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ANTIMICROBIAL EFFECT OF SOME PLANT SEEDS EXTRACTS

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ABSTRACT

A large number of plants have therapeutic potentials. Of these plants black cumin, *Nigella sativa L*. (*Ranunculacea*e); onion, *Allium cepa L*. (*Alliaceae*) and leek , *Allium porrum L*. (*Alliaceae*) were examined in this investigation in form of methanolic and aqueous extracts. Two different concentrations were prepared and examined for each plant extract. Eight microoraganisms were examined in this investigation. Two strains of Gram positive bacteria(G+) , *Bacillus subtilis* and *Micrococcus luteus* ; two strains of Gram negative bacteria(G-), *Salmonella typhi* and *Eschrichia coli* ; two strains of yeasts, *Candida lipolytica* and *Saccharomyces cerevisae* and two fungal strains, *Aspergillus niger* and *Penicillium natatum*. Results of chemical analyses proved that all methanolic extracts of three examined plants contained terpenes, flavonoids, saponins and alkaloids except the methanolic extract of black cumin contained tannins . Furthermore, the extracts of the tested plants seeds were found to contain some minerals such as Zn,Fe,Mn,Na,K,Ca,Mg and P. Results of microbiological examination exhibited that methanolic extracts either in 0.05% or in 0.1% was more effective than the aqueous extract for all the tested microbial strains.

Keywords: Methanolic and aqueous extracts,onion,leek,black cumin, Gram negative bacteria, Gram positive bacteria, fungi, yeasts .

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INTRODUCTION

Most essential oils consist of mixtures of compounds such as phenolics , polyphenols, terpenoides, saponins, quinines, esters, flavones, flavonoids, tannins, alkaloids and nonvolatile residues and their chemical composition and concentration of compounds are variable. These components have many effects as antimicrobial, stimulating animal digestive systems, antioxidants, anticoccidial, increase production of digestive enzymes and improve utilization of digestive products by enhancing liver functions (Hernandez *et al*., 2009).

There have been some studies on the antifungal activity of plant extracts (Wilson *et al*., 1997), inhibitory effects of aqueous extracts of garlic and onion (Shams *et al*., 2003), antimicrobial effects of garlic, ginger and lime (Onyeagba *et al*., 2004), antibacterial and antifungal activity of senecio (Loizze *et al*., 2004), antimicrobial activity of garlic and onion extracts (Elnima *et al*., 1983) and effects of aromatic plants essential oils, lime and garlic skin on birds intestinal bacteria (Davis *et al*., 1994).

Plant extracts represent a rich potential source of alternative and environmentally acceptable control agents for infectious organisms due to their antimicrobial properties. Plants possess essential oils, which could be utilized for killing microorganisms. (Ahmad *et al*., 2011).

Aspergillus sp. are the most common fungal species which are able to produce mycotoxins in food and feedstuffs. Mycotoxins are known to be potent hepatocarcinogens in animals and humans. The presence and growth of fungi may cause spoilage and result in a reduction in quality and quantity of foods (Rasooli and Abyaneh, 2004). Natural plant extracts may provide an alternative chemical preservatives. Over the years much effort has been devoted to the search for new antifungal materials from natural sources for food preservation (Boyraz and Ozcan, 2005; Haciseferogullari *et al*., 2005) .

Allium genus has over 500 members, each differing in maturing, color and taste, but with similar biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess anti-bacterial and anti-fungal activities (Reyhan and Mihriban ,2007).

The aim of this study is to evaluate some plant seeds (black cumin, onion and leek) and their aqueous and methanolic extracts via Phytochemical screening, total polyphenols and flavonoids determination ,element determination such as (Zn,Fe,Mn,Na,K,Ca,Mg,P) and antimicrobial activites against eight different microorganisms.

MATERIALS AND METHODS

Experimental plant seeds :

Seeds used in this study were black cumin (*Nigella sativa*), family Ranunculaceae , onion (*Allium cepa*), family Alliaceae, Egyptian leek (*Allium ampeloprasum var.egypticaum*), family Alliaceae.These seeds were purchased from local market in Mansoura city ,Dakahlia governorate,Egypt.

Phytochemical screening :

Terpenes, tannins, flavonoids ,saponins, resins and alkaloids were detected in methanolic extract sample according to the method described by Harborne (1988) .

Extraction with methanol (methanolic extraction) :

The method of Lis-Balchin *et al*. (1998) was carried out as follows: Each powdered seeds sample was extracted by soaking in methanol at ratio of 1:1 (w/v) for 48 h .The extract was filtered through cheese cloth under a strong hand pressure and the solvent was removed under vaccum at 60-65˚C to give a crude methanolic extract using a rotary evaporator .The crude extract was kept in a refrigerator until use and serial concentration were made up .

Extraction with water(aqueous extract) :

The extraction technique was carried out according to Wilson *et al*. (1997) with slight modification .Dried plant seeds were ground into fine powder.The powder of plant seeds were soaked in distilled water at the ratio of 1:1 (w/v)and kept for 20-24 h at room temperature (25± 3 ˚C). Then ,the mixture was filtered through cheese cloth under a strong hand pressure .The resulted solution was kept in a refrigerator at 5 ˚C until using .

Minerals content :

Ash of each plant seeds sample was individually dissolved in 1ml. of conc. HCL solution and the volume were completed to 100ml with distilled water. Sodium(Na) and potassium(K) were determined according to Hesse (1971) using flame photometer (Bwb-Xp20090018). Magnesium(Mg), calcium(Ca), zinc(Zn), manganese(Mn) and iron(Fe) were determined using atomic absorption (Perkin-Elmer 2380) according to Cottenie *et al.* (1982). Phosphorous(P) was colorimetrically determined as described by Page (1982).

Determination of total flavonoids :

Total flavonoids content of seeds were quantitatively determined colorimetrically using aluminum chloride as described by Chang *et al.*, (2002). Absorbance was measured at 415nm against distilled water as blank, using a Spekol 11(Carl Zesis-Jean). Quercetin was chosen as a standard of flavonoids to make the standard curve (0–50mg/l). The concentration of total flavonoids contents was expressed as mg. quercetin equivalent (QE)/g based on dry weight.

Determination of total polyphenols :

Total polyphenols were determined according to Lin and Tang (2007).The absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena). For quantitatively determination, a standard curve of gallic acid (0-200mg/l) was prepared with the same manner. Total phenol contents were expressed as mg. gallic acid equivalent (GAE)/g based on dry weight.

Microorganisms investigated :

Eight microbial strains which used in this investigation were obtained from Microbiol.Dept., Faculty of Agric., Damietta University.These strains were four bacterial strains two of them were G + the first was long spore forming bacteria namely *Bacillus subtilis* and the second was coccoid shaped bacteria namely *Micrococcus luteus* .The other two strains were G- short rod bacteria namely *Salmonella typhi* and *Escherichia coli* . Two fungal strains which were *Aspergillus niger* and *Penicillium notatum* and two yeast strains called *Candida lipolytica* and *Saccharomyces cerevisiae* .These microbial strains were chosen for their economic importance and human health.

Assessment of antimicrobial activities:

The plate diffusion methods were used here, holes with a cork borer were punched in a cultivation medium seeded with a standard inoculum of 0.5 ml cells suspension in case of bacteria and yeasts or 1.0 ml of spores suspension of fungi of young microbial cultures using vortex mixer (No. 602550, Taiwan) under aspectic conditions. These cultivation media were nutrient agar (NA) or potato dextrose agar (PDA) in case of bacterial cells and yeasts or fungi, respectively. A concentrated 0.1 ml of the tested natural extract was put into the hole, left one hr. to allow diffusion, then incubated at appropriate temperature and period of time. At the end of incubation period, the diameter of inhibition zones (DIZ) of microbial growth were measured and recorded (Bagamboula *et al*.,2003).

RESULTS AND DISCUSSION

I. Phytochemical screening of different plant methanolic extracts :

Crude methanolic extracts of the investigated plant seeds were subjected to Phytochemical screening (Table 1).Results showed that black cumin contained terpenes, tannins, flavonoids, saponins and alkaloids . However onion seeds and leek seeds contained the same components as black cumin except tannins.

Table 1. Preliminary phytochemical screening of crude methanolic extracts of the tested plant seeds .

Examined component	Methanolic extracts of plant seeds		
	Black cumin	Onion	Leek
Terpenes	+	+	+
Tannins	+	-	-
Flavonoids	+	+	+
Saponins	+	+	+
Resins	-	-	-
Alkaloids	+	+	+

II. Total polyphenols and total flavonoids content of investigated seeds:

Total polyphenols include several classes of phenolic compounds that are secondary plant metabolites and integral part of human and animal diets. Flavonoids are large group of the phenolic compounds consisting mainly of flavonols, flavanols and anthocyanins. Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damage by lipid peroxides and scavenging or neutralizing free radicals (Sroka and Cisowski, 2003).

It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular diseases (Cai *et al.,* 2004).

Table 2 showed the total polyphenols determind as mg gallic acid equavalent/g (mgGAE/g) and total flavonoids determined as mg quercetin equavalent/g (mgQE/g) contents of black cumin, onion and leek seeds . Data in Table 2 illustrated that these seed samples contained average values of 288.88, 170.00 and 143.33 mgGAE/g on dry weight basis for total polyphenols, respectively. It was clear that black cumin seeds had the highest content of total polyphenols (288.88 mgGAE/g). While, leek seeds had the lowest values (143.33 mgGAE/g) .

Total flavonoids as shown in Table (2) ranged from 53.57 to 4.28 mgQE/g on dry weight basis for black cumin seeds, onion seeds and leek seeds respectively. It can be observed that black cumin seeds contained the highest amount of total flavonoids. Whereas, onion seed came in the second level at a value of 14.42 mgQE/g .Finally, leek seed contained 4.28 mgQE/g as the lowest total flavonoids content .

Table 2.Total polyphenols and total flavonoids content of investigated plant seeds samples .

Constituents / Plant seeds	Total Polyphenols (mgGAE/g)	Total Flavonoids (mgQE/g)
Black cumin seeds	288.88	53.57
Onion seeds	170.00	14.42
Leek seeds	143.33	4.28

III. Elements content of investigated plant seeds samples :

Data illustrated in Table 3 cleared that potassium, calcium and phosphorus were the main elements in 2 samples (onion seed and leek seed) which ranged from 1431 to 984, 215 to 156 and 251 to 172 ppm on dry weight basis , respectively .

On the other hand black cumin seed have the highest amounts of iron, sodium and calcium which were 102 , 111 and 869 ppm on dry weight basis , respectively . Data in Table 3 clearly showed that black cumin seeds, onion seeds and leek seeds contain some elements in considerable amounts. These elements are sodium, potassium, calcium, magnesium, phosphorus, zinc, iron and manganese. Concentrations of these elements are found in Table3.

Table 3.Elements content (ppm) of investigated plant seeds samples .

element	Investigated plant seeds		
	Black cumin	Onion	Leek
Zn	61	1.505	1.035
Fe	101.85	1.875	1.235
Mn	0.905	1.235	0.845
Na	111.45	27.85	17.6
K	199.75	1430.55	984.4
Ca	869.45	214.65	155.7
Mg	48.3	94.55	59.45
P	32.35	251.1	172.35

IV. Evaluation of antimicrobial activities of the obtained extracts

The antimicrobial activities of the obtained extracts of the tested plant seeds were evaluated against eight microorganisms .These activities were assessed by the presence or absence of inhibition zones and obtained diameter in which no growth observed were measured after incubation at 37^{o}C/24h, 30^{o}C/48h or 28^{o}C/7 days for bacteria, yeasts or fungi, respectively.

Applying the tested seeds extracts of black cumin *(Nigella sativa*) against eight microbial strains was carried out and obtained results were recorded . Results listed in Fig.1 show the effect of black cumin in form of methanolic or aqueous extracts on the microbial strains either Gram positive or Gram negative bacteria, yeasts and fungal strains .Results illustrated that methanolic extracts either in 0.05% or in 0.1% was more effective than the water extract on all the tested microbial strains .

Data showed that *Bacillus subtilis*, Gram positive (G+) long spore forming bacteria was more resistant than *Micrococcus luteus*, (G+) spherical

cells with the methanolic extracts .On the other hand, the aqueous extract gave the same effect on the two bacterial strains that equal to 3.2mm as illustrated in Fig.1.

For Gram negative (G-) bacterial strains ,results proved that *Salmonella typhi* was more resistant than *E. coli* towards all the tested extracts as can be seen in the same Fig.1. Furthermore, the aqueous extract gave the same trend since *Salmonella typhi* inhibited by zone diameter equal to 3.0mm while 4.3mm diameter was observed for *E. coli* as shown in Fig.1 . The higher resistance of G- bacteria to external agents has been earlier documented. It is attributed to the presence of lipopolysaccharides in their outer membranes as reported by Suresh *et al*.,(2010). In case of yeast strains results proved the superiority of methanolic extracts over the aqueous extract as shown in the same Figure.

Candida lipolytica was more resistant than *Saccharomyces cerevisiae* since the inhibition zones were higher with the second strain than the first one. In addition, the aqueous extract gave the same trend since *Candida lipolytica* inhibited by zone diameter equal to 3.2mm while *Saccharomyces cerevisiae* affected by 5.2mm as shown in Fig.1.

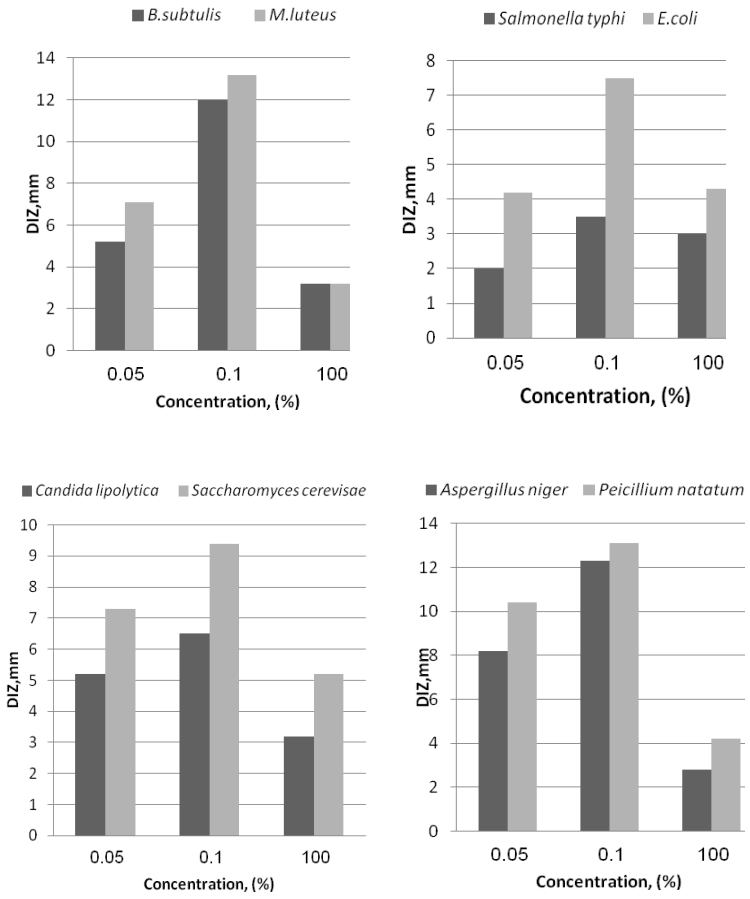
Regarding the fungal strains, *Penicillium notatum* showed to be more sensitive than *Aspergillus niger* towards all the tested extracts as illustrated in Fig.1. Addilionally, the same trend was obtained with the aqueous extract since *Aspergillus niger* was affected by 2.8 mm as a diameter of inhibition zone while 4.2mm while was found in case of *Penicillium notatum* as shown in Fig.1.

The seeds of black cumin contain tannins, which can be extracted by methanol (Eloff, 1998). A number of studies have reported antimicrobial properties of tannins (Scalbert, 1991).

Applying the tested extract of onion seeds(*Allium cepa* seeds) against eight microbial strains was also carried out and obtained results were recorded.Results listed in Fig.2 showed the effect of onion seeds in form of methanolic or aqueous extract on the microbial strains,either Gram positive (G+) or Gram negative(G-) bacteria, yeasts or fungal strains. Results illustrated that methanolic extracts either in 0.05% or in 0.1% was more effective than aqueous extract on all the tested microbial strains .

Data showed that *Bacillus subtilis*,G + long spore forming bacteria was more resistant than *Micrococcus luteus* ,G + spherical cells with the methanolic extracts .On the other hand, the aqueous extract gave negative effect on the two bacterial strains equal to zero as illustrated in Fig.2 .

For G- bacterial strains, results proved that *Salmonella typhi* was more resistant than *E. coli* towards all tested extracts as can be seen in the same Figure .The negative effect was also found by the aqueous extract against the two tested G- bacterial strains as shown in Fig.2 .



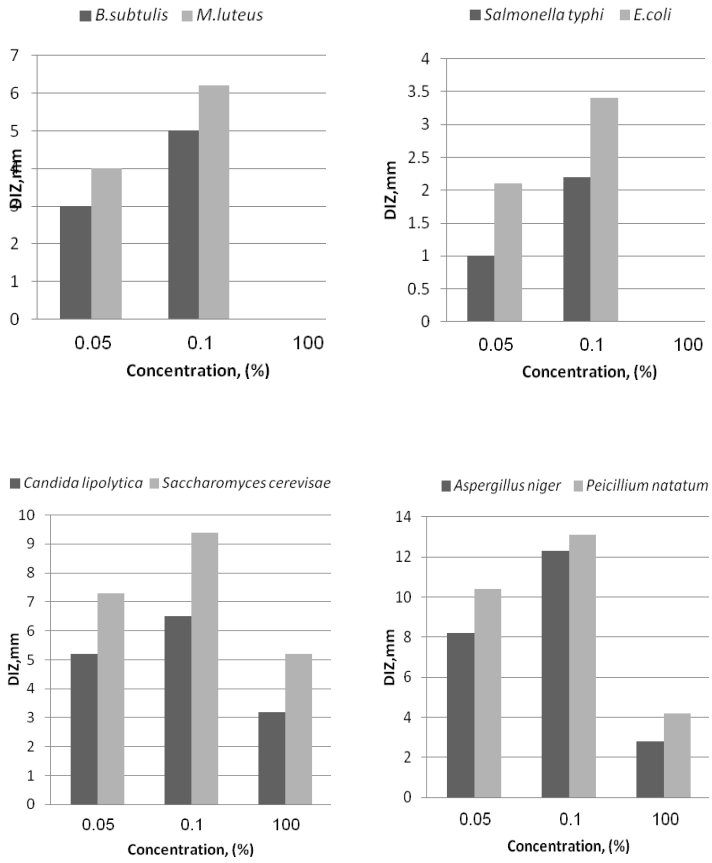
Concentrations: methanolic extract (0.05,0.1%) and aqueous extract (100%).

Fig.1. Values of inhibition zones of microbial growth as a result of treatment by black cumin seeds extracts.

In case of yeast strains, results proved the superiority of methanolic extracts over the aqueous one as shown in the same Figure .

Candida lipolytica was more resistant than *Saccharomyces cerevisiae* since the inhibition zones were higher with the second strain than the first one. In addition, the two tested yeast strains gave the same behaviour towards the aqueous extract expressed in diameter of inhibition zones that equal to 1.0mm for both strains as can be seen in Fig.2 .

Regarding the fungal strains, *Penicillium notatum* showed to be more sensitive than *Aspergillus niger* towards all tested extracts as illustrated in Fig.2 . Furthermore, the aqueous extract of onion seeds gave the same effect against the two tested fungal strains equal to1.0mm as shown in Fig.2.



Concentrations: methanolic extract (0.05,0.1%) and aqueous extract (100%).

Fig.2. Values of inhibition zones of microbial growth as a result of treatment by onion seeds extracts.

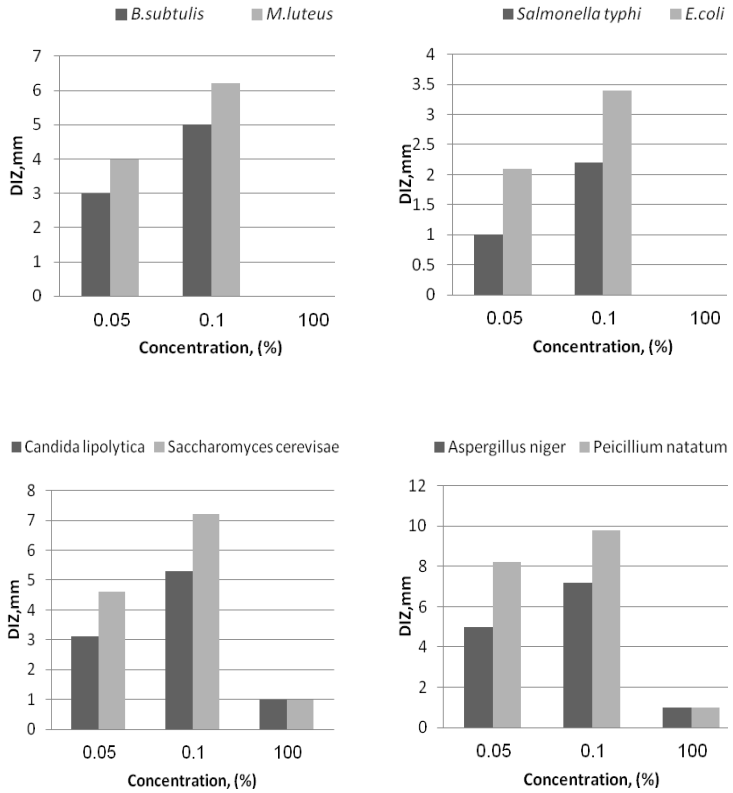
The inhibition of onion extracts may be contributed to the presence of tannins, flavonoids, as reported by Vamshi *et al*.,(2010).

Using the tested extracts of leek seeds *(Allium porrum* seeds) against eight microbial strains was carried out and obtained results were also recorded. Results listed in Fig.3 . showed the effect of leek seeds extracts in form of methanolic or aqueous extract on the microbial strains either Gram positive or Gram negative bacteria, yeasts or fungal strains. Results illustrated that the methanolic extracts either in 0.05% or in 0.1% was more effective than the aqueous extract on all the tested microbial strains .

Data in Fig.3 showed that *Bacillus subtilis*,G + long spore forming bacteria was more resistant than *Micrococcus luteus* ,G + spherical cells with the methanolic extracts .On the other hand, the aqueous extract gave negative effect on the two tested bacterial strains equal to zero as illustrated

in Fig.3 .With respect to G- bacterial strains, results proved that *Salmonella typhi* was more resistant than *E. coli* towards all tested extracts as can be seen in the same Figure . Again, the aqueous extract gave negative effect against the two tested G- bacterial strains as shown in Fig .3.Generally ,the antimicrobial activity would be active against G + than G - bacteria as decided by McCutcheon *et. al.,* (1992).

In case of yeast strains results proved the superiority of methanolic extracts over the aqueous extract as shown in in the same Figure .
Candida Lipolytica was more resistant than *Saccharomyces cerevisiae* since the inhibition zones were higher with the second strain than the first one. In addition, the aqueous extract gave similar effect against the two tested yeast strains that equal to 1.0mm as diameter of growth inhibition as can be seen in Fig.3 .



Concentrations: methanolic extract (0.05,0.1%) and aqueous extract (100%).

Fig.3. Values of inhibition zones of microbial growth as a result of treatment by leek seeds extracts.

Regarding the fungal strains, *Penicillium notatum* showed to be more sensitive than *Aspergillus niger* towards all the tested extracts as illustrated in the same Figure . Furthermore, similar effect was found by using the aqueous extract giving 1.2mm as diameter of inhibition growth zone against the two tested fungal strains as shown in Fig.3 .

As a result, increase interest is being shown in developing alternative methods for microbial contamination control to reduce or elimimate reliance on synthetic chemicals. One of this such method involves the use of plant derived products such as plant extracts that have antimicrobial effect .

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التأثير المضاد للميكروبات لبعض مستخلصات البذور النباتية

رمضان أحمد حسن, نظمي صبحي عريان, أحمد محمد يوسف و بسام أحمد زايد
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هناك أعداد كبيرة من النباتات لها تأثير مضاد للكائنات الحية الدقيقة. من هذه النباتات بذور حبة البركة, بذور البصل, بذور الكرات و التي تم دراستها في هذا البحث من خلال المستخلص المائي و المستخلص الكحولي لهذه النباتات . و قد تم عمل تركيزات مختلفة من كل نبات لدراسة تأثيرها علي الكائنات الحية الدقيقة .

في هذا البحث تم اختبار ٨ ميكروبات, ٢ سلالة بكتيرية موجبة لصبغة جرام و هي *Bacillus subtilis* و *Micrococcus luteus* , ٢ سلالة بكتيرية سالبة لصبغة جرام و هي *Salmonella typhi* و *E. coli* , ٢ سلالة فطر و هي *Aspergillus niger* و *Penicillium notatum*, ٢ سلالة خميرة و هي: *Candida lipolytica* و *Saccharomyces cerevisiae* .

و قد اظهرت النتائج ان المستخلص الكحولي لبذور الثلاث نباتات يحتوي علي تربينات ,فلافونيدات ,سابونينات و قلويدات بالاضافة الي ان مستخلص حبة البركة يحتوي علي التانينات . علاوة علي ذلك فان مستخلص هذه البذور يحتوي علي بعض المعادن مثل الزنك ,المنجنيز ,الصوديوم ,الكالسيوم ,المغنسيوم و الفسفور . كما اظهرت النتائج ان المستخلص الميثانولي بتركيز ٠,٠٥% او ٠,٠١% كان له تاثير مضاد علي الكائنات الحية الدقيقة اقوي من المستخلص المائي علي كل سلالات الكائنات الحية الدقيقة المستخدمة في هذا البحث .

قام بتحكيم البحث

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أ.د / حسين عبد الله الفضالى	**كلية الزراعة – جامعة المنصورة بدمياط**