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3,5,7,3',4'- PENTAHYDROXY FLAVONE, AN ANTIFUNGAL PHYTOCHEMICAL COMPOUND FROM *RUMEX CRISPUS* L.

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ABSTRACT :

Extraction, separation and bioassay of ethanolic extract of *Rumex crispus* L. was carried out. The most potent bioactive fraction was purified using column chromatography packed by Sephadex LH-20. The identification of that active fraction was achieved using UV and GC/MS as 3,5, 7,3', 4'-pentahydroxy flavone (Quercetin).

INTRODUCTION :

Genus Rumex is a commonly distributed herb in cultivated land, canal banks & desert in Egypt [1]. Plants belonging to the Polygonaceae are known to produce a large number of biologically important secondary products, such as flavonoid glucosides, anthraquinones [2&3] steroids, leucoanthocyanidins and phenolic acids [4].

The genus Rumex has attracted the attention of many investigators because of its medicinal properties [5&6]. Some species of this genus have been reported to contain C-glucosyl flavonoids and anthraquinones. For example, *R. acertosa* & *R.. cyprius* contain acetyl derivatives of iso-orientin and orientin [7&8]. Cyanidin -3-glucoside have been found in *R. crispus* and *R. thrysiforus* [9,10&11]. *R. chalepensis* have been reported to contain flavonoid glycosides and anthraquinones [12]. Many natural substances as chrysolphanol, emodin and quercetin-3-glucuronic acid were isolated from *Rumex crispus* L. [13&14].

In this plan it is intended to isolate the bioactive natural secondary products (biophytochemicals) from the plants native to Egypt. The present study aims to isolate the antidermatophytes agent from *Rumex crispus* L.

MATERIALS AND METHODS :

1-Plant material. Fresh shoot system of *R. crispus* L were collected from Shebin El-Qantar 1994. It was open dried at room temperature and then the powdered samples were packed in plastic bags until using.

2-Preparation of organic extract and fractions. Two hundred grams of dry shoot system of *R. crispus* L. were percolated with 70% ethanol (crude organic extract). The yield extract was partitioned using n-hexane, methylene chloride, ethyl acetate and n-butanol. Each fraction was evaporated under reduced pressure and kept under refrigeration until further antifungal investigation [15].

3-Fractionation. Active crude extracts were fractionated using column chromatography (CC) packed by silica gel. Elution was carried out at slow rate using methanol followed by increasing concentration of water. Each fraction (150 ml) was biologically investigated after evaporation of solvents under reduced pressure. Several consecutive fractions were carried out before reaching pure compound [16].

4-Isolation of Quercetin. The bioactive fraction was concentrated and chromatoghraphed on sephadex LH-20 column using ethanol with increasing the ratio of distilled water. Fractions desorbed from the column were separately collected through inspection under UV light. The solvent from each fraction was distilled off under reduced pressure. The material left was first paper chromatographically investigated. In most cases, fractions were refractionated using elution techniques, until pure components were obtained [17].

5-Antifungal assay. In vitro, the antifungal activity of plant extracts was tested against *Trichophyton ruburm*, *Microsporium gypseum* and *Chrysosporium tropicum*. The tested fungi were grown on sterilized potato-dextrose broth. The medium seeded with inoculum of each tested fungal strains and poured into sterile petri dishes (9 cm diameter) with 20 ml of potato-dextrose agar (PDA) per plate [18]. Different concentrations from crude aqueous and organic extracts of 1,2,3 & 4 mg/disk were used.

6-Acid hydrolysis. Complete acid hydrolysis was carried out for 40-50 minutes at 100°C using 2N hydrochloric acid. The hydrolysate was then extracted with ethyl acetate, and the extracts subjected to paper chromatographic investigation to detect the agylcones. The mother liquor was carefully neutralized, and then subjected to paper chromatographic studies to detect the sugars [19].

7-Spectral analysis. UV and MS spectroscopic investigation were measured on Schimadza spectrophotometeric model UV 240 and GC/MS (Finnigan mat SSQ 7000) system.

RESULTS AND DISCUSSION :

Antifungal activity of crude and partitioned organic extracts were examined against some fungal tested strains (*Trichophyton ruburm*, *Microsporium gypseum* and *Chrysosporium tropicum)* as dermatophytes fungi. The results indicated that crude organic extract (70% ethanol) showed the most potent antifungal activity than other extracts.

Chromatoghraphic separation (PC & CC) of the most potent biologically active fraction of aqueous ethanolic extract of air-dried shoot system of *Rumex crispus* L. afforded many sub-fractions. The antifungal activity of sub-fractions were again re-examined against the same tested fungal

species. The results indicated that there is one sub-fraction, which showed the strongest antifungal activity against the most tested fungal species.

The purification of bioactive fraction was carried out using sephadex LH-20 column chromatography using methanol: water by different ratio as eluent.

The pure fraction was again tested against *Trichophyton ruburm* and *Microsporium gypseum*. The result proved that pure fraction inhibited the tested fungal species.

The identification of pure antifungal fraction was achieved using acid hydrolysis with 2NHCL (5ml) for 1h. The aglycones was extracted with ethyl acetate (EtoAc), and through Co-chromatography with authentic sample of Quercetin and Kampferol the aglycones was identified as Quercetin (3,5, 7,3', 4'-pentahydroxy flavone).

The identification was confirmed using R_f values (Table 1) on paper chromatography using different elution system, UV spectral analysis (Table 2) and GC/MS analysis. GC/MS analysis was carried out by injection the pure bioactive fraction onto GC/MS [Finnigan MAT SSQ 7000 mass spectrometer coupled with a varian 3400 gas chromatography]. The injection temperature was 250 °C using DB-5 column (5% phenyl) methyl polysiloxane, 30 m,I.D. 25 mm, helium was the carrier gas, with programmable temperature 50 °, 3min; 50-300°, 5° /min; 300°, 15 min. The mass spectra were recorded in Elmode at 70 ev. The replication rate was 0.5 scan over a mass rang of 40-500 amu.

Peaks were identified by computer search of use-regenerated reference libraries and incorporation mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity; mixed peaks were resolved by computer program aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another.

By comparing the obtained UV data of the isolated bioactive compound with those reported in the literature of Quercetin they were identical [20]. So, the antifungal phytochemical compound of *Rumex crispus* L. was identified as Quercetin (3,5, 7,3', 4'-pentahydroxy flavone) (Figure 1).

Table (1): R_f values on paper chromatography of 3,5, 7,3',4'-pentahydroxy flavone isolated from *Rumex crispus* L.

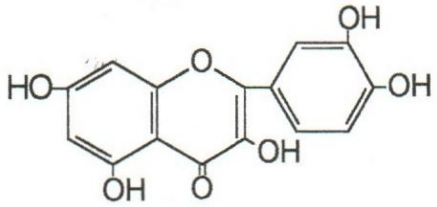
Elution system	R_f values
BAW	48
CAW	50
AcOH 50%	25
PhoH	30

BAW = n-butanol : acetic acid : water (4:1:5).
CAW = Chloroform: acetic acid : water (15:7.5:1).
AcOH 50% = Acetic acid : Water (1:1).
PhoH = Phenol : Water (4:1) (wt. Vol.)

Table (2): UV data of 3,5, 7,3',4'-pentahydroxy flavone.

MeOH	372, 305, 256
NaOMe	412, 327, 272
$AlCl_3$	444, 337, 270
$AlCl_3$+HCl	271, 262, 333_{sh}, 366_{sh}, 438,418
NaOAc	380, 324, 257
NaOAc+$H3BO_3$	260_{sh}, 262, 317_{sh}, 377, 382

Figure (1): Structural formula of 3,5, 7,3',4'-pentahydroxy flavone



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٣، ٥، ٧، ٣ ،٤'-بنتا هيدروكسى فلافون ، مركب كيميائى نباتى ضد فطرى من نبات روميكس كريسبس ال.

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يعتبر جنس الروميكس أحد أهم الأجناس النباتية التى تنمو فى عديد من المناطق الجغرافية المختلفة بالأراضى المصرية.

ولقد سُجل فى عديد من المراجع أن بعض الأنواع المختلفة لنبات الروميكس تتميز بوجود عديد من المركبات الكيميائية مثل : جلوكسيدات الفلافونيد ، الانثراكينونات والستروييد.....الخ. وفى الطب الشعبى المصرى (طب الأعشاب أو الطب النباتى) فإن عديداً من أنواع نبات الروميكس تستخدم فى علاج كثير من الأمراض المختلفة.

من أجل ذلك فإن هذه الدراسة تهدف إلى دراسة النشاط الضد فطرى للمستخلص العضوى لنبات الروميكس كريسبس ال ضد بعض الأنواع الفطرية التى تسبب بعض الأمراض الجلدية مثل : تريكوفيتون روبرم ، ميكروسبورم جيبسم ، كريرزوسبورم تروبيكم . ومن خلال التجزئة المتتابعة وعزل وتنقية الأجزاء المختلفة للمستخلص العضوى لهذا النبات ، تم عزل مركب كيميائى نباتى ذى نشاط ضد فطرى فعال وجيد.

وباستخدام طرائق التعريف الحديثة الطبيعية والكيميائية مثل التميؤ الحمضى ومطياف الأشعة فوق البنفسجية ومطياف كروماتوجرافيا الغاز/ الكتلة تم تعريف المركب الكيميائى النباتى الفعال على أنه ٣,٥,٧,٣ ،٤'-بنتا هيدروكسى فلافون.