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CHEMICAL AND BIOLOGICAL STUDIES OF CHROZOPHORA VERBASCIFOLIA

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ABSTRACT

The petroleum ether soluble fraction of the alcoholic extract of the aerial parts of C. verbascifolia afforded after saponification and column chromatography : lupeol, stigmasterol, ß-sitosterol and ß-sitosterol-D-glucoside. Their identity was established through comparison with reference materials. Fatty acid analysis was performed by GC after methylation, to indicate the presence of 15 acids. The anti-inflammatory, antipyretic, analgesic, anticonvulsant and the effect on sleeping time of thiopental anaesthetized rats of the lyophilized aqueous extract of the plant was also studied and discussed.

INTRODUCTION

The genus Chrozophora (Euphorbiaceae) is represented in Saudi Arabia by five species : C. plicata (Vahl), C. obliqua (Vahl) (= C. oblongifolia (Del) C. verbascifolia (Willd.) (= C. hierosolymitanu Spreng.), C. brocchiana Vis. and C. tinctoria (L.)(1). Plants of the genus are monecious herbs or shrubs, often covered with stellate indumentum (1,2). The species C. verbascifolia grows wildly and is locally known as Tannoum(1). The species is reported to be used as a laxative, a blood purifier and in cases of leprosy(3,4).

Reviewing the current literature, it was noticed that the plant has not received any chemical or biological investigation. Recently, we have studied the subchronic effects of the oral administration of the lyophilized aqueous extract of the plant in rats(5) and reported the isolation of rutin, kaempferol 3-0-rutinoside and the biflavonoid amentoflavone from the plant(6).

In the present study, a trial to investigate other chemical constitutents analgesic, anticonvulsant and the effect on sleeping time of thiopental anaesthetized rats of the lyophilized aqueous extract of the plant was undertaken.

It is worth mentioning that many flavonoids, including those previously isolated from the plant (6), as well as their aglycones, exhibited significant biological activities. Quercetin was one of three flavonoids responsible for the antispasmodic and anti-inflammatory activities of Achyrocline satureioides(7). Quercetin, its 7-0-glucoside as well as its 3-0-galactoside exerted anti-inflammatory effects in croton oil induced oedema and cotton pellet granuloma(8,9). The anti-inflammatory principle of Wrightia tinctoria and Delonix elata have been identified as quercetin, rutin and quercetin 3-0-galactoside(10,11).

Kaempferol, as well as its 7-0-rhamnoside and 3,7-0-dirhamnoside showed anti-inflammatory effects against

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cotton pellet granuloma[12]. Qurcetin 3-0-rhamnoside and kaempferol 3-0-rutinoside showed anti-inflammatory activity [13]. Rutin and quercetin were found to inhibit aggregation of human platelets[14-17]. The flavonols quercetin, kaempferol and their glycosides rutin and isoquercitrin exhibited antifungal activity[18-22]. Quercetin, quercitrin and the biflavonoid amentoflavone administered in a single dose of 100 mg/kg in mouse caused interferon induction in the serum, which may be the possible mechanism of their antiviral activity[22].

EXPERIMENTAL

General experimental procedures :

TLC and CC separations were performed on silica gel G (Merck) and silica gel 60 (230-400 mesh, Merck), respectively. Solvents used were reagent grade. Visualization of spots on plates was achieved by spraying with vanillin-sulfuric acid followed by heating at 100°C for 10 min. Fatty acid methyl esters were analysed on a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors and fitted with a coiled glass column (1.5 m x 4 mm) packed with chromosorb R (100-120 mesh, WAN) and coated with 10% PEGA. The oven temperature was programmed at 8°C/min. from 70°C to 190°C, then isothermally at 190°C for 30 min. Detector and injector temperatures were 220°C and 300°C, respectively. Gas flow rates for N_2, H_2 and air were 30, 33 and 330 ml/min., respectively.

Plant material :

The aboveground parts of C. verbascifolia in the flowering and fruiting stages were collected in October 1989 and May 1991 from the Qassim province, Saudi Arabia. The plant material was identified by Dr. H.M. Hassan, King Saud University, Riyadh. A voucher specimen is deposited in the Department of Veterinary Medicine, King Saud University, Al-Qassim Branch, Saudi Arabia.

Extraction and fractionation :

Powdered air-dried aerial parts (1 kg) was exhaustively extracted with 95% ethanol to give after evaporation under reduced pressure, a dark reddish-brown semisolid residue (190 g). The residue was dissolved in 20% aqueous alcohol (500 ml) and successively extracted to give the pet. ether (60-80°) extract (A, 38 g), $CHCl_3$ extract (B, 5 g), EtOAc extract (C, 5.9 g) and the remaining aqueous layer (D).

For biological experiments, the lyophilized aqueous extract was prepared as follows : 200 g powdered air-dried aerial parts were extracted with hot water for (4 x 500 ml). The combined aqueous extract was freeze dried (using a Labconco freeze dryer-18 model 75018) to give 42 g of dark shining purple granules. A 10% w/v solution in normal saline was used.

Saponification of the petroleum ether extract (A) :

The petroleum ether extract (A, 18 g) was treated with alcoholic KOH (200 ml, 15%) and refluxed for 2 hrs. Alcohol was distilled off extraction with ether till exhaustion followed by washing with water and drying (anhydrous Na_2SO_4) gave 2.7 g of unsaponifiable matter (E).

Column chromatography of the unsaponifiable matter (E) :

The unsaponifiable matter (E, 2.6 g) was chromatographed on a column of silica gel (170 g, 230-400 mesh, Merck) activated at 110°C for one hour. Elution was started with pet. ether 60080°, then increasing concentrations of benzene, then increasing concentrations of methanol in benzene.

Gas chromatographic analysis of fatty acids :

The aqueous alkaline layer was acidified with conc. HCl then extracted with ether. The combined ethereal extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated to give 7.8 g of fatty acids (F).

Table (1): GC analysis of fatty acid methyl easters of C. verbascifolia.

Fatty Acid	Retention time (min.)	% Composition
Caproic	4.36	0.95
Enanthic	5.90	0.08
Caprylic	7.53	0.74
Pelargonic	8.68	1.53
Capric	10.69	0.15
Indecilic	12.65	0.36
Lauric	13.77	1.11
Tridecilic	15.69	2.26
Myristic	16.61	5.42
Palmitic	19.94	37.62
Margaric	20.81	3.16
Oleic	21.89	3.10
Linoleic	23.21	1.82
Linolenic	25.99	9.37
Arachidic	28.42	32.32

This was treated with 5% sulfuric acid in anhydrous MeOH under reflux for 2 hrs. Excess water was added and the fatty acid methyl esters were extracted with ether, washed with water, dried over anhydrous Na_2SO_4 and evaporated under vacuum to give 8.1 g of fatty acid methyl esters. GC analysis indicated the presence of 15 acids (Table 1).

Analgesic activity :

The hot plate method[24] was used. Thirty mice of both sex (20-25 g body weight) were divided into three equal groups, each of ten. One group was kept as control, whereas the other two groups were i.p. injected with the lyophilized aqueous extract of C. verbascifolia in a dose of 50 mg/100 g body weight and paracetamol (5 mg/100 g body weight), respectively. After administration, each mouse was placed in a two liters beaker immersed in a water bath thermostatically controlled at 56.5°C. Times (in seconds) elapsed until the mouse licks the paws or jumps was considered as the reaction time and was taken as a measure for the analgesic effect. This time was recorded after 10, 20, 30, 60, 90 and 120 minutes after administration.

Antipyretic effect :

Three groups of male wistar rats (200-250 g body weight), each of five were made hyperthermic by S/C injection of Brewer's yeast suspension 12% (1 ml/kg body weight)[25]. After 15 hours, the rectal temperature of each rat was recorded by a medical thermometer, and one group of animals served as control hyperthermic. The other groups were i.p. injected with dipyrone (5 mg/100 g body weight), and C. verbascifolia aqueous extract in a dose of 50 mg/100 g body weight, respectively. The rectal temperature was then recorded hourly for a period of 3 hrs.

Anti-inflammatory activity :

This was carried out according to the method of Winter et al.,[26]. Three groups of male Wister rats (each of five) were used and 0.1 ml of Brewer's yeast suspension (20%) was injected in the paw skin of the hindlimb. After 4 hours, the thickness of the paw was measured by a skin caliber to detect the inflammation achieved by the yeast. One group was kept as control, whereas the other groups were i.p. injected with phenylbutazone (3 mg/100 g body weight) and the plant extract in a dose of 50 mg/100 g body weight, respectively. The paw thickness was measured after 3 and 6 hours after injection.

Effect on sleeping time of thiopental anaesthetized mice[27] :

Two groups of male Albino mice, each of ten, were used. The first group was i.p. injected with thiopental sodium in a dose of 10 mg/100 g body weight and left as control. Whereas, the second group was i.p. injected with the same dose of thiopental sodium and the plant extract in a dose of 50 mg/100 g. body weight. The time elapsed from loosing to

Table (2): The anti-inflammatory activity of C. verbascifolia aqueous extract given i.p. in a dose of 50 mg/100 g body weight of rats.

Treatment	Control (Normal)	Control (yeast)	Thickness of paw skin in millimeters after	
			3 hrs.	6 hrs.
C. verbascifolia (50 mg/100 g)	4.26±0.15	9.0±0.04	8.08*±0.30	7.82**±0.24
Relative potency %	---	---	78.6%	78.4%
Phenylbutazone (5 mg/100 g)	4.12±0.12	8.82±0.39	7.65*±0.24	7.42**±0.20

* P < 0.05
(Mean + S.E.)

** P < 0.01
n = 5 rats

Table (3): The analgesic activity of C. verbascifolia aqueous extract given i.p. in a dose of 50 mg/100 g body weight of rats.

Treatment	Reaction time in seconds after					
	10 min.	20 min.	30 min.	60 min.	90 min.	120 min.
Control (normal)	11.8 ± 0.58	11.8 ± 0.58	11.8 ± 0.58	11.8 ± 0.58	11.8 ± 0.58	11.8 ± 0.58
Paracetamol (50 mg/100 g)	11.7 ± 0.9	16.16 ± 0.4*	16.2 ± 2.33*	16.33 ± 1.47*	11.7 ± 0.88	13.33 ± 1.78
C. verbascifolia (50 mg/100 g)	17.75 ± 1.1*	16.5 ± 0.5*	16.25 ± 1.1*	18.75 ± 0.25*	16.25 ± 1.36*	17.5 ± 1.32*
Relative potency %	152	102	100	115	139	131

* (P < 0.001)
(Mean + S.E.)

n = 10 rats

regaining the righting reflex was recorded as the sleeping time.

Anticonvulsant activity :

Two groups of Albino mice (20-25 g each of ten), were used for studying the anticonvulsant activity (28). The first group (control) was i.p. injected with 0.2 ml saline, while the second group was i.p. injected with the lyophilized aqueous extract of C. verbascifolia in a dose of 50 mg/100 g body weight. After ten minutes later, the two groups were i.p. injected with pentylenetetrazol in a dose of 10 mg/100 g body weight. The animals were kept in separate cages and observed for the appearance of seizures.

Statistical analysis : All the above data were statistically analysed using Student's "test" (29).

RESULTS AND DISCUSSION

The petroleum ether soluble fraction of the alcoholic extract of C. verbascifolia was saponified with alc. KOH. The unsaponifiable matter was chromatographed on a column of silica gel, it afforded 4 steroidal and triterpenoidal materials namely: lupeol, stigmasterol, ß-sitosterol, and ß-sitosterol ß-D-glucoside. The identity of the isolated compounds was established through comparison of their physical and spectral characteristics with those of reference materials.

From the saponification reaction, the fatty acids were recovered and esterified with methanol and sulfuric acid. The prepared methyl esters were subjected to GLC to indicate the presence of 15 acids (Table 1). An interesting finding is the high contents of palmitic (37.62%), arachidic (32.32%) and linolenic (9.37%) acid.

Concerning the biological activity of the aqueous extract of the plant, it has been found that the extract elicited a marked anti-inflammatory activity (Table 2). Where it significantly decreased the thickness of the paw skin in the treated animals after 3 ($P < 0.05$) and 6 hours ($P < 0.01$), as compared with the control, with a relative potency of 78.6% and 84% when compared with that of phenylbutazone after 3 and 6 hours, respectively. This marked anti-inflammatory effect of the plant extract is probably due to the presence of the flavonoid glycosides rutin and kaempferol-3-0-rutinoside, previously reported from the plant(6). These findings are in accordance with those reports which indicated that rutin, kaempferol, quercetin, as well as glycosides (of the last two compounds) exhibited anti-inflammatory activity(7-13).

The plant extract also exhibited a significant analgesic activity ($P < 0.001$) with a relative potency greater than that produced by paracetanol where it increased the reaction time (Table 3). This finding is also in agreement with the reported analgesic effect of rutin(30). However, the plant extract failed to protect mice against convulsion induced by phenylenetetrazole. Moreover, it showed no antipyretic activity and did not significantly affect the sleeping time of thiopental anaesthetized rats.

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دراسة كيميائية وبيولوجية لنبات كروزوفورا فبرباسيفوليا (تانوم)

فتح الله محمد حراز - السيد أحمد عبد العزيز

قسم الطب البيطرى بكلية الزراعة والطب البيطرى - جامعة الملك سعود

(فرع القصيم) - بريده ص .ب ١٤٨٢ - المملكه العربية السعودية

فى هذا البحث تم تجزئه الجزء الدهنى من الخلاصة الكحولية لهذا النبات على عمود كروماتوجرافى ليعطى المركبات : لوبيول - ستجما ستيرول - بيتا سيتوستيرول وسيتوستيرول جلوكوزيد. وتم التعرف على هذه المواد بواسطه المقارنة بمواد قياسية معروفة .

كذلك تم تحليل استرات الأحماض الدهنية لتبين وجود ١٥ حامض دهنى. وقد أجريت دراسة التأثيرات البيولوجية للخلاصة المائية لنبات كمضاد للألتهابات وخافض للحرارة ومسكن للألام ومضاد للتشنج وكذلك للتأثير على وقت النوم فى الفئران المخدرة.