

Scientific Studies on *Citrullus Colocynthis* with Special Reference to Anti-hyperglycemic Activity

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Abstract: *Citrullus colocynthis* is a plant used as a traditional medicine for the treatment of diabetes mellitus, one of the most prevalent endocrine disorder throughout the world with special reference in the Maghreb region and in the Middle East. The present study is based on laboratory experiments for pharmacognostic, physicochemical, phytochemical, elemental analysis, TLC (thin layer chromatography) fingerprinting, pharmacological & toxicological studies with special reference to anti-diabetic activity, along with an exhaustive scientific review on *Citrullus colocynthis* phytochemical and pharmacological activities described in literature. We investigated the anti-hyperglycaemic effect of aqueous extract of *Citrullus colocynthis* seeds administered intraperitoneally in normal Wistar rats and streptozotocin in diabetic rats. The toxicity of this extract was also evaluated using lethal dose 50 methods (LD₅₀).

Key words: *Citrullus colocynthis*, pharmacognostic, physicochemical, phytochemical, elemental analysis, TLC fingerprinting, pharmacological & toxicological studies, anti-hyperglycemic activity.

1. Introduction

The plant is very common in sandy places. *C. colocynthis* presents a curious look with patches here and there in the extensive blazing deserts of all the Arab countries. The plants are suffering from winter and recovering again in summer. They have wonderful adaptation ability to grow upon the hot sandy soil of summer months where scarcely any plant can survive. The tap root is succulent and very deep [1]. Throughout Arabia [2], the plant is wide spreading in UAE (United Arab Emirates) except in the western and southern part of the country.

Scientific name: *Citrullus colocynthis* (L.) Schrad.

Synonymy: *Colocynthis vulgaris* Schrad, *Cucumis colocynthis* L.

Local name: Handal, Murrah, Shary, Serew, Hanzal, Suri, Hedge.

Arabic name: Handalm Shary, Serew, Hanzal, Suri, Hedge.

Common name: Colocynth, Bitter apple, Bitter gourd, Desert squash.

Family: Cucurbitaceae (see Fig. 1).

2. Traditional and Medicinal Uses

The leaves are diuretic and used in treatment of jaundice and asthma. The root is useful in inflammation of breasts, amenorrhea, rheumatism, joint pains and is used externally in ophthalmia and uterine pains. The fruit is pungent, cooling purgative, anthelmintic, antipyretic and carminative. It cures, tumors, leukoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemias, and throat diseases. The fruit pulp is purgative, diuretic, anti-epileptic, and is used against gonorrhea [1].

The leaves, seeds, roots and dried fruit are used to treat dog, insect and snake bites, as a laxative, to relieve pain in joints and as a hair colour. Powdered leaves mixed with water are taken as a laxative; crushed roots are mixed with goat's milk and used as a purgative and to treat colic. In Yemen the seeds which are also used as a purgative, are mixed with other food to reduce its strong laxative action. Seeds crushed with water are used as shampoo to darken hair colour and crushed fruit mixed with oil is rubbed to

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relieve pain in joints [2]. Very powerful purgative or hydragogue cathartic, emetic and gastrointestinal irritant. It is usually given with hyoscyamus to prevent gripping. It is not given in case of pregnancy. It stimulates hair growth [3].

3. Pharmacognosy & Phytochemistry

A transverse section of the seed shows that the seed coat (testa) consists of a thick palisade-like epidermal layer of long thickened cells of anticlinal walls having brown rod-like structures giving a characteristic flame-like appearance. The rod-like structures do not extend to the outer part of the testa. The inner side of this layer is adjacent to a grayish yellow layer of small sclerenchymatous cells with thick pitted walls.

This is underlain by a number of other sclerenchymatous layers which gradually become large starting from the layer below the epidermis towards the inner part of the seed. Larger sclerenchymatous cells have yellow colors and they are strongly packed

together giving characteristic block-like structures. They are heavily thickened with narrow longitudinally branching lumens. The yellow endospermic cells are polygonal and they contain oily droplets.

4. Powdered Plant Material

The material consists of the crushed seeds of the plant. It is a greasy yellow brown coarse gritty powder that has a slight characteristic odour and a very bitter persisting taste. Microscopically, the powder shows light brown thick sheet-like fragments of seed testa layers. It shows the testa palisade-like epidermal layer with brown rod-like structures having flame-like appearance in addition to isolated groups of light yellow small sclerenchyma cells of the testa; occasional individual cells are also observed. It also shows larger yellow-colored sclerenchyma cells strongly packed and heavily thickened with narrow longitudinally branching lumens. Yellow endospermic cells are polygonal and they contain oily droplets (Fig. 2).



Fig. 1 Whole plant and fruit.

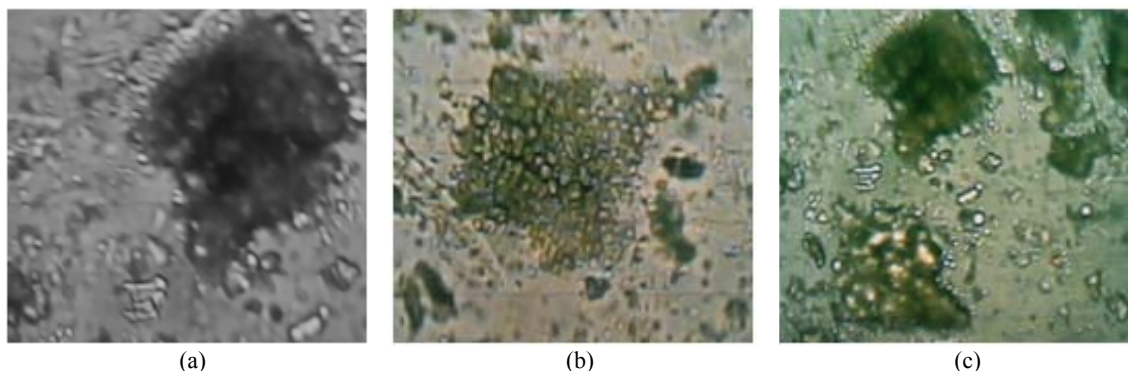


Fig. 2 (a) fragment of the seed showing the seed testa with the characteristic epidermis composing of yellowish to brown palisade cell thickened with rods giving a flame-like appearance (flame cells); the other dark part of the fragment is composed of layers of the sclerenchyma of the testa with thick pitted walls. (b) A fragment of the seed endosperm showing its yellow-coloured polygonal cells that contain oil droplets. (c) Fragments of the seed showing different tissues, different types of cells and, cell contents and oil droplets.

5. Chemical Constituents

Constituents of seeds: alkaloid, steroid glycosides and flavonoids [4]. Sterols, citbittol, Glucose and α -spinasterol from fruits and seeds.

(α -elaterin-2D-glucopyranoside) and α -elaterin (cucurbitacin E) and citrullol from pulp. Glycosides in fruit: elaterin, elatericin B (cucurbitacin I) and dihydroelatericin B (cucurbitacin L). Myristic, palmitic, stearic, myristoleic, palmitoleic, oleic, linoleic acids and citrullonol from seed oil. Hentriacontane, n-octacosanol and 1,26-hexacosanediol. Linoleic (51.3%), oleic (24.1%), palmitic (12.8%) and stearic acids (9.8%) in seed oil.

Cucurbitacins B, E, I, cucurbitacin E-2 glucoside, Quercetin and kaempferol in leaves, fruits and flowers. hentriacontane, elateridine, hexanorcucurbitacin I and its 16-O-acetyl derivative and Cucurbitacin T from fruits. glycoside as 2-O- β -D-glucopyranosyl-22,23,24,25,26,27-hexanorcucurbitacin I and 2-O- β -D-glucopyranosylcucurbitacin I, 2-O- β -D-glucopyranosylcucurbitacin E and 2-O- β -D-glucopyranosylcucurbitacin L [5-9]. glycosides: elaterin (cucurbitacin E), elatericin B (cucurbitacin I) and dihydroelatericin B (cucurbitacin L) [10].

Cucurbitacin glycosides: 2-O- β -d-glucopyranosyl-cucurbitacin I, 2-O- β -d-glucopyranosyl-cucurbitacin E,

2-O- β -d-glucopyranosyl-cucurbitacin L and the novel glycoside, 2-O- β -d-glucopyranosyl-[5-9] hexanorcucurbitacin I [11]. Flavones C-glycosides in the fruits which contained: isovitexin, iso-orientin, iso-orientin 3'-methyl ether [12]. Flavone glucosides in the fruits: isosaponarin, isovitexin and isoorientin 3'-O-methyl ether. Two cucurbitacin glucosides: 2-O- β -D-glucopyranosylcucurbitacin I and 2-O- β -D-glucopyranosylcucurbitacin L [13] in fruits: 2-O- β -D-glucopyranosylcucurbitacin E [14].

The following chemical studies have been carried out at ZCHRTM on the seeds of *Citrullus colocynthis*.

(1) Physicochemical constants (%)

Loss of weight in drying at 105 °C: 6.20

Absolute alcohol solubility: 18.80

Water solubility: 6.85

(2) Successive extractives (%)

Petroleum ether (60-800): 1.96

Chloroform: 1.70

Absolute alcohol: 2.85

(3) Ash values (%)

Total ash: 2.49

Water soluble ash: 1.17

Acid insoluble ash (10% HCl): Nil

(4) pH values (aqueous solution)

pH of 1% solution: 5.846

pH of 10% solution: 5.697

(5) Elemental analyses (Table 1)

Table 1 Elemental analyses.

Ash values (British Herbal Pharmacopeia-Reference)					
Assay and identification of metal (AOAC International- Reference)					
Apparatus	(AA-6800 Shimadzu-Flame method)				
Element	Std. conc. $\mu\text{g/mL}$ (ppm)	Sample conc. mg/mL	Samples absorbance	Actual conc. mg/mL	Actual conc. (%)
Cr	1, 2, 4	20.056	0.0000	0.00000	0.00000
Zn	0.5, 1, 2	20.056	0.1055	0.050555	0.0050555
Cu	0.5, 1, 2	20.056	0.0198	0.0094450	0.009445
Fe	1, 2, 4	20.056	0.0104	0.0199350	0.0019935
K	1, 2, 4	20.056	1.5608	0.883215	0.0883215
Pb	1, 2, 4	20.056	0.0024	0.01265	0.001265
Cd	0.25, 0.5, 1	20.056	0.0000	0.0000	0.0000

1 ppm conc. = 1 $\mu\text{g/mL}$; Actual conc. (%) = Actual conc. (ppm) $\times$ 0.0001 [1 ppm = 0.0001%].

(6) UV spectral studies (Table 2)

Table 2 UV spectral studies.

Ultraviolet Spectrum (USP reference)				
Apparatus	Milton Roy Spectronic Genesys 5 Spectrophotometer - Milton Roy.			
Sample conc. (mg/mL)	Solvent	λ_{max} (nm)	λ_{min} (nm)	Abs. ($\lambda_{\text{max}}\text{-}\lambda_{\text{min}}$)
4.6	Intestinal fluid simulated without pancreatic pH = 7.5 ± 0.1 (Fig. 3)	274	259	1.008-0.957
-	Gastric fluid simulated without pepsin pH = 1.2 ± 0.1	No shift	No shift	-

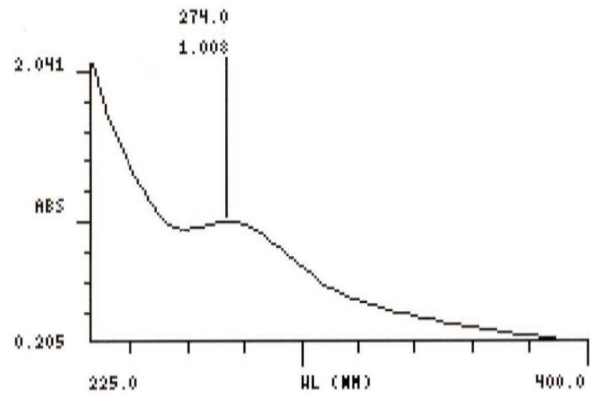


Fig. 3 Intestinal fluid simulated without pancreatic pH = 7.5 ± 0.1

(7) TLC (thin layer chromatography) (Fig. 4)

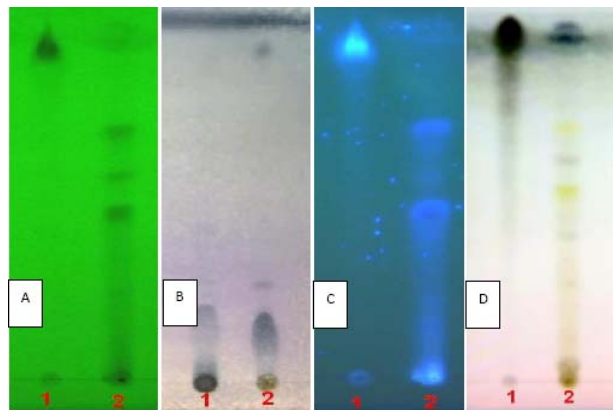


Fig. 4 TLC fingerprint of Pet. ether (60-800) extract (track 1) and MeOH extract (track 2).

Mobile phase Fig. A, C & D: Ethyl acetate, methanol, water (100:13.5:10), B: Toluene, ethyl acetate (93:7), Detection A: UV 254 nm C: UV 365 nm, Derivatization B & D: Vanillin-Sulphuric acid-vis.

6. Pharmacological and Toxicological Studies

The important pharmacological and toxicological activities of the plant *Citrullus colocynthis* reported in

various scientific journals have been presented in the present brief review:

The methanolic extract from the fruit of *C. colocynthis* showed an inhibitory effect on ear passive cutaneous anaphylaxis reactions as a type I allergic model in mice [15]. *C. colocynthis* pulp extract is a long-serving laxative [16]. In UAE many traditional plants such as the *Citrullus colocynthis* (Handal) are used as antidiabetic remedies. The plasma level of ALT (alanine aminotransferase), ALP (alkaline phosphatase), AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase), and LDH (lactic dehydrogenase) increased significantly after the onset of diabetes. Oral administration of the plant extract reduced the plasma level of AST and LDH significantly. However, the plant extract failed to reduce the increased blood level of GGT and ALP in diabetic rats. BUN (blood ureanitrogen) increased significantly after the onset of diabetes. No significant difference was observed in the blood creatinine, K^+ , Na^+ , Ca^{2+} and P levels of normal and diabetic rats. The plant extract did not have any effect on BUN level; however, it caused an increase in the level of K^+ , Na^+ in diabetic rats. In conclusion, oral administration of the aqueous extract of the *C. colocynthis* can ameliorate some of the toxic effects of streptozotocin [17].

A crude 50% ethanol extract of *C. colocynthis* was administered orally to male albino rats for evaluation of antifertility effects. A 50% ethanol extract of *C. Colocynthis* showed an anti-androgenic nature, thereby reduced reversible infertility in male albino rats [18].

Table 3 Pharmacological and safety evaluation studies on the *Citrullus colocynthis* seeds (aqueous extract).

Activity	Results			
	Strong	Moderate	Mild	Negative
Anti-diabetic	√			
Analgesic	√			
Antidepressant				√
Effect on rabbit jejunum	√			
Effect on rat fundus	√			
Effect on Guinea pig ileum	√			
Effect on right rat atria		√		
Locomotor↓	√			
Motor co-ordination & grip strength↓	√			
Rectal temperature↓	√			
Lethality↑	√			

Feeding the mixture of *C. colocynthis* caused more pronounced effects and death of rats. Vital organlesions accompanied by anemia and leucopenia were correlated with changes in serum ALP, AST and ALT activities with alterations in concentrations of total protein, albumin, urea and other serum constituents. Serum bilirubin concentration did not change [19]. *Citrullus colocynthis* extract was found to be free of hepatotoxic effects in concentrations up to 100 mg/mL incubation mixture when liver slices were incubated in William's medium E for 22 h. Carbon tetrachloride induced hepatotoxicity could not be prevented or alleviated. Moreover, the damage was sometimes enhanced by higher extract concentrations [20].

Infusions of *C. colocynthis* Schrad (Cucurbitaceae) fruits are traditionally used as antidiabetic medication in Mediterranean countries. The present study showed that different *C. colocynthis* seed extracts have an insulin tropic effect which could at least partially account for the antidiabetic activities of these fruits. Aqueous extract of the rind of *C. colocynthis* possesses a hypoglycemic effect and its hypoglycemic action could be attributed for more extent to the presence of saponin [21]. Plant has been reported to possess anti-diabetic; diuretic, anti-epileptic, anti-blennorrhoea, and effects against gout and ascites [22].

Acute toxicity and histopathological effects of saponin (extracted from the plant *C. colocynthis*) on mice was assessed. The median lethal dose (LD₅₀) of

the saponin was 200 mg/kg. The histological changes were confined to the small intestine, liver and kidney, whereas the stomach, large intestine and heart appeared normal [23].

Oral administration of 0.25 g/kg/day dose of *C. colocynthis* fruits or 0.25 g/kg/day of *R. stricta* leaves for 42 days proved to be not fatal but, that the mixture of the two plants (0.25 g + 0.25 g/kg/day) proved fatal within 26 days with profuse diarrhea, dehydration, loss in condition, ataxia and decumbency, prior to death [24]. Feeding the mixture of *C. colocynthis* and *N. oleander* caused more marked effects and death of rats [25].

The following pharmacological and safety evaluation studies were carried out on the *Citrullus colocynthis* seeds (aqueous extract) in our lab (Table 3).

7. Summary of the Results

The extract showed significant anti-nociceptive activity in the hot-plate test. The plant extract caused a strong spasmogenic activity which provides a scientific basis for its use in regulation of bowel function through rapid transit and relieving constipation (laxative activity). Overdose may produce gastrointestinal disturbance (Stomach upset; Diarrhea). The effect on isolated rat fundus showed the purgative nature of the plant extract. The plant extract revealed positive inotropic agent increasing myocardial contractility are used to support cardiac function. The plant extract

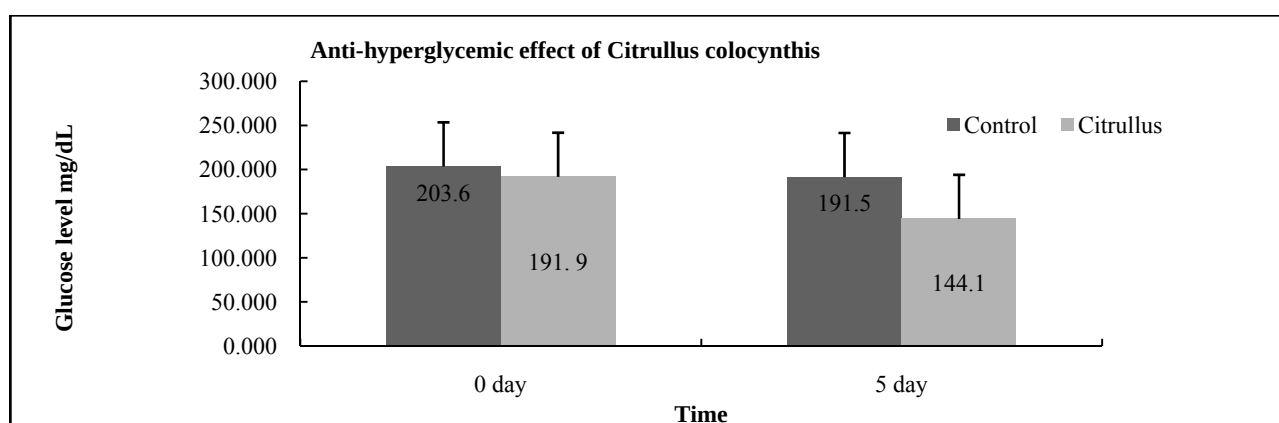


Fig. 5 Anti-hyperglycemic effect of *Citrullus colocynthis*.

failed to show the anti-depression activity. Oral administration of the plant extract 200 and 250 mg/kg proved not fatal but showed profuse diarrhea, gross lack of coordination of body movement and showed state of leaning, resting, or reclining. Significant anti-hyperglycemic activity was observed as shown in conclusion (Fig. 5).

8. Conclusions

The results obtained show an anti-hyperglycaemic effect of *Citrullus colocynthis* seed extracts on diabetic rats, significant hypoglycemic effect in mice and a stability of the blood glucose level to normal values on the normal rats.

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