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RESEARCH ARTICLE

Extraction of Poly Phenols from *Cladium Mariscus* Seeds and studying its Antifungal Activity with Isolation and Identification of one Compound of Flavone Glycosides

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Abstract

Cladium mariscus is one of the plants belonging Cyperaceae family. It is a common plant in AL-Hammar marshes, eastern - south of Iraq. The aim of the present study was extraction poly phenols from *C. mariscus* seeds by using an ultrasonic-assisted extraction method. The yield of poly phenols extracted by this method was (0.43% w/w). One compound was isolated from the methanolic extract of the plant seeds. The structure of the new compound was identified via the basis of extensive spectroscopic analyses (UV-Vis, IR, ^{1}H NMR, and Mass), and some chemical test. Acacetin glycosides were expected compound, and the methods of spectroscopy showed that there are three molecules of hexagonal sugars linked with aglycone. The antifungi activity of methanolic extract was examined against fungus: *A.flavus, A.fumigatus* and *C.albicans*, by using the well disk diffusion method. The extract showed a clear inhibition effect against fungal at (50 %, 75 % and 100 % w/v) concentrations.

Key words: *Ultrasound-assisted extraction, Cladium mariscus, Acacetin glycosides, Antifungal.*

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 [8], including functional derivatives (esters, methyl ethers, glycosides, etc.).

Flavonoids are a major group of polyphenols, which occur in plants mainly in the form of glycosides. Flavonoids (C6-C3-C6) consist of two phenyl rings linked through three carbons that form an oxygenated heterocycle of three rings commonly labeled as A, B and C. The basic structure of flavonoids is shown in Figure 1. Flavonoids account for 60% of the total dietary phenolic compounds [9, 10, 11].

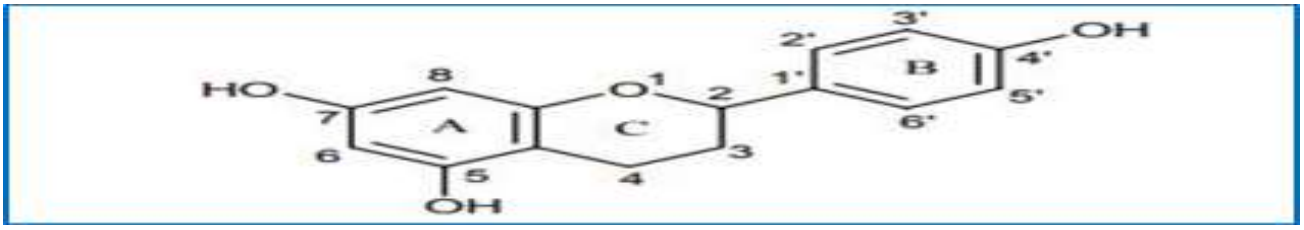

Figure 1: Basic structure of the flavonoids

Recent studies such as [12, 13] have shown that phenols are one of the secondary metabolites of some plants belonging to Cyperaceae family, such as *Cyprus rotundus* and *Cyprus esculents*. This study is found results demonstrated that the poly phenols were one of the secondary metabolites of *C.mariscus*, who belongs to the same family. In this study, ultrasonic irradiation was applied to extract poly phenols from *C. mariscus* seeds. Ultrasound-assisted extraction (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 [8]. The present study intends to characterise the poly phenolic extract of *C. mariscus*, by the UV- Vis spectrophotometer (UV-Vis 3000 nano) in between range 200 nm to 1000 nm, Fourier transforms infrared (FT-IR) spectra. Phenol's contains were isolated by Column Chromatography, over Si-gel column and eluted with ethyl acetate : hexane, after isolation one compound, which identification by LS Mass/ Mass, proton nuclear magnetic resonance analysis (^{1}HNMR) techniques and the methods were used in characterize the extract. Acacetin glycoside was expected isolated compound.

Materials and Methods

Chemicals and Solvents

Chloroform, ethyl acetate, dimethyl sulphoxide, methanol, n-hexane, silica - Jel and ultrasonic.

Samples of Cladium Mariscus

The seed 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, and then the seeds were kept in dark glass containers for further use.

Extraction Procedure

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). This method has been modified by us, effects by the extraction temperature (35°C), the quantity of C. mariscus seeds (1ooo g), and the extraction time (48hr) were studied [14]. Ultrasonic irradiation was applied by means of (250 volts, 150 AMP, 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 phenol by ultrasound-assisted extraction (UAE):

- Each 50g from C. mariscus seeds were crushed and put in brown bottles (500 mL) added 350 mL (70 %) CH3OH.
- The mixture was placed in the ultrasonic bath and the ultrasonic treatment done under the conditions mentioned above.
- After treatment, ultrasound and traditional extraction samples were filtered (Whatmann no.1).
- The filtered liquids were collected and placed into a rotary evaporator under vacuum at 40 °C to reduce solvent volumes to one-third the size.
- The filtered liquids were extracted with chloroform in the separatory funnel (three times), with half volume to get two layers (aqueous and chloroform layers).
- 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.

Isolation Phenol's Contains by Column Chromatography (CC)

Harborne (1984) [15] method was used. The extract was isolated by column chromatography over Si-gel column and eluted with ethyl acetate: hexane (5:2) ratio [16].

Column Chromatography

Length of column: 150 cm

Diameter of column: 1.5 cm

Weight of the crude extract: 2 gm

Weight of Si-gel (60-120 Mesh): 100 gm

Two gram of the methanolic extract was dissolved in 50 ml of (5:2) ethyl acetate:

hexane and applied to the column. The column was eluted by the elution was described above as a mobile phase. The column was developed by adding 500mL of eluent with collecting 15 ml fractions, and then monitored by TLC. A total number of 27 fractions were obtained. Those consecutive fractions, which have the same number of spots with the same R_f values, were combined and concentrated to dryness to get major fraction.

Results and Discussion

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.

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, and 1114.6-1076.28 and 945.12-1257.59.

Isolation of Flavonoids Glycosides by Column Chromatography

A total number of 27 fractions were obtained. Those consecutive fractions, the fractions (5-10) collected were of same R_f values, for the same reason, the fractions (11-14) were collected. Therefore mixed together. Removal of the solvent by drying it in the air to giving a yellow and very light brown respectively, the weight compound of fraction (5-10) was 0.129 g.

Figure 2: Isolated compound

Identification the Isolated Compound by Analytical Methods

UV-Visible Spectra

The UV-Vis spectra for isolated compound with λmax at (315nm) and another peak at (275 nm) and (204) respectively.

The IR Spectrum

The important peaks observed in the IR spectrum of the isolated compound are recorded in Table 1.

Table 1: Characteristic FT-IR Absorption Band (cm-1) of the isolated compound

No.	Wave number cm^{-1}	Assignment
1.	3275.13	-OH group (s)
2.	2927.94	C=C-H(C-H stretching of aromatic ring)
3.	2854.65	$-OCH_3$ group
4.	1658.78	C=O
5.	1604.77	Aromatic ring system
6.	1211.30	C-O-C vibration
7.	833.25	C-H of aromatic group out of plane

Characterization of Isolated Compound by (^{1}H NMR) Analysis

The spectrum displayed by a series of 1D spectrum data, including ^{1}H NMR. The ^{1}H NMR showed characteristic shift value at (12.98) ppm, which arises from hydroxyl group proton at C-5. The signals of one methoxyl [δ H =3.8 (3H, s)]. The other clear signals due to ortho and Meta coupled A_2B_2-type, aromatic protons at δH =7.79 ppm and 7.45 ppm. (Each 2H, d) assigned to H-2′, 6′and H-3′, 5′ respectively. The H-8 and H-6 resonance appear at δH 6.8 and 6.9 ppm respectively, and they show meta-coupling.

The two protons occur as a doublet. In addition, the singlet proton at δH =6.83 (1H, s) was assigned to H-3. The ^{1}H NMR spectra showed the signals of protons of sugar moieties were appeared in the range 2.75 – 4.16 ppm. After detailed comparison of 1H NMR with those published in literature,[17] and [18], the compound was identified as acacetin glycosides.

Mass Spectrum

The Figure 3 shows the important fragmentation pattern obtained in the Mass/ Mass of the isolated compound were at [M-H]$^-$ 770, 607, 445, 283, 268, 240, 152, 149, 120, 96, 78. Which further confirmed it's a aglycone identity is acacetin (Figure 4), and the compound was acacetin tri hexosyl combined with the literature [19 and 20].

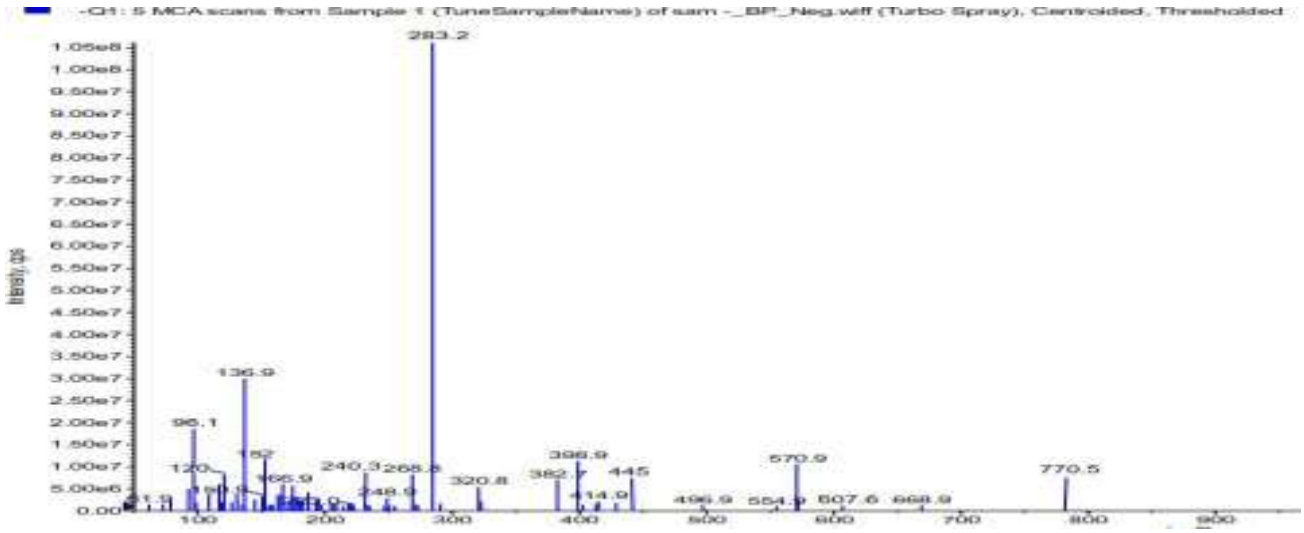

Figure 3: Mass spectra of isolated

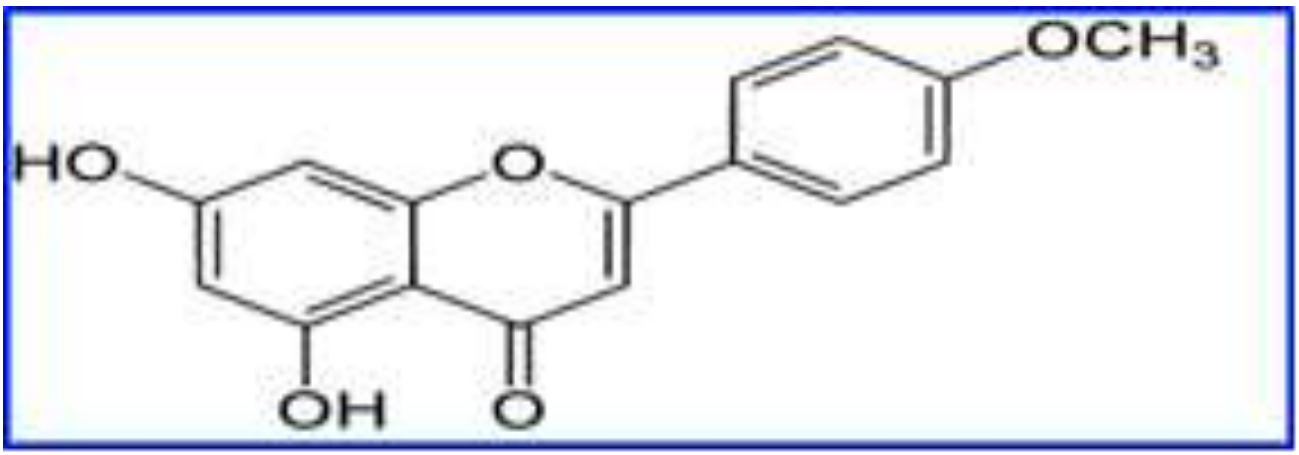

Figure 4: Acacetin compound

Antifungal Properties of Poly Phenolic Extracts

As shown in Table 2, the extracts of poly phenols have antifungal activity against (*A.flavus, A.fumigatus, and C.albicans).* On the other hand, poly phenols extract showed maximum growth activity inhibitory of fungi against pathogens like *A. flavus, A.fumigatus* , (25mm), (28mm) respectively, at concentration (100 % w/v) and the maximum growth activity inhibitory was against *C.albicans*, (18mm) at concentration (100% w/v), compare with control. The other concentrations (75%, 50%, 25% w/v) of the phenolic extract indicate that the range of inhibition increases with increasing

concentration of extract, where the range of inhibition was (22, 25, 15mm) at concentration 75%, (13, 20, 13 mm) at concentration 50% and (12, 17, 11mm) at concentration 25% to the fungus in this study.

Table 2: The growth inhibitory activities of fungi

Type of Fungi	Zone of inhibition in mm				
	Concentration of poly phenol extract(w/v)				
	25%	50%	75%	100%	H_2O
A.flavus	12	13	22	25	-
A.fumigatus	17	20	25	28	-
C.albicans	11	13	15	18	-

Conclusions

Compounds that are exist in extract identified by using UV-Visible and Fourier transforms infrared (FT-IR) spectrum appearance of several peaks indicating to the existing of phenolic compounds. After the extraction and isolated one compound from the extract, the compound identification using UV-Visible, Fourier transforms infrared (FT-IR), ^1H spectrum and Mass technique, which aglycone was acacetin bonding with three molecules of hexosyl sugars. 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.

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