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Original Article

Antioxidant and Antimicrobial Properties of Some Wild Aizoaceae Species Growing in Egyptian Desert

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Abstract

Plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as a product of photosynthesis. *M. nodiflorum* extract showed strong scavenging activity (IC_{50} < 1 mg/ml), while extracts of *M. crystallinum*, *A. canariense* and *M. forsskaolii* exhibited moderate scavenging activity (IC_{50} ≥ 1 and ≤ 2 mg/ml). The four plant extracts expressed various inhibitory activities against the tested bacterial strains. The methanolic extract of *M. nodiflorum* showed the broad spectrum against the tested bacteria except *E. coli*. While the extracts of *M. crystallinum* were potent activity against *Klebsiella pneumonia* only. *A. canariense* extract showed the different inhibitory activities against the tested bacterial strains except *E. coli* and *S. aureus*, while *M. forsskaolii* extract showed the inhibitory activities against *K. pneumonia* and *S. pyogenes*. The antifungal activity of methanolic extracts of the investigated four plant species were tested against the tested fungi. The *A. canariense* extract showed the highest activity against *A. fumigatus* (1.97 %), while the *M. nodiflorum* extract exhibited the lowest inhibitory activity against *A. fumigatus* (0.7%). In case of *M. forsskaolii* extract showed only the inhibition effect against *C. albicans* (0.93%), while *A. nigar* and *Mucor* spp were not affected by plant extracts.

1. Introduction

Antioxidants are compounds that protect our cells from damage by free radicals. They are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Free radicals are unstable molecules that can damage our cells. Our bodies synthesize them when we digest food or breath in pollution. These radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Percival, 1998). This cell damage may increase our risk for cancer, heart disease, cataracts, diabetes, or infections. Free radicals may also affect brain function (Kovacic and Jacintho, 2001; Ridnour *et al.*, 2005).

The ability to utilize oxygen has provided humans

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with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals."

The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve for the development of novel drugs (Huang *et al.,* 2004). There is a great interest in the search for new antimicrobial drugs from nature, because of the resistance that pathogenic build against antibiotics (Al-Fatimi *et al.,* 2007). In the world medicinal plants are used for antifungal, antibacterial and antiviral activities. These plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, and gastrointestinal disorders (Caceres *et al.,* 1990).

Plant-based drugs have been used worldwide in traditional medicines for the treatment of various diseases. Approximately 60% of world's population still relies on medicinal plants for their primary healthcare. Currently a large and ever-expanding global population base prefers the use of natural products in treating and preventing medical problems. This has influenced many of pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs. Herbs are natural plants and therefore often considered harmless compared with western medicines (Hsieh *et al.,* 2008). Plant species still serve as a rich source of many novel biologically active compounds. However, very few plant species have been thoroughly investigated for their medicinal properties. Thus, there is renewing interest in phytomedicine during last decade and nowadays many medicinal plant species are being screened for pharmacological activities (Gautam *et al.,* 2007).

In the present study, four plants from Aizoaceae family were screened for their antibacterial and antioxidant activities. The species *Aizoon canariense, Mesembryanthemum crystallinum, M. forsskaolii* and *M. nodiflorum.* In Egypt it is represented by 9 species included in 8 genera, all species are considered succulent but cover a wide range of life styles from pebble-like leaf succulents to small succulent shrubs. Distinctive seed capsules are valuable for identifying species (Boulos, 1999).

The species *Mesembryanthemum* is traditionally used as a medicinal plant. The leaves contain flavonoids (rutin, neohesperidin, hyperoside), catechin, ferulic acid and catechol tannins (Van der Watt and Pretorius, 2001). According to Roberts (1995) the leaf juice is also effective in soothing itching caused by spider and tick bites. The leaves also contain an astringent antiseptic juice which can be taken orally for treating sore throat and mouth infections (Rood, 1994). Reports have shown that genus *Mesembryanthemum* has long been used as food and in traditional medicine for the treatment of liver diseases and diabetes (Al-Faris *et al.,* 2010; Falleh *et al.,* 2011).

In the present paper, we investigate the potential antioxidant and antibacterial properties of both plants collected from coastal and inland desert of Egypt, in order to evaluate their medicinal potentiality and their future industrial uses.

2. Materials and methods

2.1. Plant material and preparation of the extract

Four plants *A. canariense, M. crystallinum, M. forsskaolii* and *M. nodiflorum* were collected from coastal desert (Deltaic Mediterranean coast) and inland desert (Cairo-Suez desert road) at the period of April 2014. The identification of the four species was done according to Täckholm (1974) and Boulos (1999).

The plants under investigation were dried at room temperature (20-23 ºC) and ground into a powder using a blender. The dried plant powder (100 g) was extracted with 80 % methanol (400 ml) by refluxing for 3 hours. The solution was filtered and evaporated to dryness. A stock solution of extract was prepared in dimethyl sulfoxide (DMSO) and kept at -20 ºC for future use (Mehraban *et al.,* 2005).

2.2. Phytochemical analysis

Studied species were collected and prepared as previously mentioned. The amount of phenol was determined spectrophotometrically (Sadasivam and Manickam, 2008). Tannins were determined using the method of Van Burden and Robinson (1969). Saponin content was estimated according to Obdoni and Ochuko (2001). Flavonoid content was determination according to Bohm and Kocipai-Abyazan (1994), while alkaloid was determined according to Harborne (1973).

2.3. DPPH free radical scavenging activity

Antioxidant activity was determined by using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH (Lim and Quah, 2007) as follows: two milliliters of 0.15 mM DPPH was added to 1 ml of various plant extracts (about 20 g of powdered samples were extracted with 200 ml of methanol 50 %) in different concentrations. A control was prepared by adding 2 ml of DPPH to 1 ml solvent (methanol 50 %). The mixture was incubated in the room temperature for 30 min; absorbance was recorded at 517 nm. The antioxidant activity was expressed as:

$$\% \text{ Radical scavenging activity} = [1-(A_{sample}/A_{control})] \times 100$$

2.4. Antibacterial assay

Bacillus subtilis, *E. coli, K. pneumonia, S. aureus* and *S. pyogenes* are bacterial strains employed in the screening. Filter paper discs (5 mm in diameter) are prepared before use and sterilized in an autoclave for 20-30 min. A sterile paper disc is taken by sterile forceps, wetted in the solution of the studied antibiotic and then placed over the surface of the inoculated nutrient agar in antibacterial assay (Cappuccino and Sherman, 2008). All Petridishes were incubated at 37 ºC for 24 h. After incubation, the diameter of inhibition zone (cm) was measured for recording the clear zone.

2.5. Antifungal assay

The plant extracts were screened for the presence of antifungal bioactivity using different species of fungi such as: *Candida albicans*, *Aspergillus niger, Aspergillus fumigatus* and *Mucor* spp. Filter paper discs (5 mm in diameter) are prepared before use and sterilized in an autoclave for 20-30 minutes. A sterile paper disc is taken by sterile forceps, wetted in the solution of the studied antibiotic and then placed over the surface of the inoculated potato dextrose agar in antifungal assay described by Cappuccino and Sherman (2008). Culture plates were incubated at 28ºC for 3-4 days.

3. Results

3.1. Phytochemical analysis

The phytochemical analysis of selected species (*A. canariense, M. nodiflorum, M. crystallinum* and *M. forsskaolii*) indicated that almost all plants are rich in secondary compounds (Table 1). *M. nodiflorum* exhibited the highest content of saponins and alkaloids. *M. forsskaolii* showed highest contents of both tannins and phenolics. *A. canariense* exhibited the highest content of flavonoids, whereas *M. crystallinum* showed the highest of tannins.

3.2. Antioxidant Activity

The evaluation of the antioxidant activity between the four plant extracts is shown in Figure 1. By increasing the plant extract concentration there was a corresponding continuous increase in scavenging activity. In case of A. *canariense, M. nodiflorum, M. crystallinum* and *M. forsskaolii* extracts the increase was up to 500

Table 1. The concentration of the active constituents in mg/g dry weight for the selected plant species.

Bioactive constituent	Tannins	Saponins	Flavonoids	Alkaloids	Phenolics
	mg/g dry weight				
A. canariense	10.23±0.06	5.50±0.02	6.30±0.03	3.33±0.05	4.91±0.05
M. crystallinum	9.44±0.05	4.19±0.05	6.10±0.04	3.82±0.05	4.19±0.05
M. forsskaolii	14.47±0.07	4.73±0.04	5.13±0.05	4.17±0.05	5.31±0.05
M. nodiflorum	13.97±0.05	5.76±0.04	5.56±0.04	4.51±0.05	4.55±0.05

μg/ml where the scavenging activity valves were 10.89%, 12.59%, 9.59% and 5.93, respectively.

The IC_{50} values ranged from 1.66 to 4.41 mg/ml (Figure 2), where the extract of *M. nodiflorum* showed the highest antioxidant activity with IC_{50} value of 1.66 mg/ml. The lowest antioxidant activity was observed for the extract of *M. forsskaolii* with IC_{50} value of 4.41 mg/ml. The extracts of *M. crystallinum* and A. *canariense* were attained IC_{50} values of 2.13 and 2.24 mg/ml respectively. Also we used a natural antioxidant catechol as standard for the determination of the antioxidant activity, it was attained the IC_{50} value of 0.15 mg/ml.

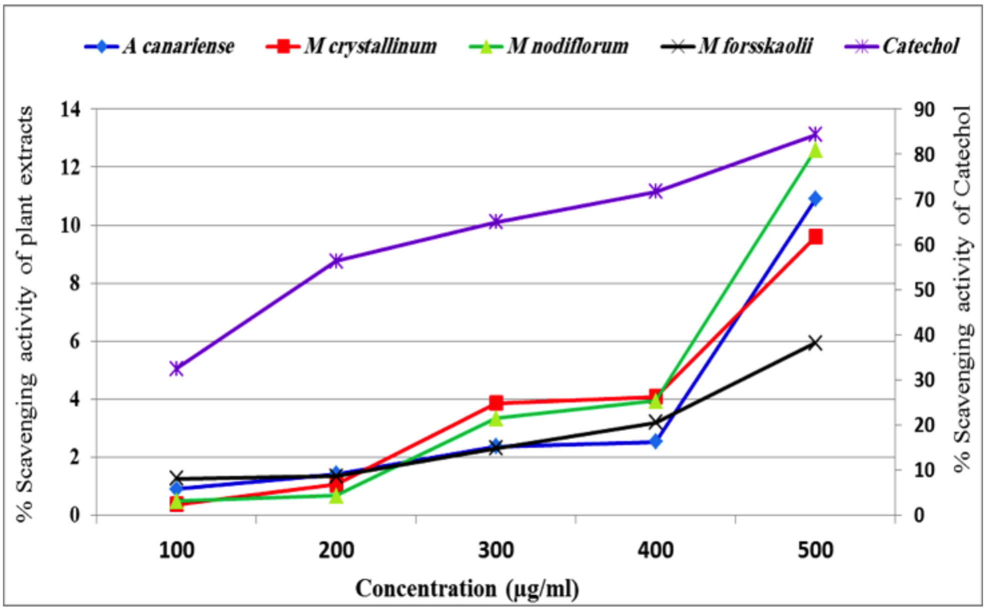

Fig. 1. Scavenging activity of methanolic extract of selected plant species and natural antioxidant catechol.

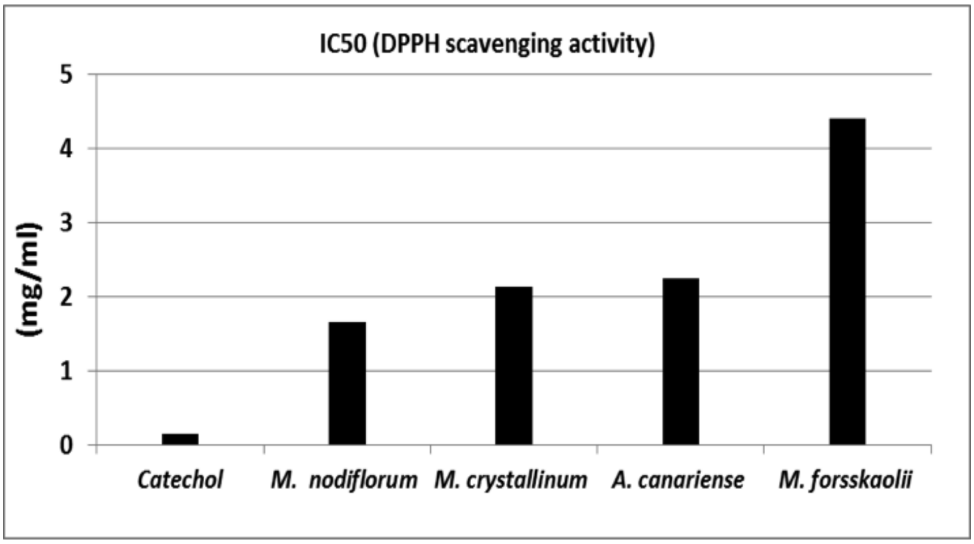

Fig. 2. The IC_{50} of methanolic extract of selected plant species and natural antioxidant catechol.

3.3. Antibacterial assay

The four plant extracts exhibited different inhibitory activities against the tested bacterial strains with different degrees as demonstrated by measuring the diameters of inhibition zones developed by the extracts (Table 2). The extract of *M. nodiflorum* showed the broad spectrum against the tested bacteria. While the extracts of *M. crystallinum* expressed an activity against *K. pneumonia* only. *A. canariense* extract showed the different inhibitory activities against the tested bacterial strains except *E. coli* and *S. aureus*, while *M. forsskaolii* extract showed the inhibitory activities against *K. pneumonia* and *S. aureus*. Also, the antibacterial spectrum of selected plant species attained highest value (80%) in *M. nodiflorum* and low (20%) in *M. crystallinum* (Figure 3).

3.4. Antifungal Assay

The antifungal activity of methanolic extracts of the investigated four plant species against the tested fungi showed different degrees of inhibition zones (Table 3). *A. canariense* extract showed the highest activity against *A. fumigatus* (1.97 %) only, while the *M. nodiflorum* extract exhibited the lowest inhibitory activity against Aspergillus fumigatus (0.7%). In case of *M. forsskaolii* extract showed only the inhibition effect against *C. albicans* (0.93%). Also, the antifungal spectrum of selected plant species is narrow and ranged from 0.0 to 20% (Figure 3).

Table 2. The inhibitory activity of the plant extract against the tested bacteria as demonstrated by diameters of the inhibition zone (cm)* .

Plant extract	**Tested bacteria**				
	B. subtilis	***K. pneumonia***	***E. coli***	***S. pyogenes.***	***S. aureus***
A. canariense	2.1	0.5	-	0.6	-
M. crystallinum	-	3	-	-	-
M. forsskaolii	-	3	-	3.03	-
M. nodiflorum	0.7	2.23	-	0.6	0.7

* The recorded value is mean value of 3 replicates.

Table 3. The inhibitory activity of the plant extract against the tested fungi as demonstrated by diameters of the inhibition zone (cm)*

Plant extract	**Tested fungi**			
	A. fumigatus	***A. Nigar***	***C. albicans***	***Mucor* spp**
A. canariense	1.97	-	-	-
M. crystallinum	-	-	-	-
M. forsskaolii	-	-	0.93	-
M. nodiflorum	0.7	-	-	-

* The recorded value is mean value of 3 replicates .

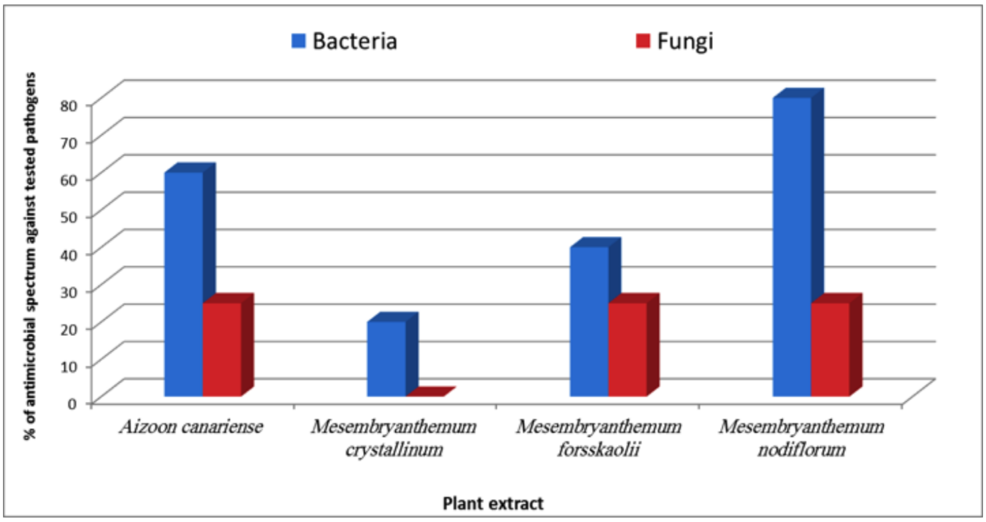

Fig. 3. % of antimicrobial spectrum of selected plant extracts.

4. Discussion

Plants produce various antioxidative, enzymes and non-enzymes, compounds to counteract and detoxify these reactive oxygen species (ROS) in order to survive. Hence, naturally occurring phytocompounds possessing antioxidative and anti-inflammatory properties appear to contribute to their chemopreventive or chemoprotective activity (Chiang *et al.,* 2004) which in turn, has been used to the benefit of human beings.

Most living organisms possess enzymatic and non-enzymatic defense systems against excessive production of ROS. However, different external factors (smoke, diet, alcohol and some drugs) and aging decrease the efficiency of such protecting systems, resulting in disturbances of the redox equilibrium established under healthy conditions (Leutner *et al.,* 2001).

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free organic radical stable at room temperature which produces a violet solution in ethanol has been widely used in the determination of antioxidant activity of single compound as well as of different plant extracts (Mensor *et al.,* 2001; Katalinic *et al.,* 2006). The DPPH free radical scavenging activity is based on the reduction of DPPH in an alcohol solution by an antioxidant, donor of hydrogen. The radical scavenging activity on DPPH was expressed as IC_{50}. This value was the concentration of the extract required to inhibit 50% of the initial DPPH free radicals.

According to Al-Ismail *et al.* (2007) who classified the scavenging activity of DPPH free radicals according to IC_{50} values as follows: group (A) showed strong scavenging capacity ($IC_{50} < 1$ mg/ml), group (B) showed moderate scavenging capacity ($IC_{50} \geq 1$ and ≤ 2 mg/ml) and group (C) showed weak scavenging capacity ($IC_{50} > 2$ mg/ml). Hence, *M. nodiflorum* extract showed strong scavenging activity ($IC_{50} < 1$ mg/ml), while extracts of *M. crystallinum, A. canariense* and *M. forsskaolii* showed moderate scavenging activity ($IC_{50} \geq 1$ and ≤ 2 mg/ml).

Drugs derived from natural products are usually secondary metabolites and their derivatives must be pure and highly characterized compounds (John, 1994). Man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Rios and Recio, 2005). Virtually all cultures around the globe have relied historically and continue to rely on medicinal plants for primary health care. There is currently a worldwide upsurge in the use of herbal preparations and the active ingredients isolated from medicinal plants in health care (Jassim and Naji, 2003). Long before, mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles (Rios and Recio, 2005).

The four plant extracts exhibited different inhibitory activities against the tested bacterial strains. The methanolic extract of *M. nodiflorum* showed the broad spectrum against the tested bacteria. Mohammed *et al.* (2012) determined similar results and found that the methanol extract of *M. crystallinum* was active against same tested bacterial strain. While the extracts of *M. crystallinum* showed an activity against *K. pneumonia* only. *A. canariense* extract showed the different inhibitory activities against the tested bacterial strains except *E. coli* and *S. aureus*, while *M. forsskaolii* extract showed the inhibitory activities against *K. pneumonia* and *S. aureus*. These results were comparable to those of *M. crystallinum* and *Carpobrotus edulis* as investigated by Bouftira *et al.* (2012) and Mohammed *et al.* (2012).

Mothana *et al.* (2008) determined similar results and found that the methanol extract of *Carthamus tinctorius* and *Centaurea pseudosinaica* were active against same tested bacterial strain and El-Amier (2010) in *Ethulia conyzoides*. These results were comparable to those of *Ocimum sanctum, Origanum majorana, Cinnamomum zeylanicum* and *Xanthoxylum armatum* as investigated by Joshi *et al.* (2009) and Iranbakhsh *et al.* (2010) in *Datura stramonium* (*E. coli, B. subtilis* and *S. pyogenes*).

The antifungal activity of methanolic extracts of the investigated four plant species against the tested fungi. The *A. canariense* extract showed the highest activity against *A. fumigatus* (1.97 %) only, while the *M. nodiflorum* extract exhibited the lowest inhibitory activity against *A. fumigatus* (0.7%). In case of *M. forsskaolii* extract showed only the inhibition effect against *Candida albicans* (0.93%). Mohammed *et al.* (2012) determined similar results in *M. crystallinum* (*A. fumigatus* and *C. albicans*) and Omoruyi *et al.* (2014) in *M. edule* (*C. albicans*).

Abdel-Sattar *et al.* (2008) determined similar results and found that the methanol extract of *Achillea biebersteinii* and *Vernonia schimperii* were active against same tested fungi strain, Sitapha *et al.* (2013) in *Terminalia ivorensis* and Al-Mughrabi (2003) in *Euphorbia macroclada* as well as Poornima and Palanisamy (2013) in *Acalypha indica* and *Tridax procumbens*.

We concluded that, the present results suggest that the various plant species tested can be used as antibacterial and antifungal agents.

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الملخص العربى

خصائص مضادات الأكسدة والجراثيم لبعض النباتات البرية من العائلة الثلجية النامية فى صحراء مصر

ياسـر أحمـد الأمـير سامية على هارون
عمر عبد السميع الشهابى عدى نعمه الحديثى

قسم النبات - كلية العلوم - جامعة المنصورة - المنصورة - مصر

تعتبرالنباتات من المصادر المحتملة لمضادات الاكسدة الطبيعية حيث انها تمتص اشعة الشمس وتقوم بتوليد مستويات عالية من الاكسجين مثل المركبات الثانوية في التمثيل الضوئي. وقد أظهر مستخلص نبات الغسول *M. nodiflorum* أقوى قيمة للنشاط المضاد للأكسدة ، في حين أظهرت النباتات الاخرى *M. forsskaolii* *A. canariense* and *M. crystallinum* نشاط معتدل في قيمة النشاط للأكسدة . وأظهرت المستخلصات الأربعة نشاط مثبط بمدى مختلف ضد السلالات البكتيرية المختارة ،حيث أظهر مستخلص نبات *M. nodiflorum* مدى واسع الطيف ضد بكتريا المختارة باستثناء بكتريا *E. coli* في حين كان مستخلص نبات *M. crystallinum*, له نشاط قوي ضد بكتريا *K. pneumonia* ومستخلص نبات *A. canariense* أظهر نشاط متباين ضد البكتريا المختارة ماعدا *E. coli* and *S. aureus* . في حين أظهر مستخلص نبات *M. forsskaolii* نشاط مثبط ضد *k. pneumonia* و *S. au-reus* . قد تم اجراء اختبار نشاط المستخلصات كمثبط ضد اربع انواع من الفطريات في المختبر والتي هي (*Mucor* spp, *C. albicans A. fumigatus, A. Nigar*) وقد أظهر مستخلص نبات *A. canariense* أعلى نشاط ضد *A. fumigatus* بنسبة (1.97%) في حين أظهر مستخلص نبات *M. nodiflorum* ادنى نشاط ضد *A. fumigatus* بنسبة .(0.7%) اما مستخلص نبات *M. forsskaolii* قد أظهر نشاط مثبط ضد *C. albicans* ,فقط بنسبة (0.93%) اما الفطريات الاخرى فلم تتأثر باي مستخلص من المستخلصات النباتية المختارة للدراسة.

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Antioxidant and Antimicrobial Properties of Some Wild Aizoaceae Species Growing in Egyptian Desert

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