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A COMPARATIVE STUDY OF THE FLAVONOID CONTENTS OF TWO *EUPHORBIA* SPECIES AT MATRUH HABITAT

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A comparative study were made on the flavonoid, total flavanoid contents and antimicrobial activities of *Euphorbia paralias* L. and *Euphorbia dendroides* L. at Matruh habitat in 2004. Results revealed that two *Euphorbia* species flavonoids, saponins, tannins, alkaloids, carbohydrates and /or glycosides contents. Percentage of the total flavonoid ratio varied between 1.89 and 1.75 in *E. paralias* and *E. dendroides*, respectively, in addition,It was found that they contained, quercetin 3-0-α-L-rhamnoside, quercetin- 7-0-β- D-glucoside, quercetin- 3-0- galactoside, luteoline- 7-diglucoside, and phenolic acids (P-coumaric acid, ferulic and chlorogenic), while kaempferol 7-rhamnoside and kaempferol 3-7-dirhamnoside in *E. paralias* and *E.dendroides* contains kaempferol 3-rhamnoside-7-glucosid, luteoline 7- glucosid, orientin and gallic acid. It was understood that biological screening of the two plant extracts (70% ethyl alcohol, ethyl acetate and water extracts) was revealed that it had a moderate effect on different microorganisms (bacteria and fungi) with different extract concentrations.

Keywords: *Euphorbia sp.,* flavonoids, phenolic acids plant extracts, chemical composition bacteria , fungi.

Rizk *et al.*(1976 and 1979) investigated the flavonoid of *Euphorbia paralias,* with isolation of quercetin 3`- xyloside quercetin, kaempferol and rhamnetic also ellagic acid, quercetin – 3- arabinoside and quercetin – 3 – galactoside. Saleh (1985) reported that besides of a number of common flavonol glycsides, kaempferol and quercetin 3- glucuronid 7- glucosides are found in *Euphorbia retusa* and *Euphorbia sanctae – catharinae.* Omurkhamziova *et al.* (1985) identified nine polyphenolic compounds from the aerial parts of seedlings of the *E. alatavica* and *E.soongarica* yielded. Among the first compounds, reported, ellagic acid, gallic acid, Me- gallate,

5, 7, 3`, 4`- tetrahydroxyflavone 3-0- α- L- rhamnopyranoside, 5, 7, 3`, 4`, tetrahydroxyflavone 3-0-β-D galactophyranoside and dehydrodigallic acid (tentative). Ling (1991) isolated from the leaves of the *Euphurbia hirta* L. Six compounds, namely, gallic acid, quercitrin, myricitrin, 3-4- di-0-galloylquinic acid, 2,4,6,- tri-0-galloyl-D-glacose, 1,2,3,4, 6 – Penta- 0-galloyl-β-D-glucose. Lwu (1993) reported that *Euphorbia hirta* is used for the treatment of acute *enteritis* and dysentery. The latex is instilled into the eye for the treatment of conjunctivity. The *Euphorbia hirta* has a reputation as a remedy for bronchitis and asthma. Other uses include lactation incursive agent, anthelminitic tonic treatment of the wounds and tumors, anticonvulsants, mild sedatives and antimcrobial agent. He reported that the *Euphorbia hirta* plant is a slightly narcotic. Ghazanfar (1994) reported that *Ricinus communis*, for treatening the bad breath, blisters ulcers, toothache and inflamed eyes. Also, plant was used as a purgative and relieve rheumatic pain. According to him, *Croton confertus* plant could be consumed for constipation and its new shoots are dipped in butter and sucked to cure cough, to purify the blood and as a tonic *Euphorbia balsanifera* is used for the chewing gum, depilator and bone fractures. Flavonoids are important dietary compounds, having a capacity to inhibit of DNA damage and lipid peroxidation, and to quench of free radicals, they also have anticarcinogenic and antiproliferative effects (Romanova and Vachalkova, 1999). Fernandez *et al.* (1998). Stated that the anit – inflammatory and antibacterial activity of P- coumaric, caffeic, ferulic, gentisic, protocachuic, syringic and isovanllic acids. These compounds were induced rat paw oedema (oral administration) and induced move ear aedema (topical administration) .

Trease and Evans (1999) declared that *Pphyllanthus emblica* (*Euphorbiacae* family) constituenty of trigallryl glucose and other tannins used its, sores, dysentery, diuretic, laxative, digestive, antiscorbutic, emetic and antinflammatory effect .

MATERIALS AND METHODS

Euphorbia paralias L. and *Euphorbia dendroides* L. collected in 2004 at Matruh and were characterized and identified by Prof. Dr. Adel Kamel Yossef who is an ecologist and phytochemist. Collected samples were cleaned and dried in the oven at 40°C and ground to fine powder then kept until processing time. Applicated progress as follows:

1. Preliminary Phytochemical Screening

E. paralias L . and *E. dendroides* L. samples were subjected to phytochemical screning included: test for flavonoids (Wall *et al.*, 1954), saponins and tannins (Balbaa, 1986), volatile oil (Balbaa *et al.*, 1981), alkaloids (Woo *et al.*, 1977) and carbohydrates and /or glycosides (Harper, 1975).

2. Estimation of Total Flavonoids

Estimation of total flavonoids in the ethanolic extract of the two plants were determined colourimetrically according to Karawya and Aboutable (1982) and calculated as qurcetin.

3. Investigation of Flavonoid Content

a. Extraction of flavonoids

The powder of aerial parts of the *E. paralias* and *E. dendroides* specimens was defatted with petroleum ether (60-80°C) and extracted with 70% ethanol.

b. Paper chromatography

The ethanolic extracts were subjected to the two dimensional paper chromatography (TDPC) using with a solvent system which was n-butanol-acetic acid- water (BAW) (4: 1: 5 v:v:v) (S_1), for the first one, followed by AcOH- 15% (S_2) for the second one. The developed chromatograms were air dried, exposed to the ammonia vapour and subjected to the ultraviolet light, afterwards, aluminum chloride reagent was sprayed (Markham, 1982).

c. Fractionation of flavonoid by column chromatography

The concentrated alcoholic extracts of the aerial parts of the plants were applied separately on the top of a polyamide 6s (radial-Dehaen, Seelze-Hannover, Germany) for each column. Elution was started with water followed by a mixtrue of water / methanol and finally with pure methanol. Similar fractions are collected together. Each fraction was subjected to preparative paper chromatography (PPC) using Whatmann paper No. 3 mm and S_1 system for 48 hours. The separated flavonoid compound, and phenolic acid, were purified using a Sephadex LH-20 (Sigma) column and system methanol/ water (1: 1 v:v) (Johnston *et al.*, 1968). The pure flavonoid compounds were ideintified by chemical and physical methods.

d. Chemical reactions (Harborne *et al.*, 1975)

i. Controlled (Mild) acid hydrolysis : (Using 0.1 N HCl)

ii. Complete acid hydrolysis (Using 2N HCl and authentic samples)

iii. Enzymatic hydrolysis (Using β-galactooxidase). Enzyme corresponds to the type of the sugar in the compound. Half of the 0.5 ml enzyme in 0.05 Aacetate buffer (P*h* = 5.1), and authentic reference markers.

e. Physical tests (Mabry *et al.*, 1970).

i. Ultraviolet spectrophotometric analysis
Chromatographically pure material was subjected to ultraviolet spectrophotometric investigation using (Shimadzu UV), visible recording spectrophotometer UV-240. In case of flavonoids, $AlCl_3$/ HCl, NaOAc/ H_3Bo_3 and NaOMe are used as shift reagents.

ii. ^{1}H-and ^{13}C-Nuclear Magnetic Resonance Analysis (NMR):
The NMR measurement were carried out on Bruker AMX- 500, Varian Inova- 500 and / or JEOL Ex-270 NMR spectrometer apparatus (270

MH_Z for ^{1}HNMR and 67.5 MH_Z for ^{13}C NMR) described by Mabry *et al.* (1970).

iii. Mass Spectrometric Analysis (MS)

The mass spectra were conducted using mass spectrometer Varian Mat 711, Finnigan SSQ 7000 ans MM 7070E spectroscopy and analysis were done according to Mabry *et al.* (1970).

4. Antimicrobial Studies

a. Successive extracts

Air-dried powders of the two plants were subjected to extraction with successive solvents using Soxhlet apparatus. Petroleum ether (B.P. 60-80°C), ether, chloroform, ethyl acetate, 96% ethyl alcohol , 70% ethyl alcohol and finally water were used in the order of increasing polarity. The effect of the two plants extract using successive extraction methods on the growth of some pollutant microorganisms were studied as follows.

b. Microorganisms

Six bacterial, *Bacillus subtilis*, *Bacillus spharicus*, *Staphylococcus aureus*, *Pseudomonas sp.*, *Echerichia coli* and *Salmonella* spp. and six fungal strains, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum*, *Fusarium oxysporium*, *Alternaria tennius* and *Microsporum fulvum* were selected.

Before, they were checked for their purity, identity and regeneration ability. Cultures were stored in the refrigerator at 5 °C and they were re-activated on the suitable media. The microorganisms were provided from the Plant Pathology and Microbiology Department, National Research Center, Giza, Egypt .

Antimicrobial activity were provided from the Animal Health Dept. and Plant Pathol. Unit, Desert Research Center, Cairo. Egypt, was tested with agar-diffusion technique using filter paper discs according to the Maruzzella and Balter (1959).

c. Preparation of the inocula

Bacterial inocula were prepared in nutrient broth media after 96 hours of incubation in the laboratory at 37°C. Fungi were grown on potato-dextrose agar (PDA) growth, the cultures were incubated at 27 °C for 10 days. The inocula concentration were 1 x 10^6 conidia/ ml. (Ismail *et al.*, 1989).

d- Agar diffusion method

Agar diffusion method was used to check the presence or absence of anti-microbial agents as described by Booth (1972).

Antimicrobial activity of different extracts (ether, petroleum ether, chloroform, ethyl acetate, 96% ethyl alcohol, 70% ethyl alcohol and water) using different dilutions (250, 500, and 1000 ppm) (by diluting the original extract with the organic solvent used for extraction in each case) of *E.*

paralias and *E. dendroides* on the inhibition of bacteria and fungi were studied

RESULTS AND DISCUSSION

1. Preliminary Phytochemical Screening

The preliminary phytochemical screening of *E. paralias* and *E.dendroides* (Table 1) indicate the presence of flavonoids, saponins, tannins, alkaloids and carbohydrates and / or glycosides.

2. Estimation of Total Flavonoids

Total flavonoid ratio was changed between 1.89 and 1.75 in the *E. paralias and E. dendroides*, respectively (Table 1).

TABLE (1). Preliminary phytochemical screening of the two plants, and total flavonoids (%).

Compounds	*E. paralias*	*E.dendroides*
Flavonoids	++	+
Saponins	+	++
Tannins	+	+
Alkalodoids	+	++
Carbohydrates and/ or glycosides	+	+
Total Flavonoid	1.89	1.75

+ = positive, ++ = strongly positive

3. Investigation of Flavonoid Content

Fractionation of the extracts revealed the presence of 9 fractions of *E. paralias* and 11 fractions of *E.dendroides,* fractions . Each fraction was subjected to PPC using Whatmann paper No. 3 mm and S_1 system and purified on Sephadex LH-20 using methanol/ water (1:1 v : v) (Johnston *et al.,* 1968). The chromatograms obtained from the two plants compounds as M_1, M_2, M_3, M_8, P_1, P_3 and P_4, while *E. paralias* contain M_4 and M_5 in the *E.dendroides* contains M_6, M_7, M_9 and P_2 (Table 2).

Identification of M_1 compound.

Compound M_1 appears as yellow colour. Its R_f-values and colour reaction were outlined in table (3) (Harborne, 1984). Acid hydrolysis revealed the presence of quercetin as aglycone by the comparison with authentic sample and the sugar moiety was identified as rhamnose by confirmed with authentic sample. UV spectrum data showed two major UV absorption bands in MeOH, they were at 358 nm and 260 nm, respectively, which indicated that it is a flavonol with 3-OH substitution (Table 4). Addition of NaOMe, causes a bathochromic shift in band I, indicating the presence of OH free at position 7. UV spectral data also revealed the presence of OH at position 3` and 4`.

^{1}H-NMR spectrum of compound M_1 showed signals characteristics for querctin (Mabry *et al.*, 1970) with additional signal for rhamnose at δ 5.35, 4.4, 3.2 , 3.7 m and at δ 1.2 for CH_3 indicating the presence of rhamnose

sugar. This was confirmed by mass spectra, showed a fragment at m/z 146 characteristic for rhamnose.

From the UV spectral data, acid hydrolysis and ^{1}H-NMR compound 1 could be identified as quercetin –3-0-α- L- rhamnoside.

Identification of M_2 Compound

The R_f values and colour reaction of the compound M_2 were outlined in table (3). Acid hydrolysis afforded quercetin as aglycone and glucose as the sugar moiety, while UV spectral data (Table 4) gave two bands at 370 and 255 nm, respectively which are typical to the flavonol with free hydroxyl group at the position 3 with MeOH (Liu *et al.*, 1989). The addition of NaOMe, gave a bathochromic shift (+ 70 nm) in band I. This suggested that position 4` has a free OH- group (Mabry *et al.*, 1970). The absence of any shoulder at 305–345 nm with addition of NaOMe indicated that the hydroxyl group at position 7. $AlCl_3$ addition gave bathochromic shift in band I (+80 nm) indicating the presence of OH group at position 5, $AlCl_3$/HCl addition gave hypthochronic shift (-28 nm) indication for the presence of free ortho-dihydroxy group. Addition of H_3BO_3 gave bathochromic shift (+ 10 nm) in band I indicated the presence of ortho–dihydroxy group in β- ring.

^{1}H-NMR spectrum of compound M_2 showed signals characteristics for quercetin with glucose (Mabry *et al.*, 1970) at δ 7.28, 6.88 , 6.2 and sugar moiety : δ 5.0, 3.35- 3.70 m(glucose proton overlapped with OH protons). These data confirmed that the isolation of M_2 as quercetin – 7-0-β-D glucoside.

Identification of M_3 Compound

Isolated compound M_3, appears as darkeep purple colour under the UV light, changing to yellow with ammonia. R_f- values and colour on PC reaction (Table 3) indicate that compound 3 of glycoside. Acid hydrolysis gave the known aglycone (quercetin and glalactos as sugar moieties), through comparison with authentic markers sample. Acid hydrolysis using β-galactosidase half ml solution in an acetate beffer (pH- 5.1) gave quercetin 3-0 galactoside. UV spectrum data of compound (3), with MeOH gave band I (370 nm) that similar to those reported for quercetin type compounds (Harborne, 1984 and Liu *et al.*, 1989). Addition of NaOAc causes shift in band II (+ 35 nm) suggesting the presence of free OH at 7 position. The bathocromic shift of band I in $AlCl_3$ indicates the presence of 5- OH group. Addition of $AlCl_3$ / HCl cause hybathacromic shift this indicated the presence of catecholic hydroxyl groups. ^{1}H-NMR spectral data showed signals at δ ppm : 8.0 (1H, d, j = 8 : Hz , H-$2^{\backprime}$) , 7.9 (1H, dd , j = 8.5 Hz , H-$6^{\backprime}$), 6.89 (1H, d, j = 5.8 Hz, H-$5^{\backprime}$), charcterisitic for Quercetin, and signal at δ 5.6 (d , J=8 Hz, H-1`` galactoside proton). Thus, from UV, ^{1}H-NMR and by comparison with authentic sample compound M_3 could be indentified as quercetin 3–0 galactoside .

TABLE (2). The flavonoids and phenolic compounds in the *E. paralias* and *E.dendroides* .

Compound	*E. paralias*	*E.dendroides*
M_1	+	+
M_2	+	+
M_3	+	+
M_4	+	-
M_5	+	-
M_6	-	+
M_7	-	+
M_8	+	+
M_9	-	+
P_1	+	+
P_2	-	+
P_3	+	+
P_4	+	+

M_1 = Quercetin - 3-0-α-L-rhamnoside,
M_2 = Quercetin 7-0-β-D-glucoside,
M_3= Quercetin - 3-0- galactoside,
M_4= Kaempferol 7- rhamnoside.
M_5= Kaempferol - 3-7- dirhamnoside,
M_6= Kaempferol 3-rhamnoside- 7 glucosid,
M_7= Luteoline 7- glucosid
M_8= Luteoline 7- diglucoside,
M_9= Orientin,
P_1= P-coumaric acid,
P_2= Gallic acid,
P_3= Ferulic acid,
P_4= Chlorogenic acid,

Identification of M_4 Compound

Compound 4, appeared as a light yellow spot. Its R_f- values and colour reactions were outlined in table (3). Acid hydrolysis gave the known aglycone (Kaempferol and rhamnose as the sugar). UV spectral analysis, adding of MeOH and different reagents showed that all absorption bands were similar to that of kaempferol. With the presence of substitution at position 7 (Liu *et al.*, 1989) identified as quercetin 3-0-galactoside (Table 4).

^{1}H-NMR spectrum of M_4 compound showed that signals at (2H, d , J= 8.5 → H-2 and H-6`), δ 6.9 (2H, d , J= 8.5 Hz→H-3` and H-5`), δ 6.4 (H, d , J = 2.5 Hz → H-8), δ 6.2 (H, d , J= 2.5 Hz → H-6), δ 5.4 (1H, d , J= 2.5 Hz → H-1 rahmnose), δ 3.5 (compex signals for sugar protons) and δ 0.8 (3H, d , J= 6Hz CH_3 rhamnose). ^{13}C-NMR showed the area for the most acidic carbon between 147 – 176 were loacted in those carbon at kaempferol position 7 appeared more upfield than normal kaempferol indicating the sugars substitution, this proved by presence of C-1^{11} at 98.2 and other four carbons between 69.8 – 70.0, also methyl carbon at 17.9. The mass spectrum, revealed a molecular ion peak M^+ and m/z 433, 275, 257, 151 and 135. Thus compound 4 was identified as kaempferol –3-0- α- L- rhamnoside.

TABLE (3). R_f- values and detection methods of the isolated compound (M_1 to P_5).

Comp.	R_f-values		Detection methods		
	BAW	AcOH-15%	Visible	UV	UV + NH_3
M_1	0.69	0.50	-	Yellow	Fl- yellow
M_2	0.38	0.11	-	Yellow	Bright yellow
M_3	0.48	0.43	-	Purple	Yellow
M_4	0.89	0.36	yellow	Yellow	Yellow
M_5	0.73	0.57	-	Brown	Fl- yellow
M_6	0 58	0.74	Brown	Brown	Brown
M_7	0.43	0.16	-	Brown	Yellow
M_8	0.24	0.28	-	Brown	Yellow
M_9	0.39	0.32	-	Purple	Yellow-green
P_1	0.92	0.65	-	Pale blue	Mauve
P_2	0.78	0.59	Fl-blue	Visible blue	-
P_3	0.88	0 56	-	Blue	Fl- blue
P_4	0.62	0.44	-	Fl- blue	Yellowish-green

Fl = fluorscence

Identification of M_5 compound

Compound M_5 appeared as yellow colour. It was subjected to acid hydrolysis as mentioned before kaempferol was detected in the ethylacetate extract as confirmed by Co- chromatography with authentic sample of kaempferol. The sugar residue released after hydrolysis was identified as usual to be rhamnose, confirmed Co-chromatography with authentic sample as reference marker. UV analysis with MeOH and different shift reagent (Table 4) showed that, band I 346 nm indicates that it's a flavonol with 3-OH substitution. Addition NaOAc/ H_3BO_3 gave no bathochromic shift in band II indicates that the 7- OH is substitution (Liu *et al.*, 1989). ^{1}H-NMR spectrum showed signals at δ 7.8 (2H, d , J= 8.5 H-2`, 6`),δ 6.9 (2H, d, J= 8.5 H-3`,5`), δ 6.8 (1H, d, J = 2.5 H-8), δ 6.4 (1H, d , J= 2.5 H-6), δ 5.55 (1H, d , J= 2.5 $H1^{III}$ rhamnose), δ5.3 (1H, d , J= 2.5 $H1^{II}$ rhamnose), 3-4 complex signals for protons, δ 1.14 (3H, d , J = 6 CH_3 rhamnose), 0.82 (3H, d , J= 6 CH_3 rhamnose).

^{13}C-NMR showed the area for the most aciodic carbon between 177.8 –156 is for quaternary carbon and most acidic carbon, carbon no 1 in both sugar attachment will appeared at 101.8 for C-1^{III} and 98.4 for C-1^{III} and other 8 carbon were shown at 69.8 to 71.6. Finally, two methyl carbon were showed at 17.49 and 17.92. This compound could be identified as kaempferol 3, 7 dirhamnoside.

Identification of M_6 Compound

Compound M_6, appeared as brown from the methanol. It could be soluble in methanol and water but insoluble in ether and chloroform. It was subjected to acid hydrolysis as mentioned before. Kaempferol was detected in the ethyl acetate extracts as confirmed by Co- chromatography with authentic sample of kaempferol. The sugars released after hydrolysis was

identified as glucose and rhamnose. UV analysis with MeOH and different shift reagents of M_6, show that all the absorption bands were similar to those of kaempferol but no bathochromic shift in band II was noticed on addition of NaOAc/ H_3BO_3 indicating that 7- OH is substituted (Table 3).

^{1}H-NMR spectrum of M_6 showed signals at δ 8.1 (2 H, d, J= 8.5 Hz,H-2`- 6`) and this is shifted more upfield than kaempferol indicating the presence of substitution at 3- OH, δ 6.96 (2 H, d, J= 8.5 Hz,H-3`- 5`), δ 6.9 (1 H, d, J= 2.5 Hz, H-8) , δ 6.4 (1 H, d, J= 2.5 Hz,H-6) , the last two signals at δ 6.9 and 6.4 were shifted more downfield than kaempferol alone this indicate the presence of substitution at C-7 . At δ 5.54 (1 H, d, J= 2.5 Hz, H-1 rhamnose), the position of this signal indicate that there is rhamnose substitution at 3- OH, δ 5.57 (1 H, d, J= 7.0 Hz,H-1 glucose), the presence of such signal doublet with J= 7 indicate the presence of glucose at 7- OH, at δ3-4 sugar protons, δ1.2 (3H, d, J= 6.0 Hz,CH_3 rhamnose). Thus M_6 could be identified as kaempferol- 3- rhamnoside- 7- glucoside.

TABLE (4). UV- spectral data (λ_{max} nm) of the isolated flavonoids compound.

Comp.	Reagents					
	MeOH	NaOMe	NaOAc	NaOAc+H_3BO_3	$AlCl_3$	$AlCl_3$+HCl
M_1	260, 300sh, 358	272, 320 sh, 430	275, 325 sh, 395	260, 325 sh, 375	272, 300 sh, 440	272, 300 sh, 420
M_2	255,262 sh, 370	246 sh, 370, 440	258, 282, 420 sh	260, 285, 380	257 sh, 273, 450	267, 304 sh, 356, 422
M_3	257, 259 sh, 362	272, 327, 409	274, 324, 380	262, 298 sh, 377	275, 305 sh, 438	268, 299 sh, 366 sh, 405
M_4	226 sh, 320, 366	226 sh, 270, 440	226 sh, 320, 420	226 sh, 320, 420	226 sh, 266 , 424	244 sh, 266, 300 sh, 422
M_5	228, 298, 320, 364	254, 298, 380	268, 294, 320, 346	228, 294, 320, 346	236, 303, 354	236, 303, 352, 400
M_6	245 sh, 264, 324 sh	245 sh, 276, 390	265, 288 sh, 398	245 sh, 264, 348	245 sh, 276, 400	245 sh, 276, 384 sh, 400
M_7	252, 264, 346	260, 335, 396	254, 350, 395	256, 369	272, 325, 420	272, 330, 383
M_8	251,266 sh, 345	260, 300 sh, 393	258, 266, 363 sh, 405	258, 370	273, 296, 327, 430	272, 292 sh, 356, 385
M_9	255, 267, 335	268, 278, 335 sh	275, 325, 382	265, 275, 325, 430 sh	275, 305, 355, 405 sh	265, 275, 302,340, 380
P_1	-	-	-	-	-	-
P_2	272, 335	275, 345	-	-	-	-
P_3	285, 312	250 sh, 290, 319	-	-	-	-
P_4	245, 300 sh	239, 265, 310 sh, 382	-	-	-	-

Identification of M_7 Compound

Compound M_7, R_f- values of M_7 and coulor reactions are outlined in table (3). R_f- values and colour reactions showed close similarity with luteolin 7-glucoside. Acid hydrolysis gave the known aglycone luteolin and glucose as sugar moiety. UV spectral analysis with MeH0 gave 346 and 252 nm. The addition of NaOMe causes bathochromic shift in band I (+ 50 nm). The addition of NaOAc cause no shift in band II. Addition of H_3BO_3 which gave bathochromic shift in band I (+23 nm), thus proving the presence of 2

catecholic OH groups. Addition of $AlCl_3$ causes bathochromic shift, this indicating the presence of free OH group at 5 position. $AlCl_3$/ HCl addition decrease the magnitude of the bathochromic shift of $AlCl_3$. From the previous discussion, compound M_7 could be identified as luteolin-7-glucoside.

Identification of M_8 Compound

Compound No. when subjected to TDPC, revealed the presence of one major spot of flavonoid nature. R_f – values of M_8 and colour reaction, outlined in table (3), indicate that M_8 may be diglycosides. UV spectral analysis with MeOH gave (345 and 251 nm), also prove the presence of free OH group at position. Addition of NaOAc cause no shift in band II, thus suggesting the occupation of 7- position. The addition of H_3BO_3 caused bathochromic shift in band I (+ 25 nm), this indicate the presence of ortho-dihydroxy groups. Addititon $AlCl_3$ causes bathochromic shift, this indicating the presence of free OH group at 5 position. $AlCl_3$/ HCl addition decreases the magnitude of the bathochromic shift of $AlCl_3$, thus proving the presence of 2 catecholic OH groups at (3`, 4`) position. Complete acid hydrolysis gave the a glycone luteolin and glucose as sugar moiety (2 mole) and by comparative on PC using authentic sample. Thus the compound M_8 could be identified as lueolin-7- digucoside.

Identification of M_9 Compound

R_f- values and colour reaction (Table 3), showed close similartiy with C-glycosides (Harborne, 1984). Acid hydrolysis showed that M_9 resisted acid hydrolysis, the Wessely – Moser effect would in fact give two isomers on acid hydrolysis: the 6- and 8- isomers: indicating its C- glycoside nature. UV spectral analysis in MeOH gave two major absorption bands at 276 and 335 nm, typical of flavon (Harborne, 1984 and Liu *et al.*, 1989). Addition of NaOMe gave a bathochromic shift in band I, indicating that the 4` position to be free hydroxyl. Addition of NaOAc exhibits a bathochromic shift in band II (+20 nm), proving that the 7- position to be free hydroxyl. $H_3BO_3$ addition gave a bathochromic shift in band I, indicating the presence of dihydroxy groups in β- ring at position 3` and 4`. $AlCl_3$ addition gave a bathochromic shift in band I, indicating the presence of 5- OH group. $AlCl_3$ /Hcl gave hypthochromic shift in band I, thus further confirming the presence of dihydroxy groups in β- ring at 3` and 4`. Thus UV analysis confirmed that the isolated compound (M_9) is orientin.

^{1}H-NMR spectrum of compound (M_9) showed signals at δ 7.9 (1 H, d, J= 8.5 Hz,H-2`), δ 7.5 (1 H, d, H= 8.5 Hz, H-6`) , δ 6.85 (1 H, d, J= 8.5 Hz, H-5`), δ 6.77 (δ, H-3), δ 6.4 (1H, s , H-6), δ 4.63 (1 H, d, J= 8 Hz, H-1`` glucose ) and δ 3.1- 3.9 (m, glucose protons). Thus $^1$H-NMR spectral analysis showed the absence of H-8 signal proton, presence of H-6 sugar proton at δ4.63 (d, J = 8 , H-1``glucose) and δ 3.1- 3.9 (m, glucose protons). H-3 appear as siglet at δ 6.77. Thus compound M_9 was identified as orientin.

Identification of P_1 Compound

Compound P_1 was obtained as white powder, soluble in methanol and aceton . R_f – values and colour reaction of compound illustrated in table (3) indicated that it has a phenolic nature, and on PC purification revealed the presence of one major spot. The compound was directly compared along with an authentic sample of p- coumaric acid in four different sdvent systems using PC, the R_f-values and colour reactions were identical. This compound P_1 can be identified as p-coumaric acid.

Identification of P_2 Compound

Compound P_2 subjected to TDPC, revealed the presones of one major spot of phenolic natur (Silverstein *et al.*, 1981). R_f- values and colour reaction (Table 3), UV spectrum analysis showed that two bands 272 and 335 nm. Also the bathochromic shift after addition of NaOMe 275 and 345 proved the presence of free OH group (Table 4). ^{1}H-NMR spectral analysis showed signals at δ (ppm) 6.98 (s, 2-H and H-6). ^{13}C-NMR spectral data of P_2 showed signals at δ 120.6 (C-l), 108.9 (C-2 and C-6) 145,5 (C-3 and C-5), 138.1 (C-4) and 176.7 (C-7). Gallic acid called 3, 4, 5- trihydroxybenzoic acid $C_6H_2(OH)_3$ COOH (170.12), which are widely used in the manufacturing azo dyes and photographic developers and to treat certain skin diseases. Gallic acid and its derivative are used in making dyes and inks, photographic developers and used as astringents medically.

Identification of P_3 Compound

Compound P_3 was obtained as long white needle shaped crystal that is soluble in methanol and acetone. The R_f- values and colour reactions (Table 3). UV spectral data of P_3 exhibits absorption bands at 285 nm and 312 nm which are characteristic for phenylpropanoids, also with adding NaOMe, gives a bathochromic shift (250, 290, 319 nm) proved the presence of free hydroxyl group. ^{1}H-NMR spectra of P_3 showed two doublets at δ (7.5 and 6.25) j= 17.0 Hz characteristic of trans olfenic protons H-7 and H-8, respectively. The two doublet at δ 7.15 and 6.95 (j =7.5 Hz) and doublet of doublet signals at δ 7.09 (dd, j = 7.09 Hz), which is corresponding to ortho and meta coupling and it is a good evidence for the presence of trisubstituted benzene. The presence of singlet at δ 9.15 ppm confinmed the UV analysis for the presence of free OH group. The ^{1}H-NMR showed signals at δ 12.2 (broad of free singlet) for – COOH proton and at δ 3.85 for 3 protons of OCH_3 group. These data suggest that (P_3) in 4-hydroxy- 3- melhoxy cinnamic acid (Ferulic acid). $HO(CH_3O)$ C_6H_3 CH= CH COOH, molecular weight of 194.19. The latter suggestion was confirmed by El-Mass spectrum, which showed a molecular ion peak M^{+1} 195, which proved that P_3 is (ferulic acid). It is an acid which contribute to the effectiveness of pycnogenol. It is also a natural for UV protection.

Identification of P_4 Compound

Compound P_4, R_f-values and colour reactions of the compound are illustrated in table (3). UV spectral data confirmed the presence of the absorption bands characteristic for phensylpropanoid. The bathochromic shift upon addition of NaOMe proved the presence of free hydroxyl groups (Table 4). ^{1}H-NMR spectral data showed signals at δ 7.45 (1H, d , J= 17 Hz → H-7$^\backslash$), 7 (1H, d , J= 2 Hz → H-2$^\backslash$), 6.95 (1H, dd , J= 7 and 2Hz → H-6$^\backslash$), 6.75 (1H, d , J= 7 Hz → H-5$^\backslash$), 6.15 (1H, d , J= 17 Hz → H-8$^\backslash$), 5.55 (1H, broad → H-3), 3.9 (1H, broad → H-5), 3.5 (1H, broad → H-4), 1.9-2.1 (4H, multiblet → CH_2-2 and CH_2-6). El- mass spectral of (P_4) showed a molecular ion peak (M^{+1}) at m/z 355, quinic acid – H_2O at m/z 157 and the base peak at m/z 217 for (M^+ - $C_8H_7O_2$). The structure as 3-0- caffoeyl quinic acid) or chlorogenic.

4. Antimicrolial Studies

a. Anti-bacterial activity

It was observed from the obtained results (Table 5) that 70% ethyl alcohol, ethyl acetate and water extracts were given the best effects on all selected bacteria strains in different dilutions (250, 500 and 1000 ppm) of *E. paralias L.*and *E. dendriodes* L. Ethyl alcohol 96% and ether extracts of the two plants had a better effect on all the tested bacteria strains at different dilutions. Except for 96% ethyl alcohol extract (250 ppm) of the *E. paralias* which produced no effect on *Pseudomonas* sp., *Echerichia coli* and *Salmonella sp.* and also (500 ppm) has no effect on *Salmonella sp.* Beside of this, 96% ethyl alcohol effect of the *E. dendroides* plant had no effect (250 ppm) on most bacteria studied and also (500 and 1000 ppm) effect on *Salmonella sp. during the E. paralias* (250 ppm) ether extract had no effect on *pseudomonas sp.* and *Echerichia coli.* While ether extract of the *E. dendroides* (250 ppm) no effect on *Bacillus spharicus* and *Echerichia coli* and also (500 and 1000 ppm) had no effect on *Echerichia coli* . Meanwhile the extracts petroleum ether and chloroform of the two plants showed the lowest effect on most of the tested bacteria strains at different dilutions. Ross and Brain (1977). Showed that ethyl acetate extract produced the best result on bacteria, which can be explained by the presence of sterol, terpens, phenolics in addition to the absence of any sugars, or amino acids. Sugars and amino acids could increase the resistance of the declared that sulphur compound are very toxic for the microorganisms because its penetrating activity of the cells or it can act as enzyme inhibitor.

b. Anti- fungal activity

Table (6) showed that 70% ethyl alcohol, ethyl acetate and water extracts produced better effect on all fungi in the different dilutions (250, 500 and 1000 ppm) of the two plants. Ethyl alcohol 96% and ether extracts had a the best effect on all the tested fungi in all dilution, except ethyl alcohol 96% extract (250 ppm) of the *E. dendroides,* which approved no

effect on *Aspergillus niger, Aspeigillus flavus* and *Alternaria tennius*. Also 500 ppm has no effect on *Alternaria tennius*.

On the other hand, chloroform and petroleum ether extracts of the two plants at different dilution produced the lowest effect on all selected fungi strains. Except, petroleum ether extract of *E. dendroides* which concentration (250 ppm) has no effect on all selected fungi strains.

It was noticed that some bacteria and fungi are pathogenic for animals and humamn and some others cause damage to plants. Some of these microorganisms; *Escherichia coli* arised from the water pollution and cause urinary infection, diarrhrea and gastroentirites. Some of the *Pseudomonas sp.* cause human ears and eyes diseases and *Salmonella sp.* cause septicimia, typhoid and food poisoning. These species considered dangerous one because they cause death in few hours.

Also, *Staphylococcus aureus* causes food poisoning that is characterized by severe diarrhea and vomitting. Such carries provide the reservoir for the spread of *staphylococcal* infections, most frequently by the way of the hands. *S. aureus* is also a major cause of impetigo, either alone or in conjgunction with group. *A. streptococci.* Such infections are seen most frequently in school children often beginning around the nose and spreading over the face. In sometimes causing piles and carbuncles. (Lippincott, 1991). *Asperigllus niger* which cause *otitis media* in human, *Asperigllus flavus* cause damage and putridity in the stored food material such as fruits, meat and jam, and also it damage the lether, papers, tobacoo, clothes and cigarettes.

Asperigllus flavus also cause asperiglloses disease which damage the lung and inhibit the respiratory system in human. *Trichoderma harzianum* and *Microsporum flavum* cause dermal disease in human.

It could be concluded from the previous results that *Euphorbia paralias* and *Euphorbia dendroides* extracts have antimicrobial activity especially its 70% ethyl alcohol, ethyl acetate and water extracts. This may be due to the different soluble biochemical com pounds in each extract.

TABLE (5). Anti-bacterial activity of *Euphorbia paralias* and *Euphorbia dendroides* extracts in growth of some bacteria (inhibition area in mm).

Extracts	Conc. ppm	Inhibition area (Diameter in mm)											
		Bacillus subtilis(+)		*Bacillus spharicus (+)*		*Staphylococcus aureus (+)*		*Pseudomonas* sp. *(-)*		*Echerichia coli(-)*		*Salmonella* sp. *(-)*	
		E.par.	*E.den.*	*E.par.*	*E.den.*	*E.par.*	*E.den.*	*E par.*	*E.den.*	*E.par.*	*E.den.*	*E.par.*	*E.den.*
Ether	250	+	+	+	0	+	+	0	+	0	0	+	+
	500	+	+	+	+	+	+	+	+	+	0	+	+
	1000	+	+	+	+	+	+	+	+	+	0	+	+
Petroleum ether	250	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	+	0	0	0	0	0	0	0	0	0	0
	1000	+	+	+	+	+	+	0	0	0	+	+	0
Chloroform	250	0	+	0	0	+	+	+	+	0	+	+	0
	500	+	+	0	0	+	+	+	+	+	+	+	0
	1000	+	+	+	+	+	+	+	+	+	+	+	+
Ethyl acetate	250	++	+	++	+	++	++	++	+	++	++	++	+
	500	++	++	++	+	++	++	++	++	++	++	++	+
	1000	+++	++	++	++	+++	++	++	++	+++	++	++	++
96% ethyl alcohol	250	+	0	+	+	+	0	0	0	0	0	0	0
	500	+	+	+	+	+	+	+	+	+	+	0	0
	1000	++	+	++	++	++	++	+	+	+	+	+	0
70% ethyl alcohol	250	++	+	++	+	++	++	++	+	++	+	+	++
	500	+++	++	+++	++	++	++	++	++	++	++	++	++
	1000	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++
Water	250	++	+	++	+	+	+	+	+	++	+	+	+
	500	++	+	++	+	++	+	++	+	++	+	++	+
	1000	++	++	+++	++	++	++	++	++	++	++	++	++

E.par. = *Euphorbia paralias*, *E.den.* = *Euphorbia dendroides*,

\+ = Refer to inhibition area in mm (1-20),

++ = Refer to inhibition area in mm (21-30),

+++ = Refer to inhibition area in mm (31-40)

TABLE (6). Anti-fungal activity of *Euphorbia paralias* and *Euphorbia dendroides* on the growth of some fungi (Diameter in mm).

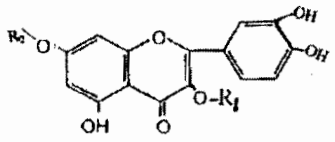
Extracts	Conc. (ppm)	Inhibition area											
		Aspergillus niger		*Asperigllus flavus*		*Trichoderma harzianum*		Fusarium oxysporium		Alternaria tenuiussima		*Microsporum fulvum*	
		E. par.	*E. den.*	*E. par.*	*E. den.*	*E. par.*	*E. den.*	*E. par.*	*E. den.*	*E. par.*	*E. den.*	*E. par.*	*E. den.*
Ether	250	+	+	+	+	+	+	+	+	+	+	+	+
	500	+	+	+	+	+	+	+	+	+	+	+	+
	1000	+	+	+	+	+	+	+	+	+	+	+	+
Petroleum Ether	250	+	0	+	0	0	0	+	0	0	0	+	0
	500	+	0	+	+	+	+	+	0	0	0	+	+
	1000	+	+	+	+	+	+	+	0	+	+	+	+
Chloroform	250	+	0	+	0	+	+	+	0	+	+	+	+
	500	+	+	+	+	+	+	+	+	+	+	+	+
	1000	+	+	+	+	+	+	+	+	+	+	+	+
Ethyl Acetate	250	++	+	++	+	++	+	++	++	++	++	++	++
	500	++	++	++	++	++	+	++	++	++	++	++	++
	1000	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
96% Ethyl Alcohol	250	+	0	+	0	+	+	+	+	+	0	+	+
	500	+	+	++	+	++	+	+	+	+	0	+	+
	1000	++	+	++	+	++	+	++	++	+	+	+	+
70% Ethyl Alcohol	250	++	+	++	+	++	++	++	++	++	+	++	++
	500	+++	++	++	++	+++	++	++	++	+++	++	++	++
	1000	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Water	250	++	+	+	+	++	+	++	+	++	+	++	+
	500	++	+	++	+	++	++	++	+	++	++	++	++
	1000	++	++	++	++	++	++	++	++	++	++	++	++

E.par. = *Euphorbia paralias*, *E.den.* = *Euphorbia dendroides*,

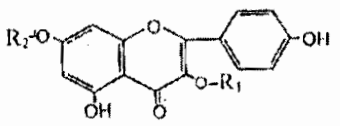
\+ = Refer to inhibition area in mm (1-20),

++ = Refer to inhibition area in mm (21-30),

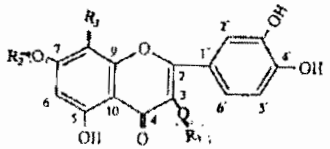
+++ = Refer to inhibition area in mm (31-40)



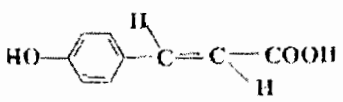
R_1 = Rhamnose, R_2 = H (M_1)
R_1 = H, R_2 = Glucose (M_2)
R_1 = Galactose, R_2 = H (M_3)



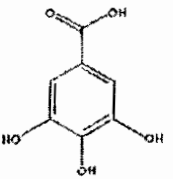
R_1 = H, R_2 = Rhamnose (M_4)
R_1 , R_2 = Rhamnose (M_5)
R_1 = Ramnose, R_2 = Glucose (M_6)



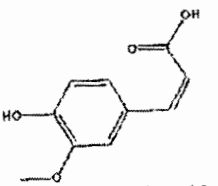
R_1 = H, R_2 = R_3 Glucose (M_7)
R_1 = H, R_2 = Diglucose (M_8)
C-3 = - R2 = H, R3 = Glucose (M_9)



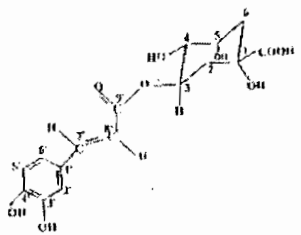
p-coumaric acid



Gallic acid



Ferulic acid



Chlorogenic acid

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دراسة مقارنة على المحتوى الفلافونيدى لنوعين من جنس الايفوربي النامى بمطروح

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أوضحت المقارنة بين نبات الايفوربيا برااليز (شجرة الحنش) ونبات الايفوربيا ديندروديس بمطروح وأن كل منهما يحتوى على فلافونيدات وصابوينات وتانينات وقلويدات وكربوهيدرات. وكانت نسبة الفلافونيدات الكلية بنبات شجرة الحنش ١,٨٩% ونبات الايفوربيا ديندروديس ١,٧٥% .

تم فصل الفلافونيدات فى كل من النباتين والتعرف عليهم وهى كالتالى: كوارستين – ٣ – أ – رامنوز ، كوارستين – ٧– أ – جلوكوز ، كوارستين –٣– أ– جالاكتوز، ليتولين –٧– داى جلوكوز بجانب الأحماض الفينولية التالية: (بـ كوماريك وفيروليك وكلوروجينك وكافيك) بينما احتوى نبات شجرة الحنش على كامفيرول –٧– رامنوز، كامفيرول – ٣–٧ – داى رامنوز، فى حين نبات الايفوربيا ديندروديس يحتوى على كامفيرول– ٣– رامنوز – ٧– جلوكوز، ليتولين –٧– جلوكوز– أورينتين والحامض الفينولى جاليك .

وتمت دراسة التاثير البيولوجى للمستخلصات النباتية لكل من النباتين باستخدام الإيثر والايثر البترولى والكلوروفورم وخلات الايثيل والكحول ٩٦% و٧٠% والماء على بعض الكائنات الدقيقة من بكتريا وفطريات التى تسبب العديد من الأمراض للنباتات والانسان وذلك باستخدام تركيزات مختلفة من المستخلصات حيث تبين انها أعطت نتائج إيجابية على البكتريا والفطريات وخاصة المستخلص الكحولى ٧٠% وخلات الايثيل والماء.