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Inhibitory effect of some plant extracts on growth of some bacteria and fungi pathogens

Hiader Radhi Malih*

ABSTRACT:

The antimicrobial activity of ethanolic extracts of Pomegranates (Punica granatium L.) pricarps and Celery (Apium graveolens L.), Sweet Clover (Melilotus officinalis L.),Alfalfa (Medicago sativa L.), Fenugreek Seed (Trigonella feonum-graecum L.) leaves and Carrot (Daucus carota L.) seeds against some pathogenic fungi , Gram-negative and Gram-positive bacteria were studied. Also, photochemical compound of extracts were determined, results of the chemical tests explain the M. sativ and P. granatium extracts contain flavonoids, tannins, alkaloids, saponins and glycosides compounds, while the other plants were varied in the kinds of these compounds. Ethanolic extracts show significant differences between most plants extracts, possibly due to deferent in the antimicrobial substance contain. All bacteria which were tested have shown susceptible to the P. granatium and D. carota ethanolic extracts. Proteus vulgaris , Klebsiella aerogenes and Streptococcus aureus the most susceptible bacteria to the D. carota and P. granatium extracts successively, whereas A. graveolens and T. feonum-graecum did not have antibacterial activity against most bacteria.

The antifungal activity of the ethanolic extracts of deferent plants show significant differences against pathogens test fungi, D. carota seed and P. granatium pericarps extracts report the most inhibited percentage than with the M.officinalis extract, also, a different level in susceptible of Geotrichum candidum and Trichophton montagrophytes fungi have shown with all extracts which had use.

INTRODUCTION:

In the present time ,interesting in the using medicinal plants to dominance the growth of the pathogenic micro-organisms, due to a large number of chemical pesticides were costly and exhibit side - effect therefore ,the award people are turning to wards herbal antimycotics [1] ,and the synthetic medicament have been banned in the world because of their undesirable attributes such as high and acute, long degradation periods [2] ,as well as,many pathogenic microorganisms increasing development of drug resistance in human pathogens as well as appearance of undesirable effect of certain antimicrobial agents [3,4] .

Some plant are known as medicinal because they contain active substance that cause certain reactions from relenting to cure of diseases, on the human and organism [5],such as diarrhea, malaria and other diseases [6], also it was widely used for its various pharmacological properties . Among compounds of antimicrobial activity interest occurring in the plant, are tannins [7] ,

*Department of Biology-College of Science-Thi-Qar University

essential oils [8] , alkaloids [6]. Number of investigations carries out on some of plant extracts , showed that they exhibit antimicrobial activity against human pathogenic microorganism [9-14].The aim of this paper was the investigating the antimicrobial activity of ethanolic extracts of P.granatium L.(Punicaceae) pricarps ,A.graveolens L. (Umbelliferae) , M.sativa L. (Leguminosae) and T.feonum-graecum L. (Leguminosae) , M.officinalis L. (Leguminosae) leaves and D.carota L. (Umbelliferae) seeds against clinical isolated Gram-negative bacteria (Eschericha coli ,Pseudomonas pseudomallei ,Klebsiella aerogenes and Proteus vulgaris) , Gram-positive bacteria (Staphylococcus aureas and Streptococcus aureus) and against pathogenic fungi, Geotrichum candidum and Trichophton montagrophytes, besides the determine the compounds which are recognized to have antibacterial active.

MATERIALS AND METHODS:

Plant material and extraction:

This study carried out during the period between March to June in 2007, The P. granatium pricarps, , M. officinalis leaves and D. carota seeds were collected from market and garden in Al-Nassiryia , after that washed well with sterile distilled water .The parts of different plant dried under shade ,then they were blending with an electric blender . Thirty grams of plant parts were soaked to separate in 100 ml of ethanol (70%) for 48 hours. The mixture was first filtered through muslin cloth, then using Watman No.1 filter paper. The ethanolic extracts were concentration with a Rotatory evaporator at 40 °C. After that, the extracts kept aseptically in sterile vials at 4 °C until use [15].

Bacterial culture and Antibacterial activity:

Fifty-four clinical isolated Gram-negative bacteria (E. coli, P. pseudomallei ,K. aerogenes ,P. vulgaris) and Gram-positive bacteria (S. aureas and Str. aureus) were obtained from Al-Nassiryia hospital.In vitro antimicrobial activity of ethanolic extracts of P. granatium pricarps, A. graveolens; M. sativa, T. feonum-graecum, M. officinalis leaves and D. carota seeds were examined. 100 mg\ml concentrations of all extracts were used, and then placed in sterile vials.A modified Agar Diffusion Technique was used to determine the antimicrobial activity of ethanolic extracts. bacterial cultures were grown in Nutrient broth (Oxoid), then placed in Muller-Hinton Agar Petri-dishes, after that 0.1 ml from clinical isolated bacterial cultures spread with L-shaped glass rod under sterile conditions, left 1 minute to dry, then bores 4 mm were made by using cork borer in the center of Petri-dishes, 0.1 ml of the deferent extracts were added. The treatment also included 0.1ml Tetracycline (250 mg) for comparison. The Petri-dishes were incubated at 37°C for 24 hours .Three replicates were formed for every bacterium [16], inhibitions zone was measured (mm).

Antifungal assays:

Twenty clinical isolated T. montagrophytes and G. candidum were obtained from Al-Nassiryia hospital.Sabouraud Dextrose Agar medium (SDA) was prepared from 10 g. of Neopepton with 10 g. Glucose and 20g. Agar, then added the distilled water to complete to 1 L. , 3 ml. of concentration 100% extracts added to flask contained 27 ml. of Sabrouraud Dextrose Agar medium (PH=6.8 \ 50°C in the water bath), the mix were

shaken, after that empty into 9 cm. diameter Petri-dishes. Bores 4 mm were made by used cork borer in the center of Petri-dishes, after that filled from the deferent extracts cylinder. The medium contains distilled water serves as control. The Petri-dishes were incubated at 26°C for 24 hours .Three replicates were formed for every extract. The colony diameter was measured; the growth was expressed as percentage of the colony diameter in control Petri dishes.

Phytochemical Tests:

1- *Tannins Test:* A modified methods stated in [17] was used to be presented of tannins on the extracts, A few drops of Ferric chloride reagent were added for 3 ml of extract. A blue-black color refereed to the present of tannins.

2- Alkaloids Test: **A few drops of Marqus reagent (prepared from mixing 0.5 ml of Formaldehyde with 5 ml of concentration H_2SO_4), added to the 5 ml of extract. Turbidity refereed to the present of alkaloids [18].**

***3-Saponins Test*: 3 ml of extract was added to the 2 ml of Ferric chloride, a white residue to be formed as evidence to the present of Saponins [19].**

***4-Flavonoids Test*: Flavonoids test were implement in conformity with [19]. 2 ml of extract mix with Alcoholic KOH (0.5 mol.), a yellow color as proofed to the present of Flavonoids.**

5-Glygosides Test: **0.5g of dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of Ferric chloride solution, then under laid with 1 ml of concentration H_2SO_4 .A brown ring indicated the present of Glycosides [20].**

Statistical analysis:

Results were statistically analyzed using Duncan Multiple Rang Test, and used Least Significance Difference to compared between the means.

RESULTS AND DISCUSSION

The phytochemical compounds of the different plants extracts are obtainable in Table 1. Results showed the ethanolic extracts of P. granatium, M. sativa contain flavonoids, tannins, alkaloids, saponins and glycosides compounds , but the A. graveolens and T. feonum-graecum did not have tannins and saponins,as well as; D. carota did not have tannins and glycosides, while M. officinalis did not contain glycosides These results agree with some studies that showed that the P. granatium pricarps extracts contain flavonoids and tannins [21, 22]. As well as, phenolic compounds was found in M. sativa [23],Whereas the tannins, saponins , phenolic compounds, flavonoids and glycosids were found in some legumes such M. sativa, T. feonum-graecum and M.officinalis [24- 27] . The results in this study showed that ethanolic extracts of P. granatium pricarps, M. sativa, M. officinalis leaves and D. carota seeds presented antibacterial activity against clinical isolated Gram-negative (E. coli ,P. pseudomallei ,K. aerogenes ,P. vulgaris) and Gram-positive bacteria (S. aureas and Str. aureus), also showed signification variation in the inhibitions zone.The results obtained of inhibition zone of prepared extracts tested and used as antibacterial against five different clinical isolated Gram-negative and Gram-positive bacteria (Table 2). P. granatium and M. sativa ethanolic extract were to be appeared signification difference in the antibacterial activity against all clinical isolated bacteria. S. aureas and

E.coli show deferent levels of susceptible to all ethanolic extracts which had been used, the extract of P. granatium pericarps have the highest antibacterial activity against all clinical isolated bacteria than with the other extracts, to follow with the antibacterial activity of D. carota extracts had been shown to be active against all bacteria , while the extract of A. graveolens and T. feonum-graecum did not have any antibacterial activity against the most clinical isolated bacteria, except (S. aureas) and (S. aureas; E. coli). Also control medium have showed the most effective against all bacteria which were used. Generally, the plant extracts have been shown antibacterial activity against some clinical bacteria such as E. coli , Str. mutans and S. aureas [28- 30], also P. granatium pricarp extract have antibacterial activity against S. aureas and Bacillus subtilis [9], in addition the ethanolic extract of P. granatium display signification function against E.coli and Helicobacter pylori [13, 14] . The antifungal activities percentages are recapitulated in Table 3. The results provide evidence of the inhibitions zone were signification variation between ethanolic extracts of P. granatium pericarps with all extracts except D. carota extract. As well as, the value of the antifungal activities percentage of A. graveolens, T.foenum- graecum, M. sativa and T. feonum-graecum extracts were similar than obtained for M.officinalis extracts.Among the six plant extracts which had used, the most inhibition percentage against clinical isolated fungi was P. granatium pericarps, followed with D. carota , Whereas the ethanolic extracts of M. officinalis have a lowest percentage of the antifungal activity against T. montagrophytes and G.candidum respectively. Some of studies were deal with the antifungal activity of plant extracts, for example Tagetes erecta and T. patula extracts signification inhibited mycelium growth of Trichophyton menatagrophyton [1], also that the essential oils of D. carota showed to be active against Alternaria alternata growth, it had varied between (66-90)% [31], while the leaf extracts of Cassia spp. were inhibited the growth of Macrosporum gypsum and T. rubrum [4].The antimicrobial effects were signification difference between the most extracts, it perhaps due to deferent in the antimicrobial substance in plant extracts, it had contain of a number of compounds, such as essential oils [31, 32], sterols , flavonoids [4] phenolic compound, alkaloids [6], tannins,proteins [33], saponinns [34]. which had antimicrobial activity and they cause cell membrane damage [35, 36],also these compounds reduce the DNA synthesis [37], and inhibited protein synthesis, which produce death cells [38],it is necessary to do experiments to define mechanisms effected of the antimicrobial compounds on metabolism activities site in the microbial cells. Results of this study showed that the ethanolic plant extracts present ability to control in the clinical isolated Gram-negative, Gram-positive bacteria and pathogens fungi T. montagrophytes and G. candidum.

Table1:Phytochemical Compounds in deferent ethanolic plant extracts.

Plant extracts / Phytochemical Tests	Punica granatium	Apium graveolens	Daucus carota	T. feonum-graecum	Melilotus officinalis	Medicago sativa
Flavonoids Test	+*	+	+	+	+	+
Alkaloids Test	+	+	+	+	+	+
Glygosides Test	+	+	-**	+	-	+
Saponins Test	+	-	+	-	+	+
Tannins Test	+	-	-	-	+	+

*** + Present of the phytochemical compounds.**

****- Absence the phytochemical compounds.**

Table 2: Diameters of inhibitions zone (mm) of the antibacterial activity of the ethanolic extracts of P. granatium pricarps and leaves of A. graveolens, M. sativa, T. feonum-graecum.and D. carota seeds.

Extracts / Bacteria	Punica granatium	Apium graveolens	Medicago sativa	T. feonum-graecum	Daucus carota	Tetracycline
Eschericha coli	24.9**	-*	3.25	3.75	18.5	31.75
Pseudomonas pseudomallei	26	-	16.33	-	22.33	36.25
Klebsiella aerogenes	28.5	-	12	-	29.25	30.75
Proteus vulgaris	22.75	-	7.9	-	29.7	30.25
Staphylococcus aureas	19.66	3.34	-	4	18.66	34
Streptococcus aureus	29.5	-	4.5	-	21.3	35.67
R.L.S.D.	5.39					

*** Absence the antimicrobial activity.**
**** Diameters of inhibitions zone express to recorder of three replicates.**

Table 3: percentage of the antifungal activity of six ethanolic extracts on the pathogens fungi Geotrichum candidum and Trichophton montagrophytes .

Extracts / Fungi	Punica granatium	Melilotus officinalis	Apium graveolens	Daucus carota	T. feonum-graecum	Medicago sativa	Control
Trichophton montagrophytes	62*	32	37.66	65.25	51.75	57.2	0
Geotrichum candidum	61.5	33.75	43.25	48	41	42	0
R.L.S.D.	11.27						

*** Diameters of inhibitions zone express to recorder of three replicates**.

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التأثير التثبيطي لبعض المستخلصات النباتية على نمو بعض البكتريا والفطريات المرضية

حيدر راضي مالح
قسم علوم الحياة - كلية العلوم – جامعة ذي قار

الخلاصة

تم دراسة الفعاليه المضاده لنمو الاحياء المجهريه في المستخلصات الكحوليه لقشور الرمان،اوراق الكرفس، الحندكوك،الجت والحلبه وبذورالجزر ضد بعض انواع الفطريات و البكتريا المرضيه (السالبه والموجبه لصبغة كرام) ، كما تم الكشف عن المركبات الكيميائيه فيها والتي شخصت احتواء المستخلصات الكحوليه في قشور الرمان واوراق الجت على المركبات القلويديه،الفلافونيديه ،الصابونيه، الكلايكوسيدات والتانينات،بينما تباينت مستخلصات بقية النباتات في وجود تلك المركبات .

وقد اظهرت الدراسه وجود تباين معنوي بين جميع المستخلصات المستخدمه ،وقد يعزى سبب ذلك لوجود تباين في محتواها من المركبات الكيميائية. وأبدت جميع أنواع البكتريا المختبرة في هذه الدراسه حساسيه تجاه مستخلصي قشور الرمان و بذور الجزر ،بينما لم يؤثر مستخلصي نباتي الكرفس والحندكوك على نمو اغلب انواع البكتريا المختبره ،بينما كانت بكتريا (Proteus vulgaris، Klebsiella aerogenes) و(Streptococcus aureus) اكثرها حساسيه تجاه مستخلصي الجزر وقشور الرمان على التوالي.

وكانت المستخلصات قد اظهرت تباينا في فعاليتها ضد الفطريات المرضيه المختبره، فقد سجل مستخلصي بذور الجزر وقشورالرمان اعلى نسب الثاثير مقارنة مع مستخلص اوراق الحندكوك، كما اظهر الفطرين Geotrichum candidum و Trichophton montagrophytes تباينا في حساسيتهما تجاه جميع المستخلصات المستخدمه.