

REVIEW ON *Citrullus colocynthis*

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ABSTRACT

Citrullus colocynthis [Family: Cucurbitaceae]. This is perennial herbs usually trailing. Commonly found wild in the sandy lands of North West, the Punjab, Sind, and Central and southern India, and coromandal coast. Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean region. Commonly *Citrullus colocynthis* known as - Indravaruni (Sanskrit), Chitrapala or Bitter apple. Medicinally root, bark and leaves are used. *Citrullus colocynthis* shows mild stomachic, bitter tonic, diuretic and antilithic property. As per Ayurveda and Siddha system of medicine it is Tikta-rasam, ushna-veeryam and katu vipakam and used as purgative, diuretic, lagu, kapharam and abortifacient. Fruit is bitter, pungent and used as purgative, anthelmintic, antipyretic, carminative, cures tumors, leucoderma, ulcers, asthma, etc. Root is useful in jaundice, ascites, urinary disease, rheumatism.

Keywords: *Citrullus colocynthis*, Cucurbitaceae, Indravaruni, Bitter apple.

INTRODUCTION

Citrullus colocynthis [Family: Cucurbitaceae]. This is perennial herbs usually trailing. Commonly found wild in the sandy lands of North West, the Punjab, Sind, and Central and southern India, and coromandal coast. Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean region. It originally bore the scientific name *Colocynthis citrullus*, but is now classified as *Citrullus colocynthis*. Tendrils are simple, 2-3fits slender and hairy. Leaves are very variable in size. Wild leaf is 3.8 to 6.3 cm in length and 2.5 cm in width while cultivated are large in size. Leaf show deltoid margin, pale green colour above and ashy colour beneath, scabrid on both surface, 5-7 lobed. *Citrullus colocynthis* shows presence of male and female flowers. Fruit are globular, slightly depressed, 5 – 7.5 cm in diameter, green in colour and get white glabrous when ripe. Fruit filled with a dry spongy very bitter pulp. Seeds are 4 – 6 mm long and pale brown.

BOTANICAL DESCRIPTION

Leaf

The angular leaves are alternately located on long petioles. Each leaf is almost 5 to 10 centimeters in length and has around 3 to 7 lobes. Sometimes the middle lobe might have an ovate structure. The leaves have a triangular shape with many clefts. The leaves have a rough, hairy texture with open sinuses. The upper surface of the leaves are fine green in color and the lower surface is comparatively pale.

Fruit

Each bitter apple plant produces around 15 to 30 globular fruits having a diameter of almost 7 to 10 centimeters. The outer portion of the fruit is covered with a green skin having yellow stripes. The fruits may also be yellow in color. The ripe fruits are characterized by a thin but hard rind. The fruits have a soft, white pulp which is filled with numerous ovate compressed seeds.

Flowers

The yellow-colored flowers appear singly at leaf axils. They are monoecious; the pistils and stamens are present in different flowers of the same plant. They have long peduncles. Each flower is also comprised of a yellow campanulate. The corolla has five lobes and the calyx is parted five ways. The female flowers are easily identified from the males by their villous, hairy ovary.

Seed

The seeds are around 6 mm in size, smooth, compressed and ovoid-shaped. They are located on the parietal placenta. The seeds are light yellowish-orange to dark brown in color.

Root

The Bitter Apple plant has a large perennial root that sends out long and slender, angular, tough, rough vine-like stems. The stems are normally spread on the ground and have a tendency to climb over herbs and shrubs by their axillary branching tendrils.

Distribution

Citrullus colocynthis found throughout India and Ceylon, both wild and cultivated. It is also indigenous in the Arabia, west Asia, tropical Africa, Mediterranean region.

Vernacular names in India

Sanskrit	→	Indravaruni
Hindi	→	Indrayan
English	→	Colocynth
Bengali	→	Makhal
Gujarati	→	Indrayan
Marathi	→	Kadu –indravani
Telugu	→	Eti-puchcha
Tamil	→	Paedikari Attutumatti
Malyalam	→	Paikumatti
Punjabi	→	Ghurunba

AYURVEDA AND SIDDHA ACTION

As per Ayurveda and Siddha system of medicine *Citrullus colocynthis* is Tikta –rasam, ushna veeryam, katu vipakam, purgative, diuretic, lagu, kaphaharam, puerperal disorders, abortifacient, ascites and dropsy. Oil from seed used in hair growth and maladu.

MEDICINAL VALUE OF CITRULLUS COLOCYNTHIS

1. The fruits are bitter, pungent, cooling, purgative, anthelmintic, antipyretic, carminative, cures tumors, ascites, leucoderma, ulcers, asthma, bronchitis, urinary discharges, jaundice, enlargement of spleen, tuberculosis glands of the neck,

dyspepsia, constipation, anemia, throat diseases, elephantiasis, joints pain.

2. Root is useful in jaundice, ascites, urinary diseases, rheumatism and given in abdominal enlargements and in cough and asthmatic attacks of children. A poultice of root useful in inflammation of the breast.
3. Fruit or root with or without nux-vomica is rubbed into a paste with water and applied to boils and pimples.
4. Past of the root is applied to the enlargement of abdomen of children^{1,2}.

PHYTOCHEMICAL CONSTITUENTS^{3,4,5}

The main chemical contain of fruit pulp colocynthin (the bitter principle upto 14 %), colocynthein (resin), colocynthetin, pectin gum. Seed contain a fixed oil (17 %) and albuminoids (6 %). Investigated chemical content is mentioned in Table 1.

PHARMACOLOGICAL ACTIVITY

1. Anti-inflammatory

Belsem Marzouk and et al study aqueous extracts *C. colocynthis* fruit and seed at immature state for anti-inflammatory activity using the carrageenan induced paw edema assay in rats. The best anti-inflammatory activities were obtained with immature fruits from south Tunisia. Therefore, *C. colocynthis* Schrad. Could be a potential useful product suitable for further evaluation for inflammatory diseases⁶.

2. Anticandidal and antibacterial

Rasool Khatibi and et al assess in vitro antibacterial and Anticandidal activity of aqueous and diluted acetone extracts of *C. colocynthis* Schrad. MIC and MBC/MFC were determined for plant organs at different maturation stages. Aqueous and diluted acetone extracts (from the plant's roots, stems, leaves and three maturation stages of its fruit and seeds) were screened for activity against Gram-negative and Gram-positive bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*) and various *Candida* spp. (*Candida glabrata*, *Candida albicans*, *Candida parapsilosis* and *Candida kreusei*). All extracts showed activity against all strains. The highest MICs and MBCs/MFCs were obtained from the fruit aqueous extracts (MIC 0.10 mg/ml against *C. albicans* and *C. glabrata*, 0.20 mg/ml against *E. coli* and *P. aeruginosa*), lowest activity from the root extracts. *C. colocynthis* Schrad shows antibacterial and Anticandidal properties.⁷

3. Antioxidant, anti-inflammatory/ analgesic or anti-proliferative

Saba AB and et al isolated Cucurbitacins are triterpenoid steroids. It is efficient antioxidant and this property lies in their ability to scavenge free-radicals such as hydroxyl radical, superoxide anions and singlet oxygen. This broad spectrum radical-scavenging capacity surpasses what had been reported for other natural antioxidants such as grape-seed extract, wheat, alfalfa and ginkgo biloba extracts. Reports also show that cucurbitacins adequately inhibit lipid peroxidation and oxidation⁸.

4. Hypoglycemic

Agarwal V and et al examine the effect of root of *C. colocynthis* on the biochemical parameters of normal and alloxan-induced diabetic rats. Diabetes mellitus was induced by intraperitoneal (120 mg/kg b.w.) injection of alloxan monohydrate for three days and the animals showing blood glucose level in the range of 175-300 mg/dL were selected for study. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 3rd, 5th and 7th day after the start of the experiment. On day 7, blood was collected by cardiac puncture under mild ether anesthesia. Aqueous extract of roots of *Citrullus colocynthis* showed significant reduction in blood sugar level (58.70%) when compared with chloroform (34.72%) and ethanol extracts (36.60%) ($p < 0.01$). The aqueous extracts showed improvement in parameters like body weight, serum creatinine, serum urea and serum protein as well as lipid profile and also restored the serum level of bilirubin total, conjugated bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP)⁹.

5. Anti-inflammatory and analgesic activities immature fruit and seed

Marzouk B and et al screen the analgesic and anti-inflammatory activities of aqueous extracts *Citrullus colocynthis* from roots and stems of the plant and from fruits and seeds at different maturation stages. Method use for testing analgesic and anti-inflammatory activities using, respectively, the acetic acid writhing test in mice and the carrageenan-induced paw edema assay in rats. All extracts displayed analgesic and anti-inflammatory activities at different doses without inducing acute toxicity. Topic

results were obtained with immature fruits followed by seeds. The stem and root extracts were shown to possess the less significant inhibitory activity against analgesic and anti-inflammatory models. *Citrullus colocynthis* Schrad. is a potentially useful drug suitable for further evaluation for rheumatoid arthritis and its folk medicinal use as an analgesic and anti-inflammatory agents is validated^{10,11}.

6. Hypolipidemic

Rahbar AR and et al investigate the hypolipidemic effect of *Citrullus colocynthis* beyond the hypoglycemic impact on human. One hundred dislipidemic patients were randomly divided into two groups namely treated (n = 50) group and placebo (n = 50) group. The subjects were treated daily by powdered seeds of *Citrullus colocynthis* (300 mg) and placebo for 6 weeks. The serums, TG, Chol, LDL-C, HDL-C, SGOT and SGPT were measured with enzymatic methods at the beginning and the end of the project. The significance of differences within these groups was calculated by Paired T-test and by analysis of covariance between them. There were significant differences within and between treated and placebo groups during our treatment in TG and in Cholesterol after intervention ($p < 0.05$). A daily intake of 300 mg day⁻¹ of powdered seeds of *Citrullus colocynthis* can lower the triglyceride and cholesterol concentration significantly in nondiabetic hyperlipidemic patients¹².

7. Anti – alopecia

Dhanotia R and et al evaluated *C. colocynthis* for hair growth activity in androgen-induced alopecia. Petroleum ether extract of *C. colocynthis* was applied topically for its hair growth-promoting activity. Alopecia was induced in albino mice by testosterone administration intramuscularly for 21 days. Its inhibition by simultaneous administration of extract was evaluated using follicular density, anagen/telogen (A/T) ratio and microscopic observation of skin sections. Finasteride (5 α -reductase inhibitor) solution was applied topically and served as positive control. Petroleum ether extract of *C. colocynthis* exhibited promising hair growth-promoting activity, as reflected from follicular density, A/T ratio and skin sections. The treatment was also successful in bringing a greater number of hair follicles in anagenic phase than the standard finasteride. The result of treatment

with 2 and 5% petroleum ether extracts were comparable to the positive control finasteride. The petroleum ether extract of *C. colocynthis* and its isolate is useful in the treatment of androgen-induced alopecia¹³.

8. Antibacterial and Anticandidal

Marzouk B and et al assess in vitro antibacterial and Anticandidal activity of aqueous and diluted acetone extracts of *Citrullus colocynthis* Schrad. MIC and MBC/MFC were determined for plant organs at different maturation stages. Aqueous and diluted acetone extracts (from the plant's roots, stems, leaves and three maturation stages of its fruit and seeds) were screened for activity against Gram-negative and Gram-positive bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*)-and various *Candida* spp. (*Candida glabrata*, *Candida albicans*, *Candida parapsilosis* and *Candida kreusei*). RESULTS: All extracts showed activity against all strains. The highest MICs and MBCs/MFCs were obtained from the fruit aqueous extracts (MIC 0.10mg/ml against *Candida albicans* and *Candida glabrata*, 0.20mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*), lowest activity from the root extracts¹⁴.

9. Mosquito larvicidal activity

Rahuman AA and et al assayed *Citrullus colocynthis* (Linn.) Schrad, for their toxicity against the early fourth instar larvae of *Culex quinquefasciatus* (Diptera: Culicidae). 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 petroleum ether extract of *C. colocynthis*. In the present 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. (LC₅₀ 8.80, 18.20 and LC₉₀ 35.39, 96.33 ppm), *Anopheles stephensi* Liston (LC₅₀ 9.79, 11.49 and LC₉₀ 37.42, 47.35 ppm), and *Culex quinquefasciatus* Say (LC₅₀ 7.66, 27.24 and LC₉₀ 30.71, 70.38 ppm). The structure was elucidated from infrared, ultraviolet, ¹H-nuclear magnetic resonance, ¹³C-NMR, and mass spectral data¹⁵.

10. Antioxidant and free radical scavenging

Kumar S and et al study methanolic fruit extract of *C. colocynthis* was screened to evaluate its free radical scavenging effect. The highest antioxidant and free radical scavenging ability of the fruit extract was observed at a concentration of 2500 microg mL(-1).¹⁶

11. Larvicidal

Rahuman AA and et al tested larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants against the early fourth instar larvae of *Aedes aegypti* L. and *Culex quinquefasciatus* (Say) (Diptera: Culicidae). The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in petroleum ether extract of *C. colocynthis*, methanol extracts of *C. indica*, *C. sativus*, *M. charantia*, and acetone extract of *T. anguina* against the larvae of *A. aegypti* (LC₅₀=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *C. quinquefasciatus* (LC₅₀=88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. The petroleum ether extract of *C. colocynthis* and methanol extract of *M. charantia* were more effective than the other extracts.¹⁷

12. Hypolipidaemic

Daradka H and et al study the effect of *Citrullus colocynthis* (70% EtOH) extract on lipid profile on Rabbits. The plant extract was orally administered to the atherogenic rabbits (atherogenic diet + cholesterol powder supplement 400 mg/kg/body weight/day dissolved in 5 mL coconut oil) at dose of 1.2 g kg(-1) body weight/day. During the hall period of the experiment blood was collected and serum was analyzed for lipid profile. Animals were sacrificed; the heart and the liver were collected and kept at -20 degrees 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 HDL-Cholesterol. All the results were statistically analyzed using students t-test. Hypolipidaemic nature of *Citrullus colocynthis* (70% EtOH) extract was studied in hyperlipidaemic Rabbits. The increased cholesterol levels were brought

to normal by administration of *Citrullus colocynthis*. Serum cholesterol levels dropped from 940.7 to 230.41 (75.55%) and further to 119.2 (87.32%) by the end of the experiment. Similarly, phospholipids and triglycerides levels were observed to be also reduced. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. *Citrullus colocynthis* possesses active hypolipidaemic constituents.¹⁸

13. Effects on reproductive system and fertility

Qazan WSH and et al study toxic effects of *Citrullus colocynthis* L. (400 mg/kg/body weight) on the reproductive system after administration to female Sprague-Dawley rats weighting 250-300 g for two time periods 4 and 12 weeks. Twenty adult female rats were divided into two groups and *Citrullus colocynthis* L. were intraperitoneally injected to experimental animals in dose of 400 mg/kg/body weight. First group containing 10 rats received treatment for 4 weeks and a second group of 10 rats received the same dose of treatment for a period of 12 weeks and compared with twenty non-exposed female rats received vehicle treatment. Female rats were allowed mating with males after 10 days prior to the last administration dose. Animals were autopsied under light anesthesia after mating and several parameters were determined including: number of pregnant rats, body and reproductive organ weight, number of implantation sites, viable fetuses and resorption sites. Assessment of pregnancies in females was measured and the significance of these results was calculated using students t and Chi-square tests. The effect of *Citrullus colocynthis* L. exposure on fertility was assessed in terms of pregnant rats number, implantation sites, viable fetuses and resorption sites. Exposure to *Citrullus colocynthis* L. for 4 weeks did not have much effect on fertility. Significant decrease in the relative ovarian weights and embryo weights in rats exposed to *Citrullus colocynthis* L. were observed. Exposure to *Citrullus colocynthis* L. for a 12 weeks resulted in a reduction in the percentage of pregnancies and in the number of implantation sites when compared with controls in both treatment periods. Rats receiving 12 weeks treatment showed a decrease in ovarian weights and a decrease in viable fetus's

number. These results indicate that long-term exposure of female rats to *Citrullus colocynthis* L. causes adverse effects on the reproductive system and fertility¹⁹.

14. Type II diabetic clinical trial

Huseini HF and et al conducted 2 month clinical trial in 50 type II diabetic patients using powder of *C. colocynthis*. Two groups of 25 each under standard antidiabetic therapy, received 100 mg *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, LDL, 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.²⁰

15. Growth inhibitory activity on breast cancer cells

Grossman S and et al study the effects of cucurbitacin glucosides extracted from *Citrullus colocynthis* leaves on human breast cancer cell growth. Leaves were extracted, resulting in the identification of cucurbitacin B/E glucosides. The cucurbitacin glucoside 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 G(2)/M phase of the cell cycle. Treated cells showed rapid reduction in the level of the key protein complex necessary to the regulation of G(2) exit and initiation of mitosis, namely the p34(CDC2)/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. The treatment caused elevation in p-STAT3 and in p21(WAF), proven to be a STAT3 positive target in absence of survival signals. Cucurbitacin glucoside treatment also induced apoptosis, as measured by Annexin V/propidium iodide staining and by changes in mitochondrial membrane potential ($\Delta\psi$) using a fluorescent dye, JC-1. We suggest that cucurbitacin glucosides exhibit pleiotropic effects on cells, causing both cell cycle arrest and apoptosis. These results suggest that cucurbitacin glucosides might have therapeutic value against breast cancer cells.²¹

16. Antifertility

Chaturvedi M and et al screened 50% ethanol extract of *Citrullus Colocynthis* Schrad in male albino rats for evaluation of antifertility effects. The animals were divided into five groups: group A was a vehicle-treated control group; treatment groups B, C, and D received 100 mg/kg/day C. Colocynthis extract for periods of 20, 40, and 60 days, respectively, and group E animals received the extract at 100 mg/kg/day for 60 days followed by 60 days of recovery. For androgenicity evaluation of the extract, the animals were divided into four groups: group F animals were castrated 30 days before the experiment to serve as controls, and group G, H, and I were subjected to castration 30 days before the experiments, followed by administration of fruit extract (100 mg/kg/day p.o.), testosterone

propionate (0.01 mg/rat/alternate day s.c.), and fruit extract along with testosterone propionate, respectively, for 30 days. Significantly reduced cauda epididymis sperm motility and density, number of pups, fertility, and circulatory levels of testosterone were observed in all treatment groups. The weights of testes, epididymis, seminal vesicle, and prostate were significantly decreased in groups B, C, and D. The weights of all organs in the different groups of the androgenicity study were markedly decreased in group F when compared with group A, in group G when compared with group F, and in group I when compared with group H, and increased in group H when compared with group F. The serum testosterone levels also showed a similar pattern. The concentration of testicular cholesterol was significantly elevated, while protein, sialic acid, acid and alkaline phosphatase concentrations were decreased. The histoarchitecture of the testes showed degenerative changes in the seminiferous epithelium, arrest of spermatogenesis at the secondary spermatocyte stage, cytolysis, and the lumen filled with eosinophilic material. Histometric parameters except Sertoli cell nuclear area and number of round spermatids showed marked alterations. All altered parameters restored to normal in group E. No changes were observed in body weight, litter size, hematology, and serum biochemistry. In conclusion, a 50% ethanol extract of *C. Colocynthis* showed an antiandrogenic nature, thereby reduced reversible infertility in male albino rats.²²

Table 1: Chemical content of *Citrullus colocynthis*

S. No	Part	Chemical content (reported / Investigated)
1	Seed	<ol style="list-style-type: none"> 1. Fatty acid like Stearic, Myristic, Palmitic, oleic, Linoleic, Linolenic acid. 2. Protein 8.25 % and rich content rich in lysine, leucin and sulfo amino acid like methionine 3. Vitamin B₁, B₂ and Niacin 4. Mineral like Ca, Mg, K, Mn, Fe, P and Zn
2	Aerial part and fruit	Flavonoid glycoside quercetin, Flavone- 3- glucoside viz iso-vitexin, iso-orientine and iso-orientine -3-methyl ether.
3	Fruit	<ol style="list-style-type: none"> 1. Cucurbitane type triterpen glycoside viz colocynthoside A & B. 2. Cucurbitane type triterpen glycoside viz cucurbitacin E 2-O-beta-D-glucoside and its aglycone Cucurbitacin E. 3. 2-O-beta-D-glucopyranosyl-16alpha-20R-dihydroxy-cucurbita-1,5,23E,25(26)-teraen-3,11,22-trione. 4. 2-O-beta-D-glucopyranosyl-cucurbitacin B and 2, 25-di-o-beta-D-glucopyranosyl-cucurbitacin L.

REFERENCES

1. Kirtikar KR and Basu BD. Indian Medicinal Plants, International Book Distribution, Dehera Dun. 2006; 4th Edn: 1147-1149.
2. Nadkarni KM. Indian Materia Medica, Bombay Popular Prakashan, Bombay, 2007; 3rd Edn : 335-337.
3. Gurudeeban S, Satyavani K and Ramanathan T. Bitter Apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. *Asian J Plant Sci.* 2010; 1: 1-8.
4. Yoshikawa M, Morikawa T, Kobayashi H, Nakamura A, Matsuhira K, Nakamura S and Matsuda H. Bioactive saponins and glycoside. XXVII. Structure of new Cucurbitane – type triterpen glycoside and antiallergic constituents from *Citrullus colocynthis*. *Chem Pharm Bull (Tokyo)*. 2007; 55(3): 428-434.
5. Nayab D, Ali D, Arshad N, Malik A, Choudhary M and Ahmed Z. Cucurbitacin glucoside from *Citrullus colocynthis*. *Nat Prod Res.* 2006;20(5): 409-413.
6. Belsem M, Zohra M, Ehsen H, Manel T, Abderrahman B, Mahjoub A and Nadia F. Anti-inflammatory evaluation of immature fruit and seed aqueous extracts from several populations of Tunisian *Citrullus colocynthis* Schrad. *African Journal of Biotechnology.* 2011; 10(20):4217-4225.
7. Rasool K and Jahanbakhsh T. Anticandidal screening and antibacterial of *Citrullus colocynthis* in South East of Iran. *Journal of Horticulture and Forestry.* 2011;3(13): 392-398.
8. Saba AB and Oridupa AO. Search for a novel antioxidant, anti-inflammatory/analgesic or anti-proliferative drug: Cucurbitacins hold the ace. *Journal of Medicinal Plants Research.* 2010; 4(25): 2821-2826.
9. Agarwal V, Sharma AK, Upadhyay A, Singh G and Gupta R. Hypoglycemic effects of *Citrullus colocynthis* roots. *Acta Pol Pharm.* 2012;69(1):75-79.
10. Marzouk B, Marzouk Z, Fenina N, Bouraoui A and Aouni M. Anti-inflammatory and analgesic activities of Tunisian *Citrullus colocynthis* Schrad. Immature fruit and seed organic extracts, *Eur Rev Med Pharmacol Sci.* 2011;15(6):665-672.
11. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A and Aouni M. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. *J Ethnopharmacol.* 2010;128(1):15-19.
12. Rahbar AR and Nabipour I. The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia. *Pak J Biol Sci.* 2010; 13(24):1202-1207.
13. Dhanotia R, Chauhan NS, Saraf DK and Dixit VK. Effect of *Citrullus colocynthis* Schrad fruits on testosterone-induced alopecia. *Nat Prod Res.* 2011; 25(15):1432-1443.
14. Marzouk B, Marzouk Z, Décor R, Edziri H, Haloui E, Fenina N and Aouni M. Antibacterial and Anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. from Medenine. *J Ethnopharmacol.* 2009; 125(2):344-349.
15. Rahuman AA, Venkatesan P and Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitol Res.* 2008 ;103(6):1383-1390.
16. Kumar S, Kumar D, Saroha K, Singh N and Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. Methanolic fruit extract. *Acta Pharm.* 2008;58(2):215-220.
17. Rahuman AA and Venkatesan P. Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitol Res.* 2008;103(1):133-139.
18. Daradka H, Almasad MM, El-Banna NM, Samara OH. Hypolipidaemic effects of *Citrullus colocynthis* L. in rabbits. *Pak J Biol Sci.* 2007; 10(16):2768-2771.
19. Qazan WSH, Almasad MM and Daradka H. Short and long effects of *Citrullus colocynthis* L. on reproductive system and fertility in female Spague-Dawley rats. *Pak J Biol Sci.* 2007;10(16):2699-2703.
20. Huseini HF, Darvishzadeh F, Heshmat R, Jafariazar Z, Raza M and Larijani B. The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of Type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. *Phytother Res.* 2009; 23(8):1186-1189.

21. Grossman S, Dovrat S, Gottlieb HE and Bergman M. Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells. Tannin-Spitz T, *Biochem Pharmacol.* 2007; 73(1):56-67.
22. Chaturvedi M, Mali PC and Ansari AS. Induction of reversible antifertility with a crude ethanol extracts of *Citrullus colocynthis* Schrad fruit in male rats. *Pharmacology.* 2003; 68(1):38-48.