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Research Article

ISOLATION OF FLAVONOIDS AND BIOLOGICAL ACTIVITIES OF *CORONOPUS SQUAMATUS*

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ABSTRACT

The flavonoids of *Coronopus squamatus* (family Crucifereae) were isolated from the alcoholic extract by partition with ethyl acetate and butanol repectively. Six flavonoids were identified, three of them are from the ethyl acetate fraction which identified as 7-methoxy luteolin, 4',5,8-trihydroxy-6-methoxyflavone-7-*O*-rhamnoside and apigenin -7-*O*-arabinoside. the other three vis. apigenin 7-*O*-rhamno-glucoside, Acacetin-7-*O*-rhamnoside and chrysoeriol -6- *O*-rhamnoside from butanol fraction. All compounds were identified using different chromatographic and spectroscopic techniques. The hepatoprotection and antioxidant activities of different extracts and isolated compounds were measured using primary culture of rat hepatocytes monolayer and DPPH scavenging activity with Trolox equivalent antioxidant capacity (TEAC). The results of hepatoprotection activity proved that, the ethyl acetate extract of *C. squamatus* showed a reasonably hepatoprotection potential to silymarin while the total alcoholic extract of total alcoholic extract of *C. squamatus* extract produced a moderate hepatoprotection potential. The results of antioxidant activity showed that the ethyl acetate extract is more active than the butanol extract (61.15% and 49.64% respectively) and the most active compounds are chrysoeriol -6- *O*-rhamnoside and apigenin -7-*O*-arabinoside (92.56% and 86.33% respectively).

Keywords: *Coronopus squamatus*, Crucifereae, Flavonoids, Hepatoprotective and Antioxidant activity.

INTRODUCTION

The genus *Coronopus* (family *Crucifereae*) was represented in Egypt by 3 species : *Coronopus squamatus* (Forssk)Asch.. , *Coronopu niloticus* (Del) Spreng. and *Coronopus didymus* (L.)[1]. The plants of this genus were subjected to many investigations which revealed the presence of many classes of compounds like flavonoids , glucosinolates , sterol glycosides, saponins and anthraquinones. many flavonoids have been isolated from *Coronopus* species like quercetin, kaempferol, chrysoeriol, Isorhamnetin, rhamnetin, Isorhamnetin-3-*O*–galactoside, luteolin-7-*O*- glucoside, luteolin-7-*O*- rutinoside and apigenin -7-*O*-glucoside [2-6] some glucosinolates as glucotropaeolin, glucoalyssin, glucobrassicanapin and 4-phenylbutyl-glucosinolatein addition to benzyl isothiocyanate[7]. It was found that coronopus genus has different activities as anti-inflammatory, anti-allergic, antipyretic, hepatoprotective, antioxidant and radio protective [8-11]. *C. squamatus* is a common herb growing in the fields of traditional crops and the farmers consider it as a weed[12]. It is used in folic medicine and has many biological activities, so the purpose of this work is the investigation of the flavonoids, antioxidant and hepatoprotective activity of this plant.

MATERIALS AND METHODS

Plant Material

Coronopus squamatus (Forssk.) is a glabrous, blue-green herb. Leaves deeply pinnatifid with narrow segments. Pods short – pedicelled to sessile. The plant was collected from the farm of Saft al-laban , Giza, governorate , Egypt, and kindly authenticated by Prof. Dr. Aza El-Hdidi , botany department , faculty of science , Cairo University , Egypt . a voucher specimen was deposited at the herbarium of national research center. the upper ground parts of the plant were air dried and grounded to fine powder .

Apparatus and Techniques

1. The UV. Absorption spectra were measured using 4-ml capacity quartz cell (1cm thick) in a shimadzu UV. 2401 UV-V recording spectrophotometer.
2. The NMR spectra were run on a Broker DRX-400 instrument operating at 300 MHz for ^{1}H using standard pulse sequences and Joel JNM-EX 270 ft NMR system measured at 270 MHz for ^{1}H at Cairo university , Cairo, Egypt .Chemical shifts reported on the δ scale in parts per million (ppm) down field TMs.
3. The fast atom bombardment mass spectra (FAB-MS) and electron impact mass spectra (EI-MS) were carried out on a Finngan MATSSQ 700 system using glycerol as the liquid matrix.

Chemical for chromatography

S1;butanol : acetic acid : water (4 : 1 : 5, upper layer), S2; ethyl acetate : formic acid : acetic acid : water (30 : 0.8 : 1.2 : 8), S3;15 % AcOH and S4; EtOAc : pyridine : H_2O (12 : 5 : 4)

Sephadex LH-20 for Column Chromatography, Pharmacia, polyamide (65 Riedel – de Haen for column chromatography), silica gel plates (Merck, type 60 F254)

Preparation of samples: About 100 gm dry powder of *C. squamatus* were extracted using 70 % methanol and evaporated under vacuum to obtain dry residue. The alcoholic residue was dissolved in hot distilled water and kept in the refrigerator overnight then filtrated. One part of the filtrate was taken for test and the other part was shacked with ethyl acetate followed by n-butanol, then all extracts were evaporated under vacuum till dryness and take about 1mg of each one. The total glucosinolates of the plant were prepared using acidic aluminum oxide column [13].

Biological evaluation

1-Hepatoprotective Activity

In vitro bioassay on primary culture of rat hepatocytes monolayer

Animals: Waster male rats (250-300 g), obtained from the animal house of the NRC (National Research Center, Cairo) were used.

Materials: The following compounds were purchased: 4-(22-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES 99%) (ACROS Co.), Potassium Chloride (KCl) (S.R.L), D(+) glucose (Merck), Calcium chloride ($CaCl_2$) (S.D), Fetal Calf Serum (FCS)(PAA Laboratories), Penicillin Streptomycin (PS) (Gibco BRL)Sodium thiopental, Silymarin (Cid Pharmaceutical Co.), 6-hydroxy 2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) (Fluka Co.), Sodium Chloride (NaCl), Sodium dihydrogen Phosphate (NaH_2PO_4), Sodium hydrogen Phosphate (Na_2HPO_4), Potassium dihydrogen Phosphate (KH_2PO_4), Ethylenebis (oxyethylenenitrilo) tetra-ethylene glycol bis (2-aminoethyl ether)-N,N,N'N'-tetraacetic acid (EGTA), Collagenase Type IV, Culture medium RPMI-1640, Trypan Blue, Insulin, Dexamethasone, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium-bromide (MTT), (1,1-

diphenyl-2-picrylhydrazyl) (DPPH) and Dimethylsulfoxide (DMSO). (Sigma Co.)

Isolation and culture of rat hepatocytes monolayer

Primary culture of rat hepatocytes was prepared according to Seglen method, 1976 [14] which modified by Kiso, *et al.* 1983[15].

Determination of LC_{50} of extracts

After preincubation of primary culture of rat hepatocytes for 22-24 hrs, the monolayer was checked under inverted microscope (Olympus) for attachment. Then, the monolayer was washed twice with (Phosphate Buffer Saline (PBS). In order to determine LC_{50}, cultured cells were incubated for 2 hours with different concentrations of the samples. The concentrations used for each of the tested samples ranged from 100-1000 µg/mL. LC_{50} was considered that concentration that induced death of half the number of cells. Samples were dissolved in (DMSO,1%). For each concentration, three replicates were carried out.

After two hours incubation of cells with the tested samples, cell viability was determined using MTT assay, that was performed according to the method of Mosmann (1983)[16] modified by Carmichael, *et al.* (1987)[17].

Evaluation of the effect of the different extracts on cultured hepatocytes was obtained by calculating the absorption of the cell viability with respect to control cells. Each experiment was carried out in triplicate to confirm validity of results.

A graph was plotted with x-axis showing different concentrations of extracts used, y-axis showing absorbance percentage of viable cells. LC_{50} was graphically determined from the concentration that yielded an absorption coinciding with 50% of cells that received no extract.

Determination of hepatoprotective activity

The concentration of the extract that was able to protect the cells from the hepatotoxic effect of Paracetamol by 100% was considered hepatoprotective. The injury model applied in this study was as follows: monolayer of cultured hepatocytes were incubated with different concentrations (12.5, 25, 50, 100 µg/mL) of tested extracts for two hours followed by incubation with Paracetamol for one hour. Silymarin 50µg/mL was used as a reference hepatoprotective. Four groups were utilized; group-1 cultured hepatocytes alone (negative control), group-2 cultured hepatocytes treated with Paracetamol for 1 hour (injury control), group-3 cultured hepatocytes treated with either extract tested for 2 hours followed by 1 hour incubation with Paracetamol and group- 4 cultured hepatocytes treated with 50µg/mL Silymarin (positive control) for 2 hours followed by 1 hour incubation with Paracetamol. At the end of the incubation the MTT reduction assay are the criteria for cell viability are determined.

2-Antioxidant activity

This activity was determined by DPPH scavenging activity, and Trolox equivalent antioxidant capacity (TEAC) as Chia-Pu L.; *et al*, (2006)[18].

DPPH scavenging activity

Radical scavenging activity against the stable radical DPPH (1,1-diphenyl -2- picryl hydrazyl) was measured using the method of Blois (1958)[19] which was modified by Duh, P. *et al*, (2001) [20]. All determinations were performed in triplicate. The radical scavenging activity of the tested samples, expressed as percent inhibition, was calculated with the following formula:

$$\% \text{ Inhibition} = [(AB - AA) / AB] \times 100$$

Where AB = absorbance of blank sample

AA = absorbance of tested antioxidant after 15 mins.

The antioxidant activity was carried out for both the different extracts (total alcoholic, chloroform, ethyl acetate and n-butanol) and the isolated flavonoidal compounds

Extraction and fractionation of the flavonoidal constituents

About 1 kg of air dried powdered plant of *C. squamatus* was defatted with petroleum ether (b.r.40- 60º C, 2 L) . The defatted plant material was macerated with 70% aqueous methanol till exhaustion. The alcoholic extract was evaporated *in vacuo* at about 50 ºC (50 g) . The residue was dissolved in hot distilled water (650 ml), left over night in the refrigerator and then filtered. The aqueous filtrate was extracted with successive portions of chloroform (3 x 500 ml) followed by ethyl acetate (5 x 500 ml) and finally with n-butanol (5 x 500 ml). The solvents were evaporated, separately, *in vacuo* at 50ºC, the chloroform, ethyl acetate and n-butanol free solvent residues amounted to 0.3 g, 1.2 g and 5.0 g respectively.

The paper chromatography of the ethyl acetate fraction using chromatographic paper Whatman No. 1 irrigated with S1 gave the best separation of the flavonoids. It revealed the presence of three main bands (R_f = 0.55, 0.38 and 0.28) .

About 1 g of the ethyl acetate fraction was dissolved in about 5 ml methanol (95 %) and subjected to preparative paper chromatography using chromatographic paper Whatman no. 3 MM, developed by S1 applying the ascending technique. The air dried chromatograms were exposed to UV light and three main bands I, II and III (R_f = 0.55, 0.37 and 0.28 respectively) were localized .the main bands were cut off as strips and eluted in descending glass jar using methanol (85 %) . The combined eluates of each band were filtered through sintered glass funnel and concentrated *in vacuo* at 45ºC.The concentrated eluant of each band was subjected for further purification.

The concentrated eluant of band - I (R_f 0.55) was applied on a small column of Sephadex LH-20 (40 x 1.5 cm, elution was carried out using methanol / water (90: 10). Fractions of 20 ml, each were collected. The column was followed using paper Whatman No. 1 developed with S1. The fractions containing compound F- I in pure form were collected and concentrated *in vacuo* till dryness to afford compound **F-I** (7 mg).

The eluants of both band - II and band-III (R_f =0.37& 0.28) were evaporated and subjected for further purification by PTLC using silica gel plates developed with S2. The main zones were localized in UV light, scrapped off, eluted with 90 % methanol, filtered and evaporated *in vacuo* at 45ºC. The eluants were further purified using Sephadex LH-20 column eluted with 90% methanol to afford compound **F-II** and compound **F-III** in pure form(9 mg and 6mg respectively).

About four grams of butanol fraction were dissolved in a minimum amount of methanol (90%) and applied on to the top of a polyamide column (90 x 4 cm) packed in water. Elution was carried out using water followed by water / methanol mixtures. Fractions of 100 ml of each were collected. The was monitored by paper chromatography developed with S1.

The combined fractions (61 – 80), (51 – 60) and (41 – 50) were further purified, separately, by PPC using Whatman no.3 MM developed with S3. The migrated main zone (R_f 0.49) was localized under UV light and eluted with 80 % methanol. The eluant was filtered and subjected for further purification by passing through Sephadex LH-20 column (30 x 1 cm) and the eluted fractions which containing compounds **F-IV**, **F-V** and **F-VI** were evaporated *in vacuo* at 45˚C .

RESULTS AND DISCUSSION

The flavonoids of *C. squamatus* were isolated from the ethyl acetate and butanol fractions of alcoholic extract. Six flavonoids were identified as follow:

F-I: *7-methoxy luteolin*, This compound was isolated as yellowish white powder, it appears as a dark brown spot changed to yellow with $AlCl_3$ under UV light.It is an aglycone in nature according to its chromatographic behavior on PC in different solvent systems (R_f = 0.59, in S1 and R_f=0.17 in S3) [21].

The data of UV absorption spectra in methanol showed band –I at 346 nm and displayed a bathochromic shift (62 nm) with high

intensity on addition of NaOMe. which indicates the presence of a free OH group at C4' [21].

The presence of an ortho-dihydroxy system was confirmed through $AlCl_3$ /HCl spectrum where it displayed hypsochromic shift in band –I, which confirmed through the bathochromic shifte in band –I of $NaOAc/H_3BO_3$ spectrum (31nm). It was found that there is no bathochromic shift in band–II of NaOAc spectrum relative to methanol which confirms the absence of a free OH group at C-7 which may be occupied by mehoxy group.

The negative FAB-MS showed a molecular ion peak at m/z=299 corresponding to M^+-H, by reviewing the chemical abstract for formula index, it was found that it agree with the molecular formula $C_{16}H_{12}O_6$ [22]. The compound undergoes retero Diels Alder rearrangement which followed by fragmentation giving rise A_1^+ fragment at m/z=166 and B_1^+-1 fragment at m/z= 133 which confirm the presence of the methoxy group at ring A not at ring B and the ortho dihydroxy system at ring B not at ring A [23].

so, the compound F-1 could be identified as 7-methoxy luteolin.

F–II: *4', 5, 8-trihydroxy-6-methoxy flavone-7-O-rhamnoside*; the compound gives red color with Shinoda's test, yellow color with $AlCl_3$ and positive Molisch's test. These tests indicates the glycosidic flavonoidal nature of compound F- II. The UV absorption spectra of the compound displayed band –I at λ_{max} =326 nm in MeOH which means that it is a flavone type structure. with a free OH group at C-4' (On addition of NaOMe, bathochromic shift of 45 nm) [21]. There is no hypsochromic shift in band –I of $AlCl_3$ /HCl spectrum relative to $AlCl_3$ spectrum which means there is no ortho- dihydroxy system .It was found that, the OH at C7 may be occupied because there is no bathochromic shift in band –II of NaOAc spectrum relative to methanol spectrum.

The negative FAB –MS showed a molecular ion peak at m/z =461 (M^+-1) which related to the molecular formula $C_{22}H_{22}O_{11}$ [24]. Two important peaks at m/z =317 and m/z=198 , which indicates that the compound lost the fragment $A1^+$ which means that, ring-A contains three OH group and one OCH_3 group .

The ^{1}H-NMR spectrum of the compound in CD_3OD showed signals at δ= 7.65 (d, 2H, H2',6'), 7.2(S, 1H, H-3), 6.85(2H, d, H-3',5'), 5.25(d,1H,H 1"), 3.7(S, 3H, OCH_3) and 0.88(d, 3H, CH_3 of rhamnose).

Acid Hydrolysis

About 2 mg of compound F - II were dissolved in 10 ml methanol, mixed with 10 % HCl and refluxed on a boiling water bath for 3 hours. The solution was diluted with distilled water and extracted with ether (4 x 25 ml). The aqueous acidic solution was neutralized with barium carbonate, filtered through a centered glass funnel. The clear filtrate was evaporated till dryness. The residue was dissolved in redistilled pyridine, filtered, and evaporated *in vacuo* at 70 ºC. The residue was dissolved in 10 % isopropanol and subjected to PC using S4 reveling the presence of rhamnose only as a sugar [25] and the aglycone was identified as 4', 5, 8-trihydroxy-6-methoxy flavone [21]. From all the above chromatographic and spectroscopic data we can concluded that the compound F-II is 4', 5, 8-trihydroxy-6-methoxy flavone -7-*O*-rhamnoside.

F- III: *apigenin -7-O-arabinoside*, The chromatographic behavior of this compound proved that, it is a monoglycosidic in nature, it appear as a brown spot in UV and changed to a yellowish with ammonia vapor. The UV absorption spectra of the compound declared that, it is a flavone type structure with free OH group at C4- [21].There is no hypsochromic shift in band –I of $AlCl_3$ /HCl spectrum relative to $AlCl_3$ spectrum that means there is no orthodihydroxy system .

The absence of free OH group at C7 was confirmed through NaOAc spectrum where there is no bathochromic shift in band –II relative to methanol spectrum.

The negative FAB- MS of the compound displayed a molecular ion peak at m/z =401 which coincided with that reported for the molecular formula $C_{20}H_{18}O_9$ [26]. The aglycone peak was appeared at m/z =270 (M^+ - pentose moiety (m/z =132))

The ^{1}H-NMR spectrum inCD_3OD showed signals at δ=7.6(d,2H, H-2',H-6') , 7.1(dd, 1H, 5') , 6.95(d,1H,H-3'), 6.9(d, 1H, H-8), 6.89(S,1H, H-3) , 6.8(dd , 1H , H-6') and an anomeric proton as a doublet at 5.35 for arabinose ,The other protons of the sugar moiety disappeared by the solvent signal.

The Acid hydrolysis revealed the presence of arabinose as a sugar moiety and apigeni as an aglycone. so that compound F-III could be identified as apigenin -7-*O*-arabinoside [26].

F-IV: *Apigenin-7-O-rhamno-glucoside;* This compound appears as a dark brown spot under the UV light and not changed with ammonia vapors and it has high R_f value in both S3 and S4 (0.65 and 0.49 respectively) which indicates that , it may be a diglycosidic flavonoidal compound. The UV absorption spectra of the compound in methanol gave band –I at 328 nm which indicates the flavone nature of the compound with a free OH group at C4'.[21] The presence of free OH group at C-5 is confirmed due to the bathochromic shift of band-I from 328nm in methanol to 352 nm with addition of the $AlCl_3$. The absence of Ortho -dihydroxy system was confirmed through both $AlCl_3$/HCl spectrum and $NaOAc/H_3BO_3$ spectrum where there is no hypsochromic shift in band –I in the first one relative to $AlCl_3$ spectrum and no bathochromic shift in band – I in the later relative to methanol spectrum. There is no bathochromic shift in band – II in NaOAc spectrum relative to methanol spectrum which indicates there is no free OH group at C-7

The EI -MS showed a small molecular ion peak at m/z =578. It was found that, this peak correspond to the molecular formula $C_{27}H_{30}O_{14}$ [27]. Another important peaks at m/z =433 corresponding to [M^+ - Deoxysugar moiety (146)] and m/z =270 corresponding to [M^+ - Deoxysugar moiety - hexose moiety (162)]. This means that, the M^+ for the aglycone is 270. Also the peak at m/z =152 confirm the fragmentation pattern, where it undegoes retero Diels Alder reaction. The acid hydrolysis gave rhamnose and glucose as and apigenin as an aglycone. So, compound F-IV may be identified as apigenin 7-*O*-rhamno-glucoside.

Compound F-V: *Acacetin-7-O-rhamnoside*; This compound was isolated as a dark purple fluorescent on paper chromatogram under UV light, gave positive tests for Molisch's test. The characteristic features of its absorption spectra in UV are band –I at 326 nm which shifted to 362 nm on addition of NaOMe. So, it is a flavone type structure. The acid hydrolysis gave acacetin as an aglycone while the is rhamnose.

The -ve FABMS of the compound gave a molecular ion at m/z 429 [M^+ - H] and other prominent ions observed at m/z 283 [m/z 429-rham] indicating the release of one

rhamnose moiety from acacetin in addition to a peak at m/z 270 related to apigenin-4'-OCH_3. ^{1}HNMR studies revealed the presence of signals at 8.05 2H, d, J= 8.8Hz H-2',6', 7.12 2H, d, J= 8.8Hz H-3',5', 6.87 1H(s) H-3, 6.75 1H, d, J=2.0Hz H-8, 6.35 1H, d, J=2.0Hz H-6, 5.70 1H, d, J=1.2Hz H-1", 3.85,3H,s, -OCH_3 3.10-4.10 (m) remaining protons of rhamnoseand 1.20 3H, d,J=6Hz $CH_3$of rhamnose [28]. So this compound was identified as acacetin-7-*O*-rhamnoside.

Compound F-VI: *Chrysoeriol-6- O-rhamnoside;* The chromatographic behavior of the compound proved that it is a glycosidic flavonoid where its R_f in different solvent systems are 0.51 and 0.58 in S2 and S3 respectively.

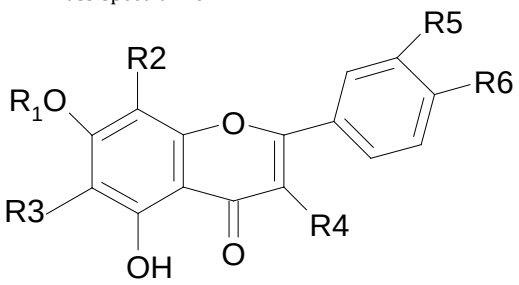
The UV absorption spectra proved that, it is a flavone type structure where it displayed band- I at 333 nm in methanol which bathochromically shifted to 380 nm (with high intensity) upon addition of NaOMe ,so, it has a free OH group at C-4' [21]. it was found that, C7 contain free OH due to the bathochromic shift in band –II (11nm) in NaOAc spectrum.

The negative FAB –MS displayed a molecular ion peak at 461which are corresponding to the molecular formula $C_{22}H_{22}O_{11}$ (M^+-H) .The peak at m/z =317attributed to M^+-146 which confirm the presence of a deoxysugar. [22]

The ^{1}H- NMR CD_3OD fig (21, P116) spectrum displayed signals at δ in ppm =7.67 (d, 2H, H-2', H-6'), 6.78 (d, 1H, H-5'), 6.7 (d, H, H-8), 6.67 (S, 1H, H-3) .The methoxy protons at C-3' appeared as singlet at δ=3.6 ppm. The anomeric proton H-1" appeared as doublet at δ = 5.1

ppm, finally the protons of methyl group of rhamnose moiety displayed as doublet at δ 1.1 ppm

The acid hydrolysis revealed the presence of rhamnose [21] as a sugar and chrysoeriol as an aglycone .The positive FAB-mass spectrum of the isolated aglycone showed the presence of a molecular ion peak at m/z =317 (M^+ +1) which referred to the molecular weight of chrysoeriol. From all the previous data, compound **F-VI** could be identified as Chrysoeriol-6- O-rhamnoside.



R_6	R_5	R_4	R_3	R_2	R_1	Compound	No.
OH	OH	H	H	H	CH_3	7-methoxy luteolin	1
OH	H	H	OCH_3	OH	rhmnose	5,8,4'-trihydroxy-6-methoxy flavone-7-*O*-rhamnoside	2
OH	H	H	H	H	arabinose	Apigenin -7-*O*-arabinoside	3
OH	H	H	H	H	Rham-gluc.	Apigenin 7-*O*-rhamno-glucoside	4
OCH_3	H	H	H	H	glucose	Acacetin-7-*O*-rhamnoside	5
OH	OCH_3	H	H	Gluc.	H	Chrysoeriol -6- *O*-rhamnoside	6

The hepatoprotection assay was applied with a broad range of concentrations of the studied samples (from 12.5-100 µg/mL) on monolayer of rat hepatocytes as well as the hepatotoxicity assay (from 100-1000 µg/mL). The biological activity index is the ratio between the concentration that induced toxicity and the concentration that achieve biological activity. Silymarin, the most known medicinal plant extract to exert hepatoprotection exhibit a biological activity index of 10 (500/50).

The data in table (1) showed that the extract **W-2** which represents ethyl acetate extract *C. squamatus* showed a reasonably hepatoprotection potential to silymarin (index >40 and >20 respectively) . This fraction was found to be considered for further bioactivity guided fractionation in order to identify the compound(s) which are responsible for such activity. Also, **W-1** which represents the total alcoholic extract of the plant produced a moderate hepatoprotection potential (index >10). The extract **W-4** showed a range of hepatoprotection potential (10-20) while **W-3** which represents the butanolic extract was produced no hepatoprotection at the range of experimental concentration (12.5-100µg/ml), these data were in accordance with that reported by Vilas etal[29]who reported about the hepatoprotection activity of a poly herbal formulation due to the presence of flavonoids and phenolic constituents.

Table 1: LC_{50} and Hepatoprotection Concentrations for Tested Extracts

Therapeutic index	Hepatoprotection	LC_{50}	Extract sample
>10	12.5 → 100	125	W-1
>20	12.5	250	W-2
ND	No till 100	250	W-3
>10-20	12.5-50	125-250	W-4

W-1 (total alcoholic extract of *C. squamatus*), W-2 (ethyl acetate extract of *C. squamatus*)

W-3 (butanol extract of *C. squamatus*), W-4 (total glucosinolates of *C. squamatus*)

*ND: no detection

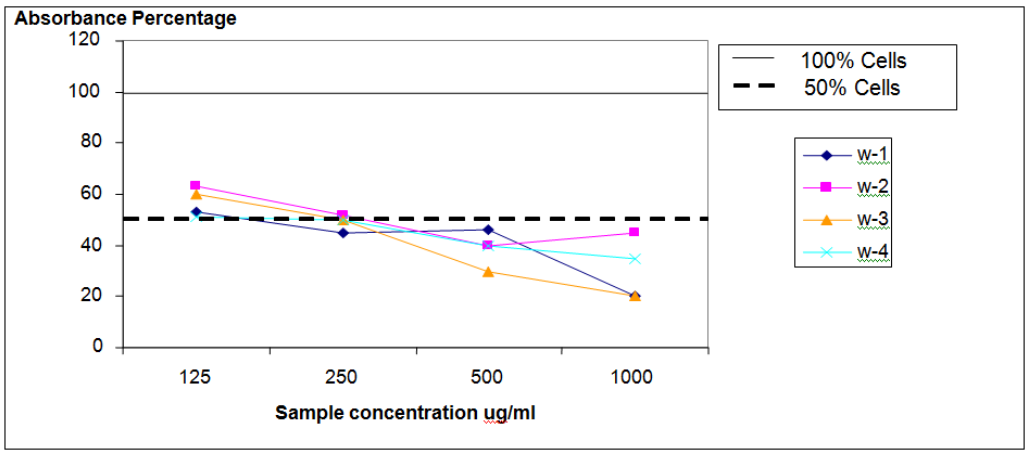


Fig. 1: Viability of Rat Hepatocytes after 2 hrs treatment with Different Concs. of the Extracts Using MTT Colorimetric Assay.

Each Point Represents the Mean ± S.D (n=3)

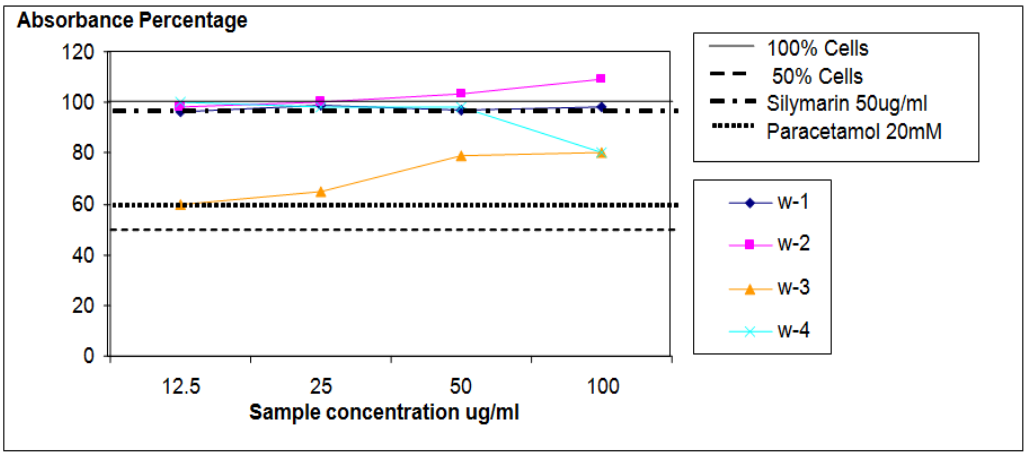


Fig. 2: Viability of Rat Hepatocyte after 2 hrs Treatment with Different Concs. of the Extracts Followed by Treatment with 20 mM Paracetamol for 2hr. in comparison with 50 µg/ml Silymarin as Control Using MTT Colorimetric Assay.

Each Point Represents the Mean ± S.D (n=3)

DPPH has been widely used to test the ability of compounds or plant extracts to act as radical scavengers[30]. The experimental data of study of antioxidant activity using DPPH (table 2) proved that as the absorption at 520 nm decreased the antioxidant activity increased. So, the best antioxidant compound is 3'-methoxy-4'-hydroxy-5,7-dihydroxy-6-*O*-rhamnoside flavone due to it represents about 90 % of trolox activity then apigenin -7-*O*-arabinoside which represents 80% . These data may be explained as by Pier in 2000 [31] who discussed the relation between flavonoids structure and their activity as antioxidants, in addition to Rumit et al [32] who relate the antioxidant activity of *Tephrosia purpurea* to the presence of flavonoids.

Table 2: antioxidant activity of tested extracts and the isolated compounds

% Activity	Abs. at λ_{max} 520nm	Sample	No.
0	0.417	Blank	0
96.9	0.013	Trolox (standard antioxidant compound)	1
17.98	0.342	*C. squamatus* total alcololic extract	C1
14.87	0.355	*C. squamatus* chloroform extract	C2
61.15	0.162	*C. squamatus* ethyl acetate extract	C3
49.64	0.210	*C. squamatus* butanol extract	C4
44.85	0.230	7-methoxy luteolin	F-I
41.24	0.245	4',5,8-trihydroxy -6-methoxy flavone-7-*O*-rhamnoside	F-II
86.33	0.057	a pigenin -7-*O*-arabinoside	F-III
51.8	0.201	Apigenin-7-*O*-rhamno-glucoside	F-IV
40.04	0.250	luteolin-7-*O*-rhamnoside	F-V
92.56	0.031	Chrysoeriol-6- *O*-rhamnoside	F-VI

CONCLUSION

This the first report about the flavonoids of *Coronopus squamatus* growing in Egypt. Six flavonoids were identified as 7-methoxy luteolin, 5,8-dihydroxy -6-methoxy flavone-7-*O*-rhamnoside and apigenin -7-*O*-arabinoside, apigenin 7-*O*-rhamno-glucoside, Acacetin-7-*O*-rhamnoside and chrysoeriol -6- *O*-rhamnoside. The results of hepatoprotection activity proved that, the ethyl acetate extract of *C. squamatus* showed a reasonably hepatoprotection potential to silymarin while the total alcoholic extract of total alcoholic extract of *C. squamatus* extract produced a moderate hepatoprotection potential. The results of antioxidant activity showed that the ethyl acetate extract is more active than the butanol extract (61.15% and 49.64% respectively) and the most active compounds are chrysoeriol -6- *O*-rhamnoside and apigenin -7-*O*-arabinoside (92.56% and 86.33% respectively).

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