The inhibitory effect of alcoholic extracts from the leaves, aerial parts and roots of *Corchorus olitorius* L. (Molukhiya), on some pathogenic bacterial species

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Abstract

In this study, the preparation of alcoholic extracts of leaves, aerial parts, and roots of the plant *Corchorus olitorius* L. in powder form have been prepared in different concentrations consisted of (30, 50, 70) mg/ml. Extracts were used to detect the inhibitory activity on gram's negative and gram's positive pathogenic bacteria, which include the following types: *Klebsielle pneumoniae*, *Escherichia coli*, *proteus mirabilis*, *Serratia marcescuns*, *Aeromonas hydrophila Staphylococcus aureus* and *Streptococcus fecalis*. The results of the inhibitory activity of alcoholic extracts depending on the different types of bacteria, and there was a clear increase in the rate of inhibition range with increasing the concentration of plant extracts against bacteria, All concentration of extracts were found to exhibit significant differences at level ($p<0.05$) by antimicrobial activity against most bacterial species. Also the results showed significant differences among the alcoholic extracts of the leaves, aerial parts and roots extracts on the growth of some types of bacteria at level ($p<0.05$).

التأثير التثبيطي للمستخلص الكحولي من أوراق وسيقان وجذور نبات الملوخية *Corchorus olitorius* L. تجاه بعض أنواع البكتريا المرضية

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الخلاصة

حضر في هذه الدراسة المستخلص الكحولي لمسحوق أوراق وسيقان وجذور نبات الملوخية *Corchorus olitorius* L. واشتملت تراكيز مختلفة (30، 50، 70) ملغم/ مل تبين فيها التأثير التثبيطي على نمو بعض أنواع البكتريا المرضية السالبة والموجبة لصبغة جرام وتضمنت: *Klebsiella pneumoniae* و *Escherichia coli* و *Proteus mirabilis* و *Serratia marcescuns* و *Aeromonas hydrophila* و *Staphylococcus aureus* و *Streptococcus fecalis*. لقد تنوعت نتائج دراسة الفعالية التثبيطية لمستخلص أوراق وسيقان وجذور نبات الملوخية باختلاف النوعية والتراكيز وكانت هناك زيادة واضحة في معدلات أقطار مناطق تثبيط نمو أنواع البكتريا المرضية بزيادة تركيز كل من المستخلصات الكحولية النباتية لأوراق وسيقان وجذور نبات الملوخية واشتملت فروقات تثبيطية واضحة بمستوى ($p<0.05$) تجاه نمو معظم أنواع البكتريا، وكذلك اظهر البحث فروقات معنوية تثبيطية مابين أنواع المستخلصات بمستوى($p<0.05$).

Introduction

Corchorus olitorius L. is known to occur in nature as it is distributed in the tropics of both hemispheres. They are used in soups under the Arabic name "Molukhyia". In Egypt and India the leaves and tender shoots are eaten (1). The leaves material contains Triterpenes, Sterols and Fatty acid (2), Phenolics (3), and Ionones (4). Per each 100 g, the leaves of *Corchorus olitorius L.* are reported to contain 4.5-5.6 g protein, 0.3 g fat, 7.6-12.4 g total carbohydrate, 1.7-2.0 g fiber, 2.4 g ash, oxydase, chlorogenic acid, saponins, and Flavones (5). An acidic polysaccharide isolated from water soluble mucilage extracted from dried leaves of *Corchorus olitorius* L. was rich in uronic acid rhamnose, glucose, galcturonic acid, and glucuronic acid (6). The root of *Corchorus olitorius* L contain Triterpenes (7), Sterols (8), a triterpenoid corosin, and sitosterol (9). This is the first research which detects the inhibitory activity against many species of pathogenic bacteria like *Klebsielle pnumoniae*, *Escherichia coli*, *proteus mirabilis*, *Serratia marcescuns*, *Aeromonas hydrophila Staphylococcus aureus*, and *Streptococcus fecalis* by alcoholic extracts of Leaves, aerial parts and roots of *Corchorus olitorius* L. *Klebsielle pnumoniae* can cause a variety of extra pulmonary infections, including enteritis and meningitis, urinary tract infections and septicemia (10). *Escherichia coli* is the most bacterial species commonly recovered in the clinical laboratories and has been incriminated in infectious diseases involving virtually every host tissue and organ system, and produce dysentery like diarrheal syndromes, Urinary tract, wound infectious and pneumococcal meningitis in neonates (11). *Aeromonas hydrophila* causes gastroenteritis (12) cellulitis and wound infections, diarrhea, septicemia, and miscellaneous (13, 14). *Proteus mirabilis* is most frequently recovered from humans, particularly as the causative agent of both urinary tract and wound infections (15). *Serratia marcescuns* causes bovine mastitis, and septicemia in chickens (16). *Staphylococcus aureus* is the most virulent species that produce several toxins and enzymes that mediate tissue invasion and survival at the infection site, wounds and deep tissue, Impetigo, necrosis of tissue, folliculitis, furuncles, scalded skin syndrome in neonates, toxic shock syndrome, and food poisoning (17). *Streptococcus fecalis* is an opportunistic bacteria that causes septicaemia in chickens, bovine mastitis, endocarditis in cattle and lambs, also urinary tract infections in dogs (18).

***Corchorus olitorius* L.**

Material and Method

The plant *Corchorus olitorius* L. was collected from the local area of Baghdad in the month of July- August 2011. This plant was identified by science Department of Biology colleges of Baghdad University. The leaves, aerial parts and roots of *Corchorus olitorius* L. were collected, washed and dried under shade, after that they were coarsely powdered with a mechanical grinder. All the powders were passed through sieve No. 40 and stored in an airtight container for extraction by Soxhlet apparatus. In this method 100 gm of leaves, aerial parts and roots powdered drugs were ground into small particles and placed in porous cellulose thimble, the thimble is placed in an extraction chamber (19), which is suspended above a flask containing the petroleum ether and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask at the end of 24 hour (20), the extracted materials was dried and then extracted with 95% methanol in Soxhlet apparatus for 72 hour. After completing the extraction, the extracts were filtered through Whatmann No.1 filter paper. The filtrates were concentrated to dryness in vacuum and stored in desiccators (21). After that, alcoholic extracts have been prepared in different concentrations of each extract consisted of (30, 50, 70) mg/ml. Extracts were used to detect the inhibitory activity to bacterial species that were identified and produced by Microbiology Department of Veterinary Medicine of Baghdad University which included: *Klebsielle pnumoniae*, *Escherichia coli*, *proteus mirabilis*, *Serratia marcescuns*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Streptococcus fecalis*. Sterile Muller Hinton agar was poured in petri plates under aseptic conditions, the test organisms 0.1 ml were spread on agar plates by sterile glass spreader, then agar disk diffusion method (22), by added the disc 6 mm in diameter that impregnated with (30, 50, 70) alcoholic extracts of leaves, aerial parts and roots of the plant with control methanol disc (5mg/disc) (23). The plates containing bacterial strains were incubated at 37 ± 0.5 º C for 48 hours, depending on the incubation time required for visible growth. After incubation, the plates were examined for the presence of zones inhibition of bacterial growth (clear ring) around the antimicrobial discs. The zone of inhibition (mm) was calculated by measuring the diameter of zone of bacterial growth around the disc; the average of three independent determinations was recorded (24). The values were considered statistically significant ANOVA (Analysis of variance) for least significant differences (25).

Results and Discussion

As in Table 1, the results of the inhibitory activity of alcoholic leaves extracts showed that the diameters of the inhibition zone were (10.6 ± 0.7) mm for *Klebsiella pnumoniae*, (7.33 ± 0.7) mm for *Escherichia coli*, then (3.44 ± 0.6) mm for *proteus mirabilis*, and (3.44± 0.6) mm for *Serratia marcescuns* at the concentration of 70 mg/ml. As for the diameter range of the inhibition zone of *Staphylococcus aureus* was (6.6 ± 0.7) mm at 50mg/ ml, but (12.7± 0.7) mm at 70mg/ml. As to *Streptococcus fecalis*, the results were (8±0.6) mm at 50 mg/ml, while (11.3±0.7) mm at the concentration of 70 mg/ml. The activity of the extract gave the best result on *Aeromonas hydrophila* as it was (12±1.2) mm at the concentration of 30 mg/ml, (16±2.4) mm at 50 mg/ml, and (20±1.2) at 70 mg/ml.

Table (1) Antimicrobial activity by alcoholic extract of leaves from *Corchorus olitorius* L.

Concentrationmg/ml / Bacterial species	30	50	70	Control 95% methanol
	Diameters of zone inhibition (mm)			
Klebsielle pnumoniae	-	-	10.6 ± 0.7	-
Escherichia coli	-	-	7.33 ± 0.7	-
proteus mirabilis	-	-	3.44 ± 0.6	-
Serratia marcescuns	-	-	3.44± 0.6	-
Staphylococcus aureus	-	6.6 ± 0.7	12.7± 0.7	-
Streptococcus fecalis	-	8 ± 0.6	11.3 ± 0.7	-
Aeromonas hydrophila	12 ± 1.2	16 ± 2.4	20 ± 1.2	

Then in Table 2, the results of the inhibitory activity of alcoholic aerial parts extracts showed the diameters of the inhibition zone arrowed the disks of alcoholic aerial parts extracts on Muller Hinton media gave significant results at level ($P<0.05$) as follows: (6.6 ± 0.67) mm for *Klebsiella pnumoniae* at the concentration of 50 mg/ml and (10.6 ± 0.7) mm in 70 mg/ml, then (6.6 ± 0.67) mm for *Escherichia coli* at the concentration 50 mg/ml and (9.3 ± 0.7) mm in 70 mg/ml. As to *proteus mirabilis* the results were (11.3 ± 0.7) mm at 50 mg/ml and (12 ± 1.2) mm at the concentration 70mg/ml, while *Serratia marcescuns* have an inhibition zone of (6 ± 1.2) mm at the concentration of 70 mg/ml, put they have no significant effect at 30 mg/ml and 50 mg/ml. The diameters ranges of the inhibition zone of *Staphylococcus aureus* were (10 ± 1.2) mm at 50 mg/ml and (10.3± 0.9) mm at 70 mg/ml, where as for *Streptococcus fecalis* the result were (8.7 ± 0.7) mm at 50 mg/ml, and (9±0.6) mm at the concentration of 70 mg/ml. Also the activity of the aerial parts extracts gave a significant results on *Aeromonas hydrophila*; as it was (14.6±0.7) mm at the concentration of 30 mg/ml, (14.6 ± 0.7) mm at 50 mg/ml, and (14.7 ± 0.9) mm at 70 mg/ml.

Table (2) Antimicrobial activity by alcoholic extract of Aerial part from *Corchorus olitorius* L.

Concentration mg/ml / Bacterial species	30	50	70	Control 95% methanol
	Diameters of zone inhibition (mm)			
Klebsielle pnumoniae	-	6.6 ± 0.67	10.6 ± 0.7	-
Escherichia coli	-	6.6 ± 0.67	9.3 ± 0.7	-
proteus mirabilis	-	11.3 ± 0.7	12 ± 1.2	-
Serratia marcescuns		-	6 ± 1.2	-
Staphylococcus aureus	-	10 ± 1.2	10.3 ± 0.9	-
Streptococcus fecalis	-	8.7 ± 0.7	9 ± 0.6	-
Aeromonas hydrophila	14.6 ± 0.7	14.6 ± 0.7	14.7 ± 0.9	

The results of an inhibitory activity of alcoholic roots extracts of *Corchorus olitorius* L. was significantly effective to all kinds of bacterial growth at level ($P<0.05$), as in Table 3.

Table (3) Antimicrobial activity by alcoholic extract of root from *Corchorus olitorius* L

Concentration mg/ml / Bacterial species	30	50	70	Control 95% methanol
	Diameters of zone inhibition (mm)			
Klebsielle pnumoniae	-	4 ± 2.1	10± 1.2	-
Escherichia coli	-	4 ± 2.03	10 ± 1.2	-
proteus mirabilis	-	7.3 ± 0.7	8.7 ± 0.7	-
Serratia marcescuns	-	-	6 ± 1.2	-
Staphylococcus aureus	-	-	8 ± 1.2	-
Streptococcus fecalis	-	7.7 ± 0.9	11.3 ± 0.7	-
Aeromonas hydrophila	12.7 ± 0.7	14.7 ± 0.7	19.3 ± 0.7	

The extracts were used to detect the inhibitory activity on different bacterial species, which depends on the type of the extract with different types of bacteria, and there was a clear increase in the rate of inhibition range with increasing the concentration of plant extracts against bacteria as I have discussed earlier. The biological effects of the flavonoids listed thirty-three different manifestations of activity under the name of "Bioflavonoids" like hesperdin, also called vitamin P or permeability factors which are used in the treatment of various diseases and symptoms of common cold, as they are generally considered antimicrobial and lipoxygenase inhibiting activity (26). The extracted material considered as anti-inflammatory (27) and antibacterial activity (28). The inhibitory activity of leaves extracts against *Aeromonas hydrophila* which was (20±1.2) mm at the concentration of 70 mg/ml, where as the test did not show any effectiveness of inhibition to *Klebsielle pnumoniae Escherichia coli, proteus mirabilis Serratia marcescuns*, *Staphylococcus aureus* and *Streptococcus fecalis* at low concentration 30 mg/ml of all kinds of extracted materials (29). Microorganisms may be resistant to antimicrobial drugs because the cellular mechanisms required for antimicrobial susceptibility are absent in the cell, this is sometimes referred to as constitutive or pre-existent resistance (30). The concentration of 70 mg/ml alcoholic extracts was found to exhibit significant antimicrobial activity against most bacterial species (31). The high concentration caused significant increase in WBC count only at a high dose level of treatment (32). The leaves, aerial parts and roots are stomachic and used in treatment of pnumoniae (33). The plant is said to possess anticancer, antipyretic, anticonvulsant, stomachic and digitalis glycosides like action, the Phenolics and Flavones compounds found in the *Corchorus olitorius* L. extracts inhibits the effect of the bacterial enzymes needed for essential metabolic reactions by interfering with the bacterial proteins (34). Therefore, the protein molecules are denatured as they become completely disorganized in a mechanism under process, the bacterial cell will stop growing and it will eventually die (35). The most important target of this study is directed towards the use of the botanic compounds as alternatives to pharmaceutical drugs as there are insufficient recent studies about this plant in Iraq. This study has shown the efficiency of alcoholic extract against some types of pathogenic bacteria when they were somehow in low concentrations, and this what lacks in the results of many researches about the alcoholic extracts efficiency. As the inhibitory effect of the leaves, aerial and roots of *Corchorus olitorius* L. is being studied, it opens the door for more researches about using the extract instead of pharmaceutical drugs.

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