ABSTRACT

Medicinal plants play an imperative role in the management of various diseases especially in the developing countries where resources are meager. Indrayan (Citrullus Colocynthis Schard.) has been reported as folk medicine in various countries. It has been traditionally used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes. The principal constituents of colocynth include alkaloids, triterpenoids, glycosides and resins which are mainly responsible for its biological activities. Considering the extensive interest and recent progress on the structural and biological activity of active constituents, the present review systematically focuses on the structures and biological activity of the plant.

Keywords: Medicinal plants, Folk Medicine, Citrullus Colocynthis, Cucurbitaceae, Activity.
Colocynthis Citrullus (Melon) is widely cultivated and consumed oil seed crop in West Africa. The seeds popularly called 'egusi' contain about 53% oil, 28% protein and some other mineral nutrients (17-18). They are consumed in "egusi soup", melon ball snacks and ogiri (a fermented condiment) (19-20). Melon seeds contain a fairly high amount of unsaturated fatty acids, linoleic acid (21), they have a hypo-cholesteronic effect. The plants contain cucurbitacin A, B, C, D and E (23).

The pulp also contains cucurbitacin E (α-elaterin) compounds, the standardized extract. The natural cucurbitacins constitute a group of tri-terpenoid substances which are well-known for their bitterness and toxicity. Structurally, they are characterized by the tetracyclic cucurbitane nucleus skeleton, namely, 19-(10→9b)-abeo-10a-lanost-5-ene (also known as 9b-methyl-19-norlanosta-5-ene), with a variety of oxygen substitutions at different positions. According to the characteristics of their structures, cucurbitacins are divided into twelve categories but we cover only those found in Citrullus colocynthis species. Cucurbitacins are noted for their cytotoxic behavior and were a hot topic within the medicinal chemistry and drug discovery community from an anti-cancer drug development perspective, particularly in the 1960's. However, the application potential of cucurbitacins was substantially hindered by their nonspecific cytotoxicity, and therefore, only very limited usage was pursued under strict medical control (24).

Cucurbitacin A (Fig. 1) is not widely distributed, but Cucurbitacin B (Fig. 2) has been found in many Cucurbitaceae species. Cucurbitacin C (Fig. 3) is rare in nature, in contrast to cucurbitacin B, cucurbitacin D (Fig. 4), which lacks the acetyl group at the 25-OH, is the most ubiquitous cucurbitacin known and cucurbitacin E (Fig. 5) found in pulp (24). The pulp was separated from the seeds. The ground pulp (500 g) was macerated in water/methanol (30/70) for 24 hours (three times), and the hydro-methanolic solution was subsequently concentrated by evaporation up to 1/3 of the initial volume. The extract was fractionated by the following solvents with increasing polarity: petroleum ether, chloroform, ethyl acetate, and butanol and water fractions. The fractions were concentrated to dryness by rotary evaporator. The butanol fraction (2 g) was subjected to Sep-Pack (ODS) fractionation using a step gradient of MeOH-water mixture (10:90, 20:80, 60:40, 80:20 and 100:0). The preparative reversed-phase HPLC analysis was employed to isolate compounds from the butanol fraction of the hydro-methanolic (70%) extract of the fruits of the locally grown C. colocynthis. Three flavone glucosides and two cucurbitacin glucosides; Isoscepin (Fig. 6); Isovitexin (Fig. 7); Isoorientin 3´-0-methyl ether (Fig. 8); 2-glucopyranosylcucurbitacin I (Fig. 9) and 2-β-D-glucopyranosylcucurbitacin L (Fig. 10) which is first time reported from this plant (11-16).

The methanolic extract of powdered C. colocynthis fruits was purified by column chromatographic on Sephadex LH20 to give two fractions, rich in flavonoids. Further purification afforded Isoorientin

**PHYTOCHEMICAL REVIEW**

The principal of colocynith include bitter, amorphous, alkaloids and resin, these are responsible for its purgative action. The plants contain cucurbitacin A, B, C, D and E (23).

<table>
<thead>
<tr>
<th>Synonyms</th>
<th>Root</th>
<th>Leaf</th>
<th>Fruit</th>
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<tr>
<td>Sanskrit</td>
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<td>Eandri, Aendri</td>
<td>Aandri, Gavadasi</td>
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<td>Bengali</td>
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<td>English</td>
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The methanolic extract of the fresh fruits was fractionated by different chromatographic techniques, which resulted in the isolation and structure elucidation of a new compound Cucurbitacin glycoside (Fig. 11), along with 2-β-D-glucopyranosyl cucurbitacin B (arvenin I, Fig. 12) and 25-di-β-D-glucopyranosyl-cucurbitacin I. (Fig. 13) which is first time reported (26-28). Equi (Citrullus colocynthis Schard) Kernels contain approximately protein (28.4%), oil (52%), ash (3.6%), fibres (2.7%) and carbohydrate; its oil contains linoleic acid (63%), oleic acid (16%) and small amount linolenic acid. Such oil composition resembles that of safflower oil and is very beneficial in human nutrition (29-32). The methanolic extract of powdered C. colocynthis fruits was purified by column chromatographic on Sephadex LH20 to give two fractions, rich in flavonoids.
3'-O-methyl ether (Fig. 8), Isoorientin (Fig. 14), Isovitexin (Fig. 7) (33, 13). The aerial parts were treated similarly on Silica Gel CC and further purification on preparative TLC results three new flavone C-glycosides viz. Hydroxibenzyl Isovitexin (Fig. 15), 6-C-p-hydroxybenzyl Isovitexin (Fig. 16), 8-C-p-hydroxybenzyl isovitexin 4'-O-β-D-glucoside (Fig. 17) (13). The methanolic extract (13.9%) of dried fruits of C. colocynthis, cultivated in Egypt, and contain Colocynthoside A (0.0036%, Fig. 18) and Colocynthoside B (0.018%, Fig. 19), were isolated together with Cucurbitacin E 2-0-β-D glucopyranoside (0.0077%, Fig. 10), 2-0-β-D glucopyranoside cucurbitacin I (0.15%, Fig. 9), colocynthoside (0.0015%, Fig. 20), 2-0-β-D glucopyranoside cucurbitacin J (0.0015%, Fig. 21), 2-0-β-D glucopyranoside cucurbitacin K (0.0007%, Fig. 22), 2-0-β-D glucopyranoside cucurbitacin L (0.032%, Fig. 23) (12, 34), hexanocucurbitacin I 2-0-β-D glucopyranoside (0.0020%, Fig. 24), khekadaengoside E (0.0014%, Fig. 25), isovitexin (0.0039%, Fig. 7), isoorientin 3'-O-methyl ether (0.0032%, Fig. 8), isosaponarin (0.0005%, Fig. 26), 4-(β-D-glucopyranoside) benzaldehyde (0.0009%, Fig. 27), 4-hydroxybenzyl β-D-glucopyranoside (0.0009%, Fig. 28), benzyl-β-D-glucopyranoside (0.0006%, Fig. 29), 4-(β-D-glucopyranoside) benzyl alcohol (0.015%, Fig. 30) (35), these are isolated from n-butanol soluble fraction (36).

Cucurbitacins are a group of highly structurally diverse triterpenes, with a rich variety of side chain derivatives and different ring A substitution patterns. They have been mainly reported from plants of the Cucurbitaceae, but are also known from a few genera within other plant families, including the Brassicaceae. They are renowned for their bitter taste, but also possess a broad range of potent biological activities. Although the cytotoxicity of cucurbitacins was known before 1800 AD, very little is known about the mechanism of the effect of cucurbitacins at the cellular and molecular level, which
Figure 7: Isovitexin

Figure 8: Isoorientin 3'-O-methyl ether

Figure 9: Cucurbitacin I 2-O-β-D-glucopyranoside

Figure 10: Cucurbitacin E 2-O-β-D-glucopyranoside

Figure 11: New Compound

Figure 12: 2-O-β-D-glucopyranosyl cucurbitacin B (arvenin I)

Figure 13: 2,25-di-O-β-D-glucopyranosyl-cucurbitacin L

Figure 14: Isoorientin

Figure 15: Hydroxybenzyl Isovitexin

Figure 16: 6-C-p-hydroxybenzyl Isovitexin
Figure 17: 8-C-β-hydroxybenzyl isovitexin 4′-0-β-D-glucoside

Figure 18: Colocynthoside A

Figure 19: Colocynthoside B

Figure 20: Colocynthoside

Figure 21: Cucurbitacin J 2-0-β-D-glucopyranoside

Figure 22: Cucurbitacin K 2-0-β-D-glucopyranoside

Figure 23: Cucurbitacin L 2-0-β-D-glucopyranoside

Figure 24: Hexanocucurbitacin 1 2-0-β-D-glucopyranoside

Figure 25: Khekadaengoside E

Figure 26: Isosaponarin
accounts for the relatively slow advance in cucurbitacin based drug discovery.

**BIOLOGICAL REVIEW**

During biblical times, fruits of the colocynth or bitter apple or bitter ground were gathered as deadly poison. Fruits were widely used medicinally, especially for stomach pains. The pulp is an effective hydragogue, cathartic and laxative because of its content of glucosides such as colocynthis. The fruits, seeds and roots of *Citrullus Colocynthis* L. Schard plants are purgative and used as an antidote to snake poison. Root is also used for the treatment of jaundice, rheumatism and urinary diseases. It locally known as Handal or Sherry, commonly found in Saudi Arabia used as an antihelmintic, purgative, molluscicide and insecticide (37). The Cucurbitacin, a class of highly oxygenated, bitter tasting tri-terpene, have been shown to produce increased rat capillary permeability (38), to demonstrate anti-fertility effect in female mice (39), to possess anti-inflammatory activity (40-41), to exhibit phago-stimulant activity on corn rootworm (42) and to act as feeding deterrents (43) or as insect steroid hormone antagonist (44).

The reported biological activities of the plant *Citrullus Colocynthis* includes:

**Anti-inflammatory Activity**

Colocynthis belongs to the mostly used plants in folk medicine because of the anti-inflammatory activity (45). The effect of chloroform and methanol extract of *Citrullus Colocynthis* on glucose homeostasis was studied in normal and diabetic rats. In addition, some pharmacological effects of an ethanolic leave extract (CCL) and the pulp (CCP) were also evaluated. Extract of seeds had no effect on fasting glucose levels or in the oral glucose tolerance test in normal or diabetic rats. Both CCL and CCP resulted in spastic contraction of the rabbit jejunum which were not blocked by atropine. No effect was observed in the rat uterus. CCL and CCP reduced the heart rate and force of contraction in isolated rabbit heart. At a dose 800mg/kg of ethanol extract, the leaves exhibited a secondary anti-inflammatory activity, but were severely toxic. At lower doses (100, 200 and 250 mg/kg), no anti-inflammatory activity was observed (46). *Citrullus extract of colocynthis* are known to stimulate intestinal peristalsis and to soften bowel contents by an irritant action on the enteric mucosa (47-48).

Owing to the purgative effect of colocynth extract when taken orally, prepare a Sodium carboxymethyl cellulose (5%) topical gel formulations containing 3% of colocynth extract, hydrolyzed extract, or acetylated extract and to study their release through cellophane membrane and their permeability.
through hairless mouse skin. Also, to study the in vivo anti-inflammatory activity of the different types of colocynth extract using the carrageenan induced paw edema model in albino rats in comparison with the commercial Voltarin Emulgel®. The acetylated extract gel showed comparatively rapid permeability through hairless mouse skin, with low release rate through cellophane membrane. The pharmacological screening revealed that the percent reduction of edema produced by Colocynth extract was 45.39%, the hydrolyzed extract produced 54.11% inhibition and the acetylated extract produced 64.95%, while Voltarin Emulgel produced 63.35%. This means that acetylated colocynth extract can be used as an effective local anti-inflammatory agent (49).

**Anti-Diabetic Activity**

Different extracts were obtained from seeds, the major free acids derivative present in the seeds. This study showed the insulinotropic effect of Citrullus colocynthis fruits. Different extracts were obtained from the seeds of this plant: RN II (crude extract), RN VI (aqueous alcoholic extract), RN X (purified extract) and RN XVII (beta-pyrazol-1-ylalanine, the major free amino acid derivative present in the seeds). All tested extracts, when perfused for 20 min at 0.1 mg/mL, immediately and significantly induced insulin secretion in-vitro in the isolated rat pancreas and isolated rat islets in the presence of 8.3 mM glucose. In normoglycaemic rabbits, oral administration of the aqueous extract (300 mg/kg) caused a significant reduction in glycaemia after 1 h and a highly significant reduction after 2, 3, and 6 h. Oral administration of components isolated from the rind of C. colocynthis (tertiary and quaternary alkaloids, glycosides and saponins) were tested in normoglycaemic rabbits at a dose of 50 mg/kg. The alkaloids did not show any hypoglycaemic effect. In contrast, the glycosidic component significantly decreased glycaemia after 2 and 3 h and even more significantly after 6 h. The saponin component reduced glycaemia after 1, 2, 3 and 6h. Oral administration of graded doses of saponin extract (10, 15 and 20 mg/kg) caused a marked hypoglycaemic effect in alloxan-induced diabetic rabbits. The saponin glycosides components could be responsible for the hypoglycaemic effect of the rind of C. colocynthis (50). The effect of the aqueous, glycosidic, alkaloidal and saponin extract of the rind citrullus colocynthis on the plasma glucose level in normal rabbits while the effect of saponin extract on the fasting plasma glucose levels were studied in alloxan induced diabetic rabbits. In normal rabbits, oral administration of aqueous extract (500 mg/kg) produced significant reduction in plasma glucose after 1hrs and highly significant after 2, 3 and 6 hrs (51).

Citrullus colocynthis (L.) Schrad fruit is an herbal medicine used by traditional herbalists for the treatment of diabetes in Iran. To determine its efficacy and toxicity, a 2 month clinical trial was conducted in 50 type II diabetic patients. Two groups of 25 each under standard anti-diabetic therapy, received 100mg C. colocynthis fruit capsules or placebos three times a day, respectively. The patients were visited monthly and glycosylated hemoglobin (HbA1c), fasting blood glucose, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, aspartate transaminase, alanine transaminase, alkaline phosphatase, urea and creatinine levels were determined at the beginning and after 2 months. The results showed a significant decrease in HbA1c and fasting blood glucose levels in C. colocynthis treated patients. Other serological parameters levels in both the groups did not change significantly. No notable gastrointestinal side effect was observed in either group. In conclusion, C. colocynthis fruit treatment had a beneficial effect on improving the glycemic profile without severe adverse effects in type II diabetic patients. Further clinical studies are recommended to evaluate the long-term efficacy and toxicity of C. colocynthis in diabetic patients (52).

In UAE many traditional plants such as the Citrullus colocynthis (Handal) are used as antidiabetic remedies. The effect of the aqueous extracts of the seed of C. colocynthis the biochemical parameters of normal and streptozotocin (STZ)-induced diabetic rats. Diabetes mellitus was induced by a single intraperitoneal (60 mg/kg body wt1) injection of STZ. Normal and diabetic rats were fed with the plant extract daily by oral intubation for 2 weeks. Blood sample were collected at the beginning and end of the experiment for the measurement of biochemical parameters. The plasma level of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactic dehydrogenase (LDH) 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. Blood urea nitrogen (BUN) increased significantly after the onset of diabetes. No significant difference was observed in the blood creatinine, K+, Na+, Ca2+ 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 (53).

Citrullus colocynthis (colocynth) seeds are traditionally used as anti-diabetic medication in Mediterranean countries. The present study evaluated the differential effects of diets enriched with C. colocynthis, sunflower or olive oils on the pancreatic β-cell mass in streptozotocin (STZ)-induced diabetes in rats. STZ injection induced rapid hyperglycaemia in all animals. However, 2 months later, hyperglycaemia was significantly less pronounced in the rats fed a C. Colocynth oil-enriched diet compared with other rat groups (7.9 mM vs 12 mM and 16 mM with colocynth versus olive and sunflower oils, respectively). Assessment of insulin sensitivity using the homoeostasis model assessment (HOMA) method also indicated less insulin resistance in the rats fed a C. colocynthis oil-enriched diet vs the other rats. Finally, 2 months after STZ injection, the pancreatic β-cell mass was similar in both the STZ-treated rats fed the colocynth oil-enriched diet and their controls fed the same diet? In contrast, the pancreatic β-cell mass remained lower in the STZ-induced diabetic rats fed with olive oil- and sunflower oil-enriched diets compared with
the C. colocynthis group. C. colocynthis oil supplementation may have a beneficial effect by partly preserving or restoring pancreatic β-cell mass in the STZ-induced diabetes rat model (54).

**Anticancer activity**

The effect of cucurbitacin glucosides extracted from *Citrullus colocynthis* leaves on human breast cancer cell growth, cell-cycle distribution, apoptosis and expression of protein involved in cell-cycle regulation, utilizing both estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) human breast cancer cell lines. Leaves were extracted, resulting in the identification of cucurbitacin B/E glucosides. The cucurbitacin glucosides combination (1:1) inhibited growth of ER+ MCF-7 and ER- MDA-MB-231 human breast cancer cell lines. Cell-cycle analysis showed that treatment with isolated cucurbitacin glucoside combination resulted in accumulation of cells at the G2/M phase of the cell cycle. Treated cells showed rapid reduction in the level of the key protein complex necessary to the regulation of G2 exit and initiation of mitosis, namely the p34CDK2/cyclin B1 complex. Cucurbitacin glucoside treatment also caused changes in the overall cell morphology from an elongated form to a round-shaped cell, which indicates that cucurbitacin treatment caused impairment of actin filament organization. This profound morphological change might also influence intracellular signaling by molecules such as PKB, resulting in inhibition in the transmission of survival signals. Reduction in PKB phosphorylation and inhibition of survivin, an anti-apoptosis family member, was observed (55).

Hep-2 (a human laryngeal cell line) cells were treated with different concentrations of cucurbitacin B for different time. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay was used to evaluate cell proliferation. Flow cytometry with PI staining and fluorescent microscopy with Hoechst 33258 staining were used to estimate cell cycle distribution and cell apoptosis. Expression of p-STAT3 (signal transducers and activators of transcription 3), cyclin B1 and Bel-2 proteins was evaluated by Western blot analysis showed that the combination effect of cucurbitacin B and docetaxel was due to suppress the expression of p-STAT3 (signal transducers and activators of transcription 3), Bcl-2, and cyclin B1. Moreover, our in *vivo* studies were reproduced in a mouse xenograft model, where, the combination of cucurbitacin B with docetaxel synergistically inhibited tumor growth. Together, this investigation suggests that cucurbitacin B combined with docetaxel may be a feasible strategy to enhance the effects of chemotherapy in patients with laryngeal cancer (57).

Glioblastoma Multiforme (GBM) is almost inevitably a fatal tumor of the brain with most individuals dying within 1 year of diagnosis. It is the most frequent brain tumor in adults. Dose-response studies showed that Cucurbitacin B inhibited 50% growth (ED50) of 5 human GBM cell lines in liquid culture at ∼10^{-7} M. Soft-gel assays demonstrated that nearly all of the GBM clonogenic cells were inhibited at 10^{-8} M of Cucurbitacin B. FACS analysis found that the compound (10^{-7} M, 24 hr) caused G2/M arrest. The GBM cells underwent profound morphologic changes within 15-30 min after exposure to Cucurbitacin B (10^{-7} M), rounding up and losing their pseudopodia associated with disruption of actin and microtubules, as observed by immuno-fluorescence.

Cucurbitacin B (10^{-7} M) caused prominent multinucleation of the cells after they were pulse-exposed (48 hr) to the drug, washed and cultured in normal medium for an additional 2 days. The drug (10^{-7} M, 3-24 hr) increased levels of p-p38, p-JNK and p-JUN in U87 and T98G GBM cell lines as seen by Western blot. Interestingly, alterations in cell morphology caused by Cucurbitacin B (10^{-7} M) were blocked by the JNK inhibitor SP600125. Cucurbitacin B has a prominent anti-proliferative activity on GBM cells; and at least in part, the mode of action is by affecting the cytoskeleton, as well as, the JNK pathway. Clinical trials of this drug should be pursued in GBM. © 2008 Wiley-Liss, Inc (58). Cucurbitacin E has also received great attention because of their cytotoxic and anticancer effects. Cucurbitacin L (23, 24-dihydrocucurbitacin I, Fig. 31) was also cytotoxic against KB and HeLa cell lines, but was less potent than cucurbitacin I (Fig. 32), which was isolated from *Citrullus colocynthis* (59-60, 24).

**Anti-spermatogenic Activity**

The antispermatogenic effect of an ethanolic extract of *Citrullus colocynthis* root was studied by Mali et al. in male albino rats. A crude 50% ethanol extract of *Citrullus colocynthis* roots was administered orally to male albino rats at dose levels of 50,
100, and 200 mg/kg for a period of 60 days for evaluation of antifertility effects. Significant decreases in cauda epididymal sperm motility, density, number of pups and fertility were observed in all treatment groups. A marked reduction in the weight of testes, epididymis and seminal vesicle was observed. The weight of the ventral prostate was non-significantly decreased. The ethanolic extract of the fruits produced stimulation, accompanied by increased motor activity, tremors, convulsions, diarrhoea and rapid irregular respiration preceding death in mice. The spermatogenic disfunction was also significant. The same extract demonstrated cytotoxic as well as mutagenic effects. It causes irritation of stomach and intestine. It is seldom prescribed alone. It induced haemorrhagic colitis. Three examples of toxic acute colitis were reported after ingestion of colocynth for ritual purposes. Its clinical feature was dysenteric diarrhoea (61).

Chaturvedi et al. have studied the induction of reversible antifertility with a crude ethanol extract of C. colocynthis fruits in male rats. A crude 50% ethanol extract of C. colocynthis was administered orally to male albino rats for evaluation of anti-fertility effects. The animal were divided in to five group; group A was vehicle treated control group; treatment group B, C, D and E received 100 mg/kg/day for C. colocynthis extract for periods of 20, 40, 60 days respectively. A 50% ethanol extracts of C. colocynthis showed an antiandrogenic nature, thereby reduced reversible infertility in male albino rats (62). The effect of C. colocynthis on reproductive system and fertility in female Spague-Dawely rats, the results indicate that long term exposure of C. colocynthis causes adverse effect on reproductive system (63).

Citrullus colocynthis is used in traditional medicine to inhibit the implantation of embryos. This study was to determine the number of embryos per pregnancy and the mortality rate in pregnant mice. 115 vaginal plug-positive mice were divided into four groups. The animals were given 30, 60 and 120 mg/kg hydroalcoholic extract of Citrullus colocynthis until 17th day of gestation. Control group was fed with solvent. At the day 17, the animals were sacrificed and the number of pregnant mice and embryos per pregnancy were counted. We found that while in 30 mg/kg group the mean number of embryos per pregnancy was around 10, no embryo was found in other groups. Furthermore, 3 out of 30 mice in 30 mg/kg group died, while in 60 mg/kg and 120 mg/kg groups the number of death was 7 and 14, respectively. A significant increase in the mortality rate was observed in groups treated with higher doses (60 and 120 mg/kg) of C. colocynthis in a dose-dependent manner (P<0.05). The fertility rate was significantly reduced as the dose increased (P<0.05), so that with higher doses, there were no pregnant mice. The total number of pregnant mice was significantly reduced in comparison to the control group, by exposure to the extract with concentrations of 60 and 120 mg/kg, although the mean number of fetuses per pregnancy was constant. According to the data, LD<sub>50</sub> of C. colocynthis was calculated to be 100 mg/kg. This dose is close to those which induce maximal infertility effects. As the results of the present study showed, hydro-alcoholic extract of C. colocynthis causes a decrease in fertility rate in a dose dependent manner but it has not any effects on the number of fetuses (64).

**Antibacterial and Antioxidant Activity**

The antibacterial screening for Citrullus colocynthis, the crude ethanolic extract of fruits, leaves, stems and root were examined for the potentialities against gram positive and gram negative bacilli. by Memon et al. Ethanolic extract of these parts to be active against gram positive bacilli, viz., Bacillus pumilus and Staphylococcus aureus, while fruit and root double strength gave positive result against gram positive bacillus (Bacillus subtilis). The negative bacillus E. coli and Pseudomonas aeruginosa showed no response (65).

The larvicidal activity of crude acetone, hexane, ethyl acetate, methanol, and petroleum ether extracts of whole plant of Citrullus colocynthis (L.) Schrad. and were assayed for their toxicity against the early fourth instar larvae of Culex quinquefasciatus (Diptera: Culicidae). The extract was concentrated under reduced pressure 22–26 mm Hg at 45°C and the residue obtained was stored at 4°C. The larval mortality was observed after 24 h exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in whole plant petrol ether extract of C. colocynthis. In this study, bioassay-guided fractionation of petroleum ether extract led to the separation and identification of fatty acids; oleic acid and linoleic acid were isolated and identified as mosquito larvicidal compounds. Oleic and Linoleic acids were quite potent against fourth instar larvae of Aedes aegypti L. (LC<sub>50</sub> 8.80, 18.20 and LC<sub>90</sub> 35.39, 96.33 ppm), Anopheles stephensi Liston (LC<sub>50</sub> 9.79, 11.49 and LC<sub>90</sub> 37.42, 47.35 ppm), and Culex quinquefasciatus say (LC<sub>50</sub> 7.66, 27.24 and LC<sub>90</sub> 30.71, 70.38 ppm). The results of this study clearly show that the extract and fraction of C. colocynthis that contain oleic and linoleic acid demonstrate a high larval mortality. However it is the first report of isolated active fraction tested for mosquito larvicidal activity. Thus, isolated compounds of the petroleum ether extract of C. colocynthis have potential to be developed as natural larvicidal agent (66).

Preliminary phytochemical screening of the plant showed the presence of large amounts of phenolics and flavonoids. Subsequent quantification showed the presence of 0.74% (m/m) phenolics (calculated as gallic acid) and 0.13% (m/m) flavonoids calculated as catechin equivalents per 100 g of fresh mass. The presence of phenolic compounds prompted us to evaluate its antioxidant activity. In the methanolic fruit extract of C. colocynthis was screened to evaluate its free--radical scavenging effect. Free radical scavenging effect of Citrullus colocynthis fruit increases with increasing concentration and maximum antioxidant activity was observed 2.5 g/mL (67).

Three flavone glucosides; isosaponarin (Fig. 6), isovitexin (Fig. 7) and isoorientin 3'-O-methyl ether (Fig. 8) showed significant antioxidant properties. The antioxidant property of the three flavonoids was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. A solution of DPPH (0.08 mg/mL) in MeOH was used. The compounds were dissolved in methanol to obtain a concentration of 1 x 10<sup>-1</sup> mg/mL. Dilutions were made to obtained concentrations of 5.0x10<sup>-2</sup>, 2.5 x10<sup>-2</sup>, 1.25x10<sup>-3</sup>, 6.25x10<sup>-3</sup>, 3.13x10<sup>-3</sup>, 1.56x10<sup>-3</sup> mg/mL.
Diluted solutions (5 mL each) were mixed with DPPH (5 mL) and allowed half hour for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and average absorption was noted for each concentration. Data were processed using EXCEL and the concentration that caused a 50% reduction in absorbance (IC50) was calculated. The same procedure was followed for the standard (quercetin). The antioxidant activity of three flavonoids was determined by this method and the IC50 values were found to be 7.13 x 10^-2, 5.62 x 10^-4 and 3.47 x 10^-3 mg/mL, respectively. The IC50 value of the positive control, quercetin, was 2.78 x 10^-5 mg/mL. Since reactive oxygen species are important contributors to tissue injury, inflammation, cancer and many other ailments, the antioxidant properties of three flavonoids probably contribute, at least to some extent, to the pharmacological and traditional medicinal uses of the C. colocynthis. (25)

The antioxidant properties of cucurbitacin B + E glucosides (cucurbitacin glucoside combination, CGC) and their direct free-radical scavenging properties, using ESR spectroscopy; Antioxidant activity was measured by the ability of the CGC to reduce preformed ABTS•+ into its native form and to inhibit MDA formation during the oxidation of linoleic acid. In both methods, the CGC exhibited antioxidant activity in a dose-dependent manner as expected from antioxidants. Using ESR spectroscopy, we found that the CGC inhibited •OH-dependent DEPMPO–OH adduct formation, O2•−-dependent DEPMPO–OOH adduct formation, and the •O2•−-dependent TEMPO adduct generated in the photoradiation–porphin system. The IC50 values were 0.38, 8, and 11 mM, respectively. Together, these data demonstrate that the CGC exhibits antioxidant properties, probably through the involvement of a direct scavenging effect on several free radicals (68).

**Miscellaneous activity**

The hypolipidaemic effect of *citrullus colocynthis* in rabbits, the plant extract was orally administered to the atherogenic rabbits (atherogenic + cholesterol + powder supplement 400 mg/kg/body weight/day dissolved in 5ml coconut oil) at dose of 1.2 g/kg body weight/day. During the period of the experiment blood was collected and serum was analyzes for lipid profile. Animals were sacrificed; the heart and liver were collected and kept -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) level were made for cholesterol, phospholipids and triglycerides. In addition blood serum was analyzed further for high density lipoprotein (HDL)-Cholesterol and all results were statistically analyzed using t-test. Hypolipidaemic nature of *Citrullus Colocynthis* (70% ethanol) extract was studied in hyperlipidaemic rabbits. The increased cholesterol levels were brought to normal by administration of C. colocynthis. Serum cholesterol levels were dropped from 940.7 to 230.41 (75.55%) and further to 119.2 (87.32%) by end of the experiment. Similarly, phospholipids and triglycerides levels were observed to be also reduced. *C. colocynthis* possess active hypolipidaemic constituents (69).

The antiallergic constituent on the methanolic fruit extract of *citrullus colocynthis* showed an inhibitory effect on ear passive cutaneous anaphylaxis as a type I allergic model in mice. The Curcurbitacin E 2-O-β-D-glucopyranoside and its aglycone exhibited antiallergic activity at a dose of 100 and 1.25 mg/kg, p.o., respectively (36). The hepato-protective activity of *citrullus colocynthis* extract against carbon tetrachloride induced hepatic damage in albino rats. Ethanolic and aqueous extract of root were used and albino rats were challenged with carbon tetrachloride (2.5 mg/kg in groundnut oil, p.o.). *Citrullus colocynthis* root and fruit extract showed significant hepato-protective action in carbon tetrachloride challenged rats (70). The effect of saponin (extracted from plant *citrullus colocynthis*) on mortality and histopathology change in mice, Divan et al. (71) evaluated the acute toxicity and histopathological effect of saponin on mice in order to assess its safety. The median lethal dose (LD50) of the saponin was 200mg/kg.

The heat-induced gelation of whole equi (*Citrullus colocynthis L*) seeds were studied by Uruskp et al. Despite the use of whole equi seeds as a food ingredient for generation, there is no report on its gelling ability (72). The immuno-stimulating activity of hot water soluble polysaccharides extracts of *Anacyclus pyrethrum*, *Alpinia galangal* and *citrullus colocynthis*. The results of the *in-vivo* effect at dose 50 and 25 mg/kg for *Anacyclus pyrethrum* and *Alpinia galangal*. While extract of *citrullus colocynthis* showed much weaker and variable immuno-stimulating activity (73). The development and evaluation of polyherbal formulation for hair growth-promoting activity; it was envisaged to preparation containing petroleum ether extract of the three herbs (*Cuscuta reflexa*, *citrullus colocynthis* Schard. and *Eclipta alba* ) in varying ratio and evaluating the formulation promoting activity (74).

A standardized extract of *citrullus colocynthis* used as oral natural laxative in folk medicine was tested for its influence on liver function parameters *in-vitro*. Cytochrome P450 (CYP) dependent production of reactive oxygen species (ROS) under the influence of *Citrullus colocynthis* extract was investigated by means of stimulated lipid peroxidation (LPO), H2O2 formation and amplified chemiluminescence in rat liver microsomes. In rat liver 9000 × g supernatants 4 monooxygenase reactions mediated by different CYP forms were measured. Putative hepatotoxic effects of *Citrullus colocynthis* extract were measured by means of potassium and GSH concentrations in and LDH leakage from precision-cut rat liver slices. For possible hepatoprotective effects the influence of the extract on carbon tetrachloride-induced changes of these parameters was investigated. *Citrullus colocynthis* extract in concentrations higher than 10 µg/ml incubation mixture proved to inhibit lipid peroxidation and ROS-production as well as CYP1A-, 2B- and 3A-dependent reactions with typical substrates. In contrast, H2O2 production was not reduced under the influence of the extract, a slight but significant increase was seen. *Citrullus colocynthis* extract was found to be free of hepatotoxic effect in concentration up to 100 µg/ml incubation mixture when liver slices were incubated in William medium E for 22 hrs (75).

The dried pulp of its fruits was used as a traditional medicine mostly for constipation (76). In Saudi Arabia, the Arabs that even treading barefoot on the squeezed fruit is enough to elicit
its purgative action but in East Africa, the seed tar was used by nomads in traditional medication applied to the skin (77). The ingestion of the cathartics fruit can have many undesired effects including bloody diarrhea (76) and even true acute toxic colitis and change in the colon similar to these other laxative abuse (47-48).

**TOXICITY**

*Citrullus colocynthis* seed was fed at 2% and 10% of the basal diet to 7-d-old Bovans-type chicks for 6 w. Average body weights and efficiency of feed utilization were markedly depressed in the chicks on 10% Citrullus feed, and the serum activities of LDH, AST and CK and concentrations of total lipid and zinc were significantly increased. The concentration of serum total iron binding capacity was particularly reduced in chicks on 2% Citrullus feed. The concentrations of other serum and blood constituents and of hepatic copper, manganese and zinc were not significantly changes. Lesions seen in the intestines, livers, kidneys and other tissues were fully reversed 4 weeks after removal from the experimental diet (78).

*Citrullus colocynthis*, which is used by diabetic patients as a hypoglycemic agent, but it, has been reported to cause gastrointestinal disorders after consumption in some patients. 50 rats were randomly divided into five groups (4 experimental and 1 controls). In the experimental groups a single daily dose of alcoholic extract of *Citrullus colocynthis* (50, 100, 200, 400 g/kg) was administered intraperitonally. Normal saline was administered in control group. After two weeks, the rats were killed and the liver, kidneys, other tissues and blood were collected. The liver, kidney, intestinal tissues and serum were analyzed for LDH, AST, CK, ALT, ALP, albumin, globulin, bilirubin, SGOT, SGPT, protein and total cholesterol. These results showed that the hepatic and renal functions in the experimental group treated with *Citrullus colocynthis* were significantly altered compared with the control group (79).

The preliminary toxicity on the individual and combined effect of *Citrullus colocynthis* and Nerium oleander in rat, the toxicity of diet containing 10% *Citrullus colocynthis* fruits or 10% Nerium oleander leaves or their 1:1 mixture. The rat treated for 6 weeks. Resulting dullness, ruffled hair, decreased body weight gains and feed efficiency and enterohepatonephropathy characterized by treatment with *Citrullus colocynthis* and Nerium oleander given alone. Diarrhoea was a prominent sign of *Citrullus colocynthis* poisoning. The combination effects of *Capsicum frutescens* and *citrullus colocynthis* on growth, haematological and pathophysiological parameters of rats. The toxicity diet contain 10% *Capsicum frutescens* and 10% *citrullus colocynthis* fruit or their 1:1 ratio mixture to rats treated for 6 weeks was evaluated. Body weight loss, inefficiency of feed utilization, diarrhoea and enterohepatonephropathy characterized *Citrullus colocynthis* toxicosis in rats (80-81). Sheep which were fed fresh *Citrullus colocynthis* fruits and leaves (0.2-10 g/kg) showed signs of poisoning (82). Doses of 10 g/kg of *Citrullus colocynthis* from 1 day to 2 weeks caused death in goats (83). The other side-effects of this plant are toxic acute colitis (47), reversible infertility (62) and hepato-toxicity in rats (75). These damages were sometimes enhanced with higher doses of *Citrullus colocynthis*.

**CONCLUSION**

Plants have potent biochemistry and have components of phytomedicine. Since time immemorial; man is able to obtain from them a splendid assortment of industrial chemicals. The medicinal actions of plants are unique to particular plant species or groups and are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. With more structurally diverse cucurbitacin-related compounds being isolated and characterized from natural sources, coupled with the advance of molecular pharmacology of cancer and inflammatory diseases, which allows activities to be assayed rapidly and molecular mechanisms deciphered, it can be anticipated that some lead compounds will be identified which could be used as templates for drug discovery.

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