The Biological effects of *uritica dioica* extraction on some pathogenic Bacteria

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Abstract:

The *Urtica dioica* is a flowering plant use in folk medicine, and as a food source. *Nettles* grow all over the world in mild to temperate climates. The study aimed to determine the antibacterial activity of alcoholic watery extracts of dry leaves of *Urtica dioica* on some pathogenic microorganisms (*E.coli, Staphylococcus. aureus*). The Plants were collected in the early time of January from Baghdad. Deride leaves of *Uritica* were extracted using co-solvent (Water and ethanol) extraction way by Soxhelt apparatus for crude extraction, and use rotary evaporator to obtain power extract. The series dilution was prepared 50%, 25, 12.5w/v that use on Mueller-Hinton agar plates against the *St.aureus, E.coli* by using well diffusion method then compared with antimicrobial activity of many antibiotics against the same isolates using Kirby-Bauer method. The crude extract of *U. dioica* was showed a significant antibacterial effect against *Staph.aureus* : at the concentration 50% the inhibition zone was 20 mm , followed by 25% (14mm) , and12.5%(10mm), and *E.coli* : at the concentration 50% the inhibition zone was18mm , followed by 25%(12mm) ,and 12.5(8mm) respectively. Based on the obtained results indicated the *U. dioica* plant is very useful used as antibacterial agent in treating bacterial infections.

Key words: Urtica dioica, Extraction, *Staph. aureus, E. coli, bioactivity.*

Introduction:

Urtica dioica or *L. Nettle* (Stinging nettle), It is consider the best-known member of the Urticaceae family , It is common worldwide wild vegetable with a medicinal properties (1), with a higher presence in Europe, North America, North Africa, and some regions of Asia (2,3). The species is divided into six subspecies, five subspecies of which bear many stinging hairs (trichomes), when trichomes touched, transforming the hair into a needle that can inject severalm chemicals: [histamine, acetylcholine 5-HT (serotonin), leukotrienes, moroidin, and possibly formic acid](4,5) causes paresthesia that may last for up to 12 hours. Its leaves, stems, and roots have unique merits in pharmaceutical application (5). The plant is enriched with many compounds such as vitamins and highly content of mineral, and proteins. 43 compounds were identified in the essential oil which are: Carvacrol (38.2%), Carvone (9.0%), Naphthalene (8.9%), E-anethol (4.7%), Hexahydrofarnesyl acetone (3.0%), E- geranyl acetone (2.9%), E-β-ionone (2.8%), and phytol (2.7%) (6). The leaves are rich sources of Terpenoids, Carotenoids, and fatty acids (7,8), It is contain various essential amino acids, Multi-Vitamins, Chlorophyll, Tannins, Carbohydrates, Sterols, Polysaccharides, Isolectins, and minerals, the most important of which is iron(98). Also, leaf of stinging nettle is highly rich in vitamins B, C, and K and minerals such as calcium, iron, magnesium, cobalt, manganese, phosphorus, potassium, and sodium, around 20% of dry mass is made of minerals (9), Also it is contain chlorogenic acid, rosmarinic acid, and caffeic acid as hydroxy cinnamic acids and quercetin as flavonoid which combinedly represents its antibacterial properties(10) it has a considerable activity against both Gram-positive, and Gram-negative bacteria. The needles release a mixture of some chemicals (Neurotransmitters – histamine, acetylcholine, serotonin, and Acids – formic, tartaric, oxalic), and also, it contains serotonin, ticoagulants, salicylic acid, and thymol (11, 12).

Almost 30% of dry mass is made of protein and the amino acid demand is taken care of by the protein on leaves (9). The amount of phenolic content in leaves is found in higher amounts as compared to root and stalk. The phenolic component mainly contains p-coumaric, kaempferol, and quercetin in roots, syringic myricetin, quercetin, kaempferol, and rutin in the stalk, and p-coumaric, isorhamnetin and quercetin in leaves (13), the nettle leaves have high antioxidant properties followed by their stalk and roots, more specifically, terpenoids, sphingolipids, steroids, lignans, flavonoids and other alkaloids represent the main bioactive constituents identified in U. dioica (14, 15, and 16). The scientist has explored many other useful characteristics of the *stinging nettle* plants as food like blood nourishment and the ability to fight against seasonal rhinitis nowadays its leaves are also consumed as juice, tea, and freeze-dried products (17). The plant U. dioica for a long time was used by the traditional medicinal practitioners for treatment various diseases such as nephritis, hematuria, jaundice, menorrhagia, arthritis and rheumatism. The leaves, and underlying foundations of plant utilized inside as a Blood purifier, Diuretic, Nasal menstrual drain, Stiffness, Skin inflammation, Iron Deficiency, Nephritis, Jaundice, menorrhagia furthermore, Hematuria, diarrhea. U. dioica has been reported to have various pharmacological activities like antibacterial, antioxidant, analgesic, anti- inflammatory, antiviral, immunomodulatory, hepatoprotective, anti- colitis and anticancer effects (18, 19, and 220.

Materials and Methods:

Leaves extraction

The leaves of U. dioica were collected from Baghdad-Iraq (Al-Kadhimiya).The plant classification has been confirmed in the Botany Department at the University of Baghdad, Ibn al-Haytham College by Dr. Israa Karim Nasrallah. The leaves were transported to the laboratory, washed, cleaned and dry at 40 ºC then grounded in a mortar and pestle, stored in dissector to be used for extraction. Alcoholic extract according to (21) with some modification using soxhlet apparatus, about 50 gm of dry leaf powder were weighed in the two filter paper , press them and put it in the extraction chamber of the soxhlet apparatus contain 250ml of 70% ethanol. The crude extraction was evaporated by the rotary evaporator by using 40ºC for 6hr, after evaporation ethanol and water eventually the extract converted to powder.

The Bioactivity Test

Nutrient agar, Brain heart infusion broth and Mueller –Hinton agar were prepared according to the manufacturing instruction. Staph. aureus, E. coli local isolates from urine in UTI patients was cultivated in brain heart infusion broth media and incubated for 18 hr Well diffusion method was used to screen the antimicrobial activity in vitro .Two replica were prepared from bacterial culture streaking in net shape on Mueller-Hinton agar , the well were prepared and the crude extract in serials dilution 50%, 25%,12.5% were aliquot in each well, the incubated for 24hr. The inhibition zone was measuring (22).

Antibiotic Susceptibility Test

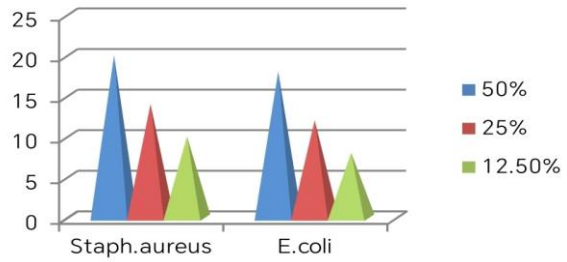
The antimicrobial susceptibility test was determined by using Kirby-Bauer disc diffusion method (22), It was utilized to detect the sensitivity of isolates to different types of antibiotics sullied from (Biofilchem, ITALY) (Vancomycin (VA30 µg), Cefotaxime (30µg), Meropenem (MEM10 µg), Ciprofloxacin (CIP5µg), Amikacin (Ak 30 µg),Ampicillin (P10 µg), Streptomycin (S 25µg), Nitrofurination (30 µg), Imipenem (IPM10µg), Azthromycin (AZM5 µg), Nalidixic (NA30µg), Cefalexin (CN10µg), and Levofloxacin (LEV5 µg).The inhibition zone were measured and the result was interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS)(23).

Results and Discussion

Crude extracts of *U. dioica* showed a significant antibacterial effect against both Gram positive and Gram negative pathogenic bacteria. When three different series concentrations of the crude extract were used against Staph. *aureus* and *E. coli* using well diffusion method. Sajfrtová et al refers to a linear relationship between the size of the inhibition zone and logarithm of the concentration tested compound which is determined by comparison the diameter of the zone with the specific standard. Increasing the concentration of the extract increased the diameter of growth inhibition zone of bacteria. It

was noted that both bacterial isolates were sensitive to the extract, and the bacteria showed the highest sensitivity at the concentration of 50%, followed by the %25concentration, then the 12.5% concentration of crude extract. Our finding that the *Staph.aureus* was more susceptible to the crude alcoholic extract than *E.coli*. When conducting a sensitivity test to antibiotics to compare it with the response of bacteria to the crude extract, it was found that the *Staph.aureus* bacteria showed sensitivity to antibiotics IPM,AK,VA,CIP while they were multiple drug resistant(MDR) to more than one antibiotics as shown in Table 1.

Figure 2: The bioactivity of alcoholic crude extract of *U. dioica* against *Staph.aureus* and *E.coli*



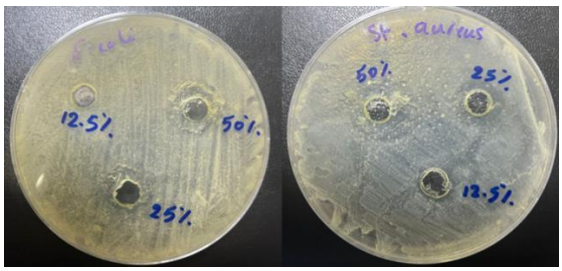


Figure 3: The bioactivity of alcoholic crude extract of *U. dioica* against *Staph.aureus* and *E.coli* on Mueller-Hinton agar after 24hr incubation

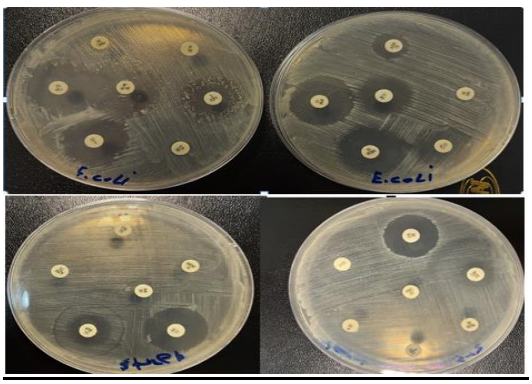


Figure 4: The antibiotic susceptibility test of *Staph.aureus and E.coli* bacterial isolates

Table1: The antibiotic susceptibility test result against *Staph. aureus* and *E.coli*

Antibiotics(µg)	***Staph aureus***	***E.coli***
AK(10)	***S***	***S***
NA(30)	**I**	**R**
MEM(10)	**R**	**S**
AZM(15)	**R**	**I**
IPM(10)	**S**	**S**
CN(10)	**R**	**S**
VA(30)	**S**	**R**
CIP(10)	**S**	**S**
NOR(30)	**R**	**S**
LEV(5)	**R**	**S**
AMC(30)	**R**	**S**
P(10)	**R**	**R**
S(25)	**R**	**S**

Nasiri et al (12) Consistent with our results indicated that the concentration of the extract may effect on antimicrobial activity. The study of Shahidi et al (13) refers the evaluated the antimicrobial effect of 45 Iranian native plants on three strains of *S. aureus* and found that increasing the concentration of the extracts increases the antimicrobial effects. According to the results, different bacteria species may be responded differently to treatment with the extract. Shariat et al. (18) concluded that the MIC of the aqueous extract of nettle against E. coli was 2.5mg/ml, while MBC was reported as 20mg/ml. Kavalali (16) reported that the watery ethanolic extract of nettle inhibited the growth of E. coli, in line with our findingsThe Gram positive bacteria *Staph. aureus* were appeared more susceptible to the crude watery ethanol extract than Gram negative bacterium *E. coli* ,which may be associated with the variation permeability of the extract through the walls and bacterial membrane. Singh , and Kali (14)

they conclude that the hydrophilic characterization, and unique structure of gram-negative bacteria membrane acts as a barrier against external agents such as hydrophilic dyes, antibiotics, and detergents, their porins ability to determine the type, and size of substances that can reach to their cytoplasm. Which explain why the permeability of this bacteria is much less than that of gram positive bacteria as mention by several, studies of Grauso, et al (2), and Esposito et al (15). In previous study in 1985, Janssen *et al* (9) mention to that nettle's extract inhibited the growth of *Staph. aureus*, and after that the Study of Kavalali in 2003(16) reported that the watery ethanolic extract of nettle was inhibited the growth of *Staph. aureus* bacteria, these finding is consistent with the findings of the present study. The difference in the effects of plant extracts on bacteria is depending on many factors: the ecological, climatic, and geographic factors, plant's age, methods of processing, drying, methods of extraction, purification of active components, concentration of compounds, and type of culture medium (4). This could be due to differences in the methods of extraction, strains studied, solvents and methods used. In this regard, Shahidi et al. (14, 18), Nettle's constituents such as terpenes and phenols are considered as effective agents in inhibition of microbial infections (20). This is consistent with Kukri´c(11), and Ahmadi (9) Notably, more advance research needs to determine and discovering new natural the compounds that inhibit the growth methicillin-resistant S. aureus (MRSA).Our conclusion that the Crude alcoholic extract of this plant has antimicrobial properties on both gram-negative and gram-positive bacteria, comparable to the effectiveness of antibiotics, such as Ak, IPM, and CIP.

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