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The Effect of *Hibiscus sabdariffa* Extracts on *Pseudomonas aeruginosa* Isolated from Wounds and Pus

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ABSTRACT

The study aimed to evaluate the efficacy of *Hibiscus sabdariffa* extracts of aqueous and alcoholic extracts on *Pseudomonas aeruginosa* using agar well diffusion method. The study enrolled 100 wound and pus patients. The results showed that 44 isolates were *Pseudomonas aeruginosa* that equivalent to 44% total isolates. The *Hibiscus sabdariffa* extract concentrations used are (10, 25, 50,75 and 100 mg/ml). The bacterial isolates identified after been growing on blood agar and purified on MacConkey Agar using many biochemical tests. The bacterial identification was confirmed using Vitek-2 compact system technique. The results showed that the aqueous extracts were more active than alcoholic extract and no inhibition at the concentrations (10, 25 and 50 mg/ml) against *Pseudomonas aeruginosa* for aqueous and alcoholic extracts, the inhibition zones were (11 and 13) mm at the concentrations (75 and 100) mg/ml respectively for aqueous extract, and (10 and 11) mm at the concentrations (75 and 100) mg/ml respectively for alcoholic. Sensitivity test was conducted for *Pseudomonas aeruginosa* using Vitec compact test. FTIR and GC/MS tests conducted for the plant extracts and the results detailed in the study.

Keywords- *Hibiscus sabdariffa*, *Pseudomonas aeruginosa*, plant extracts, Wound and pus, GC/MS.

I. INTRODUCTION

Ancient Civilizations, industrial and developing countries use medicinal plants as a source for medicines. Medicinal plants used as a food preservative, flavor enhancing and protect from diseases. Plant secondary metabolites have biological properties that used in different purposes such as treating different diseases since the plant metabolites have antimicrobial activities. Microorganisms gain resistance to most of the antibiotics at the same time the plant metabolites compounds restrict the microorganisms' growth and treat specific diseases [1].

Hibiscus sabdariffa is a shrub and one of the medicinal plants that belongs to the family of *Malvaceae*, it is wide spread plant that containing Mucilage substances [2]. The native land for this plant is Asia and Africa and seeding especially in Philippine, Malaya, Indonesia and India. In Arab countries the plant is cultivated in Egypt, Iraq and Sudan [3].

The medicinal part of the *Hibiscus sabdariffa* placed in the calyx leaves and sub calyx leave and containing different chemical substances such as Anthocyanin, Yaniding-3-Sambubioside, Delphinidin-3-Sambubiosid, Delphinidin-3-Glucose [4]. The plant contained organic acids such as Ascorbic acid, Hibiscus acid, Malic acid and Tartaric acid that gave the plant the acidic flavor [5]. *Hibiscus sabdariffa* used for medical and food purposes and the researches proved that the tea of Hibiscus lowering the blood pressure, restricts the cancer tumors in the human body, diuretic and restricts the growth of microorganisms in the human body [6]. According to the latest chemical discoveries that 100 gm of the flower calyx contain 49 calories, 48.5 gm water, 1.9gm protein, 0.1 fat, 12.3 g carbohydrate, 2.3 gm fibers, 1.2 ash, 1.72 mg Calcium, 57 mg Phosphorus, 2.9 mg Iron, 300 mg beta carotene and 14 mg Ascorbic acid [7].

Due to the importance found in the previous studies, this study aims to evaluate the activity of Hibiscus aqueous and alcoholic extracts to inhibit the growth of *Pseudomonas aeruginosa* isolates.

II. METHODOLOGY

Isolation and Identification

Bacterial isolates collected from wounds and pus using sterile cotton swabs that cultured on blood agar and then the isolates stained with gram stains and tested under microscope, it was reddish/pink rods that's mean it were gram negative and then cultured on MacConkey agar media for purification. many biochemical tests experimented, bacterial identification confirmed using Vitek-2 compact system.

Plants collection and preparation

The plant samples were collected from the local markets in Tikrit city, about 500gm, to be sufficient to conduct the required experiments, as they were placed in sterile plastic containers and transported to the laboratory and were cleaned of impurities and dust. The laboratory mill was used to grind the plant samples to obtain the powder, then put it again in sterile and airtight plastic containers, and then kept it at the temperature of the refrigerator until it is used in preparing the plant extracts.

Aqueous extraction preparation

The aqueous plant extract was prepared by mixing 40 gm of plant powder in 160 ml of distilled water, 1:4 w/v. The plant sample was set in magnetic blender with water bath for 1 hr. to degrade the plant cell membrane, the mixture kept in fridge for 24 hrs. to be well soaked then infiltration the mixture using layers of gauze then filtered again using Whatman No.1 to dispose the remnants of fibers and undissolved parts. The stock solution is prepared as dried by cooling under claustrophobic pressure using lyophilizer then the sample stored in a sealed bottle in dry conditions and frozen [8].

Alcoholic extraction preparation

The alcoholic plant extract was prepared by washing the sample by distilled water then by Sodium hypochlorite at 1% and the benefit of using this solution is to disinfection from microorganisms, then the sample washed by distilled water. 20 gm of plant material placed in food processor to grind the plant material and then added 100 ml of ethanol at 95% that mixed for 2-3 minutes then used the shaker for 24 hrs. to dissolve in ethanol then filtered using layers of gauze and finally filtered using Millipore at 0.45 µm to kill the microorganisms. The mixture is placed in oven at 40 º C to evaporate alcohol, then stored in a sealed bottle and stored in the fridge at 4 º C to be ready for use in the experiment [9].

Testing the plant extract using GC/MS

Gas chromatography/Mass spectrometry was used to evaluate the composition of the plant extracts using the data available at the national institute of standards and technology (NIST). The results were compared with data to identify the name, molecular weight and structure of the extracted compounds [10].

Antibiotic sensitivity test

The sensitivity of *Pseudomonas aeruginosa* evaluated using Vitec test. The results of antibiotics compared with standard ratios of CLSI 2020 [11].

Fourier transforms infrared spectroscopy of plant extract (FTIR)

This technique was conducted at the university of Tikrit-college of science/department of chemistry. Reflective infrared spectroscopy of plant extracts was evaluated at the range (400-4000 cm^{-1}) using potassium bromide disks to extend the range to 25 Micron (400 Cm-1). 0.01 g of plant extract dissolved in 10 ml of dimethyl sulfoxide and exposed to ultrasonography to dissolve well and then mixed with potassium bromide disks then turned into pellets using high pressure caused by hydraulic press [12].

III. Results and Discussion

Isolation and identification

Among 100 wounds and pus samples 44 isolates were *Pseudomonas aeruginosa* that representing 44% of total isolates. The identification conducted using the results of biochemical tests that explained in table (1).

Table 1: Biochemical tests used to diagnose *Pseudomonas aeruginosa.*

No	Biochemical Test	Result
1	Gram-stain	-ve
2	Oxidase test	+ve
3	Catalase test	+ve
4	Pigments production	+ve
5	Hemolysis (β-hemolysis)	+ve
6	Indole test	-ve
7	Methyl-red	-ve
8	Voges-Proskauer	-ve
9	Simmon's citrate	+ve
10	Urease production	-ve
11	H_2S production	-ve
12	Kligler's Iron agar	K/K
13	Growth on MacConky	+ve
14	Growth on Citramide	+ve
15	Gelatin decomposition	+ve
16	Motility	+ve

The identification has been confirmed using Vitek-2 compact system test explained in figure (1):

Fig 1: Results of Vitek-2 Examination Reports.

Plant extract GC/MS

Gas Chromatography and Mass Spectrometry (GC/MS) analytical method was used to identify the plant extract compounds, the results showed in the table (2).

Table 2: GC-MS analysis report for ethanol extract of *Hibiscus sabdariffa L, RT: Retention Time*

No	RT (min)	Area%	Name	Quality	CAS Number
1	15.589	1.16	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	49	000645-10-3
2	16.403	3.06	1,1,3-Trimethylcyclopentane	46	004516-69-2
3	20.445	5.41	3-Eicosene, (E)-	94	074685-33-9
4	21.893	74.55	Trinitrotoluene	91	000000-00-0
5	23.828	4.31	1-Octadecene	99	000112-88-9
6	27.165	6.33	(trans)-2-nonadecene	94	000000-00-0
7	32.929	2.38	1-Octadecanol	68	000112-92-5
8	34.429	2.8	Bis(2-ethylhexyl) phthalate	38	000117-81-7

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FTIR results of Hibiscus extracts

The results of Fourier transforms (FTIR) of hydrophobic extract, a powerful package was observed at (3477) cm-1 belonging to the hydroxyl group (OH) hydration and the emergence of a suction package at range (2928) cm-1 belongs to the aliphatic CH group, and the appearance of two suction packets at (1797 and 1741) cm-1 dates back to two carbonyls (C = O) matches with the emergence of two suction packages (1618, 1431) cm-1 backs to the C = C aromatics, plus the appearance of a package at (1226) cm-1 belongs to C-O, and the appearance of a package at (1022) cm-1 belongs to a determined mat (C-N), since these packages are an approach to the results of [13], fully consistent with the study of [14] and as illustrated in figure (2).

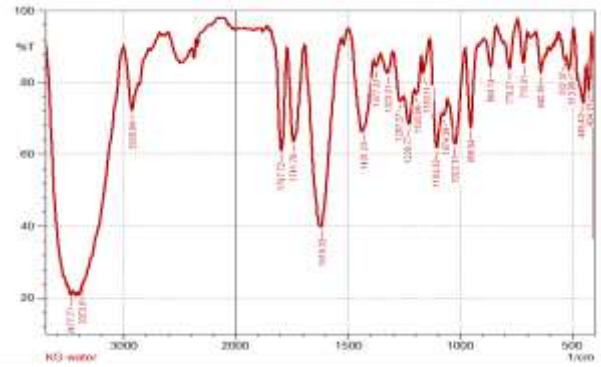

Fig 2: FTIR results of Hibiscus aqueous extract

The results of IR spectrum of alcoholic extract, a powerful package was observed at the moment. (3429) cm-1 belongs to the hydroxyl group (OH) alcohol and the appearance of a suction package at range (2924) cm-1 belonging to the aliphatic group (CH), and the appearance of packages at (1793 and 1745) C = O with the emergence of two suction packages at range (1618, 1429) cm-1 backs to the C = C aromatics, plus the appearance of a package at (1224) cm-1 goes back to C-O, and the appearance of a package at (1024) cm-1 goes back to a determined mat. (C-N), as these packages are consistent with the results of [15] study, as showed in figure (3).

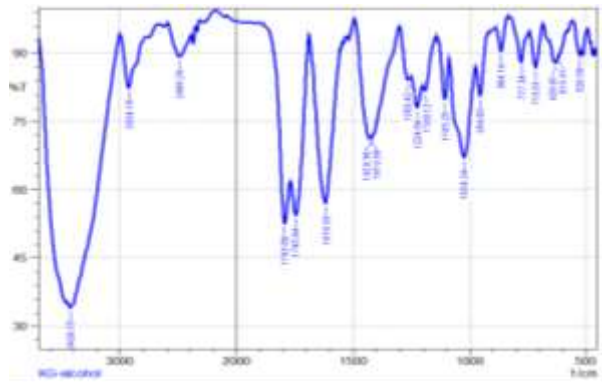

Fig 3: FTIR results of Hibiscus alcoholic extract

Antibiotic sensitivity test.

Pseudomonas aeruginosa sensitivity test was performed using Vitec compact test and the results are shown in the table 3.

Table 3: *Pseudomonas aeruginosa* sensitive to antibiotics.

Classification information	Analysis time: 6.98 hours	Status: Final			
Tested organism	99% probability: Pseudomonas aeruginosa Bionumber 0003053003660242				
Analysis messages Susceptibility information		Status: Final			
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Ampicillin	16	S	Amikacin	4	S
Amoxicillin-Clavulanic acid	16	S	Gentamicin	2	S
Piperacillin/Tazobactam	8	S	Tobramycin	<=1	S
Ceftazidime	<=1	S	Nefilmacin		
Cefotaxime			Ciprofloxacin	0.5	S
Imipenem			Minocycline		
Meropenem	>=16	R	Colistin	<=0.5	S
Cefepime	2	S	Rifampicin		
			Trimethoprim/Sulfamethoxazole		

R: resistant, S: susceptible, MIC: minimum inhibition concentration

Inhibitory effect of hibiscus extracts on Pseudomonas aeruginosa bacteria

The inhibition zones of the Hibiscus extract showed that no inhibition at the concentrations (10, 25 and 50) mg/ml for the aqueous and alcoholic extract against *Pseudomonas aeruginosa* using well diffusion method. Aqueous extracts showed inhibition activity and the diameters zones were (11 and 13) mm at the concentrations (75 and 100) mg/ml respectively. Aqueous extracts showed inhibition activity and the diameters zones were (10 and 11) mm at the concentrations (75 and 100) mg/ml respectively as shown in figures (4 and 5). As shown in the results the aqueous extract more efficient than alcoholic extract.

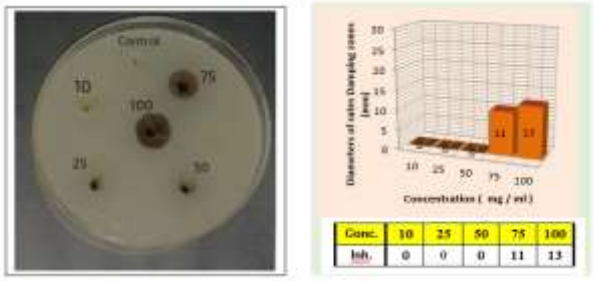

Fig 4: *Pseudomonas aeruginosa* Sensitivity to different concentrations of aqueous *Hibiscus* extracts.

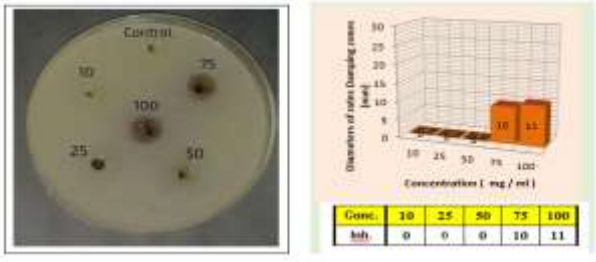

Fig 5: *Pseudomonas aeruginosa* Sensitivity to different concentrations of alcoholic Hibiscus extracts.

The ability of the *Hibiscus sabdariffa* plant to inhibit different types of bacteria in this study is due to the fact that it contains many secondary metabolic products. The flowers of the plant contain phenolic acid, flavonoids, and anthocyanins, which have proven effective against antibacterial, antioxidative, anticarcinogenic, and also contain many organic acids such as citric, malic, tartaric, and hibiscus also contain a high percentage of ascorbic acid [16].

Based on the results the antibacterial properties of *Hibiscus sabdariffa* against *Pseudomonas aeruginosa* was concentration dependent and inhibition zones increase with the concentration [17]. Hibiscus rich in phytochemicals compounds such as polyphenols especially anthocyanin, polysaccharides and organic acids that have uses in modern therapeutic [18].

The difference in the results between extracts and their inhibition efficiency on the bacterial isolates due to the difference in the difference in the type of extract that affects the active compounds [19]. Flavonoids considered as the inhibitive compound for many microorganisms due to the ability of forming a complex with the bacterial cell wall that works to interrupt the cell wall and make it permeable, that affecting the internal constituents [20].

Previous studies proved that the medicinal plants secondary metabolites inhibit the pathogenic bacteria growth by disrupting and lysis of the bacterial cell wall, prevent the formation of biofilm, inhibiting the construction of bacterial cell wall, the replication of microbial DNA and the synthesis of energy pathways [21].

IV. CONCLUSION

— Aqueous and alcoholic *Hibiscus sabdariffa* extracts showed growth inhibition against *Pseudomonas aeruginosa.*

— *Pseudomonas aeruginosa* Isolated from pus and wounds showed high resistance for some antibiotics used in this study.

— The bacterial growth inhibition zones were concentration dependent.

— *Hibiscus sabdariffa* extracts were inactive against bacterial growth at the concentrations 10,25 and 50 mg/ml.

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sabdariffa Calyx and Leaf Calyx and Leaf on Performance, Egg Quality, Immune System and Antioxidant Balance of Laying Hens. *Iranian Journal of Applied Animal Science*, *10*(2), 317-325.

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