Study The Antimicrobial Activity of Ethanolic Extract of *Lepidium draba* on Some Skin Infectious Agents

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Abstract

Background: medicinal plants are abundant in phytochemicals, which represent the richest bioresource of drugs are used against various diseases.

Objective: The present study included an in-vitro antimicrobial investigation for one of wild Iraqi plant *Lepidium draba* on some skin infectious agents.

Methods: The fresh aerial plant parts were macerated in 80% ethanol and subjected to phytochemical general test to investigate the plant active contents. Totat flavonoids were isolated through plant reflex in acidic aqeous solution to obtain the aglycone flavonoids using ethylacetate as an organic solvent. Qualification and quantification of the isolated total flavonoids were done in coressponding to standard s flavonoids. An antimicrobial activity for the crud ethanolic extract and the isolated total flavonoids had been carried out on some skin infectous agents using the following strain: *Staph aureus, Pseudomoneus aerogenosa* and *Candida albicans.*

Results: the outcome showed that the plant contain major active constituent included flavonoids Tannins, polysaccharides, alkaloids,saponines and Polyphenolic compounds.The plant contains many types of flavonoids including Rutin, Qurecetin, Kampferol and Luteolin, and each 100 g fresh aerial parts will contain 28 mg total flavonoids. The amount of each type of flavonoids were detected by HPLC technique. the extracted flavonoids at concentation of 4mg/ml showed potent effect upon the gram bacteria negative *pseudomonas aeruginosa* which is known to be more virulence than the gram positive strains but has no effect on *Staphylococcus aureus*. Also the extracted flavonoids appeared to be affected against the *Candida albicans* growth.

Conclusion: the ethanolic extractv of locally plant *L.draba* is effecient to inhibit the growth of pathogenic bacteria and decrease the chances of skin infection that provided the justification for therapeutic potential as supplementary or alternative medicine.

Key words: *Lepidium draba,*Total flavonoids, *Staph aureus, Pseudomoneus aerogenosa, Candida albicans.*

Introduction

The use of higher plants and their extracts to treat infection is an old age practice in traditional medicine. Traditional medicinal practice has been known for centuries in many parts of the world. Numerous plants and herbs are used. Numerous plants and herbs are used all by traditional medicine practitioners. It observed that these practices vary from one country to another. Because of the clinical efficacy of many presently used antibiotics is being threatened by the emergence of multidrug resistant (MDR) pathogens thus numerous herbal medicines reported in the traditional medicine and evaluated for efficacy and safety .Also, No chemical substances can be found without having adverse (1).

Cardaria draba ordinarily known as whitetop or hoary cress, is a perennial herb that reproduces by seed and by horizontal creeping roots. This plant is common in western Asia specially middle east, Eastern Europe, North America and Africa. It can be grow in different types of soil where wetness is sufficent, in various habitats including cultivated land, rangeland, pastures, along roadsides, waste areas and is known to particularly thrive in riparian or irrigated zones (2). Skin infections are caused by many pathogenic microbes, and signs vary from mild to serious. Mild contagion can be treatable with counter medications and

home remedies, whereas other infections may require medical attention. Skin infection is commonly found in the tropical areas around the globe (3,4).

Aims of the study:

- Study the antimicrobial activity of ethanolic extract of *Cardaira draba* on the skin infectious cases.

Methodology

Specimens collection:

Specimens collection is done by swabbing the involved areas of the skin. The pus or exudate is spread as a thin layer as possible on a clear slide for Gram staining. Specimens are gathered from patients who attended Al- Yarmouk hospital during the period from Oct.-1-2018 to Feb.-30 -2019.

Identification of Skin Infectious Agents

A- Cultural identification

Identification had been made according to the (shape, color, size, edges and height) of the colony on surface of agar plate (Nutreint agar for bacteria and Sabouraud agar for mold) .

B- Microscopical identification

A loop full of suspected isolate was fixed on microscopic slide then stained by Gram stain to examine (cell shape, grouping, size, Gram reaction and spore forming) (5).

C-Biochemical identification

Biochemical tests were more specific in the identification of bacteria which included many tests had been done according to (6).

Plant Collection and Classification

The aerial parts of the plant were collected from the fields in Abu Greeb in Iraq and were classified in the Faculty of Science/ Biology department at Baghdad University. The plant was classified as *Lepidium draba*. The plant was taken to the laboratory after cleaning. The wet samples were cut into small pieces at room temperature (25^{o}C) manually by using a sharp knife, packaged in the sterile containers, and kept away from direct light and humidity until use.

-Preperation of Ethanolic Extract (7)

About 150 g plant materials were macerated in 80% ethanol for a week in the cool dark place , filtered and dried with rotary evaporator at 45^{0}C. The yielded weight was designated as crude residue

-Phytochemical Investigation of the Ethanolic Crud Extract (8)

The following chemical test were proceeded to investigate active components in the crud ethanolic extract.

a. Detection of Tannins tests

A few drops of the 1% Lead acetate solution were added to the plant extract. A gelatinous or white precipitate was formed that indicated the presence of tannins.

b. Detection of polysaccharides

A liquate of 1 ml of the plant extract was mixed with 2 ml of the Benedict reagent, place the mixture in a boiling bath for 5 minutes and left to cool. The red deposit indicated a presence of this group.

c. Detection of alkaloids (Dragangroff test)

About 60mg of Bismuth sub-nitrate were dissolved in 0.2ml HCl (solution). Solution B contains 600mg potassium iodide in 1 ml Distell water.The solution [A + B] was mixed and added to the plant extract, an orange to brown color will indicate the presence of alkaloids.

d. Detection of the Saponins

The detection process will be proceeding by shaking the solution of the plant extract well. Formation of foam at the top of the extract will indicate presence of saponins.

e. Detection of Flavonoids

Alkaline reagent test: by using Sodium hydroxide solution which mixed with few amount plant extract solution and left, a bright yellow color is obtained in presence of flavonoids.

f. Detection of Polyphenolic Compounds

Few drops of 1% ferric chloride solution were added to the plant extract solution a brown deposition will formed.

-Extraction of the Total Flavonoids (7)

About (250) g of fresh plant (aerial part) were placed in a 1 liter glass flask after cutting to small pieces, then added 600 ml distilled water with(10% v/ v) HCl. Reflex extraction was performed for 8 hours continuously to ensure that the cleavage and broken of glycoside linkage of the flavonoids and the a glycon part was obtained. The plant extract was Filtered and cooled. The a glycan portion that is the biologic active part of flavonoids, is extracted by an organic solvent such as ethyl acetate by adding 50 mL per each 50 mL extract and repeated three times using a separating funnel. The acetate layer is collected in the separating funnel again and an equal amount of distilled water is added to remove HCl residues used in extraction. The acetate layer is then dried using rotary-evaporator at 45 ° C. The output is saved to complete the rest of the analysis.

-Determination of Total Flavonoids.

i. Qualitative assay (9)

A stock solution of total flavonoids extract was prepared by dissolving (5) mg residue in ml of 50% ethanol to get a stock solution 5mg/ml. A standard Rutin, Quercetin, Kaempferol, and luteolin solutions were prepared in 50% ethanol also. Thin layer chromatography (TLC) was carried out using a silica coated silica60 plate with a thickness of 0.1 mm,which represents the stationary phase in the chromatography separation process and for the mobile phase: Chloroform(5): Ethyl acetate(4): Formic acid(1) was used.

The kind of flavonoids had been separated , detected in the corresponding to standard flavonoids spots in their distance that called RF value. This value is derived from dividing the distance travelled by each flavonoid in each model phase to the distance traveled by the solvent:

$$\text{Rf value} = \frac{\text{The distance traveled by each flavonoid}}{\text{Distance traveled by the mobile phase}}$$

Each Flavonoid can be detected separately by the exposure of the silica plate to the UV light as a colored spot. The silica plate is covered with Fluorescent material, which flashes when it binds to the active groups of flavonoids under UV at a wavelength of 254nm. The result is shown as bright spots under the UV light.

ii. Quantitative Assay for Total Flavonoids (10)

Several Rutin standard flavonoid solutions were prepared with concentration of (0.3125, 0.15625, 0.625, 1.25) mg / ml in 50% ethanol solution. The following interaction is performed:

Aliquot of 1 ml of stock total flavonoids extract solution(5mg/ml) is transferred glass tube, and 1 ml Rutin standard solutions of each concentration is placed in separated glass tube, then 1 ml of 5% sodium nitrite solution was added to all tubes, to be stirred and left in room temperature for 5 minutes, then 2 ml of aluminum chloride10% were added to all tubes and mixed well and left for another 5 minutes at room temperature. Finally,5 mL of 1N NaOH solution was added to the mixture and the resulting color was read

with spectrophotometer at 510nm wavelength . A standard curve is then performed between absorbance reading of each standard solution verses their concentration to get the straight line equation and then calculating the concentration of the total amount of flavonoids in extracted plant.

iii. High performance Liquid Chromatography (HPLC) for Total Flavonoids (11)

To identify and quantify some flavonoids from plant total flavonoids, HPLC (Shimadzo apparatus) method was applied in the current study with the following conditions:

Column:(250 X 4.6Id)mm; 5µl particle size
Volume inj: 20 µl
Detector=:U.V-Vis. At 220nm
Mobile phase:(Methanol: 1% K2HPO4) (30%: 40%)
Standard Solutions: Rutin,Querecetin, Apigenin, Kaempferol, Luteolin(5mg/L).

Antimicrobial Activity of the Crud Extract and Total Flavonoids

This method was done by at ministry of industry and minerals at Ibn Sina laboratory /Research Department, using the following strain: *S.aureus, P. aerogenosa* and *C. albicans*. by using nutrient and Sabouraud agar for bacterial and fungal isolates respectivelly .

-Preparation of *L.draba* Crud Ethanolic and Total flavonoids test solution

From the crud and total flavonoid residue, about 100 mg was re-dissolved in 25 ml distilled water to get a stock solution (4mg/ml), and sterile with millipore 0.22mm filter unit into sterile tubes then stored at 4°C in refrigerator prior to use. The crud alcoholic plant extract was labeled as (1), and the total flavonoid was labeled as (2)

-Preparation of Bacterial and Fungal Working Samples

Bacterial Inoculums were cultured in a sterile broth media(Nutreint broth)and incubated at 37°C for 24 hours while *C. albicans* cultured in (Sabouraud broth) then incubated at 28°C for 72 hours.

- **Antimicrobial Activity by Agar Well Diffusion Method**

  Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants extracts . The agar plate surface is inoculated by spreading100 µL of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (100 µL) of the extract solution at desired concentration is introduced into the well. Then, agar plates are incubated overnight at 37°C for bacteria and at 28°C / 72 hours for *C.albicans* . The antibacterial and antifungal activity determined by measurement of inhibition zone(6,12) .

The Results and Discussion

- The yield of Ethanolic Crud Extract and Total Flavonoid

The ethanolic crud extract yielded 6.586 g residue from 150 g wet plant, that mean about 4.4% residue while the total flavonoids residue was 0.423 g yielded from 250 g wet aerial parts of the plant, which about 0.17% residue .The plant was high water content that make the residue for both ethanolic crud and total flavonoids lower than expected.

-Phytochemical Tests of Ethanolic Crud Extract

The results of tests were illustrated in Table (1) which showed the plant is rich with many active constituents that give an explanation about ancient plant usage as medical plant even in folk and traditional medicine (13).

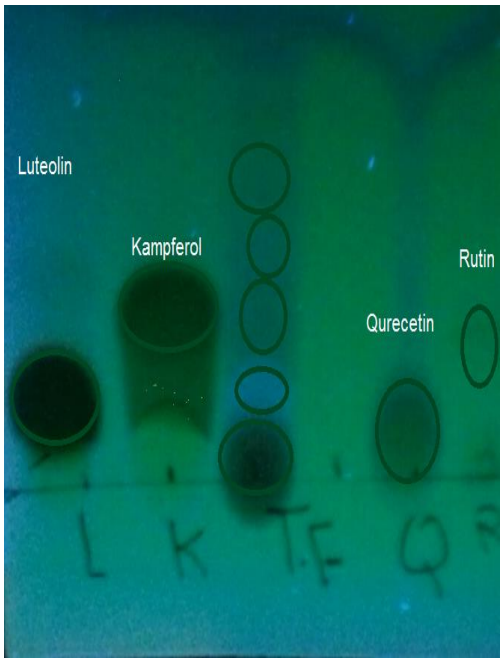
Table(1): Results of Phytochemical Tests of Ethanolic Crud Extract

Test	Result	Comments
Detection of Tannins	+	**White p.p.t.**
Detection of polysaccharides	+	**Orange-Red p.p.t.**
Detection of alkaloids	+	**brown p.p.t.**
Detection of the saponines	+	**Foam formation**
Detection of Flavonoids	+	**Bright yellow**
Detection of Polyphenolic compounds	+	**Brown p.p.t**

Determination of Total Flavonoids

i- Qualitative Assay results

Many flavonoids were detected in the plant extract as shown as in figure(1). The plant has rich contains of many types of flavonoids including Rutin, Qurecetin, Kampferol and Luteolin. Each of these flavonoids have high efficiency in biological activities such as antibacterial and antifungal (14). The chromatogram indicates the presence of Luteolin, Kaempferol, Quercetin Rutin and other flavonoids in comparison with standard flavonoids. The figure shows that both Quercetin and rutin are more abundant in the plant extract.



Figure(1): The Chromatogram of flavonoias in *L.drapa* total flavonoids :TF and the standard Flavonoids:L=Luteolin,K=Kaempferol.Q=Qurecetin,R=Rutin

The R_f values for each standard and the corresponding spots that appeared in chromatogram is shown in Table (2).

Table (2) : R_f Values for Each Standard Flavonoids and Total Flavonoids of *L.darpa*

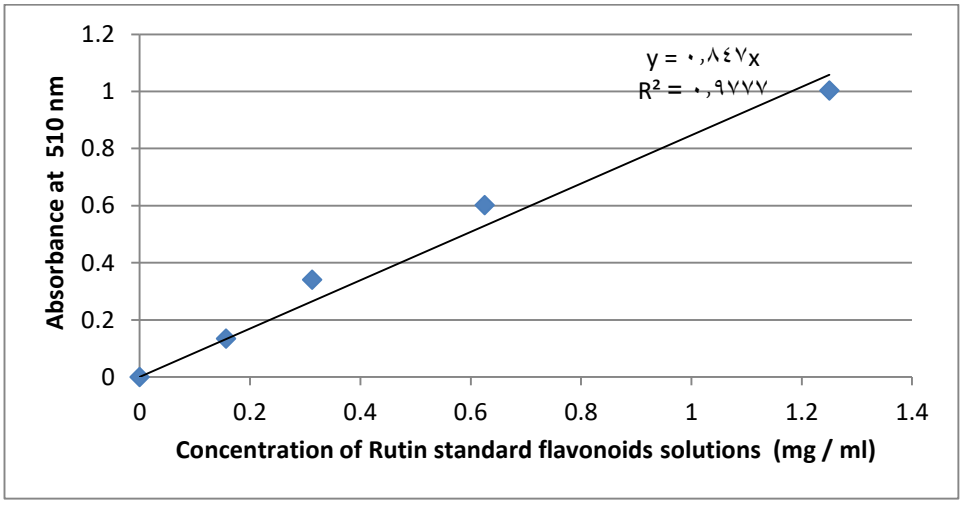
Flavonoid	Luteolin	Kaempferol	Quercetin	Rutin	Plant Flavonoids
R_f Value	0.2	0.3	Base line	0.25	All spots & others

ii. Quantitative Assay

The amount of total flavonoids found in the aerial part of the plant was estimated by using the visible spectrometer at 510 nm depending on absorption of the different concentrations for the standard rutin curve as shown as in Table (3) where the straight line equation is obtained as shown in Figure (2).

Table (3): Absorption Values of Different Concentrations of Standard flavonoids Rutin and Total Flavonoids of Plant Extract

Rutin Standard Solution(mg/ml)	Absorption at(510 nm)
0.15625	0.135
0.3125	0.341
0.625	0.602
1.25	1.003
2.5	2.750
Total flavonoids of the plant extract	0.6915



Figure(2): Rutin Standard Curve

From the equation of the straight line of the standard flavonoid curve of the Rutin with different concentrations, the concentration of the total flavonoids for the extract is as follows:

Y = 0.847X

So: 0.8164 X 0.4228 /5 X 1000 = 69 mg total flavonoid in each 250 g wet aerial part of the plant. That means each 100 g wet aerial part of the plant should contain 28 mg total flavonoids as rutin. (15) found in his study that the plant specially the aerial part is rich 16 types of flavonoids among them Querecetin and Kaempeferol , Also (16) was reported that the plant is rich with many active constituents such as phenolic compounds as many kinds of minerals.

iii- HPLC Assay Results

As shown in Figure (3a,b) and Table(4), the plant contained different flavonoids in different quantity. In a comparison with standard flavonoids, results showed that the plant extract rich with rutin, Quercetin and luteolin. The amount of each flavonoid as percentage can be calculated as follow:

Flavonoid concentration (mg/ml) =Area under the curve for sample flavonoid/Area under the curve for standard flavonoid X Standard concentration (0.005mg/ml).

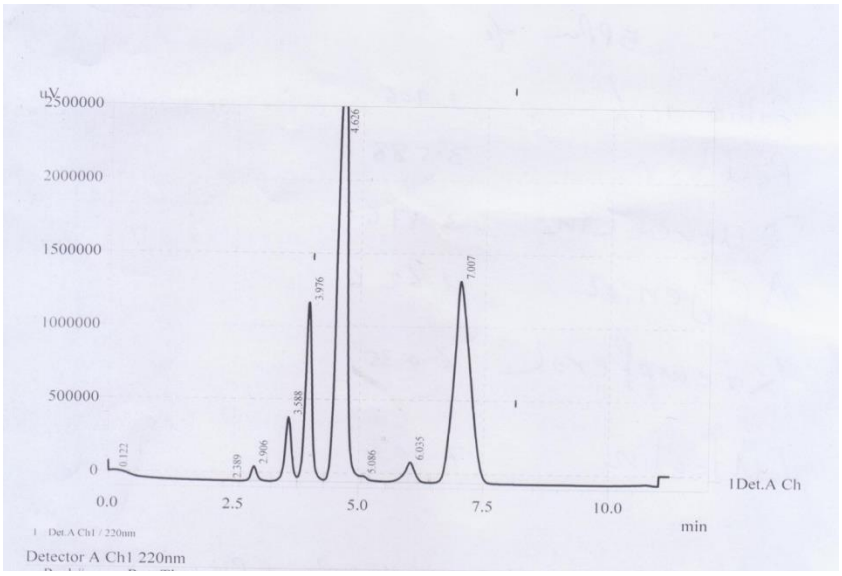


Figure (3a): HPLC Chromatogram for Standard Flavonoids

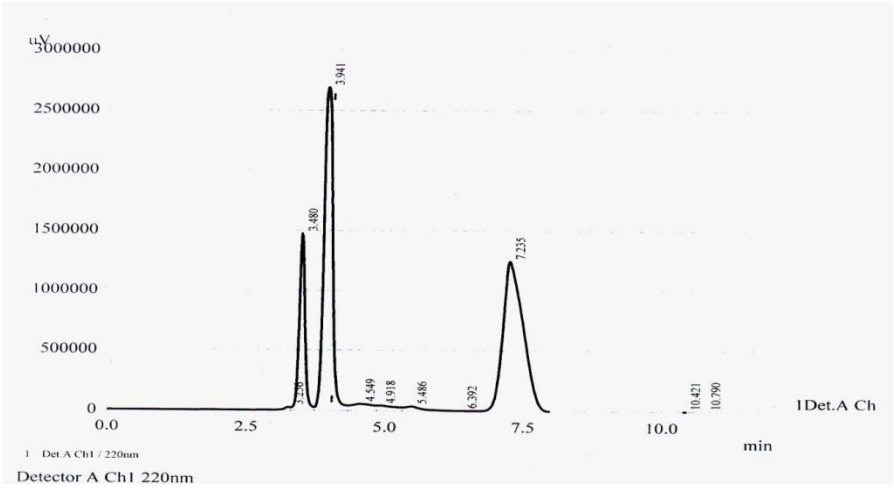


Figure (3b): Chromatogram of Plant Total Flavonoids

The retention time of the standard flavonoids and the amount of each one in the plant total flavonoids were shown in Table(4).

As shown in the Table(4), the plant is rich with Rutin and Quercetin and fewer amounts of Luteolin , Apegnine and Kaemperol and these results are emphasize that result get in the present study during TLC application process which insure the plant is good source of flavonoids. These results agreed with results reported (14) and (17).

Table(4): Retention Time in Minutes and Area Under the Peak for Each Flavonoid in Standard and Sample Solutions

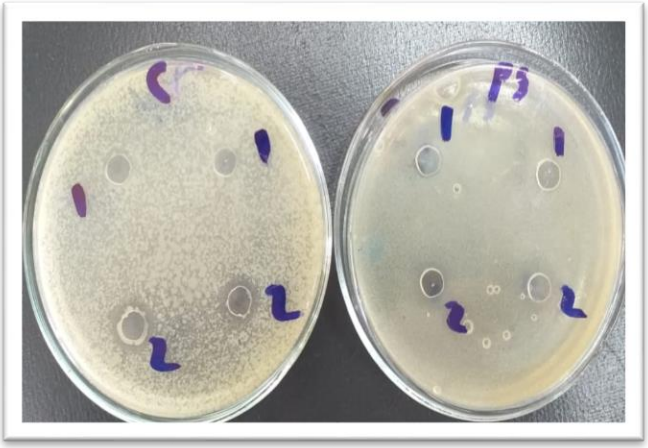
Flavonoid	Standard	Standard	Sample	Sample	Conc.mg/5mg	Conc.mg /250g
	Ret.Time	Area	Ret.Time	Area	plant Extract	plant Extract
Rutin	3.588	3980061	3.480	10862242	0.01365	0.942
Quercetin	3.976	11121824	3.941	29686384	0.013346	0.921
Apegnine	4.626	39817950	4.549	1506975	0.0001892	0.01305
Kaempferol	6.035	1965441	6.392	2391	0.000006	0.000414
Luteolin	7.007	31312350	7.235	31754413	0.00507	0.35

Antibacterial and Antifungal Activity for the Ethanolic Crud and Total Flavonoids Plant Extracts

As shown in Table(5) and Figure (4), the small amount of the extracted flavonoids of this plant has the potent effect upon special bacterial strain such as the gram bacteria negative *pseudomonas aeruginosa* which is known to be more virulence than the gram positive strains but has no effect on *Staphylococcus aureus*. Also the extracted flavonoids appeared to be affected against the *Candida* growth.The crud ethanolic extract should be investigated for antimicrobial activity in higher concentration within the safety range of the plant usage.

Table(5): Antibacterial and Antifungal activity for the Ethanolic Crud and Total Flavonoids Plant Extracts

Microbial Isolates	Crude Extract (4mg/ml)	Total Flavonoids (4mg/ml)
S. aureus	-	-
P. aeruginosa	-	+ (12 mm)
C. albicans	-	+ (13 mm)



Figure(4): The Antimicrobial Activity of *L. draba* Crude Extract Total Flavonoids in concentration of 4mg/ml

Bacterial adhesion and subsequent colonization of surfaces are the first step toward biofilm formation. Biofilm consisted of microcolonies encased in extracellular polysaccharide material which formed under selected conditions. The microorganisms differ in it's ability to produce biofilm, thickness of biofilm differ according to the genus and species of producing bacteria, conditions like temperature, pH and type of disease. The *S. aureus* isolates formed biofilm with different thickness . Biofilm considered important factor which responsible for bacteriaa pathogensity and resitance .

As shown in Figure(4) and Table(4) that the extract of total flavonoids in comparing with the alcoholic crude extract shows inhibition for each of *P.aeuroginosa* and *C. albicans* although it was used in low concentration and this results agree with Asif and co worker results about the importance of rutine and quercetin and other flavonoids in different biological activity (18).

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