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The Phenolic Contentand Antibacterial activity of Cladium Mariscus Seeds Extract

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Abstract

Cladium mariscus is one of the plants belonging Cyperaceae family. Itis a common plant in AL-Hammar marshes, eastern - south of Iraq.Polyphenols constitute one of the largest and widely distributed groups of natural products in the plant kingdom. So the aim of the present study was measurement of total phenolic content in the poly phenolic extract of the plant seeds, by using Folin–Ciocalteau reagent, and study the antibacterial activity of the poly phenolic extract. Cladium mariscus seeds were extracted in methanol: water (70:30 v/v). The optimum conditions for wavelength, and standard substance were 550 nm, and Gallic acid, respectively.Theantibacterial activity of methanol: water extract was examined against two Gram positive bacteria(S. aureus and S. coccus) andtwo Gram negative bacteria (E.coli and P. aeruginosa) pathogenic bacteria strains, by using the well disk diffusion method, showed that the methanol: water extract had a clear inhibition effect against microbes at (50%, 75% and 100% w/v) concentrations.

key words:*Cladium mariscus* ,total phenolic content, antibacterial activity

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تحديد محتوى الفينول الكلي ودراسة الفعالية البايلوجية لمستخلص البولي فينول من بذور الحلفا السبخية

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الخلاصة

الحلفا السبخية هو احد النباتات التي تنتمي للعائلة السعدية, وهو نبات شائع في هور الحمار جنوب شرق العراق. البولي فينولات تشكل احد اكبر مجاميع نواتج الايض الثانوي الواسعة الانتشار في مملكة النبات, لذا كان الهدف من الدراسة الحالية هو قياس محتوى الفينول الكلي في مستخلص البولي فينول لبذور هذا النبات باستخدام كاشف Folin–Ciocalteau ودراسة فعالية المستخلص كمضاد للميكروبات .

استخلصت بذور نبات الحلفى السبخية باستخدام الميثانول : الماء (٧٠:٣٠), أما الظروف المثالية للطول الموجي والمادة القياسية التي طبقت لقياس المحتوى الفينولي الكلي في المستخلص كانت ٥٥٠ نانومتر, وحامض الكاليك.

إن فعالية المستخلص كمضادالبكتريا الممرضة درست تجاه) والبكتريا الموجبة الغرام (S. aureus and S. coccus) والبكتريا السالبة الغرام (E.coli and P. aeruginosa), باستخدام طريقة الانتشار بالحفر وقد أظهرت النتائج أن المستخلص البولي فينولي له تأثير مثبط واضح تجاه البكتريا عند التراكيز (٥٠, ٧٥, ١٠٠ % غرام / لتر).

الكلمات المفتاحية: الحلفا السبخية, المحتوى الفينولي الكلي, مضاد البكتريا

1 . Introduction

Phytochemicals secondary metabolites, i.e. substances that in plant have little or no role in photosynthesis, respiration or growth and development, but which may accumulate in surprisingly high concentrations[1]. Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. As stated by Harborne [2] Phenolic compounds are of considerable physiological and morphological importance in plants. Tannins, lignans, flavonoids, and simple phenolic compounds serve as defense against herbivores and pathogens[3]. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant anti-thrombotic cardio protective and vasodilatory effects[4, 5, 6, 7].

The term "phenolic" or "polyphenol" can be precisely defined chemically as a substance which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives (esters, methyl ethers, glycosides, etc.).

Recent studies such as[8],[9] have shown that phenols are one of the secondary metabolites of some plants belonging toCyperaceae family, such as *Cyprusrotundus* and *Cyprusesculents*.This study is found results demonstrated that the poly phenols were one of the secondary metabolites of *C.mariscus* seeds,who belongs to the same family. In this study, ultrasonic irradiation was applied to extract poly phenols from *C.mariscus* seeds.UAE of natural products has been widely investigated, and considered one of the simplest extraction techniques because it is easy to perform in common laboratory equipment (i.e. ultrasonic bath). In this method, the crushed sample is mixed with the suitable solvent and placed into the ultrasonic bath, where the working temperature and extraction time are set [10].The current study wasundertaken to estimate the total phenolic content of the methanolic extract of plant seeds with expected the antifungal, and antibacterial activities. Additionally, a UV- visspectrophotometer (UV-3000 nano) in between range 200 nm to 1000 nm, and Fourier transforms infrared(FT-IR) spectrastudy of *Cladium mariscus* seeds extract.

2 . Materials and methods

2.1. Chemicals and solvents:

Chloroform, ethyl acetate, Folin–Ciocalteau reagent , methanol, n-hexane,

2.2. Samples of *Cladium mariscus*

The seeds of *C.mariscus* was employed for the development of the ultrasound-assisted extraction method. They were obtained from AL-Hammar marshes, east of Nasiriyah city in Iraq.The seeds were collected and crushed ,then the seeds were kept in dark glass containers for further use.

2.3. Extraction procedure

2.3.1. Ultrasound-assisted extraction(UAE)

The extraction of phenolic compounds originating from *C.mariscus* by means of ultrasound was performed employing water– methanol mixture (30:70). effects by the extraction temperature (35°C), the quantity of *C. mariscus* seeds (1000 g), and the extraction time (48hr) were studied[11]. Ultrasonic irradiation was applied by means of (250 volts, 150 watt, 50/60 Hz) (Decon G , England).Which was immersed in a water bath coupled to a temperature controller, the following steps have been taken to extraction poly phenols by ultrasound-assisted extraction (UAE):

a. Each 50g from *C. mariscus* seeds were crushed and put in brown bottles (500 mL) , and added to it 350 mL(70%) CH_3OH.

b. The mixture was placed in the ultrasonic bath and the ultrasonic treatment done under the conditions mentioned above.

c. After treatment, ultrasound and traditional extraction samples were filtered (125mm).

d. The filtered were collected and placed into a rotary evaporator under vacuum at 40 °C to reduce solvent volumes to one – third the size.

e. The filtered were extracted with chloroform in the separatory funnel (three times), withhalf volume to get two layers(aqueous and chloroform layers).

f. The aqueous layer was separated with half volume of ethyl acetate (five times), to get two layers, an ethyl acetate layer which was separated and evaporated reduced pressure at a temperature not exceeding 40C° to give 4.3g of yellow / brown residue

2.4. Determination of the total phenolic content

The total phenolic contents of the methanolic extract of *C. mariscus* seeds were estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi[12]. The calibration curve (Figure 3) was plotted by mixing 1 ml aliquots of 10, 50, 100, 150, and 200 ppm Gallic acid solutions with 5.0 ml of Folin Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured at 550 nm. The totalamount of phenolic compounds was calculated and expressed asmg Gallic acid Equivalents (GAE mg/g).

3. Results and Discussion

3.1. UV-Visible Spectra

The UV-Visible analysis of the polyphenolic extracts were recorded at the range of 190-800 nm, with λmax at (205nm) and another peak at (280 nm) respectively.

3.2.Fourier transforms infrared(FT-IR) spectra

FTIR spectra was measured to the identification of phenolic extract in the range (250 -4000 cm^{-1}). In general the bands in the spectra of FTIR were expected to appear (1716.65, 1654.92, 1608.63, 1114.6- 1076.28, 945.12-1257.59.

3.3. Total phenolic content

The total amount of phenolic content present in *C. mariscus* seeds extract was 117 mg/g. The total phenol content was lower for *C.mariscus* seeds extract.

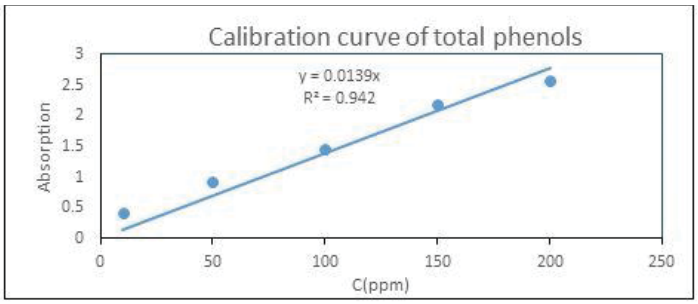

Figure 1: total polyphenols content

3.4. Antibacterial Properties of Polyphenolic Extracts

As shown in Table 3, the extracted polyphenols have antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Gram - negative), *Staphylococcus aureus* and *Strepcoccus* (Gram-positive). polyphenolic extract showed maximum activity against pathogens *Strepcoccus* (30mm) at concentration(100% w/v), and equal inhibition zone against, *Staph* and *Pseudomonas aeruginosa* (21mm) at concentration (75% w/v).

Table2: Diameters of inhibition zone (mm) for extract

Name of Organisms	Zone of inhibition in mm					
	Concentration of poly phenol extract(w/v)					
	Gram Stain	25%	50%	75%	100%	Control (Ciprofloxacin)
E.coli	-	20	23	25	27	30
Pseudomonas	-	17	20	21	22	27
Staph.aureus	+	20	20	21	23	28
Strep coccus	+	20	23	28	30	0
DMSO		0	0	0	0	

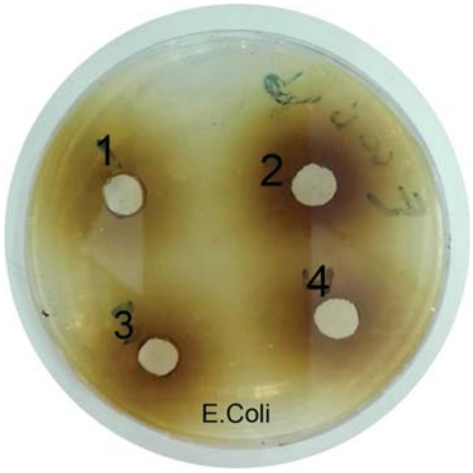

Figure 2: The activity of Polyphenolic extracts against (E.Coli) bacteria

1: 25%; 2: 50%; 3: 75%; 4: 100%, Concentrations of poly phenolic extract (w/v).

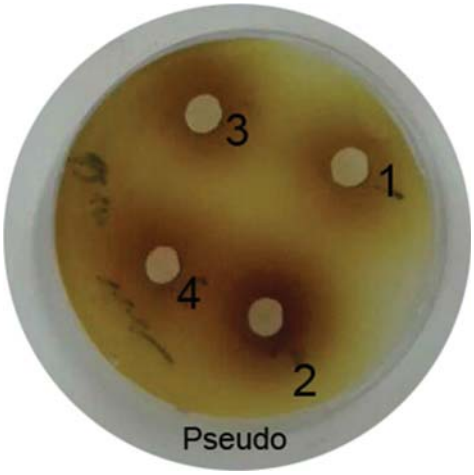

Figure 3: The activity of Polyphenolic extracts against (Pseudo.) bacteria.

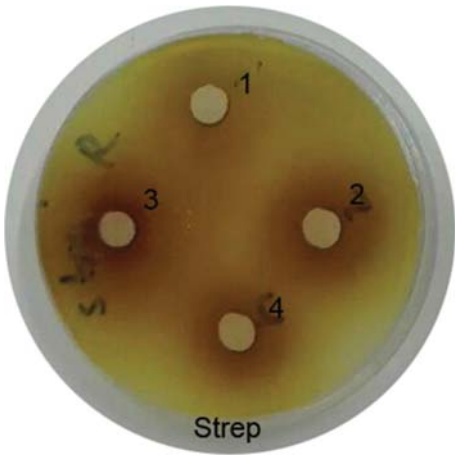

Figure4: The activity of Polyphenolic extracts against (Strep.) bacteria.

Figure 5: The activity of Polyphenolic extracts against (Staph.) bacteria.

4. Conclusions

After optimization of the conditions for the spectrophotometric determination of phenolics usingthe Folin-Ciocalteu reagent,The UV-Vis spectrophotometric method described here was demonstrated a significantly, that the total phenol content was lower for *C.mariscus* seeds extract.. The extracted compounds have been tested for their growth inhibitory activities in Comparison with various fungi, these extracts have irregular effects on various fungi compared with control fungi. The extracted compounds have been tested for their inhibitory activities in comparison with various bacteria, these extracts have irregular effects on various bacteria compared with control bacteria.

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