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CHEMICAL CONSTITUENTS AND PRELIMINARY ANTHELMINTIC ACTIVITY OF FICUS PLATYPHYLLA(DEL)

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ABSTRACT

A phytochemical study of Ficus platyphylla Del cultivated in Egypt is presented. A 7-methoxy coumarin (herniarin), two unidentified coumarins, α-and β-amyrin, ceryl alcohol, β-sitosterol, ursolic acid; in addition, chrysoeriol, rutin and unknown flavonoid aglycone were isolated. The organic acids content of the fruits was identified. The amino acids as well as the curdling effect and proteolytic activity of the latex were investigated. The anthelmintic activity of the 40 % ethanolic extract of the fruits was studied in vivo. A pronounced decrease in number of Ascaris worms in relation to the injested embryonated eggs was noticed in treated Fayomi chickens. This effect increases markedly with the increase of the given dose.

INTRODUCTION

Ficus platyphylla Del, broad-leaf fig (Moraceae) is a tree indigenous to South Africa and cultivated for its shade in public and private gardens[1]. Different species of *Ficus* have been used in folkloric medicine, for treatment of leprosy ulcers, scrofula,

chest conditions and cough[2,3]. They are also used as astringent, antidysentric and increases the flow of milk in cows[2]. The latex of various species has been used laxative , emollient, diuretic, anthelmintic and in treatment of warts[2]. The dried latex is used as chewing gum in South Africa[4]. The authors noticed that, locally, the native use the freshly cut fruits for treatment of buccal cavity ulcers and several skin diseases.

The available literature reported the presence of β-sitosterol, stigmasterol, taraxasterol, hentriacontane, tiglic acid ester and lupeol in the fruits of *Ficus glomerata*[5]. Alkaloids[6], as well as bergapten, psoralen, β-amyrin and β-sitosterol were also isolated from *F. hispida*[7], bergapten, bergaptol, β-amyrin and β-sitosterol from *F. aspirma* leaves[7], xanthotoxin, xanthotoxol and marmesin from *F. carica* leaves[7] and herniarin from *F. platyphylla*[8].

Accordingly, it was found of interest to study the chemical constituents of the different organs of *F. platyphylla* and to screen the anthelmintic action of the fresh fruits.

EXPERIMENTAL

1- Study of Chemical Constituents

Melting points were detected with Kofler hot stage apparatus and were uncorrected. UV spectra were recorded in methanol using Unicam SP 1750 UV spectrophotometer and Pye-Unicam AR 55 linear recorders. IR spectra were determined in K Br discs using Perkin-Elmer 720 spectrophotometer. ^{1}H-NMR spectrum was in $CDCL_3$ at 60 MHz. Using Varian EM 360 A, chemical shifts are given in ppm. Mass spectrum was measured at 70 ev using MS-50, Kratos A.E.I. spectrophotometer.

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^{13}C-NMR was in $CDCl_3$ using Varian CFT-20 spectrophotometer. Silica gel G (Merck) and cellulose (Merck) for TLC, aluminum oxide (Merck) and Whatmann No. 3 for PC.

Plant Material:

Collection was made from trees cultivated in the University campus and identified by Dr. N.El-Keltawi, Associate Professor of Floriculture, Faculty of Agriculture, Assiut University. Leaves and stem bark were collected during March 1983, dried and powdered.

Solvent Systems:

System	I :	chloroform-methanol	(95:5)
System	II :	chloroform-glacial acetic acid-water	(50:45:5)
System	III:	chloroform-methanol-water	(30:15:2)
System	IV :	n-butanol-formic acid-water	(4: 1:5)
System	V :	n-butanol-glacial acetic acid-water	(4: 1:1)

Extraction and Separation :

1- Leaves and Stems:

One Kg. of the dried powdered leaves and stem bark was separately extracted to exhaustion with ethanol 70 %. The concentrated ethanolic extracts were fractionated successively with pet. ether, chloroform and ethyl acetate.

A- Petroleum ether Fraction :

Crystalline precipitates were obtained from pet.ether concentrates of both leaves and stem bark, when left at room temperature for 24 hours. The crystalline residues were filtered, washed with pet.ether, dissolved in chloroform and examined by TLC using syst.I.It revealed the same 3 fluorescing spots (under UV) in each extract. The corresponding components (designated 1,2 & 3) were separated and purified by preparative TLC and recrystallised

from methanol as white plates. Their physical and chromatographic characters are given in Table 1 .

The remainder of pet.ether fractions after filtration was concentrated (30 & 10 g.) and examined by TLC using system I. A similar picture was obtained. The mixed concentrate was chromatographed over alumina column (1.2 Kg., 150 x 8 cm) using solvents : pet.ether then pet.ether-ethyl acetate mixtures in increasing polarities. Fractions (1/2 liter each) were collected and examined by TLC (system I). Five compounds (labelled 4-8) were isolated and identified. (Table 1).

Compound I :

mp. 118-20°, $C_{10}H_8O_3$, M.S. m/z (rel. int. %), M^{+1} 177 (11) , 176 (100), 148 (55) (M-CO), 133 (63), 105 (4), 91 (5), 89 (7), 77 (16), 69(5), 63 (10), 51 (61). It showed dark orange colour with Dragendorff's reagent and pink fluorescence under UV light, UV λ_{Max}^{MeOH} (nm) : 240 (sh), 250,322,not affected by addition of alkali, IR (cm^{-1}) : 3065, 3030, 2920, 2820, 1710 (coumarin C=O), 1600 (aromatic) 1550 (α-pyrone double bond), 1460, 1430, 1230,910. ^{1}H-NMR ($CDCl_3$) ppm : 6.22 (1 H,d, H-3) (J=9.5 Hz), 7.65 (1H,d, H-4) (J=9.5 Hz), 7.35 (1H,d,H-5) (J=9 Hz), 6.82 (1 H,dd, H-6) (J_{6-5}=9,J_{6-8}=2 Hz), 6.78 (1 H,S, H-8), 3.85 (3H,S, OCH_3). ^{13}C-NMR ($CDCl_3$) ppm: C-2 (160.99), C-3 (112.96), C-4 (143.23), C-4a (112,41), C-5 (128.7), C-6 (113,25), C-7 (162.76), C-8 (100.75) C-8a (155,79) and at 55.65 ppm (OCH_3).

Compound 2 :

m.p. 148-50°C, it has blue fluorescence in UV light, UV λ_{max}^{MeOH} (nm) : 254 (sh) , 295, 346, not changed by addition of alkali. IR cm^{-1} : 2960, 2860, 1715 (coumarin C=O), 1650 (aromatic), 1550 (α-pyrone double bond), 1460, 1380, 1250. Lack of material prevented more analysis.

Compound 3 :

m.p 87-90°C, it showed bluish-violet fluorescence in UV light. UV λ_{max}^{MeOH} (nm) : 254 (sh), 280 (sh), 326 not affected by addition of alkali. IR (cm^{-1}) : 2960, 2880, 1725 (coumarin C=O), 1665 (aromatic), 1620, 1560 (α-pyrone double hond), 1460 , 1380, 1250,

B-Chloroform Fraction :

Chloroform concentrate from the leaves was chromatographed over cellulose plates, using system II. It revealed one flavonoid aglycone, with R_f 0.4 (compound 9) which was isolated by preparative TLC. Another flavonoidal aglycone (R_f 0.86, syst. II) was isolated from the stem bark by the same method, (compound 10).

Compound 9 :

UV λ_{max}^{MeOH} : 258, 270 (sh), 340 (nm); + NaOMe : 256, 270 (sh), 342,380 (nm); + $AlCl_3$: 230 (sh), 255 (sh), 340, 380 (sh) nm;+$AlCl_3$/HCl : 246 (sh), 255 (sh), 340 (nm);+NaOAc: 261, 309, 320 (sh) 376 (nm); + NaOAc/H_3BO_3=256, 314 (sh) 340 (nm).

Compound 10 :

m.p. 329-32°, UV λ_{max}^{MeOH} : 248, 269 (sh) 346 (nm); + NaOMe : 262, 269, 275,405 (nm);+ $Alcl_3$: 250, 272 (sh), 405 (nm); + $AlCl_3$/HCl: 255, 405 (nm);+ NaOAc: 297, 321(sh), 359 (nm);+ NaOAc/H_3BO_3: 254, 269, 346 (nm).

C- Ethyl Acetate Fraction :

The ethyl acetate concentrate of the leaves was chromatographed over cellulose for TLC using syst. III. Three flavonoidal spots were detected. the major was isolated by preparative TLC and PC (compound 11) Chromatographic examination of the ethyl acetate fraction of the stem bark by the same methods, revealed the presence of faint flavonoidal components.

Compound 11 :

m.p. 188-90°C, UV λ_{max}^{MeoH}: 360, 300 (sh), 260 (nm); + NaOMe : 272, 327, 412 (nm); + $AlCl_3$: 270, 300 (sh), 440 (nm); + $AlCl_3$/HCl : 271, 300, 360 (sh) 410 (nm); + NaOAc : 268, 320, 385 (nm); + NaOAc/H_3BO_3: 268, 292, 385 (nm). Acid hydrolysis : quercetin, glucose and rhamnose.

2- Fruits :

Fresh fruits, 100 g. were exhaustively extracted with ethyl alcohol 70% the extract was concentrated under reduced pressure. Investigation of the organic acid content was done using PC (Whatmann No.3, syst. IV) and sprayed with bromothymol blue[9].

The latex was prepared by squeezing fresh unripe fruits and collecting the exuded white latex. Amimo acids content of the latex was studied by PC (Whatmann No.3 syst. V) and sprayed with ninhydrin reagent[9].

Curdling effect:

Two samples, each 5 ml, of boiled and unboiled milk were treated separately with 0.5 ml freshly collected latex at 37°C. Curdling occurred immediately in the case of boiled milk. Fresh milk showed a delayed and week action

Proteolytic Activity :

The albumin of an egg was mixed with 1 ml freshly collected latex, diluted with equal volume of distilled water and left at 37°C with frequent shaking. Samples were withdrawn every 10 minutes and monitored by PC (syst. V , ninhydrin reagent)[9].

An increase in the number of spots by prolonged time of reaction was noticed.

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B- Study of anthelmintic Activity of the Fruit Extract:

Preparation of Fruit Extract:

Half Kg. of the fresh fruits was cut into small pilces extracted to exhaustion by ethanol (40%) and the mixed extracts were dried under reduced pressure (30 g.) .

Preparation of the Embryonated Eggs of Ascaridia Galli:

Ascaris worms were locally isolated, minced to make eggs free and left at 30°C in enough humidity to allow embryonation. Embryonated eggs were counted by the aid of dissecting microscope. These eggs were infested to 25 Fayomi chickens (60-days old). The chickens were previously checked for beig free from Ascaris infection. Each bird received 1000 embryonated eggs per oss. The chickens were divided into five groups. The first was considered as a control. After 40 days of infection, the other groups were given orally, 2,4,6 and 8 g. of the powdered fruit extract previously emulsified in an equal amount of water, respectively. The birds were slaughtered after two days treatment and eviscerated. The intestine was opened and the Ascaris worms were counted. Results are recorded in Table 2 .

RESULTS AND DISCUSSION

The air-dried powdered leaves and stem bark of *Ficus platyphylla*(Del.) were extracted with ethanol 70% and the concentrated extracts were successively fractionated with pet.ether, chloroform and ethyl acetate. Each fraction was subjected for TLC examination.

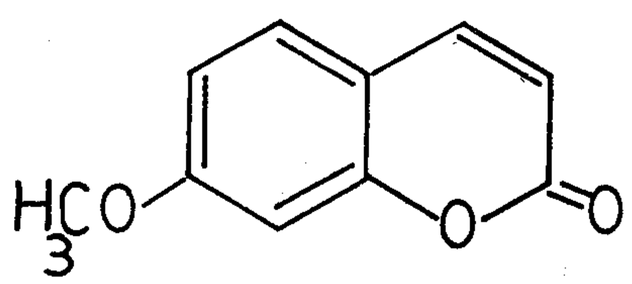
The pet.ether fractions of the leaves and stem bark gave crystalline precipitates, which upon TLC examinstion showed three, similar UV fluorescing spots in each case. The major of

them is Dragendorff's-positive (orange colour). The corresponding compounds were separated and purified by preparative TLC and subsequent crystallisation.

The major compound (I),m.p 118-120°C, $C_{10}H_8O_3$ (M^+176), gave negative reaction with $FeCl_3$ and exhibited UV absorption maxima at 240 (sh), 250, 322 (nm) and showed no shifts with alkali. This suggested a non-phenolic 7-O-substituted coumarin[10,11]. Such assumption is supported by: the ^{1}H-NMR spectrum in $CDCl_3$, lack of any hydroxyl absorption in the IR and a carbonyl absorption of a coumarin at 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) showed the characteristic coumarin doublets at 6.22 (H-3) and at 7.65 ppm (H-4). These are clearly seen in the ^{13}C-NMR at 112.96, 143.23 ppm respectively[8].

A pair of doublets at 7.35 and 6.82 ppm in ^{1}H-NMR are attributable to H-5 and H-6 protons[12,13], this is further supported by the appearance of signals at 128.7 and 113.25 ppm in ^{13}C-NMR respectively. C-8 showed signal at 100.75 ppm and a proton at 6.78 ppm in ^{1}H-NMR. Three protons appeared as a singlet at 3.85 ppm in ^{1}H-NMR and a peak at 2820 cm^{-1} in IR, suggest the presence of a mothoxy group. This is verified by a signal at 55.65 ppm in the ^{13}C-NMR[8].

In comparison with the published data of herniarin[8,14] it could be concluded that (1) is 7-methoxy coumarin (herniarin)



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Compound (2), m.p. 148-50°C , UV absorption maxima at 254(sh), 295, 346(nm) (not affected by alkali) characteristic of a 7-O-substituted coumarin chromophore[10,11]. The presence of of coumarin C=O at 1715 cm^{-1} was indicated from IR. It did not produce any colouration with $FeCl_3$ indicating the absence of phenolic OH function (C.f.IR spectrum and UV shifts).

Compound (3), m.p. 87-90°C, UV absorption maxima at 254 (sh) 280 (sh), 326 (nm), (not affected by alkali) and gave negative reaction with $FeCl_3$. This suggested a non-phenolic 7-O-subistituted coumarin[10,11]. This is verificd by the appearance of coumarin C=O at 1725 cm^{-1} and lack of OH absorption in IR.

These components (2,3) are suspected to be of coumarin type. Lack of material prevented complete analysis, further isolation and identification of them are in progress.

The mother liquor of petroleum ether fraction was subjected to column chromatography where α- and β- amyrin, ceryl alcohol, β-sitosterol, and ursolic acid (compounds 4-8 respectively) were isolated and identified by direct comparison, m.p., m.m.p., acetate m.p., Co-chromatography and IR with authentic samples.

The chloroform fraction of the leaves afforded, after preparative TLC, an unknown flavonoid aglycone (still under investigation) while that of the stem bark showed the presence of chrysoeriol (identified by direct comparison with authentic sample and comparison of data[15].

Concerning the ethyl acetate fraction, the leaves proved to contain three flavonoidal components, one of them was isolated in a sufficient quantity and identified as rutin (by comparison with authentic sample). Meanwhile, two flavonoidal

spots were noticed in the stem bark ethyl acetate fraction, but the isolated quantity prevented informative study.

The fruits studied for its organic acids content. The ethanolic extract was chromatographed over Whatman No. 3 PC, where tartaric, ascorbic, citric, malic, kojic, maleic and malonic acids were detected.

The prepared latex of the fruit was white in colour, viscous and nearly tasteless. Its amino acids content was studied and found to be aspartic, hydroxy proline, tryptophane, tyrosine arginine, lysine, alanine, histidine and two unidentified acids.

Pilot study of its curdling function showed that boiled milk is readly curdled than the fresh. The proteolytic activity of the latex is investigated by treating a sample of egg albumen at 37^{o}C. The reaction was accompanied by a subsequent chromatographic control of withdrawn samples at 10 minuted intervals. The number of the components of each monitored sample increases positively by the reaction time giving an indication that the latex has a proteolytic activity. On the other hand, the current literature[2] mentioned that the latex of some *Ficus* species exerts an anthelmintic action which may be due to the presence of a proteolytic enzyme.

Concurrently, the hydroalcoholic extract of the fresh fruits with its latex was tried in this investigation to test for an anthelmintic activity. The results in Table 2 showed clearly that the powdered fruit extract has an anthelmintic action which increases markedly by increase of dose. This may be explained by the effect of a proteolytic enzyme activity of the drug on Ascaris worm itself .

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Table 1 : Characters of Compounds Isolated from Pet. ether Fractions from the Leaves and Stem Bark.

Comp. No.	R_f* Syst I	Amounts isolated im mg .	Colour with H_2SO_4	Colour in UV	M.P.°	acetate M.P.°	Identification
1	0.89	230	---	Pink	118-20	----	Herniarin
2	0.76	4	---	Blue	148-50	----	Unknown
3	0.68	7	---	B.V.	87-90	----	Unknown
4	0.88	70	R.B.	----	198-200	201-203	β-amyrin
5	0.71	95	R.B.	----	184-86	225-27	α-amyrin
6	0.52	10	R.B.	----	80-82	66-8	Ceryl alcohol
7	0.47	180	Violet	----	135-37	125-27	β-sitosterol
8	0.32	25	Blue	----	277-79	----	Ursolic acid

B.V. = bluish-violet, R.B. = reddish-brown

* R_f = on silica gel G plates.

Table 2 : Anthelmintic Activity of Fruit Extract of *Ficus platyphylla* in Fayomi Chickens.

Groups (n=5)	Number of Ascaris Worms			
	Control	Treated Chickens		
	Chickens	2 g.	4 g.	6 g.	8 g.
1	350	144	25	21	8
2	270	110	22	26	11
3	260	134	21	19	13
4	220	152	28	23	6
5	300	160	24	41	22
mean	280	140	24	22	12

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دراسة المكونات الكيميائية لنبات الفيكس بلاتيفيلا (لينيه المنزرع فى مصر وتأثيره كمضادات للديدان

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نبات الفيكس بلاتيفلالينه يزرع فى الحدائق العامة والطرق للظل وهو عبارة عن اشجار ذات اوراق عريضة .

وقد ذكر فى المراجع ان انواع كثيرة من جنس الفيكس لها عدة استعمالات طبية شعبية وكذلك لاحظ الباحثون استعمال العامة للثمار فى علاج بعض القرح الجلدية

وبدراسة نتائج المسح الكيميائى لخلاصات الاجزاء المختلفة للنبات المنزرع فى منطقة الجامعة باسيوط تبين انها تحتوى على عدة مركبات تم فصلها والتعرف عليها بدراسة خواصها الفيزيائية والكيميائية والطيفية (الرنين النووى المغناطيسى ومطياف الكتلة والرنين النووى المغناطيسى للكربون ١٣ لاحد المركبات المفصولة بالاضافة الى الاشعة فوق البنفسجية وتحت الحمراء لبقية المركبات) .

فمن الاوراق وقلف السيقان تم فصل الهرنيارين وهو عبارة عن ٧ ـ ميثوكسى كومارين كذلك مركبين آخرين من مجموعة الكومارين مازلا تحت الدراسة بالاضافة الى بيتا اميرين ، الفا اميرين ، كحول سيريلى ، بيتا ستيوستيرول وحمض الاورسليك ، كريزو ريول ، وفلافونويد حر لم يتم التعرف عليه وجارى استكمال دراسته .

ومن ناحية اخرى تم التعرف على بعض الاحماض العضوية الموجودة فى الثمار وتم تحضير المادة اللبنية التى تفرزها الثمار غير الناضجة وعمل دراسة اولية للاحماض الامينية بها .

وبالرجوع الى المراجع وجد ان اصناف نباتات الفيكس لها تأثير طارد للديدان يعزى الى وجود نشاط انزيمى على البروتينات مما شجع الباحثون على دراسة هذا النشاط فى اللبن المحضر كذلك تأثير الخلاصة الهيدروكحولية للثمار على ديدان الاسكارس فى الكتاكيت الفيومى ومن نتائج هذه الدراسة يتضح ان هناك تأثير قوى على الديدان يزداد بازدياد الجرعة المعطاة من الخلاصة .

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