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# The Phytochemical Compounds and the Antimicrobial Activity of Portulaca grandiflora Aqueous & Methanol Extracts in Iraq

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#### ABSTRACT

Inhibitory activity against bacterial and fungal pathogens is one of the many diseases for which phytochemical compounds are useful in medicine. By using gas chromatography-mass spectrometry (GC-MS) on the floral parts of *Portulaca grandiflora* s in Iraq, several phytochemicals were extracted for this study. The study's objective is to determine the active substances found in the species' vegetative parts and evaluate their ability to inhibit pathogens. In the current study, various medical phytochemical compounds that have been isolated from Portulaca grandiflora from the floral parts that showed antifungal activity as Candida albicans and antibacterial as Proteus mirabilis. The phytochemicals of Portulaca grandiflora floral parts were exposed to gas chromatography-mass spectrometry (GC-MS) analysis. The results revealed the highest activity against fungal and bacteria. The study used six concentrations of the floral parts (5,10,20,40,60,80) mg/ml for both aqueous and methanolic extracts. The aqueous extract showed high inhibition against Candida fungi, It was (0.00mm, 0.00mm, 0.00mm, 0.00mm, 12.00mm, 22.00mm) respectively in the diameter of colonies Thus, the aqueous extract indicated high inhibition against bacteria Proteus mirabilis . It was (0.00mm, 0.00mm, 0.00mm, 10.00mm, 14.00mm, 18.00mm) separately in the diameter of colonies. The methanolic extracts of floral parts have lower inhibition rates compared to the aqueous extract.which was giving results against Candida fungi (0.00mm, 0.00mm, 4.00mm, 6.00mm, 10.00mm, 24.00mm) respectively in the diameter of colonies, so that in a result with the inhibition against bacteria Proteus mirabilis. It was (0.00mm ,3.00mm, 6.00mm,10.00mm, 15.00mm, 25.00mm) respectively in the diameter of colonies. analysis of compounds to P. grandiflora floral parts by GC-MS showed the presence of five bioactive phytochemical compounds as: Benzaldehyde: Cyclohexene, 1-methyl-4-(1-methylethenyl)-: (S), Linalool: Naphthalene: and Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate.

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#### 1. INTRODUCTION

Microbes cause many diseases and increase the mortality rate worldwide. Their resistance to conventional medicines has been a major reason for the interest in medicinal plants as a reliable alternative for managing diseases resulting from bacterial infections in recent times. Many research efforts have been directed towards exploring plant components with antimicrobial potential that are used as antimicrobial agents due to their high therapeutic performance, low toxicity and reasonable prices (Ojah EO , 2021).

The family Portulacaceae consists of one genus and 116 species (Christenhusz MJ& Byng JW, 2016) based on previous molecular studies that reduced the family to a single monophyletic group, which included one genus, *Portulaca L.*, and 100 species (Nyffeler R & Eggli U,

2010). The word genus is taken from a Latin word composed of two syllables; the first is Portare, which means carry, and the syllable Lak, which means milk and gives the meaning of the milky liquid secreted by the plant. Its types are commonly used in general to treat infections, ulcers, colds, coughs, urinary tract diseases, and many others (Philips SM, 2000). The ancient Egyptians and the English in the Middle Ages used plants of this genus medicinally, as food, or as vegetables (Boulos L, El-Hadidi MN, 1984).

Portulaca grandiflora is a plant that is cultivated by humans for its beautiful colorful flowers. It is widely spread due to its rapid reproductive property. In Iraq flora, it has been established as a cultivated plant and its original homeland is South America. In Iraq, it is called Yaldis, after its Turkish name Aldoz. It is known in

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Europe and America as the eleven O' clock rose, moss rose, sun plant and in India lyauiya and Goddu pavelli, and Sokkare Kamma in Canada(Netala S, Pravallika R, et al., 2015)

It was found that the chemical extracts of P. grandiflora samples contain alkaloids, hydroquinols, phenols, flavonoids, saponins, tannins, terpenoids and steroids, as the different effectiveness of these complex compounds variations in antioxidant significant capacity(Aisyah SI, et al., 2023). In addition, P. grandiflora plants showed antioxidant activities, indicating that they would have variable flavonoid content and therefore they had variable wound healing activity depending on flower color(Budiawan A,et al., 2023). A similar study also revealed that n-hexane extract of P. grandiflora significantly enhances wound healing by increasing cell migration and wound closure compared to wounds treated with ointment(Mus'hib HK, Abdul-Jalil TZ, 2024). The use of methanolic, ethanolic and n-hexane extracts of the P. grandiflora plant contains antibacterial, antifungal, anticancer and antioxidant properties(Salahuddin H,et al.,2024). A study showed that flavonoids, one of the compounds of P. grandiflora, can protect the skin from ultraviolet UV rays. Accordingly, it was possible to develop a sunscreen that protects the skin from the risk of skin cancer and prevents premature skin aging(Kirana BC,et al., 2023). And as stated by experiment that the methanolic extract of the green parts of the morning flower P. grandiflora contains a high percentage of flavonoids, which act as an antihyperlipidemic agent induced by a high-fat diet (HFD) and as an antioxidant(Wang X,et al.,2023).

Candida albicans is a fungus that lives in the human body in normal conditions, in the intestines and oral cavity. It then turns into an opportunistic organism in special cases, such as weak immunity, or when dealing with some animals, such as birds, cows, cats, dogs, and pigs, if hygiene conditions are not observed, as it is a fungus present in their bodies(Edelmann A,et al.,2005).

Proteus bacteria, including Proteus mirabilis, form a symbiotic relationship between higher organisms, including humans and some animals. This is indicated by the contamination of soil and water with the feces of infected animals, which constitutes a source of infection for humans when consuming this water or marine organisms contaminated with the feces of these infected animals(Drzewiecka D,2016).

#### 2. MATERIALS AND METHODS

## 2.1. Study Area And Sampling

The studied fungi and bacteria were isolated from some infected patients in Karbala, the fungi and the bacteria were identified in the pure sciences college laboratory, Kerbala university.

#### 2.2. Plant Extract Preparation

The dried aerial parts were pulverized and extracted in a Soxhlet. The polar compounds were extracted with 1.4 ml methanol + 60ml distal water for the alcoholic extraction and 200ml of distal water for the aqueous extract, then 6iltered and evaporated. After that, the compounds were weighed, and their yield percentage expressed as a percentage of their dry weight, was calculated. The yield of the dark green extract from methanol was 60 grams (0.095%). The plant extracts were placed in the dark and refrigerated at a temperature of 4 °C, Wahid & Jafar method(Wahid AZ & Jafar FN, 2005)

# 2.3. Cultivated Method of Aqueous & Alcoholic Extract *P. Grandiflora* of Plant on Pathogenic Fungi Growth

To perform the inhibition zone assay for *Proteus* and *Candida* using your plant extract and nanoparticles in a Mueller-Hinton agar setup, follow these steps:

## **Materials Needed**

- -Mueller-Hinton agar plates
- -Plant extract at concentrations of 80 mg, 60 mg, 40 mg, 20 mg, 10 mg, and 5 mg
- -Ciprofloxacin (positive control)
- -Distilled water (negative control)
- -Sterile wells or cork borer
- -Inoculating loop
- -Sterile swabs
- -Incubator

# **PROCEDURE**

- 1 .Preparation of Inoculum:
- Inoculate a single colony of Proteus and Candidain sterile broth and incubate overnight at 37°C (for *Proteus*) and 30°C (for *Candida*).
- Adjust the inoculum to match a 0.5 McFarland standard.

# 2 .Inoculating the Plates:

- Pour Mueller-Hinton agar into sterile Petri dishes and allow it to solidify.
- Use a sterile swab to evenly spread the inoculum across the surface of the agar plates.

#### 3 .Creating Wells:

- Once the agar is inoculated and dried, use a sterile cork borer or well-maker to create wells in the agar plates.

#### 4 .Adding Plant Extracts and Controls:

- Using a micropipette, add 100  $\mu$ L of each concentration of the plant extract (80 mg, 60 mg, 40 mg, 20 mg, 10 mg, and 5 mg) into separate wells.
- Add 100  $\mu L$  of ciprofloxacin into one well as the positive control and 100  $\mu L$  of distilled water into another well as the negative control.

#### 5 .Incubation:

- Incubate the plates at the appropriate temperature (37°C for Proteus and 30°C for Candida) for 24-48 hours.

#### 6 . Measuring Inhibition Zones

- After incubation, measure the diameter of the inhibition zones around each well using a ruler or caliper.
- Record the results for each concentration of the plant extract and the controls.

## **Data Analysis**

-Analyze the inhibition zones to determine the effectiveness of the plant extract at different concentrations.

- Compare the inhibition.

#### 2.4. Collection And Preparation of Plant Materials

*P. grandiflora* plant were located from various places from Karbala city in Iraq. Then plants were well washed and dried at room temperature. 10gm of plant powdered had taken in 1.4L methanol and 60ml distal water for the alcoholic extract solution and then filtered, and 10gm of plant powdered in 200ml of distal water and also had been filtered.

# 2.5. Constituents Identification Of Extract By Gas Chromatography - Mass Spectrum (GC/MS)

Phytochemical identification of *P. grandiflora* were carried out by GC-MS analysis in (Scion Instruments Company.Model: SCiON 436) GC-MS Column: SCION-5MS { ID = 0.25mm, length = 15m, df = 0.25µm } Carrier gas: Helium. Instrument under computer designed control at 60eV. About 1µL of them ethanol extract was injected into the GC-MS column using a micro syringe and the scanning was done for 10 minutes. Column oven: initial temperature is 50 °C increase by 5 °C / min to 240 °C, Sample Preparation: 50 µL of the sample is diluted with 2.5ml of Methanol (HPLC-Grade) before injection. Gas flow ratio: 1 ml/min. Pressure: 10 psi. Range: 1 – 2000 m/z, split ratio 1:40. (This method is innovative in Al-Zahraa Center for Medical and

Pharmaceutical Research Sciences (ZCMPRS)) (Purwanto A,et al.,2022).

#### 3. RESULTS AND DISCUSSION

#### 3.1. Antifungal Activity

The current study Candida albicans was selected to test the activity of the aqueous and methanol extract of P. grandiflora partss. The extract showed a high antifungal activity against Candidia albicans. Six concentrations of extract (5,10,20,40,60,80 mg/ml) were used for the floral parts of the plant each as aqueous and methanol extracts. The results showed (00.00, 0.00, 0.00, 0.00, 12.00, 22.00 mm) diameter of colonies in Candida albicans for aqueous extract, the results showed in Table 1, and (00.00 , 0.00, 4.00, 6.00, 10.00, 24.00 mm) diameter of colonies in Candida albicans for alcoholic extract. Table 2 offered the results . We conclude from the results of our current experiment that the inhibitory ability of the alcoholic extracts of the floral parts of the plant against Candida albicans was higher than the inhibitory ability of the aqueous extracts of the same parts against Candida albicans.

**Table 1.** Antifungal activity of aqueous extracts from *P. grandiflora* floral parts against *Candida albicans* 

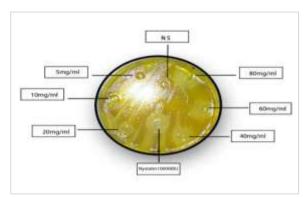
Mean of	Mean of Inhibition zone (mm									
Compa rison with distille d water (0.00) mg/ml	Compa rison with Nystat in 10mg	Conce ntrati on (5mg/ ml)	Conc entra tion (10m g/ml)	Conce ntrati on (20m g/ml)	Conce ntrati on (40m g/ml)	Conce ntrati on (60m g/ml)	Concent ration (80mg/ ml)			
0.00	8.00	0.00	0.00	0.00	0.00	12.00	22.00			

**Table 2.** Antifungal activity of methanolic extracts from *P. grandiflora* floral parts against *Candida albicans* 

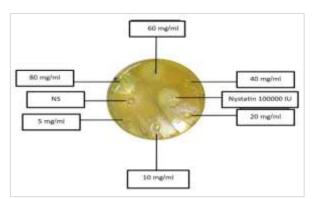
0.00   8.00   0.00   0.00   4.00   6.00   10.00   24.00	Compa rison with distille d water (0.00) mg/ml	Compa rison with Nystat in 10mg	Conce ntrati on (5mg/ ml)	Conc entra tion (10m g/ml)	Conce ntrati on (20m g/ml)	Conce ntrati on (40m g/ml)	Conce ntrati on (60m g/ml)	Concentration (80mg/ml)
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In support, scientists proved in an experimental laboratory study using the disk diffusion method that the extract of the morning glory plant *P. grandiflora* had different inhibition rates against the growth of the fungus

C. albicans(Purwanto A,et al.,2024). Based on differences in polarity of extract of P. grandiflora (aqueous, ethanolic, distilled water, ethyl acetate, n-hexane) the aqueous extract had the strongest effect against C. albicans based on disc diffusion method(Abu-Serag NA et al., 2019).



**Figure 1**: Candida albicans growth grown in varying concentrations of P. grandiflora ethanol extract.



**Figure 2**: Antifungal activity of aqueous extracts from *P. grandiflora* floral parts against *Candida albicans* 

## 3.2. Antibacterial Action

In this study *Proteus mirabilus* was selected to test the activity of the aqueous and methanolic extracts of *P. grandiflora* floral parts. The extracts showed a high antibacterial activity against *Proteus mirabilus*. Six concentrations of extract (5,10,20,40,60,80 mg/ml) were used for the floral parts of the plant each as aqueous and methanol extracts. The results indicated (00.00mm, 0.00mm, 0.00mm, 10.00mm, 14.00mm, 18.00 mm) diameter of colonies in *Proteus mirabilus* for aqueous extract. The results were displayed in Table 3, and they wrer (00.00 mm, 3.00mm, 6.00 mm, 10.00mm, 15.00mm, 25.00 mm) diameter of colonies in *Proteus mirabilus*. For alcoholic extract, the results wre showed in Table 4.

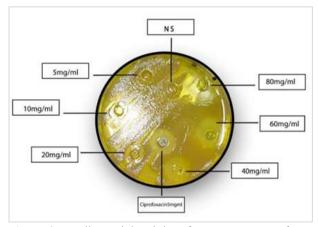
We conclude from the results of our current experiment that the inhibitory ability of the alcoholic extracts of the floral parts of the plant against *Proteus mirabilus* was higher than the inhibitory ability of the aqueous extracts of the same parts against *Proteus mirabilus*. Further, the alcoholic extracts showed inhibitions even at low concentrations.

**Table 3.** Antibacterial activity of aqueous extracts from *P. grandiflora* floral parts against *Proteus mirabilus* 

Mean of Inhibition zone (mm )									
Compa rison with distille d water (0.00) mg/ml	Compa rison with Ciprofl oxacin 10mg	Conce ntrati on (5mg/ ml)	Conc entra tion (10m g/ml)	Conce ntrati on (20m g/ml)	Conce ntrati on (40m g/ml)	Conce ntrati on (60m g/ml)	Conce ntrati on (80m g/ml)		
0.00	8.00	0.00	0.00	0.00	10.00	14.00	18.00		

**Table 4.** Antibacterial activity of methanolic extracts from *P. grandiflora* floral parts against *Proteus mirabilus* 

Compa rison with distille d water (0.00) mg/ml	Inhibition Compa rison with Ciprofl oxacin 10mg	Conce ntrati on (5mg/ ml)	Conc entra tion (10m g/ml)	Conce ntrati on (20m g/ml)	Conce ntrati on (40m g/ml)	Conce ntrati on (60m g/ml)	Conce ntrati on (80m g/ml)
0.00	8.00	0.00	3.00	6.00	10.00	15.00	25.00



**Figure 3**: Antibacterial activity of aqueous extracts from *P. grandiflora* floral parts against *Proteus mirabilus* 

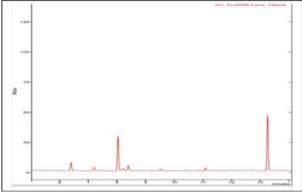


**Figure 4**: Antibacterial activity of methanolic extracts from *P. grandiflora* floral parts against *Proteus mirabilus* 

# 4. ASSESSMENT OF BIOCHEMICAL COMPOUNDS OF *P. grandiflora*

The analysis GC-MS ethanolic extract of *P. grandiflora* floral (Figure 1) partsrevealed the presence of five components found in Table 5 as: Benzaldehyde, Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S), Linalool, Naphthalene, and Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate.

It is noted that the percentage of terpene compounds is high (Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S: Linalool: Cyclohexanol, 1-methyl-4-(1-) and it is considered to be one of the highly efficient compounds in inhibiting microbes, especially pathogenic ones in the experimental samples, such as Candida (Candida albicans) and bacteria (Proteus mirabilus) (AlAmery SF & Al-Garaawi NI,2020).



**Figure 6.** Chromatography plot of *P. grandiflora* floral parts

**Table 5.** Major phytochemical composites in ethanolic extra of P. grandiflora floral parts

No.	R. t. (min)	Area	%Tota	M.w t	Prob %	Name
1	6.399	2.214e +7	13.713	63.0	106	Benzaldehyd e
2	8.041	9.134e +7	19.067	20.4	136	Cyclohexene , 1-methyl- 4-(1- methylethen yl)-, (S)
3	9.522	4.645e +6	12.359	33.6	154	Linalool
4	10.842	2.181e +6	12.169	16.4	128	Naphthalene
5	13.257	1.371e +8	22.606	13.8	196	Cyclohexan ol, 1-methyl-4-(1-methylethen yl)-, acetate

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