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Phytochemical Study with Evaluation the Antimicrobial Activity of *Cressa cretica* Plant Against Some Gram-Positive and Gram-Negative Bacteria

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Abstract

The antimicrobial activity of the alcoholic extract of the *Cressa cretica* plant was evaluated against six Gram-positive and Gram- negative bacteria: *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella aerogenes* using well diffusion method. The maximum antibacterial activity was achieved with the concentration of 500 mg/ml alcoholic extract of *Cressa cretica* . *E. coli* was showed the highest inhibition of the growth followed by *Klebsiella aerogenes* and *Staphylococcus aureus*, while the other concentrations of (250,125) mg/ml did not show any inhibiting effect for all kinds of the bacterial used. Moreover, the preliminary phytochemical analysis revealed that there were a present of tannins, saponins, terpenoides and flavonoids using different solvents.

Keyword: Phytochemicals, Antibacterial activity, *Cressa cretica* extract.

1. Introduction

Antibiotics are one of the most important human achievements in the field of medicine, which contributed to the treatment of wide range of infectious diseases caused by bacteria. However, the over and random use of antibiotics may enhance the resistance of bacterial species that cause human diseases [1], in addition to the side effects of antibiotics which cause the death of many patients. The increasing antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [2]. In the last decade, there has been exponential growth in the field of herbal medicine and the popularity of herbal drugs for the management of various diseases in the developing and developed countries due to its natural origin with lesser side effects [3]. All parts of the plant can be used as herbal medicine such as leaf, fruit, stem, bud,

rhizome, latex, peel, essential oil. Cells of plant accumulate a large variety of phytochemical compounds such as steroids, saponins, tannins, glycosides, phenolic compounds, flavonoids, etc… [4]. Medicinal plants have an immune modulatory and antioxidant effect which stimulate specific and non- specific immunity and lead to antimicrobial activities [5]. According to the World Health Organization (WHO), plant extracts or their active constituents would be the best source to obtain a variety of drugs [6].

Cressa cretica ,is known in Arabic as: Molleih, Nadewa; Bengali as: Rudravanti; English as: Alkali weed, Rosin weed, Cressa; French as: Cresse de Crète, Cresse à feuilles d'herniaire; Hindi as : Rudravanti; Poland as: Erva molhada[8,11] , belonging to Convolvulaceae family, it is a small, dwarf shrub, up to 38 cm height, roots are germinating horizontally with lateral branches. Stems are at first erect and then become decumbent. The leaves of the plant are small and compressed. The flowers are small with white color [7]. *Cressa cretica* grows in sandy or muddy saline habitats [3], such as some region of Iraq, Qatar, Bahrain, Oman, Sudan, Tunisia, Jordan, Turkey, India, Australia, Pakistan, Afghanistan, and many other countries in the Mediterranean basin, Southeast Asia and South America, in addition to Central and North Africa [8].

In the folklore medicine, the plant *Cressa cretica* was used for combating ulcers, diabetes, leprosy, anthelmintic, asthma, urinary disorders, and constipation and as a stomachic, tonic, aphrodisiac, and enriches the blood [9]. It was used as an expectorant and anti- aging, it is reported to be antibacterial, antifungal [10], and antiviral infections [11]. The pharmacological activity of *Cressa cretica* was not limited to humans only. Rather, it has proven effective as a parasitic herbicide such as *Cuscuta campestris.* L [12]. In Sudan the dry leaves of *Cressa cretica* were mashed with sugar and used as emetic [13]. The fruits of *Cressa cretica* are possible source of edible oil which is free from any unfavored compounds [14]. The phytochemical compound of *Cressa cretica* was reported to contain: phenolic compounds, terpenic compounds, coumarins, sterols, alkaloids, tannins, glycosides, protein, carbohydrate, flavonoids, unidentified sugars, and high salt contents [15].

The present study aimed to detect the active compounds of *Cressa creticca* plant and the antibacterial activity of alcoholic extract against some bacterial species.

2. Materials and Methods

Plant Collection

Plant materials were collected from Al- Musaib town at (70 km) south of Baghdad in middle region of Iraq. After collection, the plant materials were washed and dried at room temperature in the shade, then pulverized by mechanical mills and weighed.

Plant Extraction

Fifty grams of air- dried powdered plant materials were packed in the thimble of soxhlet apparatus and extracted with (500) ml of hexane for 18 hours. The resulting extract was filtered and the solvent had been evaporated using a rotatory evaporator to get a dry extract. The residue was packed again in the thimble of soxhlet apparatus and extracted with (500) ml of chloroform for 24 hours. The extract was filtered and the solvent had been evaporated using a rotatory evaporator to get a dry brown extract. The residue was packed again in the thimble of soxhlet apparatus and extracted with e 70% ethanol, for 16 hours. The extract was filtered, and the solvent was evaporated using a rotary evaporator to get a dry brown extract [16].

Phytochemical Screening

Investigation for chemical and bioactive compounds has been done for saponins, trepenoids, tannins, flavonoids. The chemical tests were carried out on the air- dried powdered plant materials [16].

Test for Saponins

Ethanolic plant extract (5) ml was mixed with distilled water (5)ml in a test tube and then shaken until persistent foam was appeared.

Test for Terpenoids

Ethanolic extract of *Cressa cretica* (5)ml was mixed with the chloroform (2ml) and concentrated sulphuric acid (3)ml was carefully added. A reddish – brown color of interface is formed to indicate the presence of terpenoids.

Test for Tannins

Powdered sample of *Cressa cretica* (0.5)g was boiled with distilled water (20)ml in a test tube and then filtered, then 1% of ferric chloride was added. The brownish – green or blue – black color indicates that tannins was present.

Test for Flavonoide

Ethanolic KOH (2) ml was added to (1)ml of ethanolic extract of *Cressa cretica.* The formation of yellow color indicates the appearance of flavonoids.

Bacterial Isolates

The microorganisms used in the study are clinically important pathogenic which are causing several infection diseases. A total of six isolates were used: *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aegenosa, E coli,* and *Klebsiella aerogenes*. All isolates were obtained from microbiology laboratory / College of Pharmacy / University of Baghdad, and identified using conventional technique [17].

Antimicrobial Activity Determination

For inoculum preparation, overnight culture of bacterial colony isolates were transferred to (5) ml tube of normal saline and turbidity was adjusted to (0.5) McFarland tube to 1×10^{8} CFU/ml bacterial culture.

Agar Well Diffusion Method

Antibacterial activity of the extract was determined in accordance with the agar well diffusion, using Mueller- Hinton Agar (MHA) as a culture media. The plates were inoculated by a swab by dibbing it into the inoculum; pressing and rotating the swab against the tube sides to eliminate of excess fluid. Swab was rubbed over the medium surface three times and the plate was rotated at 60 angle after each one application. Finally, the swab was passed across the edge of the agar surface, left the plate to dry for 5 minutes at room temperature with closed lid. Five wells at the distance of approximately (3) cm were made by using sterile (7) mm diameter cork borer [18,19], Three wells were used for samples, (100) µl of different extract concentration (500, 250, 125) mg/ml was poured into each well. Two wells were loaded with control, one with (100) µl doxycycline (50) mg/ ml as a positive control, and the negative control was media only. All petri dishes were incubated at 37Cº for 24h. After this period, the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed.

3. Results and discussion

The results of preliminary phytochemical analysis indicated the presence of flavonoids, tannins and saponins in 70% ethanolic extract, and terpenoids in hexane and chloroform, as shown in **Table (1)**

Table 1. Qualitative profile of the phytochemicals found in *Cressa cretica* plant.

Extract	Flavonoids	Tannins	Saponins	Terpenoids
Aerial part	+	+	+ +	+ +

(+) low ; (+ +) average

Flavonoids are considered as one type of phenols which happened naturally and have multi biological activities including antibacterial action and anti-inflammatory through inhibiting the activity of some enzymes which are responsible for metabolic processes in bacteria [24]. While saponins have an ability to degrade the plasma membrane of bacteria. Terpinoids can degraed the cell membrane of bacteria as well [25]. Tannins are water- soluble polyphenols have both bacteriostatic and bactericidal activities [26], which can inhibit protein synthesis inside cell of bacteria[27]. All these phytochemical components may inhibit the activities of microorganisms. Various concentrations of plant extract have showed a very poor antimicrobial activity. The data presented in **Table (2)** indicated that alcoholic extract of *Cressa cretica* inhibits the growth of some microorganisms and **Figure (1)**.

Table 2. Antibacterial activity of alcoholic extract of *Cressa cretica* showing zones of inhibition at 500, 250 and 125 mg/ ml of the extract.

Concentration of extract / Tested Bacteria	500 mg / ml	250 mg / ml	125 mg / ml
Pseudomonas aeruginosa	_	_	_
Klebsiella aerogenes	10 mm	_	_
Escherichia coli	13 mm	_	_
Bacillus cereus	_	_	_
Staphylococcus aureus	10 mm	_	_
Enterococcus facalis	_	_	_

(-) no inhibition zone

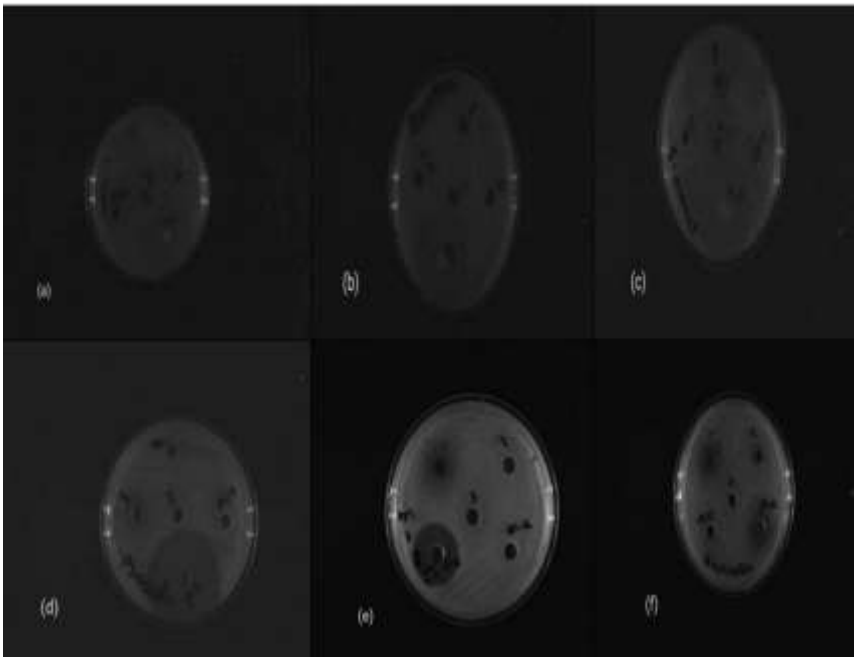

Figure 1. Antibacterial activity of alcoholic extract of *Cressa cretica* showing zones of inhibition in pathogenic bacteria: **(a)** *Enterococcus facalis* ; **(b)** *Bacillus cereus*; **(c)** *Staphylococcus aureus*; **(d)** *Pseudomonas aeruginosa*; **(e)***Escherichia coli*; **(f)** *Klebsiella aerogenes*

These results screen different concentrations of *Cressa cretica* extract for antimicrobial activity against gram- negative bacteria and gram- positive bacteria, it was found that the maximum effect at the highest concentration of (500)mg/ml and the inhibition zones were (13, 10, 10)mm for *Escherichia coli*, *Klebsiella aerogenes* and *Staphylococcus aureus* respectively, while other concentrations of extract using (125,150) mg/ml have no effect on the growth of any kind of all used bacteria. All gram negative bacteria are sensitive to the concentration of (500) mg/ml extract, except of *Pseudomonas aeruginosa* which is responsible for nosocomial infections, this was attributed to the permeability barrier of outer membrane which prevents or decreases the penetration of numerous antimicrobial agents [28].

These results were in disagreement with [29] which has reported a good effect of *Cressa cretica* extract against Gram –positive and Gram –negative bacteria. The antimicrobial activity may include complex mechanisms, such as the inhibition of cell membrane and cell wall synthesis, protein and nucleic acid synthesis, and inhibition of nucleic acid metabolism [30]. Numerous studies showed various results about the action of any kind of bacteria which was used against different plant extracts [29,31,32,33].

The results in **Table (2)** were compared with antibiotic (doxycycline 50 mg /ml) which used as a control, this antibiotic is a type of tetracycline that inhibits protein synthesis inside cells of bacteria and their growth, and it is common used as antibacterial agent against gram- positive and gram- negative bacteria [34]. In addition, tannins were one of the phytochemical components in this plant which have the same action of doxycycline in the inhibition of protein synthesis [27]. It was found that inhibition zones of plant extract were smaller than those of antibiotic, and the mean of inhibition of *Klebisella aerogenes* was closed to the diameter of antibiotic inhibition zone (**Table 3**). This can be due to the less effect on the suspension of

bacteria or the nature of cell membrane capability of bacteria using virulence factors which are weakened the inhibition of microorganism.

Table 3. The diameter of inhibition zone of bacteria using doxycycline (50 mg/ml)

P. aeroginosa	*K. aerogenes*	*E. coli*	*B. cereus*	*S. aureus*	*E. facals*
50 mm	14 mm	25 mm	32 mm	40 mm	36 mm

From this results it was found that the biological effect of *Cressa cretica* extract against the inhibition of bacterial growth was very poor, this may be due to the lower concentrations of extract used and thus the inability of these concentrations to induce significant inhibition of bacterial growth or may be that the concentrations of the bioactive compounds were very low which was referred to the preparation method of extract and the storage method that plays a greet role in the activity of extract, taking into consideration, the organic solvent properties which were used for the extraction[35]. However, this can be increased by increasing the concentration of extract, or through the increase the number extract dosage because the treatment using plant extract needs a long period and there was not enough information about the effect of plant on the physiological human compared with the antibiotic which revealed the treatment through few days.

The results obtained from this study indicated the development of different resistant mechanisms of bacterial strains used, that enable them to resist some of the bioactive substance of used extract.

5. Conclusion

The preliminary phytochemical analysis of *Cressa cretica* indicated the presence of bioactive compound such as flavonoids, tannins , saponins and terpenoids , and the antibacterial activity of different concentration of alcoholic extract show lower activity compared to the positive and negative control . Some more studies and research can support the antimicrobial activities of *Cressa cretica* extract using different solvents for extraction, different parts of plant , and other bacteria.

References

1. Hamza, N.H. Genetic Detection about integrons for *Psedomonas aeruginosa* isolated from different clinical cases. M.SC. Thesis. Al-Iraqi University, College of Education. Iraq. **2018**,20.

2.El-Seedi,H. R., Ohara,T., Sata, N., Nishiyama, S. Antimicrobial diterpenoids from Eupatorium glutinous (Asteraceae), *J.Ethnophamacol*. **2002**,*81(2)*,293-6.

3. Rani, S., Chaudhary S., Singh, P., Mishra, G., Jha, K. K., Khosa, R. L. *Cressa cretica* Linn: An Important Medicinal Plant-A Review on Its Traditional Uses, Phytochemical and Pharmacological Properties. *J. Nat. Prod. Plant Resour*. **2011**,*1(1)*,91-100.

4.Hill, A.F. Economic Botany. A textbook of useful plants and plant products.2nd edn. McGarw-Hill Book Company Inc. NewYork. **1952**.

5.Pandy, A. K. and Chowdhry, P. K. Propagation techniques and harvesting time on productivity and root quality of *Withania somnifera. Journal of Tropical Medicinal Plants*. **2006**,*7*,79-81.

6. Sunita, P., Jha, S., Pattanayak, S. P., Mishra S. K. Antimicrobial Activity of a Halophytic Plant *Cressa cretica* L. *Journal of Scientific Research*. **2012**,*4 (1)*, 203-212.

7.Khare, P., Chaudhary, S., Kumar, A., Yadav, Gh., Thakur, N. A Study on the Standardization Parameters of a Halophytic Plant (*Cressa cretica* L.). *Middle-East Journal of Scientific Research.* **2013**, *15 (10)*,1472-1477.

8-. Al-Snafi, A. E. The chemical constituents and therapeutic importance of *Cressa cretica* – A review. *IOSR Journal of Pharmacy*. **2016**,39-46.

9. Chopra, R. N., Nayar, S. L., Chopra, I. C. Glossary of Indian Medicinal Plants. National Institute of science communication and information resources, New Delhi, 80. **2008**.

10. Rizk, A. M.., and El-Ghazaly, G. A. Medicinal and Poisonous Plants of Qatar. University of Qatar. Scientific and Applied Research Centre.101. **1995**.

11. Shahat, A. A., Abdel-Azim, N. S., Pieters, L., Vlietinck, A. J. Flavonoids from *Cressa cretica.* Pharmaceutical Biology. **2004**,*42:4-5*, 349-352.

12. Al-Hadeethy, O. N., Saadedin, S. M. K., and Al-Nadaawi, B. A. Use of *Cressa cretica* extract to control dodder. *Iraqi Journal of Agricultural Science*. **2007**,*38(5)*,62-67.

13. Macdonald, H.G. A Dictionary of Natural Products. MedFord.:N.J.7 Plexus Publishing. **1997**,187

14. Weber, D. J., Ansari, R., Gul, B., Khan, A. M.. Potential of halophytes as source of edible oil. *J Arid Environ*. **2007**,*68*,315-21.

15. Shahat, A. A., Nazif, N. M., Nahla, A. S., Luc, P., Arnold, V. J. Flavonoids from *Cressa creticca*. *Qatar Uni Sci J*. **2005**, *25*,72-7.

16. Harborne, J. B. Phytochemical Methods. Chapman Hall, London, **1998**, 60-66.

17. Clinical and laboratory standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 19th information supplement, CLSI document M100-S19. Wayne, PA: CLSI**; 2009**, 29(3).

18. Olurinola, P. F. A laboratory manual of pharmaceutical microbiology. Idu. Abuja. Nigeria. **1996**, 69-10.

19. Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., Yolken, H. R. Manual of Clinical Microbiology, 6th Ed. ASM Press. Washington DC. **1995**, 15-18.

20. Woodford, N., Livemore, D. N. Infections caused by Gram-positive bacteria: a review of the global challenge. *Journal of Infection.* **2009**,59,4-16.

21. Guleria, S., Kumar, A. Antifungal activity of some Himalayan medicinal plants using direct bio autography. *J. Cell Mol. Bio*. **2006**,*5*,95-98.

22. Zakaria, Z., Sreenivasan, S., and Mohammed, M. Antimicrobial Activity of *Piper ribesoides* Root Extract against *Staphylococcus aureus. J. App. Biol. Sci*. **2007**,*1(3)*,87-90.

23. Cowan, M. M. Plant products as antimicrobial agents. *Clin Microbial Rev.***1999,***12*, 564-58.

24. Al- Zubaidi, Z. N., Baban, H. A., Flaih, F.K. Iraqi Medicinal Herbal Treatment Guide. Aab Technical Printing Company Ltd. Iraq. Baghdad. **1996**.

25. Hussain, N., and Ameer, N. The Antimicrobial Activity of *Eucalyptus* camaldulensis Leaves against Bacteria Cause Urinary Tract Infection. Al-Mustansiriya *Journal of Science*. **2017**,*28(1)*,116-123.

26. Scalbert, A. Antimicrobial properties of tannins. Phytochemistry. **1991**,*30*, 3875-83.

27. Atlas, R. M., Brown, A. E., Parks, L. C. Experimental Microbiology Laboratory Manual. Mc Graw. Hill Companies Mosby Company. St. Louis. **1995**.

28. Djeddi, S., Bouchenah, N., Settar. I., Skaltsa, H. D. Composition and antimicrobial activity of the essential oil of *Rosmarinus officinalis* from Algeria. Chem. *Natural Comp*.**2007,** *43(4)*,487-490.

29. Thirunavukkarasu, P., Ramanathan, T., Manigandan, V., Dinesh, P., Vasanthkumar, A., Kathiresan, K. Antibacterial Effect of Costal Sand Dune Plant of *Cressa cretica*. Inventi Rapid: Pharma Biotech & Microbio. **2013**, *4*,1-3.

30. Oyaizu, M., Fujimoto, Y., Ogihara, H., Sekimoto, K., Naruse, A., Naruse, U. Antioxidative and antimicrobial activities extracts from several utility plants. Food Preserv Sci.**2003**,*29*,33-38.

31. Dogrouz, N., Zeybek, Z., Karagos, A. Antibacterial Activity of Some Plant Extracts. *IUFS. J. Biol.* **2008**, *67(1)*,17-21.

32. Shohayeb, M.,Abdel-Hameed, E., Bazaid, S. Antimicrobial Activity of Tannins and Extract of Different Parts of *Conocarpus erectus* L. Int. *J. Pharm. Bio, Sci*. **2013**, *3(2)*,544-553.

33.Basheer, A.A. Effect of alcoholic extract of rosmarinus against some type of entrobacteriaceae. *Tikrit Journal of Pure Science.* **2018**, *23(7)*,18-21.

34. McManus, P., Hammond, M.L., Whicker, S.D., Primrose, J.G., Mant,A., Fairall, S.R. Antibiotic Use in the Australian Community, 1990-1995. Med. *J. Australia*. **1997**,167,124-7.

35. Nostro, A., Germano, M. P., and Marino, A. Plant antimicrobial activity. Lett. Microbial. **2000**, *30(1)*,379-384.

36. Balasubramanian, S., Kala, S. M. J., Pushparaj, T. L., Kmae, P. V. Biofabrication of Gold Nanoparticles Using *Cressa cretica* Leaf extract and Evaluation of Catalytic and Antibacterial Efficacy. Nano. Biomed. Eng. **2019**,*11(1)*,58-66.

37. Sunita, P., Jha, S., Pattanayak, S. P., Mishra, S. K. Antimicrobial Activity of a Halophytic Plant *Cressa cretica* L. *Journal of scientific Research*.**2012**,*4(1)*,203-212.

38. Rani, S., Chaudhary, S., Singh, P., Mishra, G., Jha, K. K., Khosa, R. L. *Cressa cretica* Linn: An Important Medicinal Plant- A Review on Its Traditional Use, Phytochemical and Pharmacological Properties. *J. Nat. Prod. Plant Resour*. **2011,***1(1),*91-100.