International Journal of Pharma Sciences
Vol. 8, No. 4 (2018): 1965-1974
Research Article
Open Access

ISSN: 2320-6810

Phytochemical Properties and Anti-diabetic Effects of *Citrullus Colocynthis* extract in Alloxan induced-Diabetic Rats

Medhat Fawzy Mahmod[1], Abdel-Sadek Abo Atta Youssif[1], El-Kalesh Moastafa El-Kalesh[2], Hamad Ameen El-Sadawy[1] and Mohammed Abdalla Hussein[3,*]

[1] *Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.*
[2] *Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.*
[3] *Biochemistry Department, Faculty of Applied Medical Sciences, October 6 University, Egypt.*

* Corresponding author: Mohammed Abdalla Hussein, e-mail: prof.husseinma@o6u.edu.eg

Received: 04 July 2018 Accepted: 10 August 2018 Online: 25 August 2018

ABSTRACT

Recent trends in controlling and treating diseases tend to prefer natural drugs rather than synthetic ones.Plants are considered to be the nature's Green pharmacy, which provide drugs to maintain good health of human being. The medicinal value of these plants lies in its constituents like alkaloids, cardiac glycosides, saponins, flavonoids, volatile oils, steroids and minerals. The seeds of Citrullus colocynthis were analysed for chemical, physicochemical and phytochemical properties using standard methods. The seeds contained moisture 6.96g,ash 1.98g,protien 22.31g, fiber 2.72g,lipid 52.37 g, phospholipids 12.75 g and carbohydrate 14.22 and in mg / 100 g DM, phosphorus 510.27 and calcium 52.17. The seeds were found to be slightly acidity with a pH value of 5.27. Other physical characters include specific gravity 1.0123, titratable acidity 1.79 , total soluble solids 8.81 and refractive index 1.4332. Oil of kernel 54.97± 0.91 %, oil of the unshelled seed 43.3±0.73%, moisture content 0.17 ± 0.04 %, free fatty acid content 0.67± 0.17% , iodine value 107.9± 1.8 , saponification value 210.8± 5.31, peroxide value 6.51±0.03 and unsaponifiable matter content 0.98 ± 0.07 g. The values of all the analysis are the average of six replicates. The identification of the active ingredients (glycosides, alkaloids and saponins) by using phytochemical technique such as chemical tests,colour reactions and thin layer chromatography is very important and support the chemical and physicochemical analysis. Also, the hypoglycemic and antihyperglycemic effects of an alcoholic extract of the fruit of Citrullus colocynthis was investigated in both normal and alloxan- induced diabetes in adult albino rats. Blood samples were collected before and at 0.5, 1, 2, 4, 6, and 8 h. after the oral administration of 100, 200, and 300 mg/kg. Graded dose of the extract given to both normal and diabetic rats produced significant reductions in blood glucose at 6 h. after extract administration (P< 0.001). The effect was found to be dose dependent with all treatments at the doses administered. The present study clearly indicated a significant antidiabetic activity of the fruit of Citrullus colocynthis.

Keywords: *Citrullus colocynthis*, phytochemical technique, antidiabetic activity, unsaponifiable matter, antihyperglycemic.

1. INTRODUCTION

Citrullus colocynthis (L), family cucurbitaceae. A family with 121 genera and 1760 species distributed in the tropical regions. It is now widely distributed in the Saharo-Arabian phytogeographic region in African and the Mediterranean region. The stem angular very much branched, covered with a depressed coarse hair-leaves narrowly triangular , 5-12 cm, long deeply 3-7 lobes, lobulated or incised with rounded sinuses. Flowers small greenish-yellow. Receptacle broadly companulate covered with white bispid hairs. Young fruits fleshy, globular, mottled with dark green , turning dry and yellow when ripe, 10 cm in diameter, fruit extremely bitter, seeds smooth shining. The fruits are widely used medicinally due to its pharmacological activities [1], they act as hypoglycemic and antihyperglycemic agent [2].Its fruit was used as antimicrobial antibacterial

agent [3], several investigators reported its local antirheumatic [4] and antispasmodic [5] especially for stomach pains, The pulp, because of its content of glycosides [6-10] such as Cucurbitacin–E–2–B–D–glucoside is an abundant tetracyclic triterpenoid glucoside, isolated from the ethanolic exract of *Citrullus colocynthis* increase capillary permeability in the rats [11] and laxative [12]. The seeds have high oil content (17-19%) [13] and there has been much interest in developing new *o*il seed crops which could be used in food [14 and 15] and for medicinal and industrial purposes [16]. Many melon seeds are rich in oil and protein [17] and although none of these oils have been utilized on an industrial scale, many are used as cooking oils in some African and Middle Eastern countries [18], especially in Nigeria [19].melon seed oil contains a large amount of linoleic acid (C18:2).which is important for human nutrition and an essential fatty acid and very little linolenic acid (C18:3) [20-22]. Such oil composition resembles safflower oil [23] and is very beneficial for human diet. Wild *Citrullus colocynthis* have been collected from the arid zones in Halayp and kept to evaluation of chemical, physicochemical and phytochemical characters. Also, the alcoholic extract of *Citrullus colocynthis* has been evaluated for hypoglycemic and antihyperglycemic activities in both normal and alloxan-induced diabetic rats and to compare these effects with tolbutamide as a standard hypoglycemic and in order to test the seeds as candidates of potential new oil seed crop in halayp-a crop adapted to arid zones.

2. MATERIALS AND METHODS

2.1 Plant materials:

Seeds of wild *Citrullus colocynthis* were collected throughout the Halayp region Desert. The seed storage facilities in sealed aluminum cans, until needed for maintenance, evaluation chemical,physicochemical and phytochemical analysis and the fatty acid composition.Also study the antihperglycemic and hypoglycemic effect of *Citrullus colocynthis* fruit in normal and alloxan- induced diabetes in adult albino rats.

2.2 Chemical and biochemical analysis:

Ash content, crude protein, crude lipid and phospholipids were analysed by standard AOAC [24] procedures.Moisture content and crude fiber were determined by methods described by pearson [25]. Carbohydrate was estimated according to Nasr and Bedir [26] Titratable acidity was determined by titrating dilute samples with 0.1 NaOH to the phenol phthalein end point [24]. Total soluble solids were estimated with A*bbe hand refactometer* by the method described by Pearson [25]. Calcium and phosphorous were estimated by EDTA titration as described by pearson [25]. Specific gravity was estimated by a 25 ml specific gravity bottle at 20°C, described by pomeranz and meloan [27], while refractive index was determined with the *Abbe refractometer (Italy). p*H was estimated using *a pH meter (JENWAY 3010, Japan)* at 25°C. Viscosity was estimated as described by pomeranz and meloan [27] by using *(FIELD BROOK DIGITAL VISCOMETER, U.S.A)*. Other parameters determined include acid value, saponification value,iodine number and peroxide value as described by the A.O.C.S. [28], on the extracted oil. The phytochemical analysis of active ingredients (glycosides,alkalodis and saponins) were carried out on ethanolic extract of all fruit. Also,the hypoglycemic and antihyperglycemic effects of an alcoholic extract of the the fruit of *Citrullus colocynthis* was investigated in both normal and alloxan-induced diabetes in adult albino rats and was analysed by glucose-oxidase method using a *UV- 1601PC- UV- Visible Spectrophotometer SHIMADZU (Japan),with COMPAQ Pentium 5 computer(USA).*

2.3 Lipids extraction:

Seeds were dried overnight at 50 °C and ground into powder in Moulinex coffee grinder. Ten grams of powder were mixed with 250 ml petroleum ether (40-60°C), and the lipid fraction was extracted in a soxhlet apparatus for 16h at 60°C. The solvent was evaporated by using *Heidolph rotatory evaporator,Germany* and the lipid fraction residues were weighed [29].

2.4 Determination of the free fatty acids of *Citrullus colocynthis* seed oil: Direct transesterification from seeds:

Seeds (400 mg) were dried overnight at 50 °C and ground into powder with a mortar and pestle, after which 0.6 ml of dichloromethane and 4.0 ml of 0.5 N sodium methoxide were added. Acidic catalysed esterification using the boron triflouride-methanol complex (14%w/ v) was added according to the method described by Christie [30]. The tube was shaken and heated for 30 min. at 50 °C. The reaction was stopped by adding 5.0 ml of water containing 0.2 ml of glacial acetic acid. The esterified fatty acids were extracted with 3.0 ml petroleum ether (40-60C°).the clear fraction was kept at -20C° until further analysis. Aliquots (1ul) were analysed by *Gas chromatography.* Standard fatty acid methyl esters; using palmitic, stearic, oleic, linoleic and linolenic acids (all from Sigma), their methyl esters were also prepared by the same method [31].

*Analysis conditions: *A satochrom gas chromatography equipped with a flame ionization detector (FID) and a CW_ 120 fused silica column (30 m x 0.32 mm i.d., film thickness 0.45 um, Germany) in* microanalytical center Faculty of Science Cairo University - Cairo - Egypt was used. Split-injection mode was employed with split ratio 1:30. The oven temperature was programmed from 100 C° (2 min.isothermal) to 160 C° at the rate of 3 °C / min. ,which was maintained for 20 min .then increased to 180 C° at the rate of 1C°/min. , and kept for 10 min. then cooled.

2.5 Phytochemical study on the active ingredient (glycosides, alkaloids and saponins) present in the *Citrullus colocynthis*(L) fruit pulp:

The phytochemical investigation of the active ingredients of *Citrullus colocynthis* fruits growing particularly because of their medical effect on human and animals.

1-Macromorphology of the plants:

Citrullus colocynthis(L) is a perennial, monoecious, spreading roughly hispid creeping herb with tendrils and pinnatisect leaves .Flowers yellow; ovary sparesly hispid, fruit globose, yellow when ripe , smooth, bitter taste. The plant flowering almost through the year (Fig. 1).

Figure 1: Egyptian *Citrullus colocynthis* fruits.

2-Extraction of the active ingredients:

Citrullus colocynthis fruit pulp were crushed, soaked overnight for 2 weeks in a suitable amount of 70 % ethyl alcohol and filtered through Whattman filter paper no.1. The obtained filtrate was evaporated under vacuum using *Heidolph rotatory evaporator, Germany,* till exhaustion. The extract for plant was weighed, labeled and kept in refrigerator at 4°C till use in further experiment [32].

3-Qualitative identification of the active ingredients:

The chemical test reactions were carried according to method described by Rentoul and Smith [33]. The reagents use were Molich's reagent,Antimony trichloride reagent, Baljest's reagent, Fehling's reagent and Benedict's Reagent for identification for the presence of glycosides.Mayer's reagent,picric acid reagent, Wagner's reagent, Dragenodorff's reagent and Tannic acid reagent for identification the presence of alkaloids where the reagents used for identification for the presence of saponins are mercurous chloride and silver nitrate reagents.

4-Thin layer chromatography:

Chromatographic technique is one of the most out standing methods for the purification, separation, detection and identification in phytochemical studies, separation and identification of the active ingredient of the plant *Citrullus colocynthis* was carried chromatographically according to methods described by Sammaya [34]. Sample of 10 ul of the extracts were spotted on silica gel GF254 (*Merck*) for TLC plates, developed in CH3OH:CHCl3,(1 : 9),and spots were visualized by spraying with 0.5 % vanillin-dil H2SO4 (1:1) reagent followed by heating at 100 0C for five min. All cucurbitacin derivatives gave reddish- brown colors with this reagent.

2.6 Animals

Adult albino rats (200 – 220 gm) of either sex obtained from animal house of Faculty of Vet. med. Zagazig University. They were divided into 10 groups of six each and were provided with standard diet and water *ad libtum.* All the rats were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained in a well ventilated animal house with 12 h. light and dark cycle. They were fasted for 18 h. prior to the experiment, allowing access to water only and were deprived of both food and water during the 12 h. monitoring period of the experiment after the treatment either with drug (or) non treated rats.

2.7 Chemicals used

Tolbutamide was a generous gift sample from *Hoechst Pharmaceuticals* and alloxan monohydrate was obtained from *Sigma-Aldrich, St.Louis, USA*. The glucose oxidase reagents kits were obtained from *Winer Lab. Argentina.*

2.8 Effect on normal rats

Groups I, II, III were given the alcoholic extract of *Citrullus colocynthis* fruit suspended in distilled water orally at dose of 100, 200, 300 mg/kg body weight, respectively. Animal in group IV received tolbutamide at a dose 250 mg/kg body weight and served as standard. Group V served as a normal control and received appropriate volumes of distilled water orally.

2.9 Induction of diabetes

Groups VI – X were rendered diabetic by injecting a freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg, i.p.) after a baseline blood

glucose estimation was done. After 48 hrs when the condition of diabetes was stabilized, animals with blood glucose level was in the range of (222 – 286) mg/dl were selected for the study.

2.10 Effect on diabetic rats

Groups VI – VIII were treated with alcoholic extract of *Citrullus colocynthis* fruit suspended in dis.water in the form of mucilage orally by gavage at doses 100, 200, 300 mg/kg. body weight, respectively. Group X served as a diabetic control and received appropriate volume of dis. Water orally while group IX received tolbutamide at a dose of 250 mg/kg.body weight and served as a standard.

2.11 Collection of blood and determination of blood glucose:

Blood samples were collected from the marginal ear vein of each rat before and at 0.5, 1, 2, 4, 6 and 8 h. after drug administration. The samples were analysed for blood glucose content by using glucose-oxidase method [35] with optical density measured by a *UV- Visible Spectrophotometer* at 520 nm.

3. RESULTS AND DISCUSSION

The results of the chemical analyses are summarized in (Table1) revealed that the seeds (100 g) of *Citrullus colocynthis* reported to contain 6.96 g H2O, 22.31 g protein ,52.37 g lipid, 12.75 g phospholipids, 14.22 g total carbohydrates,2.72 g fiber,1.98g ash , 52.17mg calcium and 510.27 mg phosphorus. The physical characteristics with respect to shape, consistency, total soluble solids, pH at 25 ˚C, refractive index and specific gravity were showed also in (Table 1).

Table 1: Average chemical composition of seeds of *Citrullus colocynthis* (Average of six replicates)

Variable	Values for each 100 g of seeds
Moisture	6.96±0.05g
Ash	1.98±0.12g
Calcium	52.17±0.2mg
Phosphours	510.27±7.9mg
Phospholipids	12.75±6.3 g
Crude protein	22.31±0.21g
Crude fibre	2.72±0.13g
Crude lipid	52.37±0.29g
Carbohydrate	14.22±1.5g

Physicochemical properties	
Shape	Ovoid
Consistency	Smooth and compressed
Total soluble solids	8.81
Titratable acidity (%)	1.79
PH (25oC)	5.27
Refractive index	1.4332
Specific gravity	1.0123

3.1 Average of physical characteristic and chemical properties of oil extracted from seed of *Citrullus colocynthis* (tables 2&3)

Results of the study (Table 2) showed that the yield of extracted *Citrullus colocynthis* oil was 54.97±0.91% of the kerenel and 43.3±0.73 of the unshelled seed by weight. The moisture content, free fatty acid contained, iodine value, saponification value, peroxide value and unsaponifiable matter content were 0.17±0.04 %, 0.67±0.17, 107±1.8, 210.8±5.31, 6.51±0.03 and 0.98±0.07 % respectively. Oil content in seeds varied from 17 to 19.5 % .The two major unsaturated fatty acids in seed oil of *Citrullus colocynthis* (Table 3) are oleic (C18:1), 16.71±0.19% ; Linoleic (C18:2), 54.3±2.7% and Linolenic (C18:3), 0.2±0.04%, while the saturated fatty acids were Palmitic (16:0), 11.7±0.53% and Stearic (18:0), 15.67±0.93% the unidentified fatty acids detected amounted to 2.2±0.13%.

Table 2: Average of physical characteristics and chemical properties of oil extracted from seeds of *Citrullus colocynthis* (Average of six replicates)

Physical properties (%)	
Parameter	**Properties**
Odour	Odourless
Colour	Dark brown to light yellowish orange
Condition at room temperature	Semi-drying soft edible oil
Refractive index	1.3526
Specific gravity	0.7158
Viscosity at 30 °C	1.85
Chemical properties	
Oil of The kernel	54.97±0.91%
Oil of the unshelled seed by weight	43.3±0.73%
Moisture content	0.17±0.04%
Free fatty acid content	0.67±0.17%
Iodine value (Wij's method)	107.9±1.8
Saponification value (mg KOH/g)	210.8±5.31
Peroxide value (mg /kg)	6.51±0.03
Unsaponifiable matter content	0.98±0.07

Table 3: The fatty acid composition of seed oil *Citrullus colocynthis* (Average of six replicates)

Fatty acids	**% of total oil**
Palmitic (C16:0)	11.7±0.53
Stearic (C 18:0)	15.67±0.93
Oleic (C 18:1)	16.71±0.19
Linoleic (C 18:2)	54.3±2.7
Linolenic (C 18:3)	0.2±0.04
Unidentified fatty acid	2.2±0.13

3.2 Coloured qualitative test for the identification of the presence of glycosides, alkaloids and saponins:

Positive Baljest's, Fehling's, Benedict's , Molich's and Antimony trichlorid tests indicate that alcoholic *Citrullus colocynthis* (L) extract contains glycosides (Table 4&Fig2). Positive Mayer's, picric acid, Wagner's,Dragenodorff's and tannic acid tests indicate that alcoholic *Citrullus colocynthis (L)* extract contains alkaloids (Table 4 & Fig.3). Positive test of mercurous chloride and silver nitrate indicate that alcoholic extract of the fruit pulp under investigation contains saponins (Table 4 & Fig.4) .

Table 4: Showing the chemical tests for detection of active ingredients of *Citrullus colocynthis* fruit pulp (ethanolic extract)

Detection of glycosides					**Detection of alkaloids**				
Molich's test	Antimony trichloride	Baljest's test	Fehling's test	Benedict test	Mayer's test	Picric acid test	Wagner's test	Dragenodorff's test	Tannic acid test
Violet ring.	White ppt.	Dark orange	Reddish brown	Brown ppt.	Brown ppt.	Yellow ppt.	Reddish brown ppt.	Orange ppt.	White turbidity
Detection of saponins									
Mercurous chloride	Silver nitrate								
White ppt.	Silver mirror								

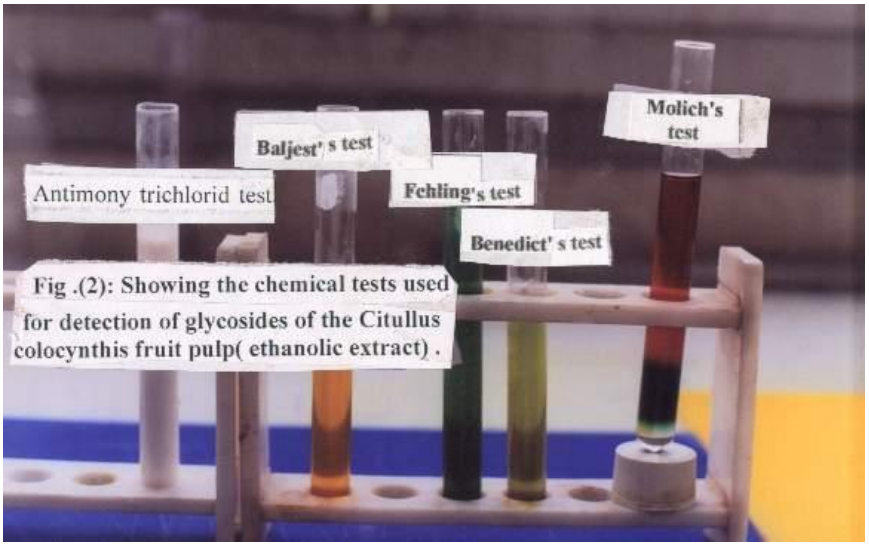

Figure 2: Showing the chemical tests used for detection of glycosides of the *Citrullus colocynthis* fruit pulp (ethanolic extract).

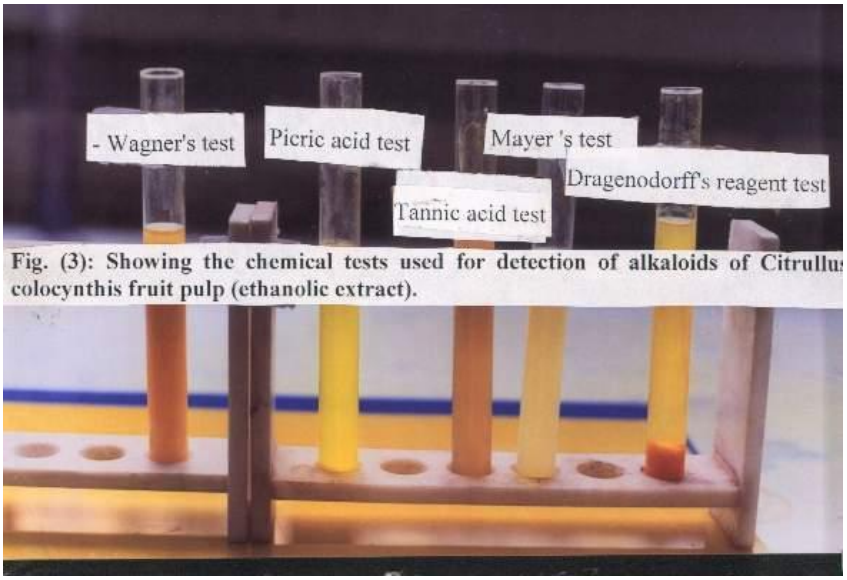

Figure 3: Showing the chemical tests used for detection of alkaloids of *Citrullus colocynthis* fruit pulp (ethanolic extract).

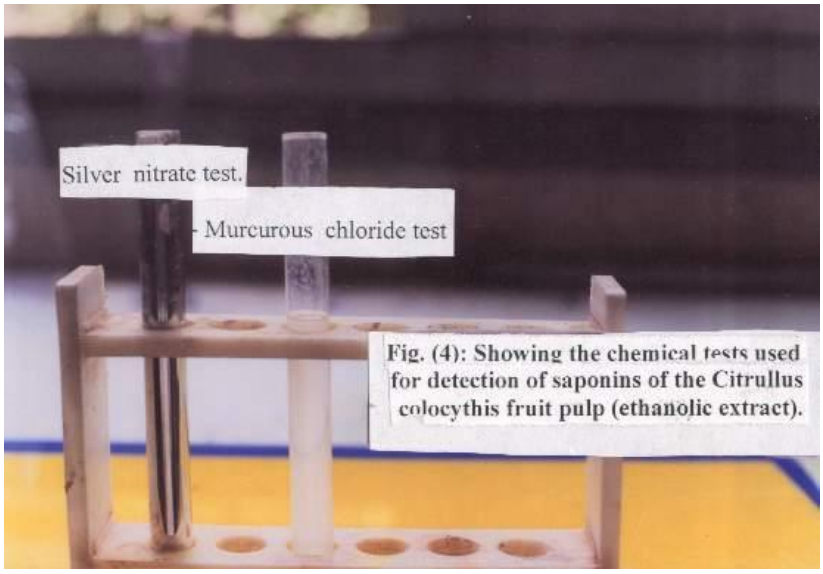

Figure 4: Showing the chemical tests used for detection of saponins of *Citrullus colocynthis* fruit pulp (ethanolic extract).

3.3 Rf values of Cucurbitacin-E-2-B-D-glucoside of coloynthis on silica gel chromato-plates using mixture of aromatic hydrocarbons (Table 5):

Thin layer chromatographic technique showed that the visualization of *Cucurbitacin* spots on silica gel GF245 for TLC . spraying the compound with spraying solution (dil H2 SO4 : vanillin, 1:1) were accomplished to help the location and characterization of the compound spots on chromatogram. The Rf value of *cucurbitacin* using different eluents are shown in (Table 5) and (Fig. 5). The solvent system chloroform: methanol (9:1) gave complete separation for all cucurbitacin derivatives.

Table 5: The Rf values of Cucurbitacin–E–2–B–D–glucoside of colocynthis on silica gel chromato-plates using mixture of some aromatic hydrocarbons

Eluents	Rf Value
Chlorofom: Methanol (9:1)	0.86
Benzene : Ethylacetate(7:3)	- ve
Toluene: Ethylacetate(8:2)	- ve
Sulphuric acid:Methanol(3:7)	- ve
Chloroform:Benzene(1:1)	- ve
Ethylacetate: Benzene(1:1)	- ve

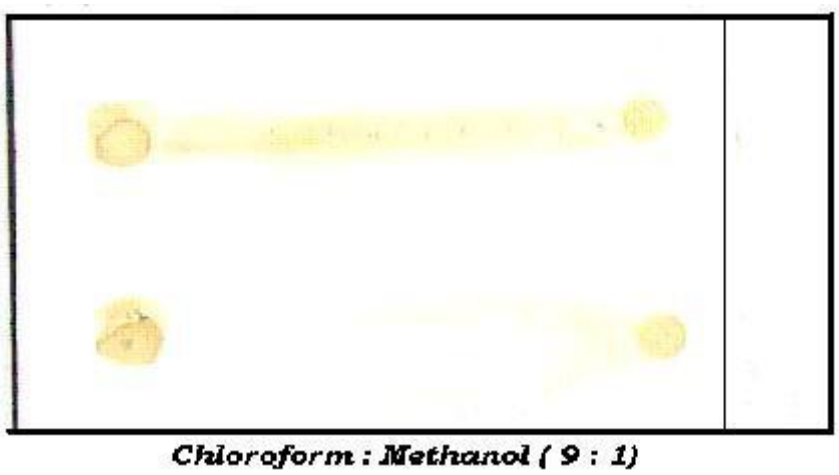

Figure 5: Showing Rf value of *Cucurbitacin–E–2–B–D–glucoside* of colocynthis on chromatogram plate.

3.4 Effect of fruit extract on blood glucose levels in normal rats

The mean blood glucose concentration of control and drug-treated animals (after oral administration of different doses of *Citrullus colocynthis* fruit extract) at various time intervals are shown in Table. 6. A dose dependent hypoglycemia was observed in animals treated with *Citrullus colocynthis* fruit extract. A significant reduction (P< 0.001) in blood glucose of 22.45%, 28.54 % and 30.21 % was observed at 6 h. with dose 100, 200, and 300 mg/kg body weight respectively. There were also significant reductions starting 1 hr. following treatment .The maximum reduction was observed 6 h. after treatment.

3.5 Effect of fruit extract on blood glucose in diabetic rats

The mean blood glucose concentration of control, *Citrullus colocynthis*-treated (100, 200, and 300 mg/kg. body weight and tolbutamide treated (250 mg/kg. body weight i.p.) rats shown in Table 7. A dose dependent antihyperglycemic activity was also observed with *Citrullus colocynthis* in alloxan-induced diabetic rats .The percentage reduction of blood glucose was higher in the diabetic state compared to normal state by the three doses of *Citrullus colocynthis*. A significant reduction (P<0.001) in blood glucose of 26.48 %, 33.52 % and 46.28 % was observed at 6 h. with the dose 100, 200, and 300 mg/kg. body weight, respectively . Also a significant reduction (P < 0.001) in blood glucose of 19.6 %, 37.26% and 36.11 % was observed at 8 h.. There were also significant reductions starting 2 h following treatment. Tolbutamide produced a significant reduction (P<0.001) in blood glucose compared to diabetic control at the 6 h. and 8 h. of 56.15 % and 42.69 % respectively.

Table 6. Effect of *Citrullus colocynthis* extract on blood glucose levels after oral administration in normal rats.

		Blood glucose levels at different hours after the treatment						
		0	0.5	1	2	4	6	8
Groups (n=6)	Control	93.84 ±4.0	92.72 ±3.1	91.06 ±3.01	91.77 ±2.4	91.67 ±1.92	92.58 ±0.98	91.53 ±1.98
	Citrullus colocynthis 100mg/kg	92.84 ±2.1	91.3 ±3.4	87.92 ±3.56	82.27 ±2.5*	82.33 ±1.19*	71.79 ±5.26**	79.65 ±4.1*
	Citrullus colocynthis 200mg/kg	88.72 ±5.2	87.73 ±6.1*	80.05 ±4.2*	77.3 ±5.1**	68.6 ±3.1**	66.15 ±2.9***	73.7 ±1.9**
	Citrullus colocynthis 300 mg/kg	81.5 ±4.2	78.8 ±3.2*	72.6 ±1.9**	69.12 ±3.2***	67.8 ±4.9**	64.6 ±2.6***	70.5 ±3.2**
	Tolbutamide 250 mg/kg.	83.1 ±1.9	77.7 ±2.6*	71.7 ±2.6**	68.55 ±2.9***	58.7 ±3.1***	62.45 ± 4.1***	72.2 ±3.5**

Values are mean blood glucose levels (±S.E.M.) of six animals.
Significant difference from control at corresponding intervals: * P < 0.05, ** P< 0.01 *** P< 0.001

Table 7. Effect of *Citrullus colocynthis* extract on blood glucose levels after oral administration in diabetic rats.

		Blood glucose levels at different hours after the treatment						
		0	0.5	1	2	4	6	8
Groups (n=6)	Control	240 ±1.9	240 ±3.05	241.1 ±4.1	243.4 ±42	241.2 ±2.9	242 ±1.2	240.8 ±3.6
	Citrullus colocynthis 100mg/kg	283.1 ±2.5	276.6 ±3.2	271.1 ±4.2**	255.2 ±2.2*	226.5 ±1.6***	177.9 ±1.99***	193.6 ±2.54***
	Citrullus colocynthis 200mg/kg	251.8 ±1.9	242.4 ±2.6	231.65 ±2.9**	224.3 ±3.56***	212.34 ±1.64***	160.87 ±1.52***	151.06 ±0.98***
	Citrullus colocynthis 300 mg/kg	271.54 ±1.6	258.3 ±2.5**	245.84 ±3.4*	230.9 ±3.02***	206.14 ±2.4***	130 ±2.56***	153.84 ±1.96***
	Tolbutamide 250 mg/kg.	275.19 ±2.4	269.35 ±2.21**	253.6 ±1.88*	215.03 ±3.34**	186.5 ±2.98***	106.1 ±1.96***	138 ±1.86***

Values are mean blood glucose levels (±S.E.M.) of six animals.
Significant difference from control at corresponding intervals: * P < 0.05, ** P< 0.01 *** P< 0.001

3.6 Statistical analysis:

All data were analyzed by means of procedures of the SAS package (SAS Institute)[36]. Statistical differences were calculated as Least Significance Difference (LSD) and evaluated with Duncan's Multiple Rang test (DMRT) at P < 0.05.

The results of the chemical analysis are summarized in (Table 1) showed that the seeds (100 g) reported the moisture content protein, lipids, phospholipids, carbohydrates, fiber, ash, calcium and phosphorus were 6.96 g, 22.31 g, 52.37 g, 12.75 g, 14.22 g, 2.27 g, 1.98 g, 52.17mg and 510.27mg respectively. These results agreed with that reported by Oresanya [37] and Nasr and bedir [26]. Results of the study (Table 2) showed that the oil of kernel and the oil of the unshelled seed by weight were 54.97 ± 0.91% and 43.3 ± 0.73 % respectively. These results agreed with that reported by Nasr and bedir [26]. Oresanya and Ebuehi [38] reported that, although the lipid or oil content is high, it is less than or nearly equal to those of other known oil seeds such as *Irvingia gabonensis*, 70% [39]; *Annona murcicata*, 44.63% [40] and beniseed, 48.2% [41].

Colors and precipitate with the different reagents [42] are summarized in Table (4) and Fig.(2),(3) and (4).These results agreed with that reported by Amer,et al. [43]. As the colour tests are not enough as a method of identification, a combination of thin layer chromatography and colour reaction have been used to identify various compounds [44]. Spraying of chromatoplate using certain reagents produced different characteristic spots. These are used to determine the presence of number of compounds. If no compounds are present in the sample no characteristic colour spots will appear. This is also of a benefit when comparing compounds with similar Rf value isolation of the studied compounds should be done by thin layer chromatography which has the advantage of being able to indicate the presence of all the compounds [33]. The

solvent system chloroform : methanol (9:1) gave complete separation for all active ingredients. Many trials were done for chosing the separating system, (dil H2SO4: vanillin 1:1) as a general developer for all active ingredient Cucurbitacin-E-2-B-D-glucoside (Table 5) and (figure 5).These results agreed with that reported by Amer, et, a [43].Also diabetes mellitus is possibly the world, s largest growing metabolic disease , and as the knowledge on the heterogenecity of this disorder is advanced [44], the need for more appropriate therapy increases [45]. Traditional plant medicines are used throughout the world of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future. In the present study ethanolic extract of *Citrullus colocynthis* suppressed blood glucose levels in normal and alloxan-induced diabetic rats ,when compared to control animals .The hypoglycemic potential of the extract was comparable with that of tolbutamide in normal and diabetic rats .On the other hand , tolbutamide caused significantly ($P<0.001$) more hypoglycemia in comparison with the plant extract at 300 mg/kg. body weight ($P<0.001$) An emphasis is laid on glucose homeostasis as severe hypoglycemia can result in life threatening situation. The mechanism of this hypoglycemic effect of the extract is not elucidated in this study. Some medicinal plants with hypoglcemic properties are known to increase circulating insulin level in normoglycemic rats.The antidiabetic action of *Citrullus colocynthis* is probably due to enhanced insulin secretion or due to increase in peripheral glucose uptake.

4. CONCLUSION

The colocynth plant is a native of dry climate and its cultivation should be evaluated under arid conditions .It is a potential source of protein and other nutrients for animal feeds and is very beneficial for human diets and considered a good source for edible oil owing to its higher oil content . In conclusion our results have showed that fruits of *Citrullus colocynthis* possess blood glucose lowering effect in normoglycemic rats and in alloxan- induced hyperglycemic rats. Thus, the folk use of these plants may be validated by this study. The fruits seem to have a promising value for the development of potent phytomedicine for diabetes. Further investigation is expected to characterize the active hypoglycemic principles and to elucidate the mechanism of action. A special attention should be given to evaluate its medicinal and diuretic potential as a source of high value by –products.

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