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Determination of Saponin Content in Certain Egyptian Plants By the Blood- Agar Haemolytic Zone Method.

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ABSTRACT:

The saponin contents in nine Egyptian plants was determined against standard saponin using the blood agar haemolytic zone method. The aqueous extract of C. coccineum which showed highest saponin content (6.62%) was further tested for hypoglycemic effect to indicate statistically significant activity.

INTRODUCTION

Obviously, saponin represents the major crop source for partial synthesis of cortical and sex hormones, oral contraceptives, diuretics as well as cytotoxic, anti-inflammatory and peptic ulcer[1,2]. Although, total synthesis of steroids and terpenoids is employed, there is a great demand for the discovery of new high-yielding strains of saponin-containing plants to assure a regular supply of pharmaceutical raw materials. Recently, more than 1000 tonnes of saponins are commercially used every year.

During a biologically-directed research program on certain Egyptian plants, some extracts demonstrated apparant saponin characters.

Although, phytochemical and structure elucidation reports have been published on most of these plants[5-9], yet non had dealt with the percentage of saponin in these species. Therefore, it seemed necessarily to estimate the total saponin contents in order to select the promising species for further study.

In spite of, several methods had been employed for the determination of saponins, the blood-agar haemolytic zone assay is considered the easiest, most accurate and highly specific one[10].

Thus, in the present study aqueous extracts of nine plants have been subjected to this method. The highest saponin-containing plant C. coccineum[15] was further subjcted to a preliminary hypoglycemic testing procedure. The phytochemical investigation of this plant is in progress.

EXPERIMENTAL

Plant Material

Fresh whole plants used in this work were collected from either Sinai deserts viz: Cynomorium coccineum L. (Cynomoriaceae), Cistanche violacea Dest. (Orobanchaceae), Atractylis carduus Forsk. (Compositae), Daucus capillifolius Gilli. (Umbelliferae) and Rhanterium suaveolens Desf. (Compositae). Other plants were obtained from the Experimental Station of the Faculty of Pharmacy, University of Zagazig viz: Hemerocallis fulva L. (Liliaceae), Bidens pilosa L. var. radiata (Compositae), Amaranthus tricolor Hill. and Amaranthus chlorostachys

Willd. (Amaranthaceae). The plants were collected in the flowering stage, grinded into coarse powder and consequently subjected to extraction.

Preparation of the Plant Saponin for Assay:

Exactly, 5 g of the powder of each pant was separately extracted by boiling with 100 ml distilled water for 10 min, then kept aside for 18 h. The extract was filtered and the filtrate was adjusted to 100 ml with dist. water, then sterilized by Seitz bacterial filter. A volume of 0.1 ml of the sterile solution was treated by the same method as the standard saponin solutions. The corresponding concentration of saponin was calculated from the standard curve (Table 1), while the percentage (w/w) of saponin was deduced from the following equation:

$$\frac{\text{conc. in } \mu g \times 100 \times 100}{\text{wt. of plant powder} \times 1000 \times 1000}$$

Preparation of the Standard Saponin Solution:

Exactly, 10 mg of reference saponin (Merck) was dissolved in 10 ml sterile distilled water to form the stock solution (0.1%). Several different dilutions (1000 µg, 500 µg, 250 µg, and 125 µg) were prepared from the stock solution and sterilized by filteration through Seitz bacterial

Calibration Curve for Stnadard Saponin: [10]

A Volume of 0.1 ml from each standard saponin dilution was poured in 8 mm circular cup, cut into the blood-agar plates and kept at 37°C for 48 h. The whole process being carried out under aseptic conditions (Laminar flow). A graph was constructed by plotting the square of radii (avarage of three experiments) of the haemolyzed zone against the

Table 1: Haemolytic Zone Data of Saponins From the Different Standard Solutions

Sample	Radii (R)	Mean R	R^2	Conc. µg/0.1 ml	Log C
Standard dilution 1	13,14,13	13.38	179.26	1000	3.0
Standard dilution 2	13,12,13	12.66	160.27	500	2.698
Standard dilution 3	12,11,12	11.66	135.95	250	2.397
Standard dilution 4	10,11,11	10.66	113.63	125	2.096

Table 3: The Effect of Cynomorium coccineum L. (200 mg/kg) on Blood Glucose Level (mg/100 ml) In Streptozotocin Hyperglycemic Rats.

Treatment	Blood glucose Level in mg/ 100 ml (Mean ± S.E)				
	Zero hour	One hour	Two hours	% Change	
				1 hour	2 hours
Control (Normal)	130.0±3.8	128.0±3.8	125.0±3.8	1.5%	3.8%
C. coccineum Aqu. Ext.	305.0±8.2	217.0±18.6*	279.0±4.2■	28.85	8.52

* Significantly different at P< 0.001

■ Significantly different at P< 0.05

logarithm of the equivalent concentration of standard saponin (mg/ml) and the results are summarized in table 1.

Preparation of the Blood Agar Plates: [11]

Sterile agar solution (2% in distilled water) was warmed to 50°C and mixed with 5 ml of sterile blood by slow swirling of the mixture. The mixture was poured into sterile plates and allowed to set, inverted and stored in refrigerator.

The Antidiabetic Activity [12,13]

The test was carried out on 30 male adult (250 ± 10 g) Wistar rats after two weeks of streptozotocin administration (I.P., 65 mg/kg) in citrate buffer (pH 4.4). A control group (10 rats) was adminstered citrate solution. Only diabetic animals showing > 300 mg/100 ml blood glucose level were used. The aqueous extract of C. coccineum was given orally (200 mg/kg body weight) and the blood samples were withdrown after one and two hours. The glucose was determined by the glucose-oxidase method and the data were analysed using Student's "t" test of the paired data[14], with significance at probability <0.05 to <0.001 and the results were presented in table 3.

RESULTS AND DISCUSSION

Although total synthesis of some medicinal steroids and triterpenoids is carried out, there is a great demand for new sources of saponins. The aqueous extract of nine different plants; most of them were previously investigated for their phytochemical constituents [5-9], showed the presence of appreciable amounts of saponin.

The percentage of saponin by the blood-agar haemolytic zone method indicated (Table 2) the highest yield in C. coccineum (6.62%) and A.

Table 2: Haemolytic Zone Data of Saponins From the Different Investigated Plants.

Investigated Plant	Radii (R)	Mean R	R^2	Log C	corresponding conc. µg/0.1 ml	% w/w of saponin
Cynomorium coccineum	15,16,14	15.00	225.00	3.52	3311.31	6.62
Cistanche violacea	12,13,14	13.00	169.00	2.82	660.69	1.32
Atractylis carduus	15,14,14	14.33	205.44	3.82	1905.46	3.81
Amaranthus chlorostachys	13,13,15	13.67	186.78	3.04	1096.48	2.19
Amaranthus tricolor	14,14,14	14.00	196.00	3.16	1445.44	2.89
Hemerocalis fulva	11,13,13	12.33	152.11	2.60	398.11	0.80
Bidens pilosa var. radiata	12,11,13	12.00	144.00	2.50	316.23	0.63
Daucus capillifolius	12,13,13	12.67	160.44	2.70	501.19	1.0
Rhanterium suaveolens	14,13,13	13.33	177.78	2.89	776.25	1.55

Chlorostachys (2.19%) while the lowest in Cistanche violacea (1.32 %). In addition, A. carduus showed (3.81%), A. Tricolor (2.89%), and H. fulva (0.8%).

The hypoglycemic activity showed noticeable decrease in blood glucose. Statistical analysis showed significant decrease of glucose at probability <0.05 to < 0.001. Evidenty, the amount of saponin in the parasitic plant C. coccineum (6.62%) and the hypoglycemic activity afforded an answer for using the pulb as decoction by the bedoins in diabetis.

This, to our knowledge is the first report about the yields and percentages of saponin in these investigated plants. A tempting study increasing the yield of saponin will be in progress.

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تقدير كمية الصابونين فى تسع نباتات مصرية

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فى هذا البحث تم تقدير كمية الصابونين فى تسعه نباتات مصرية (صحراوية ومنزرعة) بطريقة تكوين المنطقة الدائرية الناتجة من تكسر كرات الدم الحمراء نتيجة كمية صابونين محددة فى أطباق الآجار بالدم .

وقد وجد أن أعلى نسبة صابونين فى نبات سينوموريوم كوكسينيوم (٦٢ر٦٪) . وكذلك جرى إختبار الخلاصة المائية على فئران التجارب المصابة بمرض السكر وبتحليل النتائج إحصائيا ثبت لهذا النبات تأثير معنوى فى تخفيض سكر الدم .