *Cladosporium* ssp, *Mucor* ssp, *Rhizopus* ssp, *Botrytis* ssp. 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 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. Study efficiently the inhibition for extraction plants against fungal. The plant extracts were added to PDA (at 45°C) to give a final concentration 250, 500, 750 and 1000,1250,1500 mg/ml for each extract were poured in petri dishes (8cm in diameter). Ethanol was added to medium in control plates. Then, inoculum discs (5 mm in diameter) Ethanol was added to medium in control Tube. from 5 days growing cultures of fungal placed in the center of petri plates containing PDA and extracts.. The plates were incubated in 27°C (Mahboubi and Kazempour, 2013).

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

3. RESULT AND DISCUSION

The antifungal activity of ethanol plant extracts were tested against *Aspergillus niger*, *Penicillium* ssp, *Fusarium* ssp, *Alternaria* ssp, *Cladosporium* ssp, *Mucor* ssp, *Rhizopus* ssp, *Botrytis* ssp. The activities of the extracts were evaluated by measuring the diameter of inhibition zone around the respective discs in the concentrations to give a final concentration 250, 500, 750 and 1000,1250,1500 mg/ml for each extract were poured in petri dishes (8cm in diameter). The results are presented in Tables 1 , 2 plant extract have potent activity against fungal. In this study, these extracts were for their antimicrobial activity against various fungal 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 extracts . 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 constitute major sources of number of primary and secondary metabolites which are the bioactive compounds of great therapeutic value(Akthar *et al.*, 2014). The majority of essential oils are composed of terpenes and terpenoids and other aromatic and aliphatic constituents, all characterized by low molecular weight. Terpenes are the major group of plant natural products characterized by an extensive variety of structural types and the most valuable compounds (Degenhardt *et al.*, 2009). The terpene compounds are hydrocarbons of

general formula (C5H8)n formed from isoprene units. These compounds could be acyclic, monocyclic, bicyclic or tricyclic (Abed, 2007). The *T. vulgaris* essential oils have been found to display different biological properties. Some papers are dedicated to the antimicrobial activity of the essential oil thymus of and of its single constituents. Moreover, the antioxidant property of thyme make its helpful for food safety(Mancini *et al.*, 2015).

Cinnamon has also been used as an health-promoting agent for the treatment of diseases such as inflammation, gastrointestinal disorders and urinary infections .Another potential medical use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents that might act against these resistant microorganisms, and cinnamon could be an interesting candidate (Nabavi *et al.*, 2015). extracted from *C. zeylanicum* demonstrated strong antifungal activity on both the species of Aspergillus. Plant oils are important source of fungitoxic compounds and they may provide a renewable source of useful fungicides that can be utilized in antimycotic drugs against *A. fumigatus* and *A. niger* infection in patients suffering from respiratory diseases. Among the plant oils tested, *Cinnamomum zeylanicum* (Cinnamon).(Uniyal *et al.*, 2012). The main compounds of cinnamon oil transcinnamicaldehyde, cinnmyl cinnamate, andbenzyl cinnamate ,which are responsible for the antimicrobial activity. Cinnamon oil and its main compounds are known to exhibit broad antimicrobial activity. The effects of cinnamon oil on the mycelia growth of *R. nigricans* .Cinnamon oil inhibited the growth of *R. nigricans* (Li *et al.*, 2012).

Table 1. Antifungal activity (inhibition zone/mm) added different concentration from extract thyme against fungal isolates.

concentration extract thyme	*Aspergillus*	*Penicillium*	*Fusarium*	*Alternaria*
250 mg/ml	17mm	12mm	13mm	14mm
500 mg/ml	15mm	11mm	11 mm	13 mm
750 mg/ml	14 mm	9mm	11 mm	12mm
1000 mg/ml	12mm	8mm	9mm	10mm
1250 mg/ml	10 mm	7 mm	9 mm	9 mm
1500 mg/ml	9 mm	5 mm	7 mm	8 mm

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

Table 2. Antifungal activity (inhibition zone/mm) added different concentration from extract thyme against fungal isolates.

concentration extract thyme	*Cladosporium*	*Mucor*	*Rhizopus*	*Botrytis*
250 mg/ml	16mm	15mm	18mm	14mm
500 mg/ml	15 mm	13mm	16 mm	13 mm
750 mg/ml	13mm	12mm	14 mm	12mm
1000 mg/ml	11mm	10mm	13mm	11mm
1250 mg/ml	10 mm	9 mm	11 mm	8 mm
1500 mg/ml	9 mm	8 mm	10 mm	8 mm

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

Table 3. Antifungal activity (inhibition zone/mm) added different concentration from extract cinnamon against fungal isolates.

concentration extract cinnamon	*Aspergillus*	*Penicillium*	*Fusarium*	*Alternaria*
250 mg/ml	19mm	15mm	14mm	16mm
500 mg/ml	17mm	13mm	14 mm	14 mm
750 mg/ml	15 mm	11mm	12 mm	14mm
1000 mg/ml	14mm	10mm	10mm	12mm
1250 mg/ml	13 mm	9 mm	10 mm	10 mm
1500 mg/ml	11 mm	8 mm	9 mm	9 mm

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

Table 4. Antifungal activity (inhibition zone/mm) added different concentration from extract cinnamon against fungal isolates.

concentration extract cinnamon	*Cladosporium*	*Mucor*	*Rhizopus*	*Botrytis*
250 mg/ml	18mm	17mm	19mm	15mm
500 mg/ml	17 mm	16mm	17 mm	15 mm
750 mg/ml	15mm	14mm	15 mm	13mm
1000 mg/ml	14mm	12mm	14mm	12mm
1250 mg/ml	12 mm	11 mm	13 mm	11 mm
1500 mg/ml	10 mm	9 mm	11 mm	10 mm

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

4.0 CONCLUSIONS

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

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