Delta J.Sci 16 (1) 1992 , 198 - 211

ISOLATION AND IDENTIFICATION OF THE PHENOLICS FROM Ricinus communis L.

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ABSTRACT

The phenolic constituents of the leaves of Ricinus communis L. have been investigated by paper, thin layer and column chromatography. Spectral (IR and UV) analysis and high performance liquid chromatography (HPLC) were used in identifying the isolated components.

Nine compounds were characterized. Three of these compounds are recorded for the first time Viz isoquercetrin, 2,5-dihydroxy benzoic acid and (-)-epicatechin. The six known compounds are rutin, hepyroside, quercetin and chlorogenic, neochlorogenic and gallic acids. Hydrolysable and condensed tannins were also detected.

All the flavonoids isolated were of the flavonol-O-glycosides.

INTRODUCTION

Plants of the Euphorbiaceae are of considerable economic importance, and the products obtained from this family

include oils, various alkaloids and flavonoids (Rizk, 1968). Castor bean (Ricinus communis L.) is naturalized and is cultivated in Egypt for its medicinal importance.

The leaf of Ricinus communis L. contains rutin (Rizk, 1986). Moreover, the presence of quercetin and hyperoside in the leaf has been reported (Khafagy et al., 1986). Chlorogenic acid, neochlorogenic acid, gallic acid and an unidentified derivative of herbacetine were also isolated from the plant (Rizk, 1986).

Phytochemists and chemotaxonomists are attracted by the importance of secondary plant products of which phenolics are the most important. In a continuation of the phytochemical studies of Ricinus communis L, the isolation and identification of some phenolics from the plant leaves are reported.

MATERIALS AND METHODS

Plant material and germination

Castor bean seeds were germinated in ordinary garden soil,* in growth pots (Mayer and Shain, 1974). The pots were taken to a thermostatically controlled growth chamber

*CO_3=Zero, HCO_3= 0.7625 mwq/l, Total organic matter=9.92%, oxidizable organic matter= 2.250%, Bluk density= 1.074 gm/cm ^{3}, Porosity = 49% and Soil texture=% of sand=73%, silt=16%, clay=11%.

adjusted to 25 ± 2°C. After complete germination (15 days) the grean leaves were obtained.

Fxtraction and isolation of phenolic compounds

The leaves were dried then powdered and defatted with ethyl acetate, extracted with methanol and concentrated. Hot distilled water was added to the extract and after cooling the aqueous/methanolic solution was filtered (Thieme and Khogali, 1975).

Flavonoids

Half the aqueous/methanolic extracts was concentrated and applied to silica gel column.

Phenolic acids, free aglycones and (-)-epicatechin

The other half was evaporated then subjected to continuous ether extraction. It was then shaked several times with water. The etherial layer contained the free aglycone.

Tannins

Tannins were detected in the aqueous extract of the leaves as described by Shmidt et al. (1955).

Standard methods of purification and identification of all compounds were followed (Mabry et al., 1970, Harborne, 1967, Markham, 1982) as well as by comparison with authentic samples. Also cochromatography with pure samples was employed. Acid hydrolysis was carried out using 2% H_2SO_4 for 120 min.

Delta J.Sci 16 (1) 1992

Isolation and Identification

Paper chromatography (Pc)

Filter paper : Whatman No. 1 and 3

Developing solvent systems:

Flavonoids (Harborne, 1984)

BAW : n-Butanol-acetic acid-Water (4:1:5)

HOAC-H_2O : Acetic acid-water (15%)

PhOH-H_2O : Phenol-water (3:1)

Spray reagent : Zirconiumoxychloride ($ZrOCl_2$) 2% in methanol

Sugar moities of flavonoids (Lewis and Smith, 1967)

BAW and

BPW : n-Butanol-pyridine-water (6:4:3)

Sprav reagent : Anilin hydrogen phthalate.

Aglycone moieties

BAW and HOAC-H_2O.

Spray reagent: ($ZrOCl_2$)

Phenolic acids (Harborne, 1973)

BAW, HOAc-H_2O and

BEW :n-Butanol-ethanol-water (4:1:2.2).

Spray reagent: Ferric chloride ($FeCl_3$) 5% in methanol

Thin layer chromatography (TLC)

Adsorbent : Silica gel plates (20x20)

Developing solvent systems:

Aglycones

BPF : Benzene-pyridine-formic acid (36:9:6)

EtOAC-MeOH-H_2O : Ethyl acetate-Methanol-water (30:5:2)

Spray reagent : $ZrOCl_2$ and $FeCl_3$

Delta J.Sci 16 (1) 1992
Khogali et al.,

Phenolic acids

C-EtOAc-f : Chloroform-ethyl acetate-formic acid (5:4:1)
HOAC-C : Acetic acid-chloroform (1:9)
EtOAC-B : Ethyl acetate-benzene (9:11)
TCA : Toluene-chloróform-acetone (40:25:35)
Spray reagent : $FeCl_3$ (5%)

The ultra-violet (UV) and infra-red (IR) spectroscopic analyses were recorded by Perkin-Elmer lambda 48/Vis and Perkin-Elmer, recording IR spectrophotometer model 1430 respectively. For High performance liquid chromatography (HPLC) Perkin Elmer series 38 liquid chromatograph was used.

RESULTS AND DISCUSSION

Extraction of the dried leaves of Ricinus communis revealed eight phenolic compounds: four flavonoids, three phenolic acids and (-)-epicatechin. However, one of the flavonoids; isoquercetin and one of the phenolic acids; 2,5-dihydroxy benzoic acid, as well as (-)-epicatechin were detected for the first time.

Neochlorogenic acid which has previously been determined in Ricinus communis (Rizk, 1986) could not be detected under the prevailing experimental conditions.

The chromatographic data of the isolated compounds in different solvent systems are given in Table 1.

Delta J.Sci 16 (1) 1992
Isolation and Identification

UV Spectra were measured after the addition of the following reagents separately to the methanolic solution of the flavonoids: aluminium chloride ($AlCl_3$), alumininum chloride/hydrochloric acid ($AlCl_3$/HCl), sodium acetate (NaOAc), sodium acetate/boric acid ($NaOAc/H_3BO_3$). The absorption maxima are shown in Table 2.

Isoquercetin (quercetin, 3-glucoside) was identified by its chromatographic and spectral analysis Chromatographic investigation of the separated aglycone showed that it possesses the same R_f value and UV-spectral analysis as quercetin. It was also undepressed upon admixture with authentic quercetin. The R_f values of its sugar moiety were identical with glucose (Table 1).

The infra-red spectrum of 2,5-dihydroxybenzoic acid revealed the presence of a phenyl group in the range 723-756 cm^{-1}), hydroxyl groups at 3121 cm^{-1} and carbonyl group at 1752 cm^{-1}. The IR-spectral analysis of the identified phenolic acids are shown in Figs. 1-3.

(-)-Epicatechin is detected for the first time in R. communis. It was characterized by its chromatographic data and IR-spectral analysis (Tab. 1 and Fig. 4).

Flavonoids, phenolic acids and (-)-epicatechin were also identified by HPLC, using pure standard for qualitative comparison (Figs. 5 and 6).

Both hydrolysable and condensed tannins were detected in the leaves of R. communis. The presence of the two groups of tannins has not been reported previously.

The data obtained, so far, show that all the glycosidic patterns are flavonol-O-glycosides of quercetin. The different phenolic patterns are related to their occurrence to the family Euphorbiaceae.

Table 1. R_f values of the phenolic compounds isolated from the leaves of R. communis.

Compounds	Pc				R_f (x100) in TLC				
	BAW	HOAc-H_2O	PhOH	BEW	BPW	BPF	EtOAc-MeOH-H_2O	C-EthCAc-F	HOAc-C	EtOAc-B	TCA
Flavonoids											
Quercetin (Q)	76	84				16	87				
Q-3-glucoside	71	39	54				63				
Q-3-galactoside	70	37	56				65				
Q-3-rutinoside	57	60	45				35				
Phenolic acids											
2,5-dihydroxybenzoic acid	77	73		50					33	45	
3,4,5-trihydroxybenzoic acid	62	50						32	70	39	
chlorogenic acid	73	65		45				09			15
(-)-Epicatechin	65	55	59								
Sugar moieties											
glucose	18				50						
galactose	16				44						
rhamnose	30				70						

Table 2. UV-spectrum of the flavonoids in Ricinus communis leaves!

Flavonoids	Spectral maxima max (nm) MeOH		NaOMe		$AlCl_3$		$AlCl_3$/HCl		NaOAc		NaOAc/H_3BO_3	
Quercetin (Q)	373	256	323	220	455	223,270	423	225,270	405	227,276	402	227,262
Q-3-glucoside	368	232	430	227,279	353,405	229	343,407	227 227	403	229,252	398	229,261
Q-3-galactoside	360	257	410	270	440	275	430	270	380	270	380	260
Q-3-rutinoside	348,370	249,258	322,413	226,272	366,402	225,266	355,404	225,267	403	226,273	393	227,263

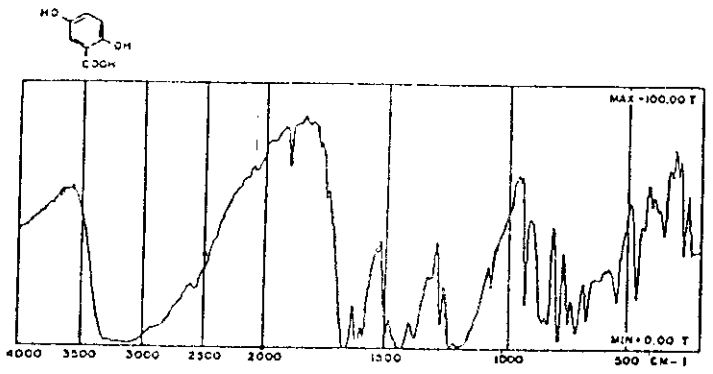

Fig.1 IR spectrum of 2,5 dihydroxy benzoic acid isolated from R.communis leaves

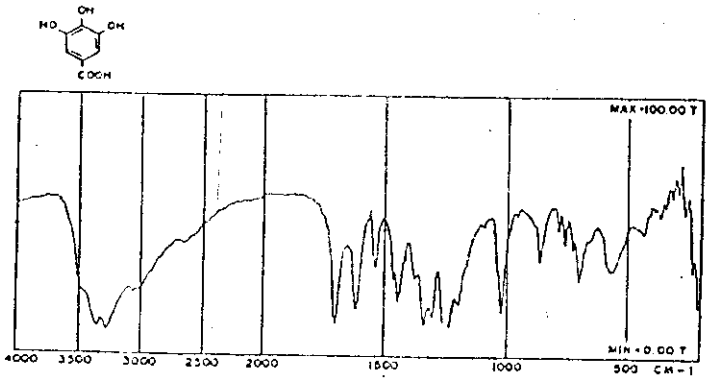

Fig.2 IR spectrum of gallic acid isolated from R. communis leaves

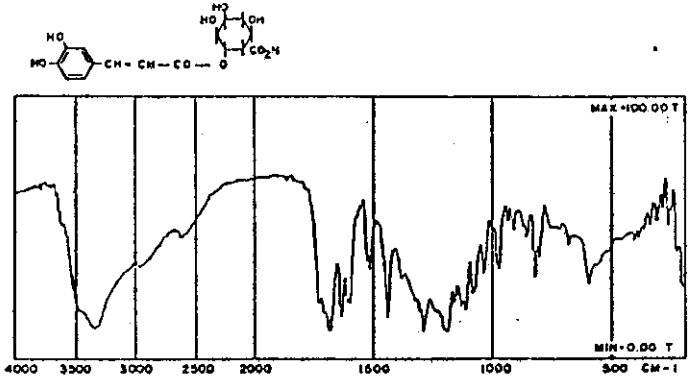

Fig.3 IR spectrum of chlorogenic acid isolated from R. communis leaves

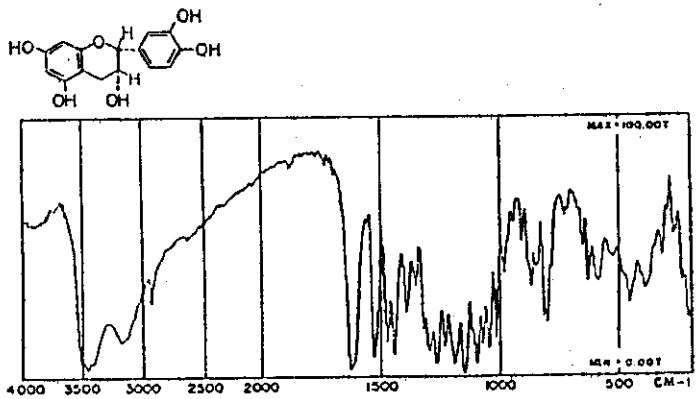

Fig.4 IR spectrum of (-)-epicatechin isolated from R. communis leaves

Delta J. Sci 16 (1) 1992

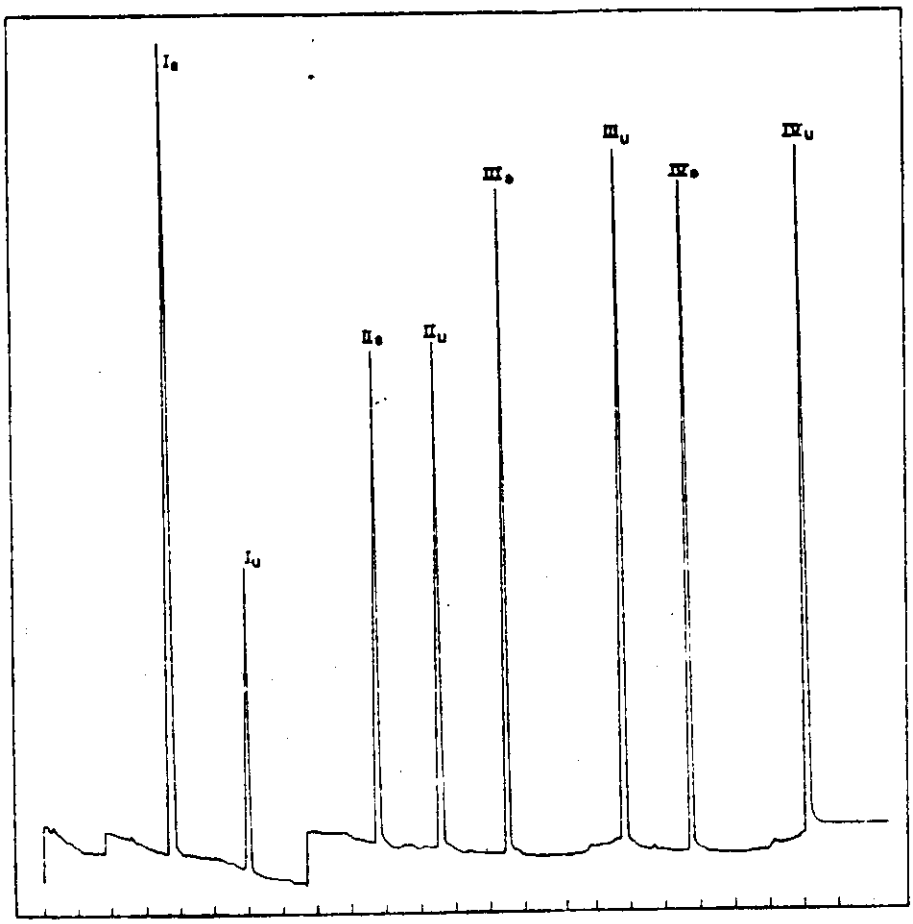

Fig. 5: HPLC – analysis of Flavonols isolated from R. communis leaves
I = Rutin ; II = Quercetin ; III = Isoquercetrin ; IV = Hyperoside
S = Standrd ; U = Unknown
Retention time t_R = 6 min. , Perkin Elmer series 3B liquid chromatograph,
Column analytical HC – ODS , Solvent 1% acetic acid : methanol (9 : 1),
rate of flow 2ml / min. ; temp. = 24 C , detection : UV detector .

Delta J. Sci 16 (1) 1992

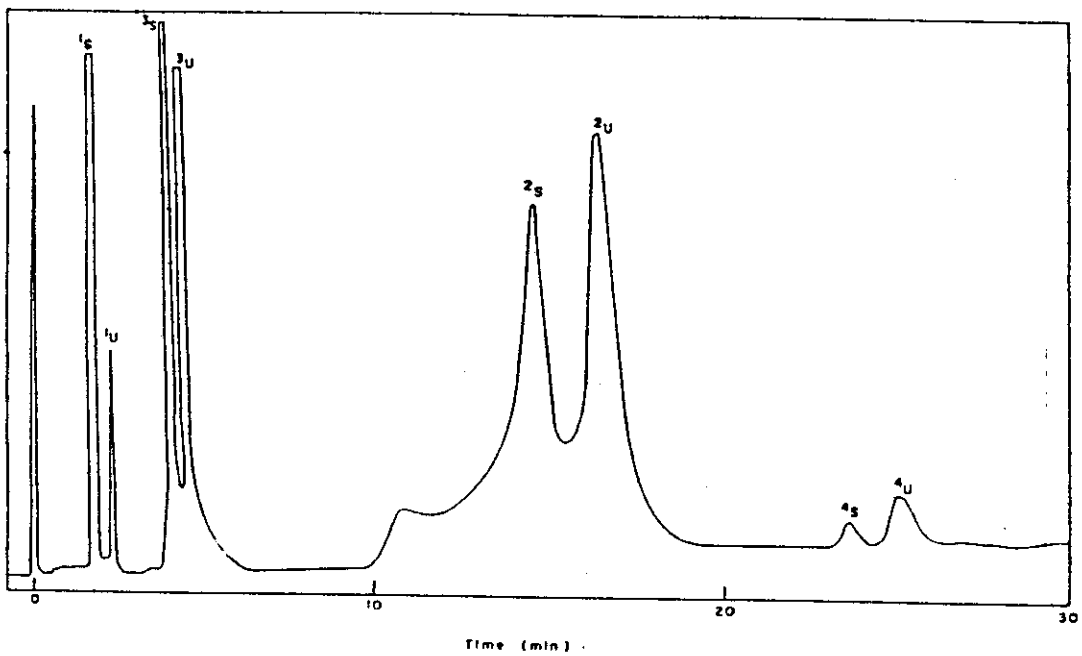

Fig.6 HPLC - analysis of phenolic acids and -(-) epicatechine isolated from R. communis leaves

S = Standard ; U = Unknown

t_R = Retention time (min) , Column analytical HC - ODS , Solvent 1 % acetic acid : methanol (9 : 1) , rate of flow 2 ml/ min ; temp = 24 °C , detection : UV detector

Compound	t_R	
	S	U
1- Gallic acid	2 16	2 18
2- 2,5 - dihydroxy benzoic acid	15 89	16 16
3- Chlorogenic acid	4 26	4 28
4- -(-) Epicatechine	24 51	25 60

Delta J.Sci 16 (1) 1992

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Delta J.Sci 16 (1) 1992

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Delta J.Sci 16 (1) 1992

استخلاص وتعريف المواد الفينولية من نبات الخروع R.Communis

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يتضمن البحث فصل وتعريف المواد الفينولية الموجودة فى اوراق نبات الخروع Ricinus communis بعد انبات البذور فى درجة حرارة ٢٥ م. وقد استخدم فى فصل وتعريف هذه المركبات طرق كروماتوجرافيا الورق والطبقة الرقيقة واعمدة الفصل بجانب وسائل الامتصاص الطيفى للأشعة تحت الحمراء وفوق البنفسجية او طريقة الكروماتوجرافى تحت الضغط العالى HPLC . وقد اوضحت هذه الدراسة ان اوراق نبات الخروع تحتوى على عدد من المواد الفينولية والتى قسمت حسب تركيبها الكيميائى الى : مواد فلافونية : (Hyperoside,isoquercetin* quercetin, rutin) واحماض فينولية (الجاليك ، الكلوروجينك ،*ا٢ه ثنائى هيدروكسى بنزويك) والمركب ابيكاتشين (*epicatechin) الذى يعتبر المادة الاولية لتكوين التانينات. ثلاثه من هذه المركبات تعرف لاول مرة فى بادرات الخروع وهى التى تحمل العلامة * . وكذلك امكن الاستدلال على وجود مجموعة التانينات المكثفة والمجموعة القابلة للتحليل.

وقد بينت الدراسة ان الكورستين يوجد فى صورة حرة والجليكوسيدات المتصلة به تنتمى الى مجموعة فلافونول -O - جليكوسيدز.