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Evaluation of the antimicrobial (antibacterial and antifungal) activity of ethanolic extracts of some medical plants.

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Abstract
The aim of the study was to evaluate the antimicrobial activity ethanolic extracts of Nigella sativa (N.sativa), Zingiber officinal (Ginger), and Trigonella foenum graecum (Fenugreek), in three concentration (50mg/ml, 100mg/ml, and 200mg/ml).The antimicrobial activities have been evaluated against two gram positive bacteria: Staphylococcus aureus, Streptococcus sp., and two gram negative bacteria: Escherichia coli, Proteus sp. And two pathogenic fungi: Candida albicans, Sacromysis sp.
Ethanolic extract of Ginger showed the maximum antimicrobial against gram positive bacteria and Candida albicans fungi while Fenugreek extract give the potent effect against gram negative bacteria and Sacromysis fungi. On the other hand N. sativa extract showed the minimum antimicrobial effects. The results indicate the efficacy of the plants as a potent antimicrobial agents.
Key words: Antibacterial, Antifungal, Ethanolic extract, Nigella sativa, Ginger, Fenugreek.

Introduction:
The microbial infection represent a critical problem to health and they are the major causes of morbidity and mortality of developing country (**1**). Antimicrobial agents are available for the treatment and management of infectious diseases **(2).** In order to overcome the effects of chemical drugs, the World Health Organization have advised researchers to investigate possible use of natural products (**3**). A large number of antimicrobial agents derived from traditional medicinal plants are available for treating various diseases caused by microorganisms (**4**) . In today world, medicinal plants are gaining attention owing to the fact that herbal drugs are cost-effective, easily available and with little or no side effects (**5**). Henriqu *et al* ., **(6)** reported that some plant extracts and phytochemicals are known to have antimicrobial properties, and can be great significance in therapeutic treatments. Numerous medicinal plants have been focused by human being for the treatment of different diseases **(7)**. Nigella sativa (N. sativa) family Ranunculaceae is a widely used medicinal plant throughout the world to fight antimicrobial diseases **(8)**. Also Zingiber officinal roscoe (Ginger) belong the family Zingiberaceae is one of the most widely used species in the world for the treatment of cold, fever, headache, digestive problem and reported to have antibacterial activity **(9, 10)** . while Trigonella foenum graecum (Fenugreek) belong the family Fabaceae is one of the oldest medicinal plants and has a long history of medical uses **(11).** The present study has been designed to assess the antimicrobial activity of ethanolic extract of three medical plants (N. sativa, Ginger, and Fenugreek) in three different concentration (50 mg/ml, 100 mg/ml, and 200 mg/ml) against most common pathogenic bacteria and fungi (Staphylococcus aureus, streptococcus sp. , Escherichia coli, Proteus sp., Candida albicans, and Sacromysis sp.).

Materials and Methods :
Plant materials: Seeds of the N. sativa , Fenugreek and Giger rhizome were purchased from the local herbs store in Wasit city, Iraq. Botanical identification was performed at the department of pharmacology and Toxicology, medicine college, Wasit university, Iraq.
Extraction of plants material: Ethanolic extract of medical plants according to Saeidi, S. *et al*., **(12)** and Karuppiah, P. and Rajaram , S. **(2)** . Plants were properly dried and pulverized into a coarse powder. Each of 20 gram grainded powders was soaked in 60 ml of ethanol (95%) separately for one day (shaken occasionally with a shaker). After one day of the dissolving process, materials were filtered with (Whatman no.1 filter paper). Then the filtrates were evaporated using a rotary evaporator. Dried extracts were obtained and then stored at 4 ^{0C} in air tight screw – cap tube. Preparation of dilution of crude extracts at different concentration (50 mg/ml, 100 mg/ml , and 200 mg/ml) for antimicrobial assay.
Determination of antimicrobial activity: the antimicrobial activity of the plants extract tested on six different strains, two gram positive bacteria namely: *S.aureus* and *Streptococcus sp*. ; two strains of gram negative bacteria including : *E.coli* , *Proteus sp.* And two strain of pathogenic fungi: *Candida albicans* , *Sacromysis sp*. All cultures Were obtained from the laboratory of microbiology – Al- Karama hospital, KUT, iraq. Antimicrobial assay was performed by Agar well diffusion method. Muller- Hinton medium for antibacterial and Sabarouid Dextrose Agar medium (SDA) were used for antifungal activity. Preparation of culture media according to Sayanthi P. and Lalitha, P. **(13)** . Microbial cell suspension of about 1.5 * 10 ^{6} CFU/ml obtained from a McFarland turbidity Standard number 0.5 was used. A plates were swabbed with microorganisms strain

and kept for 15 minutes for adsorption. Well were made aseptically (three in the surface for the different plant extract concentration and one centrally for negative control ethanol -95%). Of each agar plate with a diameter 5mm. (with exception of plates that were used for antibiotic or antifungal which is used as positive control).; 0.1 ml of each concentration of each extract was poured into the wells (at the surface) beside 0.1ml ethanol 95% (centrally) considered as negative control. All the plates were incubated at 37^{0C} for 24-48 hours. Antibacterial and antifungal potential of extracts were assessed in term of zone of inhibition. The antibacterial activities of extracts were comparable with that of standard antibiotics: Ampicillin, Ceftriaxan, Ciprofloxacin, Chloramphenicol, and Impanem. While the antifungal activities were comparable with standard antifungal drugs: Nystatin and Amphotricin B.

Statistical Analysis:

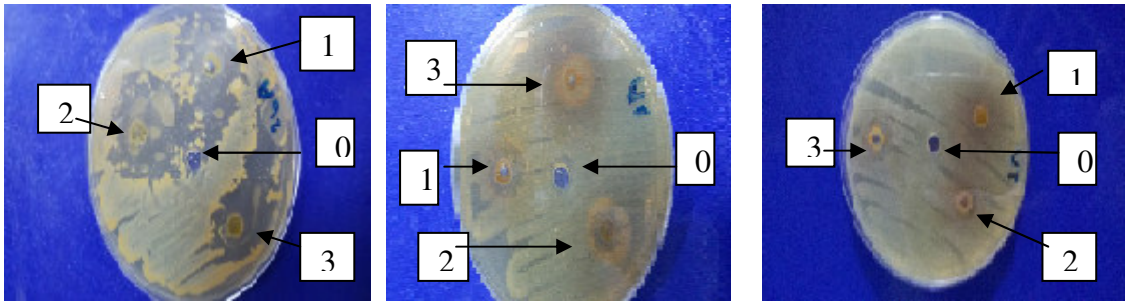
The results are presented as mean $\pm$ standard error. One way ANOVA test with Least Significant differences (LSD) at significant level of ($P \leq 0.01$).

Results:

Present study investigate the antibacterial and antifungal effects of ethanolic plants extract of N. sativa, Ginger, and Fenugreek. The all three extracts exhibited insignificant degree of inhibitory activity against most of six tested micro-organisms when compared with the standard antibiotic and negative control , are presented in Table **(1), (2)** and **(3).**

Antibacterial and antifungal activity increased linearly with increase in concentration of crude extracts (mg/ml).Ginger rhizomes ethanolic extract showed highest antifungal activity against *Candida albicans* with diameter zone of inhibition (3.24mm), followed by Fenugreek extract with mean zone of inhibition (1.08mm) (Figure: **2**, **3**). On the other hand Fenugreek extract recorded the potent antifungal activity against *Sacromysis* fungi with mean zone of inhibition (2.62mm), followed by Ginger ethanolic extract with mean zone of inhibition (0.82mm) (Figure: **2**, **3**). While N. sativa ethanolic extract recorded the least zone of inhibition against both fungi when compared with other extracts (Figure: **1**).Ginger ethanolic extract demonstrated antibacterial activity against four clinical pathogenic bacteria with zone of growth inhibition ranging from (1.78mm) to (2.98mm) (Figure: **2**). The maximum zone of inhibition was showed against gram positive bacteria with potent antibacterial activity. While Fenugreek ethanolic extract recorded the potent antibacterial activity against gram negative bacteria with mean zone of inhibition (2.48mm) against *E. coli* and (1.5mm) against *Proteus* sp. Bacteria (Figure: **3**).Ethanolic extract of N. sativa also recorded the minimum diameter zone of inhibition against all tested bacteria among other extracts (Figure: **1**).

Ethanol 95% as negative control , standard antibiotic drugs (Ampicillin, Ceftriaxan, Ciprofloxacin, Chloramphenicol, and Impanem) as positive control for tested bacteria (Table:**4)** and Nystatin , Amphotricin B as positive control in case of pathogenic fungi were used (Table: **5**).



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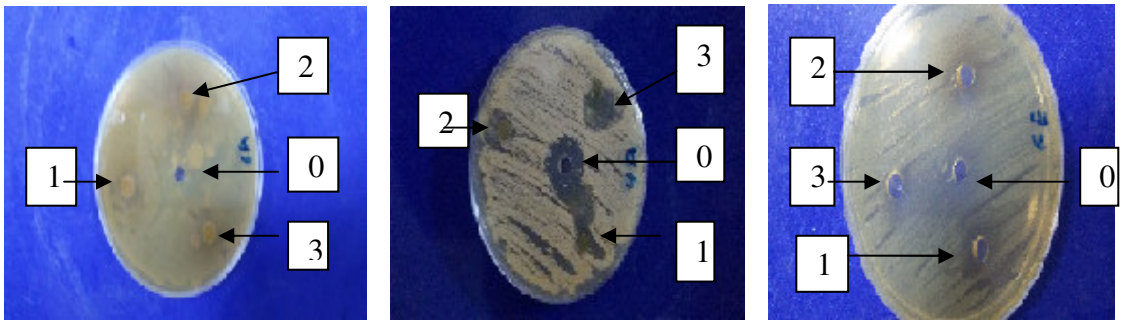
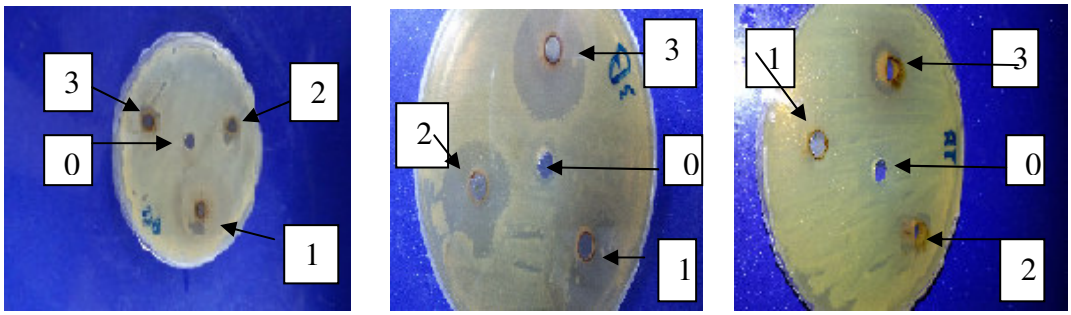


D E F

Figure (1): Effect of N. sativa extract on tested pathogenic bacteria at the different concentration:0,1,2 and 3 (negative control, 50, 100 and 200 mg/ml). **A:**effect on the *S. aureus*, **B:** effect on the *Streptococcus*, **C:** effect on the *E. coli* , **D:** effect on the *proteus,* **E:** effect on C. albicans fungi , and **F:** effect on Sacromysis fungi.



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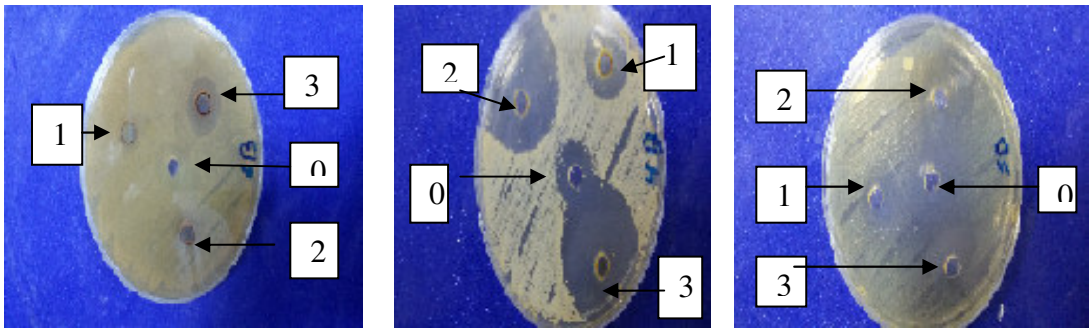

J K L

Figure (2): Effect of Ginger. extract on tested pathogenic bacteria at the different concentration:0,1,2 and 3 (negative control, 50, 100 and 200 mg/ml). **G:**effect on the *S. aureus*, **H:** effect on the *Streptococcus*, **I:** effect on the *E. coli* , **J:** effect on the *proteus,* **K:** effect on C. albicans fungi , and **L:** effect on Sacromysis fungi.

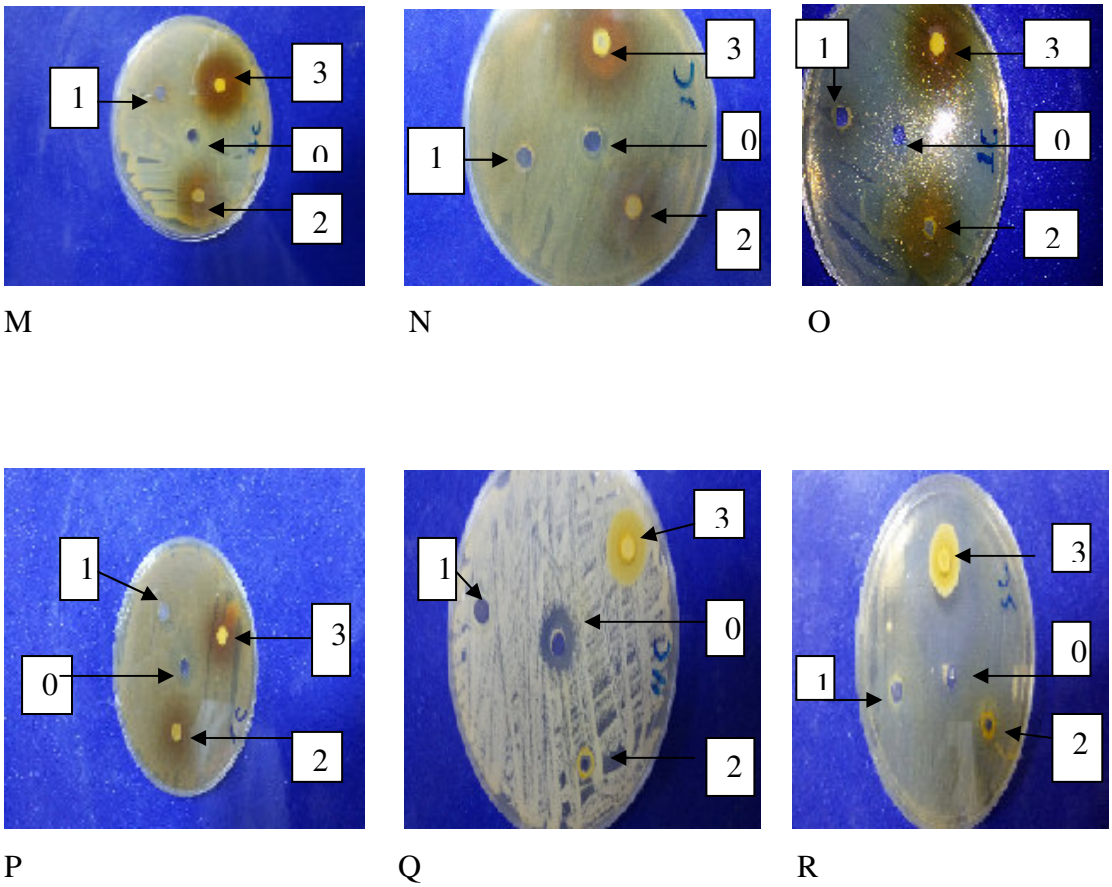

Figure (3): Effect of Fenugreek. extract on tested pathogenic bacteria at the different concentration:0,1,2 and 3 (negative control, 50, 100 and 200 mg/ml). **M:**effect on the *S. aureus*, **N:** effect on the *Streptococcus*, **O:** effect on the *E. coli* , **P:** effect on the *proteus,* **Q:** effect on C. albicans fungi , and **R:** effect on Sacromysis fungi.

Table(1): Antibacterial activity of ethanolic plant extract of N. sativa against gram positive , gram negative bacteria and fungi.

Plant extr. / bacteria	Negative cont.	N. sativa 50mg/ml	N.sativa 100mg/ml	N. sativa 200mg/ml
S. aureus	0.32± 0.1 a	0.6 ± 0.08 a	0.92± 0.14 a	1.9± 0.17 a
Streptococcus sp	0.44 ± 0.06 a	1.12 ± 0.08 a	1.46± 0.09 a	2.28± 0.08 a
E. coli	0. 24± 0.04 a	1.0 ± 0.15 a	1.28 ± 0.15 a	1.62± 0.16 a

Proteus sp.	0.38 ± 0.06 a	0.62± 0.1 a	1.14± 0.05 a	1.52± 0.04 a
C. albicans	1.46 ±02 a	0.62 ±0.06 a	0.84 ±0.11 a	1.18 ±0.17 a
Sacromysis sp.	1.1 ±0.19 a	0.56 ±0.11 a	0.66 ±0.08 a	1.04 ±0.13 a

-The value represent zone of inhibition (mm) Mean ±Standard Error
-The different small letters (horizontally) show significant effect while the same small letters show insignificant effect between different groups.

Table(2): Antibacterial activity of ethanolic plant extract of Ginger against gram positive , gram negative bacteria and fungi .

Plant extr. / bacteria	Negative cont.	Gin. 50mg/ml	Gin. 100mg/ml	Gin. 200mg/ml
S.aureus	0.56± 0.1 a	1.48 ± 0.08 a	1.86± 0.05 a	2.68± 0.06 a
Streptococcus sp	0.6 ±0.05 b	2.1 ± 0.04 a	2.56± 0.04 a	2.98± 0.07 a
E. coli	0.16± 0.04 a	0.28± 0.06 a	1.3± 0.09 a	1.68± 0.1 a
Proteus sp.	0.36± 0.07 a	0.84 ± 0.05 a	1.34± 0.07 a	1.78± 0.08 a
C. albicans	1.44 ±0.07 b	2.14 ±0.1 a	2.9 ±0.11 a	3.24 ±0.09 a
Sacromysis sp.	0.42 ±0.07 a	0.44 ±0.06 a	0.54 ±0.05 a	0.82 ±0.04 a

-The value represent zone of inhibition (mm) Mean ±Standard Error
-The different small letters (horizontally) show significant effect while the same small letters show insignificant effect between different groups.

Table(3): Antibacterial activity of ethanolic plant extract of Fenugreek against gram positive , gram negative bacteria and fungi.

Plant extr. / bacteria	Negative cont.	Fen. 50mg/ml	Fen. 100mg/ml	Fen. 200mg/ml
S. aureus	0.68± 0.1 a	0.66 ± 0.05 a	1.58± 0.13 a	2.34± 0.09 a
Streptococcus sp	0.54 ±0.05 a	0.6 ± 0.04 a	1.5± 0.08 a	2.56± 0.07 a
E. coli	1.52± 0.15 a	1.26± 0.1 a	2.16± 0.09 a	2.48± 0.08 a
Proteus sp.	1.3± 0.09 a	0.56 ± 0.08 a	1.16± 0.09 a	1.5± 0.03 a
C. albicans	1.26 ±0.1 a	0.58 ±0.04 a	0.78 ±0.08 a	1.08 ±0.13 a
Sacromysis sp.	0.6 ±0.08 a	0.62 ±0.06 a	1.46 ±0.08 a	2.62 ±0.15 a

-The value represent zone of inhibition (mm) Mean ±Standard Error
-The different small letters (horizontally) show significant effect while the same small letters show insignificant effect between different groups.

Table(4): Antibacterial activity of standard antibiotic (positive control) against gram positive and gram negative bacteria.

Bacteria / Standard antibiotic	S. aureus	Streptococcus	E. coli	Proteus sp.
Ampicillin (Amp10)	10.98± 0.42 a	10.24 ± 0.28 a	5.26± 0.28 a	11.72± 0.22 a
Ceftriaxon (CTR)	20.62 ±1.56 b	19.2 ± 0.84 b	6.48± 0.12 b	10.94± 0.19 b
Ciprofloxacin (Cip)	21.72± 2.25 c	22.08 ± 0.89 c	7.06± 0.25 c	18.76± 0.16 c

Chloromphenicol (C30)	26.5± 1.26 d	26.02 ± 0.51 d	16.9± 0.2 d	20.66± 0.29 d
Impenem (IPM)	26.34± 1.52 d	29.62± 0.45 e	19.04± 0.26 e	23.32± 0.27 e

-The value represent zone of inhibition (mm) Mean ±Standard Error
-The different small letters (vertically) show significant effect while the same small letters show insignificant effect between different groups.

Table(5): Antifungal activity of standard drugs against Candida and Sarcomysis fungi.

Fungi / Standard antibiotic	C. albicans	Sacromysis sp.
Amphotricine-B	18.88± 0.14 a	15.96 ± 0.3 a
Nystatin	17.4 ±0.36 b	13.88 ± 0.12 b

-The value represent zone of inhibition (mm) Mean ±Standard Error
-The different small letters (vertically) show significant effect while the same small letters show insignificant effect between different groups.

Discussion:

Present result indicated that ethanolic extract of N. sativa , Ginger, and Fenugreek have antimicrobial activity against tested micro-organism, which is in close agreement with Mashhadian and Rakhshandeh., **(14)** who reported concentration dependent of N. sativa ethyl ether extract to inhibit gram positive and gram negative bacteria. Our results agree with Bakathir and Abbas., **(15)** who recorded the inhibitory effects of N. sativa seed at concentration 300 mg/ml against S. aureus. Also agree with Morsi N., **(16)** who investigate the antibacterial effects of crude extracts of N. sativa against various bacteria isolates which include 16 gram negative and 6 gram positive bacteria . On the other hand present research agree with Anian and Yunus., **(17)** who reported high activity of 10% ethanolic extracts of Ginger against Streptococcus mutance , Candida albicans and Enterococcus faecalis. Agree with Jain *et al*., **(18)** who investigated the prevention and treatment usage of Ginger against dental infection. And agree with Kader *et al*., **(19)** that evaluate the antibacterial and antifungal activity of Ginger against thirteen pathogenic bacteria and three fungi. Also agree with Mohammad *et al*., **(20)** and Yadav and Baguer., **(21)** that recorded the usage of Fenugreek seed extract to treat sever skin inflammation , antibacterial, antifungal and other medicinal effects. The antimicrobial effects of the all used plants extract may be due to its Containing more number of secondary metabolites which may be interfere with the antibacterial and antifungal activity of the extracts **(22).** Shafiq *et* al., **(23)** recorded that most important active compound of N. sativa are Thymoquinone (30-48%) which exhibited a significant bactericidal activity against various human pathogenic bacteria. Or may be due to the presence of another compounds in the n. sativa like alkaloid, limonene, flavonoids, essential oil in a adequate concentration and many other compounds, which damage microorganisms **(16, 24).** Ginger contains a number of different active compounds which are mainly 6-gingerol, 6-shagaol, 8-gingerol, zingerone, phenolic and flavonoids **(25, 26)**. Although Jain *et al*., **(19)** recorded that active components present in the Ginger extract have the capability to destroying bacterial cell wall. While the antimicrobial effects of Fenugreek extract due to alkaloid content, trigonllin, galactomannas, amino acids,

flavonoids, resins, saponins, terpenes, tannins, phenol, and steroid which may play a role as antimicrobial substances **(27, 28, 29).**

conclusion:

the present study showed that there is a higher antimicrobial activity of ethanolic extracts of Ginger, Fenugreek, and N. sativa. In the concentration 200 mg/ml.

So this study support the traditional usage of this plant as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogenic bacteria and fungi.

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