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Antifungal Efficacy of the crude Flavonoid, Terpenoid, and Alkaloid Extracted from *Myrtus communis* L. against *Aspergillus* species isolated from Stored Medicinal plants seeds in the Iraqi Markets

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Abstract

The present study was conducted to investigate the effect of the crude Flavonoid, Terpenoid, and, Alkaloid compounds were extracted from the leaves of (*Myrtus communis* L.) plant against *Aspergillus* species isolated from stored medicinal plants seeds collected from different local markets in the province of Babil 2020 in Iraq. Antifungal activity was achieved in vitro by using food poisoning method against *Aspergillus* species by preparing three concentrations for each crude compound (5, 10, and 20) mg/ml and compared with positive control represented by fungicide Quinoleine 50% and negative control represented by 10% dimethyl sulfoxide. The current study was aimed to control of *Aspergillus* species isolated form stored medicinal plants seeds by using secondary metabolites extracted from leaves of *Myrtus communis* L. The collected data from this study shown that, the crude Flavonoid, Terpenoid, and, Alkaloid compounds extracted from (*Myrtus communis* L.) leaves revealed significant inhibition at P≤ 0.05 in the growth of *Aspergillus* species at 20 mg/ml compared with negative control especially in Flavonoid compounds. Finally, it can be concluded that Flavonoids of *Myrtus communis* L. is most effective in controlling *Aspergillus* species.

Keywords: Antifungal, *Myrtus communis* L., Flavonoids, Terpenoids, Alkaloids.

Introduction

Myrtus communis L. is a perennial aromatic evergreen plant belonging to family *Myrtaceae*. Small tree with height about 1.8-2.4 or shrub, small leaves and bark with deep-fissured. Southern Europe, North of Africa, ad West of Asia are its native habitats. It is also found in South of America, Australia, and North western of Himalaya. It is very common in the regions of Mediterranean. It is often grown in the gardens, particularly northwestern of India regions Because of the beautiful smell of flowers (1). There are one hundred genera and three thousand species in family *Myrtaceae* (2). The fruits are also distinguished by their dark colour and small size (3). The leaves are evergreen with long about 2-5cm It emits an aromatic smell when broken like in a Eucalyptus and myrrh plants (4). For a long time, individual parts of this plant, including as the berries, branches, leaves, and fruits, have been utilized widely as a traditional medicine. Its leaves' astringent, tonic, and antibacterial properties explain its use for wound healing and digestive and urinary system diseases. Because the oil is antimicrobial and anti-catarrhal, it has been used to treat chest problems (5). All over the world *Myrtus communis* L. is a medicinal plant that is widely used in traditional folk medicine. There are many bioactive compounds are extracted and isolated from this plant such as Polyphenols, myrtucommulone (MC), semimyrtucommulone (S-MC), 1,8-cineole, α-pinene, myrtenyl acetate, limonene, linalool and α-terpinolene. Experimental and Clinical research indicates that it has a broader range of pharmacological and therapeutic effects, including peptic ulcer, diarrhea, inflammation, hemorrhoid, skin and pulmonary disorders. In addition to use as antifungal, antibacterial, antiviral, anticancer, antidiabetic, antioxidant, neuroprotective and hepatoprotective (6). *M. communis* contained 1, 8-Cineole 28.62%, α-Pinene 17.8%, Linalool 17.55%, and Geranylacetate 6.3% as the major chemicals substances and Geraniol 1.6%, α-Humulene 1.5%, eugenol 1.3%, isobutyl-isobutyrate 0.8%, and methyl chavicol 0.5% as minor chemicals substances (7). The resistance of pathogenic fungi to antifungal drugs is one of

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the major public health problems. Plant extracts have shown inhibitory effect on the growth of wide range of fungi. They are represented a good alternative for prevention and treatment of fungal diseases (8). From this standpoint, humans should search for natural sources that are less harmful and environmentally friendly in order to control fungi and reduce as much as possible the use of fungicides and pesticides. However, the present study aimed to control of *Aspergillus* species isolated form medicinal plants seeds by using the crude Flavonoid, Terpenoid, and Alkaloid compounds Extracted from leaves of (*Myrtus communis* L.) plant.

Materials and Methods

Plant material collection: Myrtle leaves were collected from gardens at University of Babylon, during October 2020, identified based on the taxonomic features in Iraqi Flora (9). (Table: 1). Leaves of this plants were cleaned, dried, and kept according to (10), Picture 1.

Table 1: Scientific, Local, English name, Family, and active parts

Scientific name	Local name	English name	Family	Active part used
***Myrtus communis* L.**	**Yas**	**Myrtle**	**Myrtaceae**	**leaves**

Picture 1: *Myrtus communis* L.

Extraction of the Crude Flavonoid Compounds: Crude Flavonoid compounds were extracted according to (11).

Extraction of the Crude Terpenoid Compounds: Crude terpenoids compounds were extracted according to (12).

Extraction of the Crude Alkaloid Compounds: Crude Alkaloid compounds were extracted according to (13). Stock solution of 200 mg/ml for Flavonoid, Terpenoid, and Alkaloid, were dissolved in (10% Dimethyl Sulfoxide DMSO) then purified from microorganisms by using Millipore filter papers (0.22µm) and kept at (-20C˚) pending usage (14).

Medicinal plants Seed's collection: Medicinal plants seeds were collected from different regions of local markets in the province of Babil/ Hillah City 2020 in Iraq.

Isolation and diagnosis of *Aspergillus* species: To isolate the *Aspergillus* fungus 100 seeds were taken randomly from each of the collected samples. It was sterilized using 2% sodium chlorate for 2 minutes and then washed with sterile distilled water twice to remove traces of sterile material and dried with sterile filter paper. It was transferred with sterile forceps to 9 cm petri-dishes containing (20 ml) of pre-prepared (Potato dextrose agar P.D.A) with antibiotic chloramphenicol (50 mg / l) to prevent bacterial growth (15), by 5 seeds per dish

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and three replicates per sample and then incubated in 25C˚ for 5-7 days. The fungi associated with the seeds were then purified by secondary cultures for identification. Isolated fungi were then diagnosed based on the taxonomic keys of both (16, 17). The fungal isolates were kept in clean, sterile glass containers containing the Nutrient Agar. Containers were incubated at 25C˚ for a week and then placed in the refrigerator at 4° C until it was used.

Antifungal activity assay of extract: PDA medium was prepared and autoclaved after that a known volume (2ml) of the each plant extracts is placed in the center of the petri plates and complete the volume to 20ml with PDA medium to obtain the required final concentrations (5, 10, and 20 mg/ml) of the medicinal plants. After the medium had completely solidified, a 5 mm disc of a seven-day-old culture of the test fungus was aseptically placed in the center of the Petri plates and incubated at 25±2C° for seven days, while 0.02ml of antibiotic solution was added to each assay petri plate to check for bacterial growth contamination, as suggested by the (18). Fungicide Quinoleine 50% was used as positive control and dimethyl sulfoxide as negative control. On the seventh day, observations were taken. The diameter of the colony was measured in millimeters. As a control, PDA media with no extract was used. Three duplicates of each treatment were kept. Using the formula, the fungal toxicity of extracts was estimated in terms of percent inhibition of mycelia growth (19).

Inhibition% = (dc-dt/dc)*100
Where:
dc = Average increase in fungal growth in control.
dt = Average increase in fungal growth in treatment.

Statistical analysis: Three replicates were used to determine all treatment data. An analysis of variance was done on the data using the SPSS 16.0 program, with a completely randomized design and the least significant difference (L.S.D) set at P≤ 0.05.

Results

The antifungal activity's results of the crude Flavonoids compounds extracted from the leaves of (*Myrtus communis* L.) against *Aspergillus* species isolated from stored medicinal plants seeds are presented in (table 2). The antifungal activity of Flavonoids secondary metabolites with three concentration (5, 10, and 20 mg/ml) was screened by food poisoning methods. The results revealed that, the crude Flavonoids compounds extracted from the leaves of (*Myrtus communis* L.) exhibited significant decrease at P≤ 0.05 in the growth of *Aspergillus* species. Antifungal activity was applied at (5, 10, and 20) mg/ml. mycelial inhibition ranging from (28% in 5 mg/ml, 67.33% in 10 mg/ ml, and 85% in 20 mg/ml) (Figure: 2) compared with negative control and positive fungicide Quinoleine 50% control where inhibition percentage was (0.00% for negative control and 100% for positive control). In the same context, the crude Terpenoids compounds showed 17% mycelial inhibition at (5 mg/ml) and 53.666% at (10 mg/ml), and 61.333% at (20 mg/ml) concentration, Thus, it differed significantly compared to the control treatment (table 3).

Table 2: An antifungal activity of the crude Flavonoid compounds extracted from leaves of (*Myrtus communis* L.) against *Aspergillus* species isolated from stored medicinal plants seeds

Concentrations (mg/ml)	Flavonoid compounds
	Inhibition percentage %
Negative Control	0± 0.00
5 mg/ml	28± 2.00
10 mg/ml	67.33± 2.51
20 mg/ml	85± 2.00
Positive Control	100± 0.00
L.S. D	3.080

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*Mean± standard deviation

Figure2: An antifungal activity of the crude Flavonoid compounds at 20 mg/ml against *Aspergillus* species

Table 3: An antifungal activity of the crude Terpenoid compounds extracted from leaves of (*Myrtus communis* L.) against *Aspergillus* species isolated from stored medicinal plants seeds

Concentrations (mg/ml)	Terpenoid compounds
	Inhibition percentage %
Negative Control	0± 0.00
5 mg/ml	17.666± 2.08
10 mg/ml	53.666± 2.08
20 mg/ml	61.333± 1.52
Positive Control	100± 0.00
L.S. D	2.698
*Mean± standard deviation	

Table 4: An antifungal activity of the crude Alkaloid compounds extracted from leaves of (*Myrtus communis* L.) against *Aspergillus* species isolated from stored medicinal plants seeds

Concentrations (mg/ml)	Alkaloids compounds
	Inhibition percentage %
Negative Control	0± 0.00
5 mg/ml	6± 1.00
10 mg/ml	10± 1.00
20 mg/ml	14± .00
Positive Control	100± 0.00
L.S. D	1.992
*Mean± standard deviation	

On the other hand, although there are significant differences at three concentrations (5, 10, and 20 mg/ml) compared with negative control against *Aspergillus* species isolated from stored medicinal plants seeds (Table 4). The crude Alkaloids compounds were less in activity in comparison with other secondary metabolites extracted from the *Myrtus communis* L. leaves. Finally, the highest percentage of inhibition (85%) was recorded at 20 mg/ml of Flavonoids. While the maximum percentage of the reduction in the crude Alkaloid compounds was reached up to (14%) at 20 mg/ml concentration and the maximum percentage of reduction in the crude Terpenoid

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compounds was reached up to (61.333%) at 20 mg/ml concentration.

Disscusion

The present study was proved that, the secondary metabolites include Flavonoids, Terpenoids, and Alkaloids extracted from the leaves of the *Myrtus Communis* L. have antifungal activity against *Aspergillus* species isolated from stored medicinal plants seeds especially Flavonoid compounds. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example, Phenolic, terpenoids, and alkaloids are bioactive chemicals derived from a variety of medicinal plants, including (*Lactuca serriola* L., *Lepidium sativum* L., *Myrtus Communis* L., *Cassia senna* L., *Ricinus communis* L., *Cassia didymobotrya* (Fresenius) Irwin & Barneby, *Melia azedarach* L., *Dianthus caryophyllus* L., and *Salvia hispanica* L.) have antibacterial efficacy against different pathogenic microorganisms (20,21,22,23,24,25,26,27,28). (29) Were used primitive plant like *Chlorella vulgaris* as antibacterial. (30) Was used *Curcuma longa* L. and *Boswellia carteri* Birdwood against *Fusarium* species isolated from maize seeds. *M. communis* has been reported to have antifungal activity against many species such as *C. albicans* (31), *Aspergillus flavus* (32), *Aspergillus fumigatus* and *Paecilomyces variotii* (33), *Aspergillus niger*, *Penicillium* sp, anthropophilic and geophilic dermatophytes *Trichophyton mentagrophytes* (34). The phenolic extracts and essential oils of *M. communis* have antifungal agent against fungi that are pathogenic to humans such as *Aspergillus fumigatus* and *Candida albicans* (35). Essential oil of *M. communis* may replace antifungal drugs in the treatment of fungal infections of the skin, mucous membranes and fight against dandruff (36). On the other hands, the mode of the antifungal action of the Alkaloids is usually pleiotropic, where protein synthesis is inhibited, and the fungal (Deoxyribonucleic acid DNA) is intercalated or by enhancing the development of fungi inhibitors (37). Terpenoids reduced the mitochondrial content, thus modified the level of (Reactive oxygen species, R.O.S) and (Adenosine triphosphate ATP) generation. It is also described that triterpenoid has more potent antifungal efficacy as compared to the tetraterpenoid (38). Terpenoids and flavonoids make their effects by disruption of microbial membranes (39). Flavonoids frequently reduce fungal growing with several principal mechanisms, including plasma membrane disturbance, the stimulation of mitochondrial dysfunction, and inhibiting the following: cell wall creation, cell division, Ribonucleic acid and protein synthesis, and the efflux mediated pumping system (40). Medicinal plants possessed antifungal effects by many mechanisms, they caused membrane disturbance leading to the loss of membrane integrity, inhibited Deoxyribonucleic acid transcription and reduced the cell populations, inhibited the activity of fungal antioxidant enzymes, and restrained fungal biofilm formation (41, 42). Finally, antifungal activity of *Myrtus Communis* L. might be belonging to secondary metabolites like Flavonoids, Terpenoids, and Alkaloids and their effect in proteins, RNA, and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

Conclusion

Flavonoid compounds of *Myrtus Communis* L. leaves have powerful antifungal activity against *Aspergillus* species.

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الفعالية التضادية الفطرية للفلافونويدات والتربينات والقلويدات الخام المستخلصة من نبات الياس ضد الاسبرجلس المعزول من بذور النباتات الطبية المخزونة بالاسواق العراقية

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الملخص

أجريت الدراسة الحالية للكشف عن تأثير المركبات الفلافونويدية والتربينية والقلويدية الخام المستخلصة من اوراق نبات الياس ضد انواع الاسبرجلس المعزولة من بذور النباتات الطبية المخزونة والمجموعة من الاسواق المحلية المختلفة في محافظة بابل في عام 2020 في العراق. الفعالية التضادية الفطرية تم انجازها خارج الجسم الحي بأستخدام طريقة التسمم الغذائي ضد انواع الاسبرجلس وذلك بتحضير ثلاث تراكيز من المواد الفعالة الخام (5و10و20) ملغم/مل ومقارنتها بمعاملة السيطرة الموجبة المتمثلة بالمبيد الفطري (كينولين 50%) ومعاملة السيطرة السالبة المتمثلة ب(داي مثيل سلفواوكساييد 10%). هدفت الدراسة الحالية للسيطرة على انواع الاسبرجلس المعزولة من بذور النباتات الطبية المخزونة عن طريق استخدام مركبات الأيض الثانوية المستخلصة من اوراق نبات الياس. اظهرت النتائج المستحصلة من هذة الدراسة ان المركبات الفلافونويدية والتربينية والقلويدية الخام والمستخلصة من اوراق نبات الياس لها تثبيط معنوي تحت مستوى إحتمالية (0.05) في نمو انواع الاسبرجلس في التركيز (20ملغم/مل) مقارنةً بعينة السيطرة السالبة وخصوصا المركبات الفلافونويدية الخام. وفي الختام فأن المركبات الفلافونويدية الخام الاكثر تأثيرا و فعالية في السيطرة على نمو انواع الاسبرجلس.

الكلمات المفتاحية: الفعالية التضادية الفطرية، نبات الياس، الفلافونويدات، التربينات، القلويدات.