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PHARMACOLOGICAL ACTIVITIES FOR THE EXTRACT AND ISOLATED COMPOUNDS OF *EUPHORBIA CUNEATA*V VHL

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ABSTRACT

The present work exploredthe chemical profile and pharmacological activities *Euphorbia cuneata*Vahl. Eight phenolic compounds were isolated and identified using IR, ^{13}C-NMR and ^{1}HNMR. as; naringenin**1**,aromadendrin**2**, apigenin**3**, , 3'-O-methoxy-luteolin-7-O-rhamnoglucoside **4**, Apigenin 7- galactoside**5**, kaempferol**6**, quercetin**7** and gallic acid **8**. The extract showed variable pharmacological activities on both*in-vitro* (on smooth and skeletal muscles) and *in-vivo* (analgesic, antipyretic and anti-inflammatory activity). No side effects were reported on liver and kidney functions.

Keywords: Antimicrobial activity, *Euphorbia cuneata*, gallic acid, kmpeferol, nargenine, phenolic compounds

1. INTRODUCTION

Family of *Euphorbiaceae* consists of almost 2000 species. The genus *Euphorbia* is the largest genus of medicinal plants widely distributed [1]. Forty species are

known to occur in Egypt[2,3]The members of this family contain many phytochemical groups and have grate economic importance and medicinal use. This genus is very important due to its various phytochemical constituents as; phenolic compounds[4-6], terpenoids [7], tannins [8], etc.

It is also well known due to its important medicinal uses. The extract of *E. prostrata* has been found to have significant anti-inflammatory, analgesic, haemostatic, wound healing properties and anti-hemorrhoid agent. The major flavonoids of *E. prostrata* extract are apigenin, luteolin, apigenin-7-glucoside and luteolin-7-glucoside. The major phenolic compounds present are gallic acid, ellagic acid and tannins [9]. Several researchers studied the anti-inflammatory activity of some *Euphorbia* species such as; *E. acaulis*[10], *E. peplus*[11],*E. prostrate* [12], E. *heterophylla*[13] and*E. Royleana*[14] they referred their anti-inflammatory activity to the flavonoids present in these species.

Others reported that there are anti-leukemic and antitumor activities for some species such as; *E. esula*[15],*E. lagasa*[16],*E. kansui*[17], *E. fischeriana*[18] and *E. serpense*[19]. There are many other reported biological activities for *Euphoria* such as; acetylcholine like action with muscarinic and nicotinicactivities (*E. dracunculoides*, Lam)[20,21], diuretic effect(*E. palutris* and *E. serpens*)[22,23] *Euphorbia cuneata*Vahl growing in the southern coast of the Red Sea in the area of Gabal Elba in Egypt[2,3]. This plant was in use by native in treatment of Gastric disorder but no scientific reported data approved this activity. So, our work aimed at isolation and identification of the biologically active compounds and some other pharmacological activities of *Euphorbiacuneata*.

2.MATERIAL AND METHODS

2.1.Plant materials: The aerial parts of *Euphorbiacuneata*, Vahl.(*Euphorbiaceae*) were collected during flowering stage in 2006, from Gabal Elba in Halayeb and Shalatin. The sample was kindly identified by Dr. Dr. M. Gebali, former researcher of Botany, and by comparison with plant description Flora of Egypt[2,3]. A voucher specimen (19356 SH) of the titled plant is kept in the herbarium of the Desert Research Center. Plant materials were air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored for phytochemical and biological studies.

2.2. Extraction &Isolation of the active compounds

The air dried powder of *E.cuneata*, was extracted by percolation in 90% ethanol at room temperature for two days and then filtered. The residue was re-percolated again; for four times. The combined ethanol extracts were concentrated under reduced pressure at a temperature not exceeding 35°C till a dry extract was yielded. Water was added and the resultant mixture successively extracted with diethyl ether, chloroform, ethyl acetate, and *n*-butanol respectively. Each extract was dried over anhydrous sodium sulphate, and concentrated under reduced pressure at a temperature not exceeding 35°C and dry extracts were obtained.

Chromatographic examinations of the successive extractswere carried out using Silica Gel G (stationary phase) and solvent systems; (a), ethyl acetate-methanol-water (30:5:4); (b), ethyl acetate-methanol-acetic acid-water (65:15:10:10); (c) chloroform-methanol (95:10); (d) butanol-acetic acid- water (4:1:5) were used for developing the chromatoplates. Visualization of chromatograms was achieved under UV before and after exposure to ammonia vapor or by spraying with aluminum chloride [24]. TLC examination of the different extractives adopting solvent systems (a and c) and visualizing reagent revealed the presence of same 2 spots in ether and chloroform extracts while ethyl acetate and n-butanol extracts were found to have 5 similar spots.

Accordingly, the ether and chloroform extracts combined together and applied on the top of column chromatography packed with silica gel and eluted gradually with chloroform - methanol, 90 fractions (100 ml each) were collected and reduced to 3 main sub-fractions in different yields 2.0, 3.7 and 4.6 respectively (according to the number, color and R_f values of the spots), these sub-fractions were reapplied on preparative thin layer chromatography (system c) for final purifications from which compound **1 -3** were isolated.

The ethyl acetate and butanol extracts were combined together and subjected to column chromatography packed with silica gel and eluted gradually with ethyl acetate –methanol, from this column 150 fractions (100 ml each) were collected and reduced into 6 mean fractions (according to number, colure and R_f of the spots), each fraction was concentrated under reduced pressure to yield 6.11, 4.55, 3.22, 4.87, 3.11 and 5.98 respectively. The residue of each fraction was separately reapplied on preparative paper chromatography (using systems d). Bands corresponding to each flavonoid were separately extracted with methanol, concentrated and re-purified on a column of

Sephadex LH-20 eluted with methanol – water where compounds 4-8 were isolated.

2.4. Biological Activities

2.4.1.Plant extract

Dried aerial parts of *Euphorbiacuneata*, Vahlwere extracted as mentioned before apparatus with ethanol 95%. The ethanol extract was completely dried under vacuum, weighed and the residue was used in testing. The dried plant extractwas freshly suspended in distilled water just before administration.

2.4.2*In-vitro* investigation

2.4.2.1. Isolated smooth and skeletal muscles:

The effect of the alcoholic extract on rabbit intestine motility and frog rectus abdominals were studied using the glass organ bath as described by the staff of Department of Pharmacology, University of Edinburgh [25, 26].

2.4.3. *In- vivo* investigation

2.4.3.1. Determination of median lethal dose (LD50)

The oral LD50 of the total alcohol extract of *E. cuneata* was determined as described BySolima*net al.*, [27]. Groups of six mice, received one of 500, 1000, 2000, or 4000 mg/kgdoses of the tested extract. Control animals were received the vehicle and kept underthe same conditions. Signs of acute toxicity and number of deaths per dose ithin 24h were recorded.

2.4.3.2. Antinociceptive activity

Anti-nociceptive were determined using acetic acid inducedand writhing test [28]. Six groups of mice each of six were used. Three groups received thetotal alcohol extract of the investigated plant at doses 100, 200 &400 mg/g, controlgroup received the vehicle, standard group received diclofenac sodium (30 mg/kg)and the last group received the isolated narigenin (100 mg/kg).

2.4.3.3. Anti-inflammatory activity

Anti-inflammatory activity was assessed using carrageenan induced paw edemamethod [29]. Thirty six rats were distributed equally into six groups.Groups 1. 2 & 3 received the total alcohol extract of the investigated plant (100, 200& 400 mg/g), group 4 (control) received the vehicle, group 5 (standard) receiveddiclofenac sodium (30 mg/kg) and the last group received the isolated narigenin (100mg/kg).

2.4.3.4. Antipyretic activity:

Antipyretic were assayed using yeast-induced hyperpyrexia method described byLoux et al., [30]. Six groups each of six rats were used. Three groups received thetotal alcohol extract of the investigated plant at doses 100, 200 & 400 mg/g, controlgroup received the vehicle, standard group received paracetamol (100 mg/kg)

and thelast group received the isolated narigenin (100 mg/kg).

2.4.3.5. Measurement of liver and kidney functions

Rats were randomly divided into two groups each of 10. Rats of the 1st group received the vehicle in a dose of 5 mL/kg and left as normal control. Rats of the 2nd, group were administered the alcohol extract of *E. cuneata* (400 mg/kg). All medications wereadministeredorally daily for 35 consecutive days. After the treatment period, serums were collected and many markers were measured. Serum activity of alanine transferase (ALT) and aspartate aminotransferase (AST) following the method of Christoph et al., [31]. Serum concentrations of ureaandcreatinine were determined [32,33].

Statistical analysis: All parametric values are given as Mean ± S.E.M and were analyzed by One-way ANOVA followed by Student Newman-Keuls test for significance at $p < 0.05$.

3.RESULT AND DISCUSSION

3.1. Chemical investigation

3.1.1.2. Isolated active compounds: Eight phenolic compounds were isolated and identifiedas;

Naringenin**1**;Aromadendrin**2**;Apigenin**3**; 3'-O-methoxy-luteolin-7-O-rhamnoglucoside **4**, Apigenin-7-galactoside**5**;Kaempferol**6**;Quercetin**7** and Gallic acid **8** (Figure 1). Identification was carried out by comparing their TLC chromatograms, UV spectra in methanol and with different shift reagents, EI-MS, ^{1}H NMR and ^{13}C NMR spectra with authentic samples [34].

3.2. *In-Vitro* Activities

3.2.1. Effect on isolated smooth and skeletal muscles: The alcohol extract in different concentrations (5, 10, 25, 50, 100, 200 and 300 mg/ml) showed initial stimulation to the rabbit intestinal followed by slight inhibition and then causes blocking of the ganglia. The effect of the extract seems reversible by washing on the motility and study of the possible site of action.

3.2.2. Effect on rectus abdominals:

No effect was observed on the muscle at doses up to 300 mg/ml.

3.3. *In-Vivo* Activities

3.3.1. Determination of median Lethal Dose (LD_{50}): The total alcohol extract *E. Cuneata*did not produce any behavioral changes and mortality in mice in doses up to 5000 mg/kg

3.3.2. Analgesic activity: the alcohol extract has a significant analgesic activity at the dose starting from 1g/ kg body weight when using both acid-induced pain and Eddy's hot plate method (Tables 1&2). The isolated compound naringenin had no significant analgesic activity.

3.3.3.. Anti-inflammatory activity

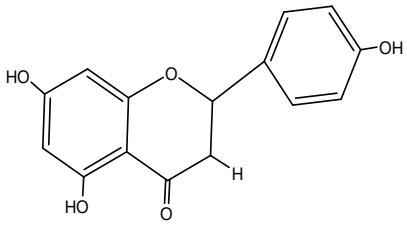
The obtained results (Table 3) revealed that there is no significant effect with the extract (1g/kg).

3.3.4. The antipyretic activity

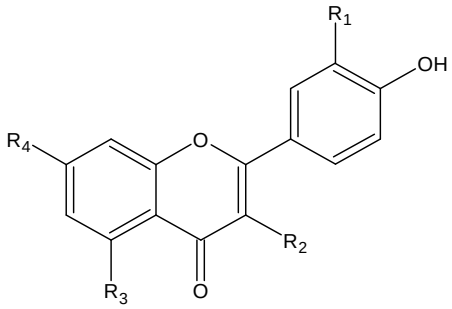
There is no antipyretic activity for the alcohol extract or the isolated compound naringenin (Table 4).

3.3.5. Measurement of liver and kidney functions

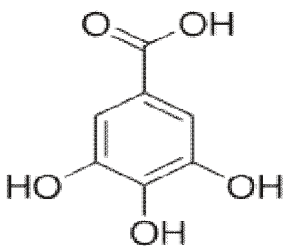
Daily administration of the alcohol extract (2 g/kg) for 15 days did not produce any observable side effects related to liver or kidney functions (Table 5).



1. R = OH (Naringenin).
2. R = OH (Aromadendrin).



R1	R2	R3	R4	
3. H	H	OH	OH	(Apigenin)
4. OCH_3	H	OH	rha.gluc.	(3'-O-methoxy-luteolin-7-O-rhamnoglucoside)
5. H	H	OH	gal.	(Apigenin-7- galactoside)
6. H	OH	OH	OH	(Kaempferol)
7. OH	OH	OH	OH	(Quercetin)



8. Gallic acid

Figure 1: The compounds isolated from *Euphorbia cuneata*Vahl

Table 1: The analgesic effect of different doses of the alcoholic extract and naringenin on acetic acid-induced pain

Groups	No. of writhing/20 minutes (mean ± S.E.)	Percentage of Inhibition
Control	20. 714 ± 1.222	
Diclofenac	15.600 ± 1.284	64.12
0.5 g/ kg	7.600 ± 0.5640	24.69
1 g/ kg	2.100 ± 0.640 *	63.32
2 g/ kg	3.100 ± 0.767*	89.88
4 g/ kg	7.430 ± 0.400*	85.05
Naringenin (100 mg/ml)	19.514 ± 0.988	5.79

*** Significant at P<0.05**

Table 2: The analgesic effect of different doses of the alcoholic extract and naringenin on by writhing method in mice

Treatments	Dose (mg/kg)	No. of writhing / 20 minutes (mean ± S.E.)	Percentage of Inhibition
Control	400	35.83 ± 0.87	01.83
Diclofenac	200	20.66 ± 1.08*	43.39
0.5 g/ kg	400	35.33 ± 1.02	03.20
1 g/ kg	400	35.50 ± 1.05	02.73
2 g/ kg	400	25.00 ± 0.73	31.50
4 g/ kg	400	36.00 ± 0.73*	01.36
Naringenin (100 mg/ml)	400	36.00 ± 0.81	01.36

*** Significant at P<0.05**

Table 3: The anti-inflammatory effect of different doses of the alcoholic extract and Naringenin on carrageenan-induced oedema

Treatments	Difference in paw thickness (mm) after		
	1h	2h	3h
Control (saline)	1.375±0.249	2.067±0.133	2.260±0.161
Extract (1 g/ kg)	1.362±0.191	1.657±0.265	2.557±0.431
Extract (2g/ kg)	1.155±0.171	0.555±0.166*	0.703±0.172*
Extract (4 g/ kg)	0.948±0.135	1.477±0.199	3.057±0.308
Diclofenac(50mg/kg)	1.300±0.120	0.610±0.160*	0.200±0.210*
Naringenin (100 mg/kg)	1.358±0.262	2.136±0.153	2.311±0.182

*** Significant at P<0.05**

Table 4: The antipyretic effect of different doses of the alcoholic extract on yeast -induced hyperthermia

Treatments	Difference in rectal temp (ºC) after			
	0 time	1 h	2 h	3 h
Control (saline)	2.833±0.325	3.317±0.282	2.900±0.459	3.233±0.406
Extract (1 g/ kg)	2.800±0.403	2.833±0.358	3.083±0.426	2.950±0.409
Extract (2g/ kg)	1.917±0.227	2.650±0.167	2.650±0.281	2.500±0.250
Extract (4 g/ kg)	2.083±0.294	2.317±0.341	2.883±0.227	3.017±0.287
Paracetamol (100mg/kg)	3.000±0.703	1.500±0.500*	1.100±0.521*	0.700±0.410*
Naringenin (100 mg /kg)	2.743±0.331	3.421±0.253	3.101±0.988	3.324±0.288

*** Significant at P< 0.05**

Table 5: Effect of fifteen daily administrations of alcohol extract on kidney and liver functions

Treatment	Liver functions		Kidney functions	
	ALT	AST	Urea	Creatinine
Control	10.800±0.583	13.000±0.707	50.812±3.568	0.970±0.016
Extract	11.600±3.999	13.800±1.019	66.556±5.208	1.078±0.045

DISCUSSION

Upon investigation of the active compounds in *Euphorbia cuneata* 8 compounds were isolated, only naringenin obtained in a higher amount which allowed us to investigate some of its biological activities with the total extract. It seems that neither the alcoholic extract nor the isolated compound naringenin has antipyretic activity. On the other hand the use of the standard antipyretic paracetamol produced significant antipyretic effect [35, 36].

The biological activities of the plant extract showed very promising activities *in-vitro* and *in-vivo*. The effect of the extract on isolated smooth and skeletal muscles shoed stimulation of the contraction followed by slight inhibition was recorded following the addition of 200 mg/ ml, same result was achieved in 10 separate experiments. In order to identify the site of action, dilute nicotine (1 µg / ml) was applied for 30 seconds then washed .The alcoholic extract was applied (1 mg / 1 ml organ bath) for 5 minutes followed without wash by the same dose of dilute nicotine (1 µg / ml). This time dilute nicotine did not produce any stimulation this means that the alcoholic extract possesses a ganglion blocking effect. Washing then addition of the same dose of dilute nicotine to the contracting muscle produced the same stimulation. It could be concluded that the alcoholic extract contain substances that initially stimulates and then causes blocking of the ganglia. The effect of the extract seems reversible by washing. On the other hand it seems that the alcohol extract of *E. cuneata*is devoid of substances that can stimulate skeletal muscle because it didn't show any effect on rectus abdominals.

The variation of antimicrobial activities which were observed from alcohol extract when it tested on 10 different microorganisms revealed the possible use of this plant for treating such organisms as total better than successive fractions.

The *in-vivo* activities of the plant extract proved that this plant is very save for human use because No deaths were recorded in mice treated with the alcohol extracts in doses up to 5000 mg/kg. This means that, the investigated extract was safe for use, since substances possessing safety test higher than 0.5 g/kg are considered non-toxic [37]. It seems that the alcoholic extract has a significant analgesic activity at the dose starting from 1g/ kg body weight. The isolated compound naringenin had no significant analgesic activity. No significant effect was produced with the extract (1g / kg). The alcoholic extract started to have an anti-inflammatory activity at a dose of 2 g/ kg after 2 and 3 hours. However increasing the dose to 4 g /

kg did not produce any significant anti-inflammatory effect. Furthermore the isolated compound naringenin had no significant anti-inflammatory activity.

Side effects of total alcohol extract showed that this plant has no adverse effect on both liver and kidney function because the results were very close to that reported to the control group of animal [38, 39].

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