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ANALYSIS OF ACTIVE INGREDIENTS IN SYRIAN MESQUITE (*Prosopis Fracta*) POWDER AND EXTRACTS WITH EVALUATION OF BIOLOGICAL ACTIVITY AGAINST *Aeromonas hydrophila*

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ABSTRACT

Achemical analysis was performed to identify the active compounds in the powder and extracts derived from the fruits of *Prosopis. fractal*. Additionally, the study investigated the effects of both the powder and extracts of *P. fractal* on the *Aeromonas. hydrophila* bacteria. The results demonstrated that the powder was more effective than both the alcoholic and aqueous extracts, exhibiting the highest antibacterial activity. Specifically, the inhibition zones measured 20.25 mm for both 50% and 100% concentrations of *P. fractal* powder. In comparison, the alcoholic extract recorded inhibition zones of 16.8 mm and 20.8 mm, while the aqueous extract showed zones of 10.4 mm and 11.9 mm. additionally, the antibiotic displayed an inhibitory activity of 30 mm against *A. hydrophila*. The results indicated that the bacteria displayed sensitivity to the antibiotic ciprofloxacin, which had an inhibition zone diameter of 33.3 mm. This was followed by doxycycline, with an inhibition zone diameter of 20.5 mm, and trimethoprim, which had an inhibition zone diameter of 10 mm. Conversely, the bacteria were resistant to the antibiotic ampicillin, which showed no inhibitory activity.

Additionally, the analysis revealed the presence of several active compounds, including flavonoids, phenols, saponins, resins, tannins, and alkaloids. The abundance of these compounds ranged from medium to high, depending on the type of material, whether it was in powder or extract form.

Keywords: Syrian mesquite, biological activity against *A. hydrophila.*

INTRODUCTION

Disease outbreaks, especially infectious diseases, have been considered a major constraint affecting sustainable aquaculture development globally. Because they have

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annually caused the loss of at least 10 % of aquaculture's production as mentioned Adams [2]. Amongst aquaculture bacterial pathogens, Aeromonas spp, including Aeromonas hydrophila, can be pathogenic to many freshwater fish species such as common carp (*Cyprinus carpio* Linnaeus, 1758). Antibiotics effectively kill bacteria, despite counter indicating harmful effects on biotics in waters and humans as mentioned Abdelkhalek [1] due to reducing the host's immune system Ahmadifar [3]. In addition, consuming fishery products containing residues presents harm, Devi [12], as it is undegradable, triggering pathogenic bacteria in the environment. The application of natural products is urgently required as an alternative to minimize the administration of chemical drugs to prevent bacterial resistance, improve the host immune response and reduce free radicals Dawood [11]. Among the various medicinal plants, those in the Prosopis genus have been widely utilized in traditional medicine. The Prosopis genus, which belongs to the *Fabaceae* (or *Leguminosae*) family, comprises approximately 45 species of spiny trees and shrubs. These plants are found in both subtropical and tropical regions around the world. In addition to their long history of medicinal use, Prosopis plants also hold commercial value. The paste, gum, leaves, and pods of Prosopis plants exhibit a range of bioactive properties, including anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antioxidant effects [17]. In recent years, the emergence of drug-resistant pathogenic microorganisms has become a significant concern due to the indiscriminate use of antimicrobial drugs typically employed in treating infectious diseases [4]. This situation has led to an increased interest in finding alternative treatments from various sources, particularly medicinal plants. The present study aims to investigate the antimicrobial properties of the powder, as well as the alcoholic and aqueous extracts of *Prosopis farcta.*

MATERIALS AND METHODS

The disk diffusion method described by Bauer was used to test the antibiotic susceptibility of *Aeromonas hydrophila* in the laboratory of the Fish Unit at the Central Veterinary Laboratories and Research Department, part of the Epidemiology Department of the Veterinary Medicine Department. The inhibitory activity of 50% and 100% concentrations of powder, as well as alcoholic and aqueous extracts of *Prosopis fracta*, was tested against *Aeromonas hydrophila*. The results were compared with the effect of antibiotic tablets containing 30 micrograms of oxytetracycline.

Powder preparation

The fruits of the *Prosopis fractal* plant were collected from the Tidan area, located southeast of Fallujah city, and from the western area of Hit. After collection, the fruits were cleaned, dried, and ground using an electric grinder in the laboratories of the College of Agriculture at the University of Anbar.

Preparation of the crude aqueous extract

The aqueous extract of the yarrow plant was prepared according to Ratheesh and Helen's method [28]. Fifty grams of yarrow powder was weighed and placed in a flask, 500 ml of distilled hot water was added to it, and it was placed in a vibrating incubator for 24 hours at room temperature. Then, it was filtered using a Buechner funnel for air evacuation. After that, the filtrate was concentrated using a rotary evaporator to get rid of the solvent at a temperature of 40 °C. After that, the concentrated filtrate was collected.

Preparation of crude alcoholic extract (ethyl)

The extract was prepared using the method of Harborne [15] by weighing 200 g of powdered leaves of the yarrow plant and placing it in a 1000 ml glass beaker, adding 500 ml of 99.9% pure ethyl alcohol, mixing the mixture well and leaving it for 24 hours with shaking from time to time. Then, the extract was filtered using three layers of medical gauze, then filtered using What Man No. (1). Filter paper and a Buchner funnel using a vacuum device, then the total filtrate was concentrated using a rotary vacuum evaporator to convert it into a thick liquid at a temperature of 40 °C.

Drying of yarrow extracts

The aqueous and alcoholic extracts were dried separately by placing the samples in glass Petri dishes and placing them in a laboratory oven at a temperature of (40°C) for 24 hours to obtain a dry extract, and it was stored in sterile laboratory bottles until use in the experiment.

Bacterial isolation

The bacterial isolates used in the test were obtained from the Fish Unit/Central Veterinary Laboratories and Research Division of the Epidemiology Department of the Veterinary Department/Ministry of Agriculture. They are *A. hydrophila* isolates. Health carp and carp with hemorrhages and dermal ulcers on their bodies were obtained from different farms around Baghdad. Samples of the gill, kidney, and skin of each fish were collected. The samples were placed on 5% sheep blood agar plates, tryptic soy agar plates, and MacConkey agar plates (all from Oxoid) and then incubated at 25-30ºC for 1-2 days under aerobic conditions. After incubation, pure hemolytic yellow colonies were isolated from the skin and internal organs of all the carp. The bacteria were identified as **Aeromonas hydrophila** based on colony morphology, Gram staining, and biochemical characteristics. Wet mounts of smears from skin, fin, and gill were also examined microscopically.

Activation of bacterial isolates

The bacterial isolates mentioned in the above paragraph were activated according to the method mentioned by Harrigan and McCance [16]. All isolates were activated in Nutrinet Broth medium which was prepared according to the manufacturer's instructions.

Antibact

Bacterial antibody test

Well diffusion assay method was used to test the antibacterial activity of both alcoholic and aqueous extracts of yarrow as mentioned before Girish and Satish [13] with slight modifications. The plates were inoculated with the bacterial isolates and then the culture medium prepared in the above paragraph was poured over them and the medium in the plate was mixed well with the bacterial inoculum and left to solidify. Then each plate was divided into four regions, three of which were perforated with a diameter of (5 mm) using a sterile cork piercer under sterile conditions, and the fourth region of the plate was left without perforation to place the antibiotic disc on it. Then the aqueous and alcoholic extracts were transferred at the concentrations (100% and 50%) into two holes with a volume of 50 microliters. As for the third hole, sterile distilled water was placed as a control solution, and the antibiotic was placed in the fourth region of the plate. Then the plates were incubated at a temperature of 37°C for 24 hours. After the end of the incubation period, the effectiveness of the aqueous and alcoholic extract powder was determined by measuring the diameter of the inhibition

zone formed around the holes in millimeters using an electronic Vernier device.
Detection of active compounds in *Prosopis fracta* powder and extract Some diagnostic tests were conducted on yarrow powder and extract with the aim of identifying some of the compounds present in it, such as flavonoids, phenols, tannins, alkaloids, saponins, and resins. This test was conducted according to the method described by Harborne [15].

Detection of Flavonoid

This detection was carried out according to the method described by Harborne [15] with some modifications to determine the presence of flavonoids in the samples (2 ml) of concentrated fennel powder and extract were added to a test tube, and a few drops of concentrated ammonia were added to it. A yellow color was observed, which is an indication of the presence of flavonoids.

Detection of phenols

Three milliliters of the plant extract were added to 2 ml of a solution (1% ferric chloride), and the appearance of a blue-green color indicates the presence of phenols Harborne [15].

Detection of the presence of resins

Ten milliliters of the powder and plant extract were taken, and 20 ml of distilled water acidified with 4% hydrochloric acid (HCL) was added to it, as the presence of resins is indicated by the presence of turbidity Harborne [15].

Detection of Alkaloids

Ten grams of the plant part was boiled in 50 ml of distilled water acidified with drops of hydrochloric acid (HCl), at a concentration of 4%, then the solution was cooled and filtered. The detection process was carried out using Mayer's reagent, as the appearance of a white precipitate indicates the presence of alkaloids Harborne [15].

Detection of the presence of saponins

This test, also called the foam test, was carried out according to the method described by Harborne [15], with some modifications to determine the presence of saponins in the test sample. 2 ml of concentrated fennel powder and extract were added to 6 ml of distilled water in a test tube, then the mixture was shaken vigorously, and foam was observed on the surface of the mixture, which is an indication of the presence of saponins.

Detection of Tannins

The detection was carried out according to the method described by Harborne, [15], with slight modifications in order to determine the presence of tannins in the samples using ferric chloride. 2 ml of concentrated lemongrass extract were added to 10 ml of distilled water in a test tube and shaken well for 20 minutes, then the mixture was filtered. After that, 2 ml of the extract were transferred to a test tube and 3 ml of distilled water was added to it and shaken vigorously, then two drops of diluted ferric chloride solution were added to it and a very dark precipitate was observed, which is an indication of the presence of tannins.

RESULT AND DISCUSSION

Antimicrobial activity test

Table 1 shows the results of the tests of the inhibitory activity of the powder and alcoholic and aqueous extracts shown by the powder and the aqueous and alcoholic extract of the *Prosopis fracta* herb at concentrations of 50% and 100% against *Aeromonas hydrophila* bacteria. It can be concluded that the *Prosopis fracta* powder and extracts have biological activity against microorganisms. The powder is superior to the alcoholic and aqueous extract as it recorded the highest activity towards the bacteria under test as the diameters of the inhibition zones for the concentrations of 50% and 100% *Prosopis fracta* powder reached 20, 25 mm, the alcoholic extract recorded 16.8, 20.8 mm and the aqueous extract recorded 10.4, 11.9 mm. In contrast, the antibiotic recorded an inhibitory activity of 30 mm against *Aeromonas hydrophila* bacteria, as shown in pictures (1, 2, 3, 4).

Table 1: The inhibitory activity of the powder, aqueous and alcoholic extract of Prosopis fracta against A. hydrophila bacteria and its comparison with the inhibitory effect of the antibiotic used.

Bacteria used	*P. fracta*	Inhibition zone diameter (mm)			
		50%	100%	Antibiotic	Inhibition Zoon
A. hydrophila	powder	20	25	Oxytetracycline	30
	Alcoholic extract	16.8	20.8	Ciproflaxin	33.3
	Aqueous extract	10.4	11.9	Doxycycline	20.5
				Trimethoprim	10
				Ampicillin	0

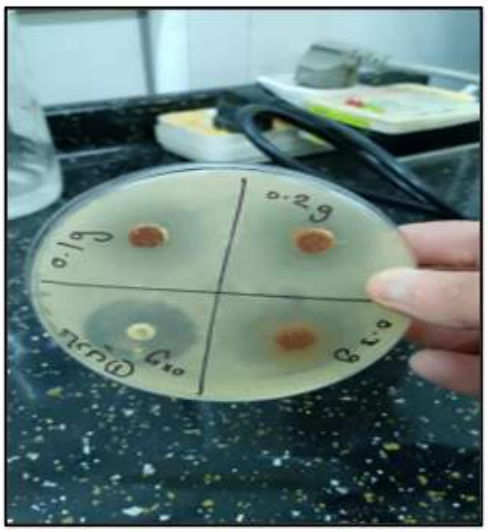

Figure1: shows the inhibitory activity of P. fracta powder against A. hydrophila bacteria.

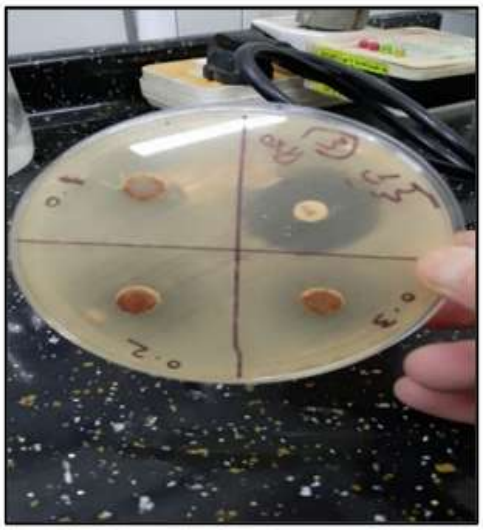

Figure 2: Ihibitory activity of the alcoholic extract of P.F. against A. hydrophila bacteria.

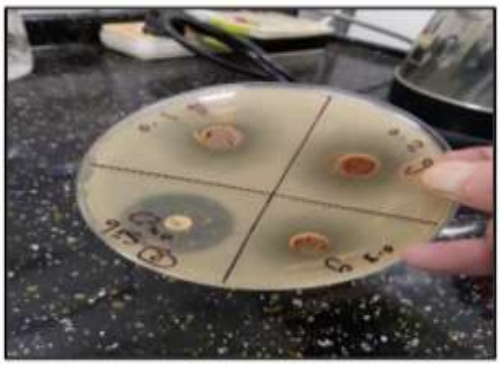

Figure 3: The inhibitory activity of the aqueous extract of linden against A. hydrophila bacteria.

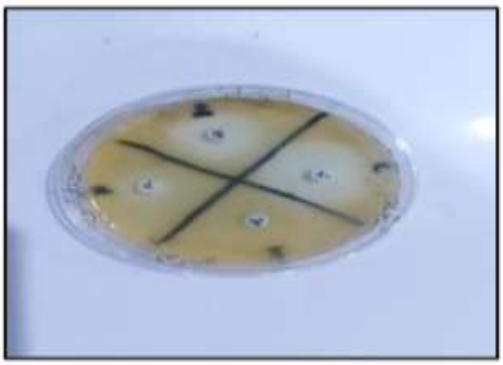

Figure 4: Inhibitory activity of antibiotics against A. hydrophila bacteria.

The sensitivity and resistance of bacteria were tested in the laboratory using several antibiotics, excluding Oxytetracycline, which was utilized in the practical aspect of the study. The bacteria showed sensitivity to ciprofloxacin, exhibiting an inhibition zone diameter of 33.3 mm. They were also sensitive to doxycycline, with an inhibition zone of 20.5 mm, and to trimethoprim, which had an inhibition zone of 10 mm. In contrast, the bacteria were resistant to ampicillin, as it did not demonstrate any inhibitory activity.

The results show that inhibition increases with increasing concentration for Powder and extracts *prosopis fracta*. The results also show that Gram-negative bacteria have an outer membrane that acts as an impermeable barrier for many small molecules. These results agree with what was mentioned by Naik [23] that the *P. fracta* herb has antibacterial activity. They also agreed with the results of the study Jahromi and Zebarjad [17], where the results of the antimicrobial test showed the highest inhibitory effect of the alcoholic *P. fracta* extract against the growth of *Staphylococcus aureus* and *Escherichia coli* with MIC values of 16 µg/ml when compared to other tested bacteria. They also agree with the results of Yaseen *et al.* [36] that all active and diagnostic substances have an inhibitory effect on the growth of bacterial strains, and the separate diameters differ according to the active substances and their concentrations. The results of this study are also consistent with Mustafa [19], whose results showed that extracts of *P. farcta* pods using the MIC method had an inhibitory effect against the isolated *S. paucimobilis* with the number S.p4, which was the most resistant isolate to most of the antibiotics studied, and the MIC was 1000 mg/ml for both methanol and ethanol extracts while it was 1200 mg/ml for the aqueous extract, which the researcher attributed to the chemical components of the pods and seeds of this plant such as flavonoids. As a result, it is clear that the powder has a higher activity against microorganisms and accordingly, it was used as a direct feed additive with testing the effectiveness of both the alcoholic and aqueous extracts. The current results are close to what was stated by Ameri [4] that the aqueous extract of *P. fracta* has antimicrobial properties. In addition, *P. fracta* consists of phenolic compounds such as tannins and several types of glycosides that act as antibacterial agents. The current results regarding the antibiotic used (oxytetracycline) are consistent with the findings of the researcher Samal [30], who stated that although chloramphenicol sensitivity was the most common, *A. hydrophila* strains were sensitive to azithromycin, ofloxacin, oxytetracycline, doxycycline, streptomycin, chlortetracycline, nitrofurazone, and norfloxacin, but resistant to ampicillin, amoxicillin, bacitracin, cloxacillin, cefuroxime, cotrimoxazole, cephalexin, erythromycin, and flumequine. The sensitivity and

resistance of bacteria were tested in the laboratory for several antibiotics other than the antibiotic (oxytetracycline) used in the practical aspect. The bacteria were sensitive to the antibiotic ciproflaxin with an inhibition zone diameter of 30.3 mm, followed by doxycycline with an inhibition zone diameter of 20.5 mm, then trimethoprim with an inhibition zone of 10 mm. The bacteria were resistant to the antibiotic ampicillin, as it did not show any inhibitory activity. These results are consistent with the study Daskalov [10], which showed that *Aeromonas hydrophila* bacteria are resistant to the antibiotic ampicillin. The results of this study are in agreement with Dang [9] which showed that the highest antibiotic resistance of *A. hydrophila* was to ciprofloxacin (45%), followed by trimethoprim/sulfamethoxazole (35%), streptomycin (25%), tetracycline, florfenicol and chloramphenicol (20% each), erythromycin and doxycycline (15% each), and rifampicin (5%), These results indicated antibiotic resistance in A. hydrophila strains in freshwater aquaculture in Vietnam. However, antibiotic treatment was associated with an enrichment in Plesiomonas, accompanied by a decline in other bacteria taxa. Oxytetracycline treatment increased the proportion of *tet*A in the distal gut of fish and tank biofilms of the treated group. Furthermore, the abundance of *tet*A along with other tetracycline resistance genes was strongly correlated with a number of microbiome members, including Plesiomonas. The findings from this study demonstrate that antibiotic treatment can exert selective pressures on the gut microbiome of fish in favour of resistant populations, which may have long-term impacts on fish health Payne [26].

Active ingredients in the plant

Table 2 shows the active compounds present in the powder sample and the alcoholic and aqueous extract of the studied *Prosopis fracta*. The results showed that the extract contained a high abundance of phenols and a moderate abundance of flavonoids, phenols and tannins in the yarrow powder, and that the alcoholic extract contained a high abundance of flavonoids, tannins and alkaloids and a moderate abundance of saponins. The results also showed a moderate abundance of flavonoids, phenols, saponins, alkaloids and tannins. The presence of flavonoids was indicated upon examination by the clear yellow color of the reaction mixture, and the presence of tannins was indicated by the formation of a precipitate at the bottom of the tube containing the reaction mixture, and the presence of saponins was indicated by the formation of a light.

Table 2: Active compounds in the herb *Prosopis fracta*

Active Ingredients	Powder of P.F.	Alcoholic extract	Aqueous extract
Flavonoids	+	++	+
Phenols	+	++	+
Saponins	-	+	+
Resins	-	-	-
Tannins	+	++	+
Alkaloids	+	++	+

Where (+++) represents high abundance of the component, (++) moderate abundance, (+) low abundance, (-) absence of the component

Foam on the surface of the mixture. The yarrow plant is a valuable and useful medicinal plant for the purpose of extracting flavonoids and is one of the most important plants

with medicinal properties WHO [35]. These results are consistent with what was found by Thorat [32], which confirmed the presence of chemical compounds in the extract of the P.F. herb.

Alkaloids have antimicrobial activity, as isolated pure plant alkaloids are used as antibacterial agents Okwu [24]. Plant saponins help reduce the activity of microorganisms, and they also lower blood cholesterol Pandey and Gupta [25]. Flavonoids are phenolic compounds that act as flavoring components. Flavonoids are also antioxidant agents Tatipamula and Kukavica [31]. Tannins are plant polyphenols with many properties, including medicinal properties, as they have antimicrobial activity Chew [8]. Tannins have also been confirmed in the herb extract, and they are also compounds with antioxidant properties, which means that they can help neutralize harmful free radicals, which are highly reactive molecules that can cause oxidative damage to cells and contribute to various diseases, consuming foods rich in plant tannins can provide antioxidant benefits and support overall health Losada-Barreiro and Bravo-Diaz [20]. The results showed the presence of phenols in the extract of the *Prosopis fracta* herb, which is a class of organic compounds with strong antioxidant properties, which help neutralize harmful free radicals, and also have antimicrobial properties, which can help prevent the growth of microorganisms such as bacteria, fungi and some viruses as mentioned Gokoglu [14].

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تحليل المكونات الفعالة في مسحوق ومستخلصات نبات (المسكويت السوري) *Prosopis fracta* وتقـــويم النشاط البايولـــوجي ضـــد البكتـــيريا *Aeromonas hydrophila*

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الملخص

تم إجراء التحليل الكيميائي لتحديد المركبات الفعالة الموجودة في مسحوق ومستخلصات ثمار الينبوت بالإضافة إلى ذلك فحصت الدراسة تأثير كل من مسحوق ومستخلصات *Prosopis fracta* في بكتيريا *Aeromonas hydrophila* بينت النتائج أن المسحوق يتفوق على المستخلصات الكحولية والمائية، اذ سجل أعلى نشاطا ضد البكتيريا، اذ بلغ أقطار مناطق التثبيط لتركيزين 50% و100% من مسحوق *Prosopis fracta* 20.25 ملم، وسجل المستخلص الكحولي 16.8، 20.8 ملم، في حين سجل المستخلص المائي 10.4، 11.9 ملم، كما سجل المضاد الحيوي نشاطاً مثبطاً بلغ 30 ملم ضد بكتيريا *A. hydrophila* ، كما أظهرت النتائج حساسية البكتيريا للمضاد الحيوي سيبروفلاكسين بقطر منطقة تثبيط 33.3 ملم، يليه الدوكسي سايكلين بقطر منطقة تثبيط 20.5 ملم، ثم ترميثوبريم بقطر منطقة تثبيط 10 ملم. في حين كانت البكتيريا مقاومة للمضاد الحيوي أمبيسلين، الذي لم يظهر أي نشاط مثبط. كما أظهرت النتائج وجود المركبات الفعالة التالية الفلافونويدات، الفينولات، الصابونينات، الراتنجات، التانينات والقلويدات. تراوحت وفرتها بين المتوسطة والعالية والحرة وفقًا لطبيعة المادة أو المسحوق أو المستخلص.

الكلمات الدالة: تحليل المكونات الفعالة، المسكويت السوري، *Aeromonas hydrophila*

[1] مديرية الزراعة في محافظة الانبار، الانبار، العراق.
[2] جامعة الأنبار، كلية الزراعة، الانبار، العراق.
[3] دائرة البيطرة، وزارة الزراعة، بغداد، العراق.

- **تاريخ تسلم البحث:** 30/كانون ثاني /2025.
- **تاريخ قبول البحث:** 13/شباط/2025.
- **متاح على الانترنت:** 30/حزيران/2025.