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Comparison of Various Bioactive Compounds in Leaves and Seeds of *Tribulus longipetalus* Viv.

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ABSTRACT

A study was conducted to analyze different bioactive compounds in Leaves and seeds of *Tribulus longipetalus* include saponins, total proteins, amino acids, fat and flavonoids with one and two dimensional, thin layer and column chromatography followed by spectrophotometric analysis. Results indicate that leaves contained high concentrations of flavonoids and fat. Whereas levels of saponins, proteins, amino acids, total minerals and other organic compounds were high in seeds. The analysis of leaves and seeds of *Tribulus longipetalus* for these valuable organic compounds will provide important raw materials that can be used for preparation of medicines in pharmaceuticals companies. A single subcutaneous dose of CCl_4 (5.93ml kg^{*1}b.wt. which represents 24h LD_{100}) severed as a toxicant, was used in this study. The results showed that ethyl acetate fraction was the most active fraction, then butanol, aqueous and dichloromethane fraction. By subjecting the ethyl acetate fraction to column chromatography it afforded fractions which are effective in reducing CCl_4 induced mortalities. The efficiency of the isolated glycosides in reducing CCl_4 –induced mortalities and some disturbances in biochemical parameters (liver transaminases AST, ALT and creatine kinas CK activities, serum urea and serum creatinine) were investigated in adult female *Bufo regularis* (Egyptian toads). The biochemical studies confirmed the bioassay studies. This suggestes that these saponin glycosides may be promoting in modulating CCl_4- induced lethality and most of its toxic effects.
Keywords: *Tribulus longipetalus*, medicinal plants, chemical analysis, biochemical activity.

INTRODUCTION

Approximately 80% of the worlds population depends exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual but in the recent years, more and more people are complementing their treatment with natural supplements (Dursum *et al*. 2004). Furthermore, motivation of people towards herbs are increasing due to their concern about the side effects of drugs, those are prepared from synthetic materials. Many botanicals and some common dietary supplements are good sources of antioxidants and anti-inflammatory compounds (Leung and Foster, 1996 and Nadkarni, 1976).

Tribulus longipetalus belongs to family Zygophylaceae, which is an annual (Tackholm, 1974). It is a source of phytochemicals hormones (saponins), proteins, amino acids, fats, flavonoids and many other organic compounds. While all of these organic compounds are important for various purposes of human population. The use of these secondary compounds for the treatment of human's illness is indeed very old (Aritomi and Kawasaki, 1984 and Miura *et al*. 1986).

The leaves, stalks and seeds (fruits) of the plant species are edible. It is used as a diuretic and as an aphrodisiac. It is used for treatments. An extract of the plant is made and taken for treatment (Ghazanfer, 1994). Therefore keeping in view the importance of *Tribulus longipetalus* the present study was undertaken with following aims and objectives; 1) to analyze bioactive compounds in leaves and seeds of *T. longipetalus*, 2) to assess the *T. longipetalus* leaves and seeds for saponin and flavonoids, 3) to highlight effects of *Tribulus longipetalus* health of human population.

Carbon tetrachloride CCl_4 is commonly used as typical toxicant reflecting the various aspects of toxicity. Doses of CCl_4 in the range of several mg kg^{-1} body weight produce liver necrosis within one or two hours (Edwards *et al*., 1993). Also, it causes damage to lungs, kidneys, adrenal and central nervous system in humans and experimental animals (Rechangel, 1989; Zhao and O'Brien, 1996; Wong *et al*., 1998)

MATERIALS AND METHODS

Collection and preparation of leaves and seeds samples

The leaves and seeds were collected from North Sinai (April/2006). Sixty samples of leaves and seeds were dried and crushed into powdered form. The prepared samples were stored at room temperature for further process for separation of chemical compounds.

Analysis of total protein, amino acid and lipids

The total protein content of leaves and seeds were determined by using kjeldahal method whereas total lipid content were determined by taking ether extraction by soxhlet apparatus. The percentage of lipid was determined by calculating initial and final weight of samples according to Farag *et al*. (1986).

Analysis of protein and amino acids

The purified samples of leaves and seeds of *T. longipetalus* were subjected to one and two dimensional thin layer chromatography. The Rf values of each amino

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acid was calculated and compared with standard values according to Steven *et al*. (1989).

Analysis of flavonoids and Saponins

Analysis of flavonoid and saponins from leaves and seeds of *T. longipetalus* were carried out in the column chromatography by using silica gel. The columns were washed with various solvents (Diethyl ether, n-propanol, ethanol and methanol etc.) on the basis of their polarity order (Aritomi and Kawaski, 1984). A hundred grains of samples had provided about 10g of purified (Flavonoid or saponin) compounds.

Isolation of the flavonoid compounds

Air-dried aerial parts (1kg) collected from spring samples and seeds (200g) of the plant were separately extracted exhaustively with 80% aqueous ethanol after defatting with petroleum ether using soxhelet apparatus. The ethanolic extract was separately evaporated under reduced pressure and low temperature, then extracted with chloroform. The obtained residue was treated with excess of ethanol and filtered to remove inorganic salts and non-phenolic compounds.

Chromatographic screening of each extract was done on paper chromatography using system BAW (butanol: acetic acid: water 4:1:5 v/v/v) and system AcOH-15% (acetic acid: water 15:85 v/v). The developed chromatograms were air dried, and examined under ultra violet (UV) light, then exposed to ammonia vapor and re-examined under UV light. For isolation of the obtained compounds, polyamide column was used. Elution was started with water and water/methanol in a gradual increasing of methanol amount. The received fractions were concentrated and subjected to preparative paper chromatography (PPC) on Whatman No.3 using BAW (Liu *et al*., 1989). The collected separated bands were eluted in methanol, purified by repeated PPC (in AcOH-15%) and over Sephadex LH-20 column using methanol water system.

For sugar identification, the glycosidic parts were examined with authentic sugar references on PC using BAW (4:1:5).

Physical tests

The purified isolated compounds were characterized by ultraviolet spectrophotometer, ^{1}H and ^{13}C-NMR (nuclear magnetic resonance) (Mabry *et al*. 1970) and mass spectrometric analysis (MS).

Chemical reaction

A known weight of the flavonoid material under investigation was subjected to controlled (mild) or complete (normal) acid hydrolysis and enzymatic hydrolysis (Harborne *et al*., 1975).

Bioassay studies

(1) Animals and treatments

Adult female *Bufo regularis* (Egyptian toad), weighing about 25±4.3g, were used throughout the studies. The animals were housed in groups of 20 in glass cages covered with a wire galvanized meshes. The bottom of these glass cages were covered with a wet sponge. Food (earth worms) was available *ad libitum*. In order to minimize possible nutritional effects, both experimental and control animals were fasted for 18h before sacrifice.

(2) Carbon tetrachloride

The CCl_4 (Analar) was used as the toxicant agents .It was diluted with com oil to deliver the proper dosage in a volume of 10ml kg^{-1} b.wt. All doses were given as a single subcutaneous injection.

(3) LD100 measurement

Animals were divided into groups of 20 and were administered CCl_4 at various doses. Mortalities were recorded at 24h after CCl_4 injection. 24h LD_{100} was estimated. It was found 5.93ml kg^{-1}.b.wt.

(4) Most effective concentration extract

The dried alcoholic extract of *T. longipetalus* was dissolved in methanol to prepare the desired dose in a volume of 1ml kg^{-1} b.wt. Preliminary experiments were carried out to determine the most effective concentration of the alcoholic extract that can reduce the mortality –induced by CCl_4. It was found to be 60mg kg^{-1}b.wt. When administered subcutaneously. The animals were divided into 5 groups, 20 animals in each group (Table 4). Mortalities were recorded after 24h of CCl_4 injection.

(5) Effect of fractionated extract

The fractions of the alcoholic extract (dichloromethane, ethyl acetate, butanol) and the aqueous fraction left after fraction of the alcoholic extract with the organic solvents were dissolved in methanol to prepare the desired doses in a volume of 1ml kg^{-1} b.wt. Preliminary experiments proved that s.c injection of 50mg of each fraction/kg b.wt. was the most effective concentration in reducing CCl_4 inducing lethality. The animals were divided into 10 groups 20 animals in each group (Table 5). Mortalities were recorded after 24h following CCl_4 injection. Ethyl acetate proved to be the most potent one in reducing the CCl_4 induced mortality.

RESULTS AND DISCUSSION

Results obtained after analysis of leaves and seeds of *Tribulus longipetalus* indicated that flavonoids, saponins, proteins, amino acid and fats were present in these samples. The leaves contain high concentration of flavonoids and fat. Whereas the level of saponins, protein, amino acids and other organic compounds were high in seeds. (Tables 1-3). The seeds of *Tribulus longipetalus* are considered as essential ingredients for many local medicines those can be used against stomach, kidney and liver infection and disorders (Etherton, 2002; Matin *et al*. 2002). The organic compounds obtained from seeds will further increase the market values of these valuable medicinal plants. The young and fresh leaves are considered as delicious and traditional vegetables in many areas of Sinai

(Karnick, 1998). Furthermore, seeds (fruits) are being use in all most all of houses of this region for many purposes of human population, whereas leaves are mostly used as vegetables either cooked or in the form of salad (Zaidi, 1998).

The simultaneous administration of the alcoholic extract of *Tribulus longipetalus* (60mg kg^{-1} b.wt.s.c.) and CCl_4 (5.93ml kg^{-1}b.wt. s.c. which represents the 24h LD_{100}) reduced the percentage of mortality to 65% (Table 4). The results indicated that the injection of the ethyl acetate, butanol, aqueous and dichloromethane extracts (50mg kg^{-1} .b.wt. s.c.) decreased the percentages of CCl_4 – induced mortality to 50, 60 and 75% respectively (Table 5). In this study, results indicated that alcoholic extracts of *Tribulus longipetalus* was effective in reducing CCl_4 –induced lethality (Table 4). Results also indicated that the ethyl acetate extract was the most effective fraction in reducing CCl_4 induced mortality. The efficacy of the extract was as follows: butanol > aqueous > dichloromethane (Table 5)

The crude extract of medicinal plant studied were found to contain one or more of the following phytochemical compounds saponins, saponin glycosides, flavonoids, fats and amino acids. The effect of these medicinal plant on the Bufo regularis may be due to the presence of above phytochemical components.

The results of the present study showed the presence of flavonoids and glycosides in fraction of *Tribulus longipetalus*. Alkaloids and steroids were absent from the fraction of *Tribulus longipetalus* although they were originally present in the crude extract. Harborn (1984) reported that the activity of plant extracts can sometimes change after fractionation and pure crystalline compound may eventually be obtained which lacks the activity of the original extract.

Table (1): The percentage of different chemical compounds in leave and seeds of *Tribulus longipetalus*.

Chemical compounds	Seeds	Leaves
Dry matter	81.10	75.6
Total protein	24.12	18.5
Total fat	20.90	25.6
Total fiber	9.50	11.5
Total mineral	9.50	12.5
Moisture	8.90	20.5

Table (2): The percentage of saponins, Flavonoids and Amino acids in leaves and seeds of *Tribulus longipetalus*.

Sample	Saponins		Flavonoids		Amino Acids		
	Seeds	Leaves	Seeds	Leaves		Rf Values of Seeds	Leaves
1	31.10	25.1	26.17	46.14	Glycine	0.45	0.48
2	33.12	15.5	17.10	37.19	Leucine	0.46	0.58
3	30.10	25.4	14.16	34.16	Isoleucine	0.78	0.75
4	29.50	11.9	9.20	39.20	Proline	0.57	0.51
5	19.20	12.5	11.25	29.25	Phynylalanine	0.47	0.49
6	38.16	20.6	8.16	38.17	Tryptophane	0.58	0.52

Table (4): Effect of alcoholic extract (60mg kg^{-1} b.wt.) on the mortality of *Bufo regularis*.

Studied groups**	Number of mortalities	% of mortality
Control	0	0
CCl_4	20	100
Methanol*	20	0
Alcoholic extract*	20	0
CCl_4 +alcoholic extract	13	65

* means that these groups served as negative controls
**means that number of animal used in each studied group n= 20

Table (3): Spectophotometric analysis (470nm) of leaves and seeds of *Tribulus longipetalus* after extraction of various compounds with different solvents.

Solvents	Leaves				Seeds			
	Compounds	Concentration	Absorbance at 470nm	pH	Compounds	Concentration	Absorbance at 470nm	pH
Diethyl ether	1	1.718	1.704	7.5	1	1.718	1.704	7.5
	2	1.556	1.566	7.4	2	1.556	1.566	7.4
	3	1.136	1.136	7.5	3	1.136	1.136	7.5
n-Propanol	1	1.398	1.308	7.3	1	1.396	1.308	7.3
	2	1.657	1.655	7.45	2	1.657	1.655	7.45
	3	1.771	1.701	7.6	3	1.771	1.701	7.6
Ethanol	1	0.413	0.388	7.2	1	0.412	0.388	7.2
	2	0.351	0.272	7.1	2	0.351	0.272	7.1
Methanol	1	0.428	0.511	7.6	3	0.228	0.211	7.6
	2	0.461	0.513	7.9	1	0.427	0.511	7.6
	3	0.431	0.524	7.7	2	0.461	0.513	7.9

1 means total proteins, **2** means flavonoids, **3** means saponins

Table (5): Effect of fractionated extract (50mg kg^{-1}b.wt.) on the mortality of *Bufo regularis.*

Studies groups	Number of mortalities	% of mortality
Control	0	0
CCl_4	20	100
Dichloromethane extract	0	0
CCl_4+dichloromethane extract	15	75
Ethyl acetate extract	0	0
CCl_4+ Ethyl acetate extract	10	50
Butanole extract	0	0
CCL_4+ Butanole extract	11	55
Aqueous extract	0	0
CCl_4+ Aqueous extract	12	60

* means that these groups served as negative controls
**means that number of animal used in each studied group n= 20

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مقارنة في المركبات الحيوية المختلفة لأوراق وبذور نبات "تريبيولس لونجيبتلس"

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الملخص العربـــى

النباتات الطبية تستخدم ضد العديد من الأمراض المعدية منذ قديم الأزل. وتستخدم الأعشاب كطعام مثل الخضروات ومكسبات الطعم منذ مئات السنين في أماكن عديدة من العالم بينما تستخدم العديد من الأعشاب كعلاجات طبيعية لمعظم ألام الجنس البشري. وفضلا عن ذلك بعض النباتات العشبية تعتبر بيت الأدوية وتلعب دورا هاما فيما يقرب كل ثقافة على الأرض بما في آسيا وأفريقيا وأوروبا وأمريكا .

أجريت دراسة لتحليل الأوراق والبذور لنبات تريبيولس لونجيبتالس والعديد من المواد الفعالة البيولوجية منها الصابونيات والبروتينات الكلية والأحماض الامينية والدهون والفلافونيدات بطريقة الطبقة الرقيقة ذات الاتجاهين وبدون عمود الفصل اللوني يتبعها التحليل الضوئي . وأوضحت النتائج أن الأوراق تحتوي على تركيزات عالية من الفلافونيدات والدهون بينما في البذور كانت النسبة أعلى للصابونيات والبروتينات والأحماض الامينية والمعادن الكلية والمركبات العضوية الأخرى . تحليل الأوراق والبذور لنبات تريبيولس لونجيبتالس أوضحت أن هدا النبات عبارة عن مادة خام جيدة لتصنيع العديد من العلاجات.

تمت دراسة تأثير الجليكوسيدات المفصولة على خفض معدلات الموت في إناث الضفادع البالغة المحقونة برابع كلوريد الكربون وتمت دراسة الاختلافات في بعض قياسات الكيمياء الحيوية مثل إنزيمات الكبد والكلى في مصل دم الضفادع ومن الدراسة تبين أن مستخلص الاثيل اسيتات + رابع كلوريد الكربون أعطى أقل نسبة وفيات وهي 50% (50 جم لكل كجم من وزن الجسم). وهذا يؤكد الاقتراح بأن الجليكوسيدات الصابونينية يمكن أن تحفز في تعديل معدلات الموت بواسطة رابع كلوريد الكربون ومعظم تأثيراته السامة.