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RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITIES OF URTICA DIOICA AGAINST SOME PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

In view of the fact that ancient time, plants have been a tremendous source of medicine. The knowledge of traditional medicine and medicinal plants and their study of scientific chemical principles may lead to the discovery of newer and cheaper drugs.Fresh Leaves of Urtica dioica was collected from different sites from Diyala , Iraq.the leaves were dried and chopped into small pieces, pulverized into fine powder in a grinder .Methanol extract :25 g of air dried powder of plant leaves extracted successively with 300 ml of methanol using a Soxhlet extractor for 72 hours. The methanol was concentrated to near dryness under reduced pressure below 40 ^{o}C..Aqueous extract : 25 g of dried of leaves powder was filled in a beaker extracted successively with 400 ml of distil water using heat magnetic stirrer at 50 ^{o}C for 24 hours.then the extract was filtered over what – man No.1 paper. The aqueous was concentrated to near dryness under reduced pressure below 40 ^{o}C, after complete solvent evaporation. 1 g of solvent residue from aqueous and methanol extract was dissolved 10%dimethylsulfoxide (DMSO) solution was used as the test extract for antibacterial activity assay. The concentrations depended in both aqueous and methanol extracts.were, 50, 100, and 200 mg/ ml. The antibacterial activity of aqueous and methanol extract of Urtica dioica was evaluated via growth inhibitory zone assay using well and disc diffusion methods, against isolated pathogenic bacterial strains of Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Bacillus subtilis, Proteus and Klebsiella. All extract established significant antibacterial activity against tested bacteria.In aqueous extract in well method Proteus was the most sensitive at the three concentration, followed by the other tested bacteria in used as they were sensitive at 200 mg / ml only . While in disc method aqueous extract exhibit inhibition to Pseudomonas, then Proteus only. While the other tested bacteria showed no significant difference in their sensitivity. when compared aqueous extract according methods(Well with disc) Bacillus was sensitive at significant level at 100 and 200 mg / ml, then Staphylococcus and Proteus at 200 mg / ml. Methanol extract of U. Dioica in well method only showed inhibition at significant level to Pseudomonas aeruginosa. other tested bacteria were not sensitive at significant difference.in disc method methanol extract exhibit sensitivity to Proteus, E. Coli, Staphylococcus and Klebsiella respectively. in comparison the inhibition of methanol extract only Klebsiella showed significant difference other test bacteria did not showed significant difference.

In comparison methanol extract with aqueous extract in well method only Klebsiella and Proteus showed a significant difference and the methanol extract was more inhibited. While in disc method there were significant difference between methanol and aqueous extract to E. Coli, Bacillus and Staphylococcus to side of methanol extract. at the three in used concentration.

INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Hedges and Lister, 2007).

Medicinal plants represent an important source of medically important compounds.since ancient time, medicinal plants are used to cure several types of health problems. Systemic analysis of the plants provides a variety of bioactive molecules for the development of newer pharmaceutical products recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine. In last few decades, many of traditionally known plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer, anti – inflammatory, anti diabetic, anthelmintic, antibacterial, antifungal, hepatoprotective, antioxidant, larvicvidal activity (Kumar,G, *et al*., 2010).

Now a days, medicinal plants have many applications in

people's lives They can be used in the Pharmaceutical compounds, cosmetic, sanitary and nutritional industries (Ramtin M. *et al*, 2012).

Urtica dioica which is a member of Urticaceae class, Nettle, has many important functions in traditional treatment because it has a lot of curable effects. There are many reports which show this plant is very effective in the treatment of blood pressure, diabetes, and prostate hyperplasia, rheumatoid arthritis and allergic rhinitis (Fathi- Azad, F.*et al*., 2005). Nettle (Urtica dioica) has medical properties and its extract have been used for hundreds of year in world traditional medicine for treating diseases such as eczema, digestion, joints, pain and anemia (Chrubasik *et al*., 2007). Antimicrobial activities of alcoholic and aqueous extracts of the separate parts of Urtica were investigated on the Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Candida albicans (Majd *et al*., 2001). Its summary illustrated that alcoholic extract of Urtica seed had the greatest influence on the gram positive bacteria, leaves extract had the maximum effect on the gram negative bacteria.(Majd *et al*., 2001). They can be used in the pharmaceutical compounds, cosmetic, sanitary and nutritional industries(Kalemba, D. and Kunnicka A. 2003).

The available literature shows that Urtica dioica L has antioxidant(Kanter *et al*., 2005);anti inflammatory, anti ulcer(Gulcin, I *et al*., 2004); anticancer, antimicrobial(Nisha *et al*., 2011); cardiovascular(Asgarpanah and Mohajerani, 2012) and hepatoprotective properties (Kanter *et al*., 2005).

The highest activity of Urtica dioica leaves extract was against K. pneumonia and B. cereus. In addition, average activity was related to S. aureus and P. aeruginosa. The effects of Urtica dioica oil was better than ampicillin except for E. faecalis and E. coli.(Ramtin, *et al*., 2012).

MATERIALS AND METHODS

Plant materials, sample preparation

Fresh Leaves of Urtica dioica was collected from different sites from Diyala , Iraq.the leaves were dried and chopped into small pieces, pulverized into fine powder in a grinder, then stored at 4 ^{o}C until use.

Preparation of extracts

Methanol extract: 25 g of air dried powder of plant leaves was filled in the thimble and extracted successively with 300 ml of methanol using a Soxhlet extractor for 72 hours. The methanol was concentrated to near dryness under reduced pressure below 40 ^{o}C, after complete solvent evaporation the solvent extract was weighed and preserved at 4 ^{o}C in airtight bottles until use.

1 g of solvent residue was dissolved 10% dimethylsulfoxide (DMSO) solution was used as the test extract for antibacterial activity assay (Karamasn et asl.2003,; Okeke *et al*., 2001). (Mingarro *et al*., 2003). The concentrations depended in the experiment were, 50, 100, and 200 mg/ ml.

Aqueous extract : 25 g of dried of leaves powder was filled in a beaker extracted successively with 400 ml of distil water using heat magnetic stirrer at 50 ^{o}C for 24 hours.then the extract was filtered over what - man No.1 paper.. The aqueous was concentrated to near dryness under reduced pressure below 40 ^{o}C, after complete solvent evaporation.

1 g of solvent residue was dissolved 10%dimethylsulfoxide (DMSO) solution was used as the test extract for antibacterial activity assay (Karamasn et asl.2003,; Okeke *et al*., 2001).(Mingarro *et al*., 2006). The concentrations depended in the experiment were, 50, 100, and 200 mg/ ml.

Test bacterial strains

The following bacterial strains were used as test organisms: Staphylococcus aureus, Bacillus subtilis; E. coli : Pseudomonas aeruginosa : Klebsiella and Proteus. All the bacterial strains were obtained from Department of Microbiology, College of Veterinary Medicine, University of Diyala, Diyala, Iraq.

Culture preparation

A loop full of 24 hr. surface growth on a NAS slope of each bacteria isolate was transferred individually to 5 ml of Brain heart infusion broth(PH 7.6) and incubated at 37 ^{o}C for 24 hr. Bacterial cells were collected by centrifugation at 3000 rpm for 15 min., washed twice and resuspended in 0.1% pepto water. turbidity was adjusted to match that of as McFarland standard(10^{8}cfu/ ML). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of(10^{7}CFU/ ml).

Antibacterial activity of methanol and aqueous extracts were determined by well and disc diffusion methods on nutrient agar medium.

Agar well diffusion method

The extract activities were carries by spreading 0.1 ml 0f bacterial suspension prepared according (Bauer *et al*., 1966).which contain $1X10^{8}$ cells / ml over the surface of Muller – Hinton agar plate, to obtain uniform growth, left the plate to dry for 5 minutes. Then well were prepared by using Pasture pipette 5 mm diameter. These well were filled by 50 µl concentrated extract of either aqueous or alcoholic extract according to dilution used. Leave the medium to settle for 1 hour in laboratory condition. Then incubate for 24 h at 37 ^{o}C and zone of inhibition if any around the well were measured in mm. each treatment consists of four repeat(Karaman *et al*., 2003; Srinivasa *et al*., 2001; Masika and Afolayan, 2002).

Disc diffusion method

Disc method: Firstly the bacteria to be tested were inoculated into Mueller Hinton broth, and incubated for 3- 6 hours at 35 ^{o}C. Petri dishes containing Mueller Hinton Agar were impregnated with these bacterial suspension. Discs of 5 mm diameter, sterile blank were impregnated with different concentration of each extracts. Blank disc of chloramphenicol (10 micro g / disc) as positive control. All test plates were incubated at 37 ^{o}C for 24 hour and zone of inhibition if any

around the well were measured in mm (millimetre). Each treatment consists of four repeat (Karman *et al*., 2003; Srinivasa *et al*., 2001; Masika and A folayan 2002).

Standard antibiotic disc Rifampin 5; Doxycycline 30; Amoxicillin 25; Kanamycin 30 and Ampicillin – cloxacillin 30, for antibacterial activity test were carried out against bacterial strains in used.

Statistical analysis

All values are expressed as the mean ± the standard error of the mean (SEM). The data were analyzed by using one way analysis of variance ANOVA, and then the test of the least significant differences between the means of inhibitory zones (Steel and Torrie, 1985). The significant level of test was $P < 0.05$.

RESULTS

The results revealed that in aqueous extract of Urtica dioica in well method there was significant difference at 200 in comparison with 100 and 50 mg / ml in sensitivity of E.Coli. while in disc method no significant differences in sensitivity was observed . in case of Bacillus there was significant difference at 200 in comparison with 50 mg / ml in well method. in disc method there was no significant difference. in comparison well with disc method there were significant difference at 100 and 200 mg / ml. In well method Staphylococcus aureus showed significant difference in sensitivity at 200 in comparison with 50. In disc method no significant difference o9bserved. in comparidson well method with disc method there was significant difference at 200 mg / ml. in Pseudomonas there was significant difference at 200 in comparison with 50 mg / ml.in disc method there were significant difference at 100 and 200 in comparison with 50 mg / ml. in case of Klebsiella there was significant difference at 200 in comparison with 50 and 100 mg / ml. While in disc method there was no significant difference in sensitivity. In case of Proteus there were significant difference at 200 in comparison with 50 and 100. And between 100 with 50 mg / ml. in disc method there was significant difference at 200 in comparison with 50 mg /ml. In comparison well with disc method there was significant difference at 200 mg / ml.(Table -1-)

Table 1 showing the sensitivity of isolated bacterial strains to aqueous extract of Urtica dioica

Bact. sp.		Well			Disc	
Concent.	50	100	200	50	100	200
E. coli	5.25± 1.30 a	6.75± 1.60a	9.33± 1.30 b	6.0± 1.25a	5.67± 0.50a	6.67± 1.20a
Bacill.	7.33± 1.10aA	9.0± 0.25aA	10.33± 0.25bA	5.67± 1.20aA	6.67± 0.60aB	8.0± 0.20aB
Staph.	8.50± 1.20a	10.0± 0.95a	11± 1.53bA	7.33± 1.03a	7.6± 1.52a	8.33± 1.61aB
Pseud.	6.0± 1.1a	7.0± 0.6a	8.67± 0.25b	5.67± 0.57a	8.0± 0.25b	8.33± 0.5b
Kleb.	7.25± 0.25a	8.0± 0.30a	11.0± 0.30b	7.33± 1.03a	8.0± 0.40a	8.6± 1.03a
Proteus	6.67± 0.20aA	8.677± 0.50bA	11.67± 1.10bcA	6.0± 0.25aA	7.33± 0.25aA	8.33± 0.25bB

Values M±SRM: a, b c, significant difference within group; A, B significant difference between groups. The significance at P< 0.05.

Alcohol extract in well method: Proteus: none significant. disc significant at 100 and 200 mg / ml in comparison with 50 mg / ml; in comparison well with disc method no significant difference. E.coli well significant at 200 in comparison with, 50 and 100mg / ml. In disc method no significant difference ; well method compared with disc no significant difference.Bacillus did not show any vbsignificant difference in both well and disc methods.Staphylococcus in well method no significant difference; In disc method there wasv csignificant difference at 200 in comparison with 50 mg / ml. In well compared with disc no difference.Klebsiella well none; disc 200 in comparison with 50. In comparison well with disc method there was significant difference at 50 mg / ml.Pseudomonas aeruginosa in well method there were significant difference 100 , and 200 in comparison with 50. In disc method no significant difference. In comparison well with disc methods there vwas no significant difference (Table -2-).

Table 2 showing the sensitivity of isolated oathogenic bacteria to alchol extract of Urtixca dioica

Bact. sp.		Well			Disc	
Concent.	50	100	200	50	100	200
E. coli	6.00± 1.00a	6.67± 0.67a	9.00± 1.16b	8.33± 1.67a	9.33± 2.19a	10.0± 1.00a
Bacill.	8.00± 1.73a	10.0± 2.00a	10.67± 1.86a	9.00± 1.53a	10.33± 1.46a	12.33± 2.03a
Staph.	8.67± 1.34a	10.33± 1.20a	11.33± 1.20a	10.0± 0.58a	11.0± 0.58a	13.0± 1.53b
Pseud.	5.67± 0.34a	7.67± 0.88b	8.67± 1.20b	7.00± 1.16a	8.67± 0.67a	9.00± 0.58a
Kleb.	6.33± 0.34aA	7.67± 0.88aA	9.33± 2.34a	8.67± 0.34aB	9.67± 0.67aB	10.33± 0.88b
Proteus	8.00± 2.52a	9.67± 2.19a	12.0± 3.22a	6.00± 1.00a	8.0± 0.58b	8.67± 0.67b

Values M±SRM: a, b c, significant difference within group; A, B significant difference between groups. The significance at P< 0.05.

In comparison between aqueous and alcohol extract the results revealed that in well method. Bacillus; staphylococcus; Pseudomonas and. E. Coli no significant difference; Proteus significant at 50 and Klebsiella at 200.While in disc method Bacillus; E. Coli and Staphylococcus showed significant difference in sensitivity at 50, 100 and 200 mg /ml; Proteus; Pseudomonas and Klebsiella no ashow significant difference.

DISCUSSION

The results of present study revealed that All extract established significant antibacterial activity against tested bacteria.In aqueous extract in well method Proteus was the most sensitive at the three concentration, followed by the other tested bacteria in used as they were sensitive at 200 mg / ml only. While in disc method aqueous extract exhibit inhibition to Pseudomonas, then Proteus only. While the other tested bacteria showed no significant difference in their sensitivity. When compared aqueous extract according methods(Well with disc) Bacillus was sensitive at significant level at 100 and 200 mg / ml, then Staphylococcus and Proteus at 200 mg / ml.

Methanol extract of U. Dioica in well method only showed inhibition at significant level to Pseudomonas aeruginosa. other tested bacteria were not sensitive at significant difference.in disc method methanol extract exhibit sensitivity to Proteus, E. Coli, Staphylococcus and Klebsiella

respectively. in comparison the inhibition of methanol extract only Klebsiella showed significant difference other test bacteria did not showed significant difference.

In comparison methanol extract with aqueous extract in well method only Klebsiella and Proteus showed a significant difference and the methanol extract was more inhibited. While in disc method there were significant difference between methanol and aqueous extract to E. Coli, Bacillus and Staphylococcus to side of methanol extract. at the three in used concentration.

Some studies obtained that U. dioica methanol extract contain flavonoids and alkaloids, phenols, sponins and Tannins and this plant was a rich source of flavonoids and alkaloids(Ahmed *et al*., 2012).

Methanol extract of nettle had obvious antibacterial activities and this may be due to high phenolic content and presence of active compounds such as alkaloids, tannins and terpenoids in nettle (Kais Kassim Ghaima, *et al* 2013).

It is obvious that the average activity of essence can be result of the reaction of its components because resultant of this reaction is positive or sometimes is negative.definitely, different effectiveness can be result of ecological, geographical, climatic factors and the age of plant on the mixing of various population of one or combined sort(Ramtin *et al*., 2012).

Ethyl acetate extract of nettle was more effective on all bacterial isolates (Aeromonas hydrophila, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Escherchia coli) than dandelion, with highest inhibition zone 24 mm towards B. cereus, A. Hydrophila was more resistant than other bacteria. Also it was found that nettle gave large inhibition zone to S. typhi (22mm). (Kais K. G.*et al*., 2013). It has been suggested that high resistant to plant extracts in gram negative bacteria is due to the outer membrane of their cell wall, acting as barrier to many substances including antibiotics (Marino *et al*., 2011). (Chahardehi *et al*. 2012) revealed that ethyl acetate , hexane and chloroform extracts showed higher antimicrobial activity than the other crude extracts, where the ethyl acetate extract showed highest inhibition against B. Cereus, Methicillin resistant Staphylococcus aureus and Vibro parahaemolyticus. Terpens and phenols of U dioica are one of the major groups associated with the inhibition of microbial infections and cancer (Dar *et al*., 2012). U. dioica is a rich source of phytochemicals such as phenolic compounds and minerals which can be used as a potential source of useful drugs (Ahmed *et al*., 2012).

The effect of chloroform extraction was more pronounced on the growth of Staphylococcus aureus rather than E. Coli and Pseudomonas. While alcoholic extraction has a marked effect on the growth of E.Coli in comparison to Staphylococcus aureus and Pseudomonas aeruginosa. Pseudomonas aeruginosa had low effect by the action of alcoholic and chloroform compared to water extraction (Ahlam, 2002).

Inhibition zone diameter of selected bacteria compared with Urtica dioica leaves essence illustrated that this essence had the highest activity against K. Pneumonia and B. Cereus. in addition, average activity was related to Staphy. aureus and P. Aeruginosa..It can be deduced that the effects of U. Dioica oils was better than ampicillin except for E. Faecalis and E. Coli.(Maryam *et al*., 2014)..

Water extract nettle exhibit antimicrobial activity against all tested microorganisms (Pseudomonas aeruginosa; E. Coli; Proteus mirabilis; Citrobacter koseri; Staphylococcus aureus; Streptococcus pneumonia; Enterobacter aerogenes; Micrococcus luteus; Staphylococcus epidermidis; and Candida albicans.). All concentration of WEN possessed noticeable antimicrobial activity against Gram – positive and – negative bacteria when compared with standard and strong antimicrobial compound as miconazole nitrate, amoxicillin – clavulanic acid (Ilhami *et al*., 2004).

CONCLUSIONS

Results of this study strongly confirm that aqueous and methanol extracts have inhibitory effect against all tested bacteria. There was significant difference according to concentration as there was concentration dependence difference. The methanol extract was morew inhibited. well method showed beter inhibition. Proteus was the most sensitive to aqueous. While to methanol extract Proteus and E.coli were the most sensitive.

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