No. 1

Antibacterial effect of nettle (Urtica dioica)

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Abstract

The antibacterial activity of aqueous and 95% ethanol extracts of nettle leaf were tested against some Gram-negative and Gram-positive bacteria isolated from hospitalized patients by the agar well diffusion method. *Staphylococcus aureus, Escherichia coli, Klebsiella spp., Bacillus Subtillus, Proteus spp. Salmonella spp.* and *Pseudomonas spp.* were used. The results indicate that both extracts showed different antibacterial activities which was in favor of ethanolic extract because of more solubility of active ingredient in ethanol than in water. *Staphylococcus aureus, Bacillus Subtillus subtillus* and *Salmonella spp.* showed the highest susceptibility to nettle extracts antibacterial effect, while *E coli, Pseudomonas* and *Proteus* were less susceptible. The only clear resistant bacteria isolate was *Klebsiella spp.*

Key words: antibacterial, nettle, Urtica dioica, Gram negative bacteria, Gram positive bacteria.

التأثير المضاد للبكتيريا لنبات القريص نادية عبد الكريم صالح ايمان ظاهر عارف دلوفان جلال علي كلية الطب البيطري - جامعة السليمانية

الخلاصة

تمت دراسة التأثير المثبط للخلاصة المائية والكحولية (الإيثانول 95%) لنبات القريص مكون من الأوراق ضد نمو أنواع من البكتريا الموجبة والسالبة لصبغة غرام, لعز لات جرثومية من المرضى في المستشفى بطريقة الانتشار في بالحفر. البكتريا المستعملة هي المكورات العنقودية الذهبية و الاي كولاي و زوائف الايروجينوزا و الكليبسيلا والسالمونيلا وباسيلص سبتيلص و بروتيس , أظهرت النتائج بان للخلاصتين تأثيرات مختلفة كمضاد بكتيري ولكن النتائج كانت بأفضلية للمستخلص الكحولي بسبب قابلية الذوبان الأكبر للمكونات العشب في المحول التي لا تذوب في الماء. الفاعلية التثبيطية لمستخلص المحولي بسبب قابلية الذوبان الأكبر للمكونات العشب في الكحول التي لا تذوب في الماء الفاعلية التثبيطية لمستخلص المعشب كانت اكبر ضد المكورات العنقودية الذهبية والسالمونيلا وباسيلص سبتيلص بينما كانت ما التثبيطية لمستخلص العشب كانت منه الاي كولاي و زوائف الابروجينوز و بروتيس قليلة , الكليبسيلا كانت الفاعلية التثبيطية لمستخلص العشب مند الاي كولاي و زوائف الابروجينوز و بروتيس قليلة , الكليبسيلا كانت جدا للعشب و لم تظهر اي نتائج متعلقة بالفاعلية التثبيطية لمستخلص العشب

الكلمات المفتاحية: مضاد البكتيريا ، نبات القريص

Introduction

Nettle (Urtica dioica) belongs to the *Urticaceae*is recommended for family complaints associated with rheumatoid arthritis, osteoarthritis and urinary tract infections, allergies, Alzheimer's, asthma, bladder problems, bronchitis, cough, bursitis, gingivitis, gout, hair growth and baldness, hives, kidney stones, prostate enlargement, sciatica, tendinitis (1,2). New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms. Interest in plant-derived drugs has been increasing, mainly due to the current widespread belief that "green medicine" is safer and more dependable than costly synthetic drugs (3,4). Nettle is stated possess to antihaemorrhagic and hypoglycemic properties. Traditionally, it has been used for uterine hemorrhage, cutaneous eruption,

infantile and psychogenic eczema, epistaxis, and melena and specifically for nervous eczema (1, 2, 5). The German Commission E approved internal use of nettle leaf as irrigation therapy for inflammatory disease of the lower urinary tract and prevention of kidney gravel. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (3). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent new antimicrobial need to discover compounds with diverse chemical structures and novel mechanisms of action for new

infectious diseases (6,7). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (4, 1, 2, 8). So the present study aimed to investigate the antibacterial effect of nettle against some selected gram positive and gram negative bacterial species.

Materials and methods

1. Samples: - one of the aerial parts which is (leaves) of nettle was taken for the both extraction procedures which obtained from Al-Kut city in Iraq.

2. The microorganisms tested in this study were isolated from sputum samples of hospitalized patients in the Shoresh Hospital of Sulaimani city- Iraq.

Ethanolic extraction:

Plant were washed under tap water, and then dried in room temperature in shade. The dried plant was crushed by a laboratory blender. Organic solvent extraction of nettle was carried out by using ethanol (95% ethyl alcohol) which is considered as a very effective in extracting the active ingredients of the plant according to method described by (10, 11). This was done by using Soxhlet apparatus, which consists of an electric heater with a thermostat regulator upon which a round bottom glass flask placed that fitted to an extraction unit. The extracting unit contains the solvent and cellulose (thumble) located inside it that contains the dry plant powder. A distiller unit is fitted on to the extraction unit. For condensation of vapor solvent, 25 g. of plant leaves powder was put inside the thumble and 250 ml of 95% ethanol was put inside the flask. The extraction was carried out for 24 hours by heating temperature that kept the solvent at 50-60 C. After that, the extract was dried by using an electric oven at temperature 40-45 C until dry extract was obtained. The dry extract was placed in an incubator under 38-40 Ċ for complete dryness of the sample. The dry ethanolic extract dissolved in Dimethylsulphoxide (DMSO) to prepare concentration of 30mg/ml that used for testing its antibacterial activity.

Aqueous extraction:

2014

Plants were washed under tap aqueous, and then dried in room temperature in shade. The dried plant was choped into small pieces and then grinded by an electric grinder into powder. Sixty grams from the grinded crude powder was mixed with 100 ml distilled water. Aqueous extract was carried out according to (11,12,13) by using magnetic stirrer at 60°C for 3 hours then filtrate was kept in incubator till complete drying .The yield of dry powder was 15 gm. The dry powder dissolved in DMSO to prepare concentration of 30mg/ml that used for testing antibacterial activity.

Media preparation and antibacterial activity:

The antimicrobial assay of aqueous and ethanol extract of nettle was performed by agar well diffusion method. The molten Mueller Hinton agar was inoculated with 50 ul of the tested microorganism (Cell suspension containing 1×10^8 CFU/ml cells) (McFarland according to standard 0.5).(6,7).For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (5mm). The plates were incubated overnight at 37 C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, the result was obtained by observation of the zone of inhibition (3, 9). **Bacterial isolate:**

Clinical isolates of *Staphylococcus* pseudomonas aureus, Escherichia coli, aeroginosa, Proteus. Bacillus subtillus, Salmonella spp. and Klebsiella spp. were used to test the effect of nettle aqueous and ethanol extract to demonstrate the antibacterial effect on these microorganisms. Inhibition zone diameter of 5mm and above was taken as significant susceptibility of each test micro organism to the extract, we considered the range of inhibition of (5-10mm)as slight and take one plus(+), range of (10-15mm) as moderate and take two plus (++), and range (=>15 mm) as high and take three plus (+++), while the negative result (-) refers to the absence of inhibitory zone.

Results

The results depicted in table (1) and figures (1, 2, 3, 4, 5, 6 and 7) indicating that the inhibition zone of both extracts is so clear with

2014

some bacterial isolate species. Seven different microbial species were used to screen the possible antimicrobial activity of both aqueous and ethanol extract of nettle. Nettle exhibited antimicrobial activity against some of tested microorganisms as shown in table (1), the species used, *Staphylococcus aureus* is one of the most common Gram-positive bacteria

causing food poisoning. Interestingly nettle showed antibacterial activity against this bacterium. As it is shown in table (1), other generations of most bacteria was inhibited by nettle are *Salmonella* and *Pseudomonas*. While *Escherichia coli*, *Proteus* and *Klebsiella* are resistant and show no antibacterial effect of nettle.





Figure 1:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *E. coli* isolate growth.





Figure 2:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *Salmonella* growth.



Figure 3:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *Proteus* growth.



Figure 4:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *Klebsiella* growth.

No. 1



Figure 5:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *Bacillus subtillus* growth.



Figure 6:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *Staphylococcus aureus* growth.



Figure 7:- Effect of nettle aqueous extract 30 mg/ml (A) and ethanolic extract at 30 mg/ml(B), against *Pseudomonas* growth.

| Microorganisms | Aqueous Extract (30mg/ml) | Ethanol Extract (30mg/ml) |
|---------------------------|---------------------------------|---------------------------------|
| E-Coli | _ | + |
| Salmonella spp. | +++ | +++ |
| Proteus spp. | + | _ |
| Klebsiella spp. | _ | _ |
| Bacillus subtillus | ++ | +++ |
| Staphylococcus aureus | + | +++ |
| Pseudomonas aeruginosa | + | ++ |

Table (1) Antibacterial effect of aqueous and ethanolic extract of nettle (Urtica dioica) against some bacterial isolate

Inhibition zone diameter of 5mm and above was taken as significant susceptibility of each tested microorganism to the extract, we considered the range of inhibition of (5-10mm) as slight and take one plus (+), range of (10-15mm) as moderate and take two plus(++), and range (=>15mm) as high and take three plus(+++).while the negative result (-) refers to the absence of inhibitory zone.

Discussion

Our results are in agreement with (8) and (11) who refer that the extracted materials from nettle have a powerful antibacterial effect. Analysis of the chemical compounds of nettle showed that nettle (Urtica dioica) contained neophytadiene. Neophytadiene is reported to be an antibacterial compound also contain aromatic compounds including carboxylic acids and esters were also. Reported in this plant .Finally, fat acids including phtalic acid, dibutyl ester, Bis (2ethyl hexyl) maleat and 1,2-benzenedi carboxylic acid were observed in this plant. These compounds are reported to have anti antimicrobial putrefying and effects. (16,22,23). Nettle have other constituents which plays major role in antibacterial effect such as alkaloids, phenols, flavonoids, tannins and saponins in these plants extract which have been claimed to be responsible for their antimicrobial activity (7,14). Alkaloid, it is antibacterial activity may be due to its ability to react with amino, carboxyl, sulfhydryl and hydroxyl groups in bacterial protein as well as nucleic acids, it highly reactive chemical compounds that combine with proteins to give intermolecular cross-links and intercalate with DNA (15).Tanninic substances are another constituents which capable are of precipitating gelatin from solution, a property is known as astringency. It has been reported development to prevent the of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (9,16).Phenolic compound is mostly hydrophobic, it has a hydroxyl group (-OH). The importance of this group on antimicrobial activity is well known, the site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative activity to microorganisms (17,18). The high activity of the phenolic components may be further explained in terms of the alkyl substitution into the phenolic nucleus, which is known to enhance the antimicrobial activity of phenols.

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2014

The introduction of alkylation has been proposed to alter the distribution ratio between the aqueous and non-aqueous including bacterial phases. phase, bv reducing the surface tension or altering the species selectivity. It was suggested that plant products act via two main mechanisms of action: the first is related to the general hydrophobicity of plant products, which facilitates their adhesion to the bacterial surface inducing un-stabilization (9,14). The second mechanism is the inactivation of different molecules of the bacteria such as enzymes or receptors by their adhesion to specific sites (13, 18, and 19).Flavonoid antibacterial activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. more lipophilic flavonoids may microbial also disrupt membranes.(20,21).The differences in antimicrobial activity against different isolate that bacterial noticed between ethanolic and aqueous extract was in favor of ethanolic extract may be because of the higher solubility of some hydrophobic components like phenols and flavonoids in ethanol than in aqueous (20,21). Many researchers preferring use of plant extract instead of antibiotics was due to attenuation of pathogens virulence by plant extract as opposed to the direct killing of pathogenic bacteria with antibiotic as a strategy to combat infections is an interesting concept. The idea that anti-pathogenic molecules that prevents for instance the production of toxins or abolish the ability of bacteria to adapt to the host environment would give а competitive advantage to the host immune system to allow clearance of the infectious organism. It is also anticipated that such virulence attenuators would not affect nonpathogenic bacteria communities or exert a selective pressure for the development of resistance as seen from the pressures exerted by conventional antibiotics that targeted vital bioprocesses in bacteria (9 and 19).

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No. 1

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