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

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Abstract

The *Urtica dioica* is a flowering plant with long history of 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* showed significant antibacterial effect against some clinically important pathogenic bacteria *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 it can be concluded that *U. dioica* is useful as antibacterial agent in treating infectious diseases.

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

Introduction

Urtica dioica or *L. Nettle* (Stinging nettle), It is 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 mpossibly 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, carotenoidsand fatty acids(7,8), as well as of various essential amino acids chlorophyll, vitamins, 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). Due to the variety of phytochemicals and their proportions they contain, 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 is widely used by the traditional medicinal practitioners for curing various diseases such as nephritis, hematuria, jaundice, menorrhagia, arthritis and rheumatism. The leaves and underlying foundations of plant a utilized inside as a blood purifier, diuretic, nasal menstrual drain, stiffness, skin inflammation, iron deficiency ,nephritis ,hematuria, jaundice, menorrhagia furthermore, 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, 20)

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 antibiotic susceptibility test was determined using Kirby-Bauer disc diffusion method (22), 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

Extracts of *U. dioica* showed significant antibacterial effect against some clinically important pathogenic bacteria. When three different concentrations of the extract were used, 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.

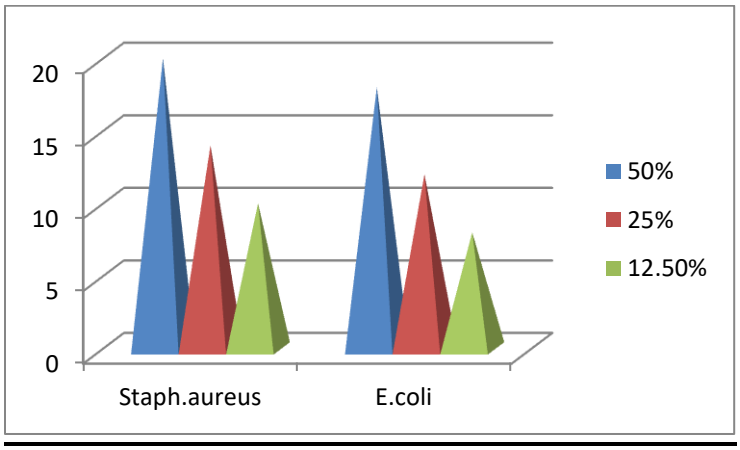

Figure 1.The bioactivity of alcoholic crude extract of *U. dioica* against *Staph.aureus* and *E.coli*

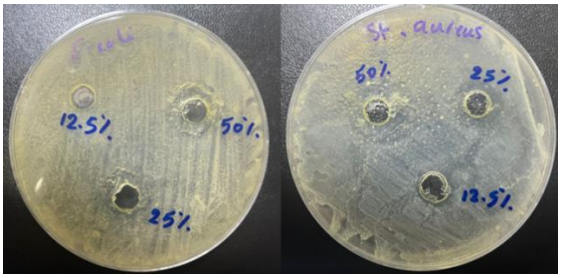

Figure 2. The bioactivity of alcoholic crude extract of *U. dioica* against *Staph.aureus* and *E.coli* on Mueller-Hinton agar after 24hr incubation

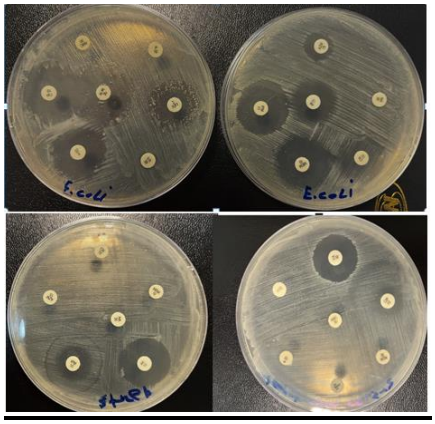

Figure 3. The antibiotic susceptibility test of *Staph.aureus and E.coli* bacterial isolates

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**

Discussion

Natural compounds are considered as important sources of new antibacterial agents (11). This study evaluated the inhibitory activity of the watery ethanolic extract of nettle leaves isolated from three different regions against *S. aureus and E. coli* using well diffusion method. The diameter of growth inhibition zone is influenced by the concentration of active compound in the plant. There is a linear relationship between size of the zone and logarithm of the concentration tested. The antimicrobial power of the compound tested is determined by measuring diameter of the zone and its comparison with the specific standard (8). Increasing the concentration of the extract increased the diameter of growth inhibition zone of bacteria. Several studies have shown that concentration of the extract affects its antimicrobial effects as mention by (12). Consistent with our results, study of Shahidi in 2004 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 effect (13). According to the results, different bacteria responded differently to treatment with the extract. The gram positive bacteria *Staph. aureus* were more susceptible to the crude watery ethanol extract than gram

negative bacterium *E. coli* ,which may be associated with the power of extract to penetrate inside bacteria. The membrane of gram-negative bacteria is highly hydrophilic and acts as a barrier against external agents such as hydrophilic dyes, antibiotics and detergents (14). There can be due to unique structure of gram-negative bacteria. They have an outer membrane envelope that act as a barrier to the entrance of some substances and their porins determine the type and size of those substances that can reach to their cytoplasm. As a result, the permeability of the membrane of these bacteria is much less than that of gram positive bacteria, which is reported by several, studies (2, 8, and 15). In various studies, the bactericidal effect of nettle on Staphylococcus has been well demonstrated. In 1985, Janssen et al. stated that nettle's extract inhibited the growth of S. aureus (9). Study of Kavalali in 2003 reported that the watery ethanolic extract of nettle inhibited the growth of S. aureus (16), consistent with the findings of the present study. The difference in the effects of plant extracts on bacteria depends on various factors including ecological, climatic and geographic factors, plant's age, methods of drying and extraction of active components, type of solvent, concentration of the extract and type of culture medium (4). Shariat et al. (18), the minimum inhibitory concentration of the aqueous extract of nettle against E. coli was 2.5mg/ml, while the minimum bactericidal concentration 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 findings. This could be due to differences in the methods of extraction, strains studied, solvents and methods used. Some studies also showed that nettle has little inhibitory effect on the growth of E. coli (14, 18,19). 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).Considering therefore mentioned issues, the intensity of the antimicrobial activity of U. dioica L. could be influenced by the amount of phenolic compounds

and secondary metabolites. Thus, the antibacterial activity of the plant could be attributed to the mentioned compounds. Considerable antibacterial activity against gram-positive species suggests that the extracts of U. dioica can be selective agents for treatment of infections caused by these organisms. Based on the obtained results it can be concluded that U. dioica is useful as antibacterial and bactericidal agent in treating infectious diseases, this is consistent with Kukri´c(11), and Ahmadi (9) Notably, excellent inhibition against Staphylococcus species can be a hope for discovering new natural antibacterial agent against coagulase-positive and coagulase-negative staphylococci, especially methicillin-resistant S. aureus (MRSA) These results showed that the target site of active constituents of *U. dioica* was bacterial cell wall. As the peptidoglycan synthesis and cell shape of bacteria is dependent to penicillin-binding proteins (PBPs), it can be concluded that these antibacterial substances inhibited these proteins and consequently could disrupt the integrity of peptidoglycans. These types of antibacterial compounds have two important superiorities over the others. First, they have bactericidal effect and recurrence of the infection due to the persistence of pathogen will be prevented. Second and most importantly, these agents affect cell wall and with regard to the uniqueness of this structure, these compounds can be regarded as safe antibacterial agent with no or less side effects on eukaryotic cells. Furthermore, the cell wall has a significant role in maintaining high internal pressure of bacterium. Those antibacterial agents that can affect this structure are suitable for killing bacteria (19). It is known that many factors affect antibacterial activity. Therefore, we believe that the bacterial inhibition or killing can vary based on the plant extract, the used solvent, and the tested organisms. Concluded 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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