

Pharmacochemical Studies of the Oil, Aerial Parts, Pulp and Peel of *Citrullus colocynthis*

¹M. A. KALHORO, ¹N. AFZA, ¹M. SALEEM AND ²A. MALIK

¹Pharmaceutical Research Centre

PCSIR Laboratories Complex, Karachi-75280, Pakistan

²H.E.J. Research Institute of Chemistry,

University of Karachi, Karachi-75270, Pakistan

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Summary: The fixed oil of *Citrullus colocynthis* Schrad seeds (*Cucurbitaceae*) has been investigated for its physico-chemical characterization and the fatty acid composition. The toxicological and hypoglycemic properties of the oil and various crude extracts of *C. colocynthis* have also been investigated.

Introduction

Citrullus colocynthis Schrad, (*Cucurbitaceae*) is a large creeping herb, which is widely distributed in Punjab, Sindh, Balochistan and on the ceremonial coast [1-2]. The plant is well reputed for its therapeutic activity in folklore. The fruits of this plant are useful in biliousness constipation, fever and intestinal parasites. The roots are used in ascites, Jaundice and urinary disease, boils, pimples, rheumatism and enlargement of abdomen of children [1]. Antiinflammatory and insecticidal activities of various parts of this plant has also been reported in literature [3].

Sifting of literature has revealed that a variety of compounds including oil have so far been reported from *C. colocynthis* [4-6]. The infusions of this plant are traditionally used in Mediterranean countries as antidiabetic medication and this activity was also confirmed by Nimila *et al.* [7]. This has prompted us to further exploit the therapeutic utility of *Citrullus colocynthis*. In this paper we have determined the diabetic and toxicological potentials of the extracts obtained from various parts of this plant. The paper also includes a detailed study of the oil of *Citrullus colocynthis* with particular reference to its extraction

in acceptable yield and determination of its various parameters.

Results and Discussion

The fatty acid composition and physico-chemical characteristics of the oil (Tables I and II) showed that the latter can find applications in paint and soap industries. The major fatty acids were found to be palmitic, oleic and linoleic, which are widely used in soap, paints and lubricant industries. The Table-III shows that the plant is practically nontoxic while the seed extracts showed the maximum tolerable dose. Therefore the plant can be classified as harmless for grazing animals. The hypoglycemic activity of the thanolic extracts of various parts of this plant (hexane extract in case of seeds) was also carried out and presented in Table IV. The hexane extract of seeds showed moderate activity and can be used as a nontoxic hypoglycemic drug.

Experimental

1. Extraction of oil

The fresh and undried seeds (100 g) of *Citrullus colocynthis* were crushed and extracted with

n-hexane (b.p. 68 ± 2 °C) in a soxhlet apparatus. The extract was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to yield 20g pale yellow oil. The physicochemical properties of the oil were determined by standard methods and are given in Table-1.

Table-1: Physico-chemical properties of the oil of *Citrullus colocynthis*

Refractive index	1.4642	Specific gravity	0.8886
Tintometer No.	20Y+2R	Acid value	21.44
Iodine value	144.47	Saponification value	175.85
Acetyl value	17.92	Peroxide value	1.18
Ester value	52.28	Ester value (after saponification)	221.2
Unsaponifiable matter	2.4%		

2. Saponification of oil:

The oil (300mg) was refluxed with 0.5 M methanolic potassium hydroxide (50ml) for 5h. The reaction mixture was taken in water and extracted with ether to remove the non saponi (table matter). The aqueous layer was acidified with 4N sulphuric acid and the liberated fatty acids were extracted with ether, the organic phase dried over anhydrous sodium sulphate and the solvent removed under the nitrogen atmosphere.

3. Esterification of fatty acid

The fatty acids obtained from the above procedure were converted to their corresponding methyl esters through reaction with ethereal diazomethane at low temperature. The esters thus formed were kept under nitrogen for gas chromatography.

4. Examination of methyl esters of fatty acids by G.C.

The percentage composition of the fatty acids was determined by GC of the esterified fatty acid on Shimadzu 221-25412 using an SP-2310-3% Sp-3100 column.

Operational conditions

Column temperature	22°C
Carrier gas	Nitrogen
Flow rate	30 ml/min
FID temperature	300 °C

The identification of fatty acid esters was carried out by running a standard mixture of methyl

esters of fatty acids under identical conditions and comparing their respective retention times.

Table-II: Fatty Acid Composition of the Seed Oil of *Citrullus colocynthis*

Fatty Acids	Percentage
Palmitic acid	15.198
Oleic acid	24.3
Linolenic acid	0.996
Tricosanoic acid	0.496
Tribecylic acid	0.21
Stearic acid	9.243
Linoleic acid	46.6
Arachidic acid	0.786
Margaric acid	0.24

5 Determination of toxicity of various extracts of *Citrullus colocynthis*

Toxicology is approached as the study of the effect of external agents on biological system with emphasis on the mechanism of harmful effects of chemicals and the condition under which these effect occur. The toxicity is either instantaneous or require

Table III

Name of Extract	Part of Plant	No. of Animals	Dose/Subject	Toxic effect
Pet ether Ext.	Leaves & stems	3+3(6)	1-2.5mg	Non toxic
	Roots	3+3(6)	1-2.5mg	Non toxic
	Fruit pulp	3+3(6)	1-2.25mg	Non toxic
	Fruit peel	3+3(6)	1-2.25mg	Non toxic
	Seeds	3+3(6)	1-2.0mg	Non toxic
Chloroform Ext.	Leaves & stems	3+3(6)	1-2.20mg	Non toxic
	Roots	3+3(6)	1-2.0 mg	Non toxic
	Fruit pulp	3+3(6)	1-2.25mg	Non toxic
	Fruit peel	3+3(6)	1-2.25 mg	Non toxic
	Seeds	3+3(6)	1-1.0mg	Non toxic
Ethanol Ext.	Leaves & stems	3+3(6)	1-2.0mg	Non toxic
	Roots	3+3(6)	1-1.8mg	Non toxic
	Fruit pulp	3+3(6)	1-2.0mg	Non toxic
	Fruit peel	3+3(6)	1-1.8mg	Non toxic
	Seeds	3+3(6)	1-1.5mg	Non toxic
Aqueous Ext.	Leaves & stems	3+3(6)	1-100 mg	Non toxic
	Roots	3+3(6)	1-100mg	Non toxic
	Fruit pulp	3+3(6)	1-95mg	Non toxic
	Fruit peel	3+3(6)	1-90mg	Non toxic
	Seeds	3+3(6)	1-80mg	Non toxic
	Fruit juice	3+3(6)	1-85mg	Non toxic

The propylene glycol is used as control for pet ether and chloroform extracts, whereas water is used for ethanol and aqueous extract. The controls were fed on normal diet with water and propylene glycol.

short delay from the absorption of an adequately large single dose of the compound.

The toxicity of the petroleum ether, chloroform, ethanolic and aqueous extracts of various parts of this plant were tested against mice for toxicology. The mice of either sex (20-30 g) were kept in plastic cages and orally administered the relevant extracts taken either in distilled water or propylene glycol. After administering the extracts, normal feed was given to the animals and the later were kept under observations for 72 hours to locate the signs of toxicity. The experiments were repeated in each case with 6 animals.

Table-IV

Identification	No. Animals	Diet schedule	Initial reading mg/dl (serum)	Final reading mg/dl (serum)
Control	3+3 (6)	Normal diet	93.5 mg/dl	92.8 mg/dl
Ethanollic ext. of aerial part	3+3 (6)	Normal diet + 5gm of extract	94.5mg/dl	81.3 mg/dl
Ethanollic ext. of roots	3+3 (6)	Normal diet -I 5gm of extract	90.0 mg/dl	76.6 mg/dl
Ethanollic ext. of pulp	3+3 (6)	Normal diet + 5gm of extract	92.4 mg/dl	70.0 mg/dl
Ethanollic ext. of seeds	3+3 (6)	Normal diet + 5gm of extract	89.9 mg/dl	65.2 mg/dl
Hexane ext. of seeds	3+3 (6)	Normal diet + 5gm of extract	89.9 mg/dl	61.5mg/dl

6. *Hypoglycemic activity of crude ethanolic extracts of aerial parts, root, pulp, seeds and the crude hexane extract of the seeds of Citrullus colocynthis*

The crude ethanolic extracts of the aerial parts (leaves & stems), roots, pulp, seeds and hexane extract of seeds of the plant, were tested against white rabbits for hypoglycemic activity. The animals of either sex of about 2 kg body weight were orally administered the respective extracts (5 g each) with the help of cannula and the test animals were kept in plastic cages. Each animal received a single dose per day for 7 days. During this period the animals were kept on normal diet. After seven days, the blood samples of all animals were obtained from the ear vein for final reading whereas the initial readings were recorded before treating the animals with extract.

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