Phytochemical analysis and pharmacological spectrum of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae)

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INTRODUCTION

Ethno medicinal studies play a major role to highlight the endemic plant species notably for the invention of recent crude medicine. Documentation of native medicinal information of ancient plant species has resulted in development of variety of recent medicine [1]. Medicinal plants are found through varied habitats and landscapes. Rajasthan state is understood for a fashionable floral diversity with 1911 wild species including 780 genera and 154 families. The most skillfully accustomed herbal medicines used by tribes of Rajasthan are plants of Fabaceae, Euphorbiaceae, Asteraceae, Apiaceae, Cucurbitaceae, Acanthaceae, Papaveraceae, Capparidaceae and Solanaceae families [2].

Chemical constituents of plants are of utmost importance for the discovery of therapeutic agents and in establishing the medicinal value of traditional plants [3]. *Citrullus colocynthis* (L.) is a member of family Cucurbitaceae, a xerophyte with huge medicative importance and a decent supply of valuable oil. It is cosmopolitan throughout Asia as well as India. It is normally referred to as bitter apple, or colosynth is employed as an abortifacient, cathartic, purgative and vermifuge, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, neoplasm and as an insectifuge. It additionally act as a conventional medicines for inflammatory disease, diabetes, inflammatory disorders, and gastralgia [4].

*Citrullus colocynthis* is annual or perennial (in wild), herbaceous, bearing monoeccic type flowers, pepo fruit and numerous seed. Its fruits are used as robust laxative [5]. This plant produces fruits known as as colocynth apples that are the same as the common *Citrullus vulgaris* and tastes bitter. It possesses solitary sterile flowers and an oversized, fleshy perennial root, that rises to from slender, tough, angular, vine-like stems and branched tendrils [6].

Aqueous pulp extract of *Citrullus colocynthis* fruits is used for treatment of kidney, liver related diseases. Isolated phenolic compounds have antioxidative and antineoplastic properties by absorption and neutralization of free radicals [7]. *Citrullus colocynthis* fruits are known for pain relieving, cathartic, anti-inflammatory, antioxidative, anti-diabetic effects. Cucurbitacins are reported to be the main constituent of fruits of this plant [8]. Infusion prepared from the seed as well as fruit of this plant are indeed recommended to diabetic patients. Since this plant has promising effect on diabetic patients and it is known that antidiabetic plants contains alkaloids, polyphenols, polysaccharides, gums and glycans [9].

This literature survey involves the documentation of data from 1950–2017 from authenticated sources like Google scholar, ScienceDirect and PubMed regarding phytochemical studies and pharmacological activities of various parts of *Citrullus colocynthis* plant. Figure 1 shows the fruits and leaves of *Citrullus colocynthis* lying in desert region while Figure 2 shows the dried fruits and seeds portion of *Citrullus colocynthis* plant.

PHYTOCHEMICAL STUDIES

Isolated compounds ursolic acid and cucurbitacin E 2-O-β-D-glucopyranoside in the methanolic fruit extract of *Citrullus colocynthis* showed antimicrobial activity. In
an experimentation isolated compounds, cucurbitacin and colocynthin from the ethanolic root extract of this species proved to be hepatoprotective against carbontetra chloride induced toxicity in experimental animals [10].

In an investigation identified with *Citrullus colocynthis* leaf extract two new triterpene glycosides were isolated from an ethyl acetic acid derivation concentrate of leaves of this plant alongside four known cucurbitacins. Compound structures were designed through spectroscopic information utilizing NMR and mass spectrometry. Two new cucurbitacins isolated were 25′-p-coumaroyl-3′-acetyl-2-O-β-D-glucocucurbitacin I and 6′-acetyl-2-O-β-D-glucocurbitacin E. The later coumaroyl cucurbitacin subordinate demonstrated huge particular cytotoxic action towards colorectal cell lines [11].

In an experimental study, the methanolic extract of the *Citrullus colocynthis* plant was divided into fractions soluble in hexane, chloroform, ethyl acetate, butanol, and water. Column chromatography of the ethyl acetic acid soluble fraction showed three new bitter principles named colocynthin A, B and C along with β-sitosterol, 3-O-β-D-glucopyranoside, elaterinide, and bryoamaride, respectively. New compounds were glycosides gave positive Molisch test result, as well as Salkowski and Liebermann–Burchard color reactions for triterpenes.

The compound colocynthin A was obtained as grayish indistinct amorphous solid and infrared spectrum showed bands for OH (3400 cm⁻¹), C¼O (1715 cm⁻¹), conjugated C¼O (1680 cm⁻¹), and olefinic (1610–1650 cm⁻¹) functionalities. The HR-FAB-MS (positive-ion mode) gave a [M+H]+ crest at m/z 659.3422, showing the atomic recipe C_{36}H_{50}O_{11}, and also a fragment ion [M-162+H] + at m/z 497.2832 because of the loss of the glucose moiety. Colocynthin B also obtained as grayish formless strong compound. The HR-FAB-MS of Colocynthin B gave signal at 691.3681 [M+H]+, and in conjunction with the ¹³C-NMR information, the sub-atomic formula decided as C_{37}H_{54}O_{12}. Colocynthin C was obtained as a grayish nebulous strong. The atomic equation got as C_{37}H_{55}O_{11} by HR-FAB-MS in the positive-particle mode, which gave an [M+H] + peak at m/z 675.3744 [12].

In a study conducted in the chloroform portion of the methanol concentrate of *C. colocynthis* natural products, dynamic constituent was isolated by silica gel segment chromatography and preparative HPLC. Structural analysis was done using spectroscopy including EI/MS, ¹H NMR, ¹³C NMR, COSY, DEPT, and HMQC NMR and then by direct comparison with an authentic reference compound. The dynamic compound segregated was 4-methylquinoline. Spectroscopy detailed around 4-methylquioline (C_{10}H_{9}N); EI/MS (70 eV) m/z M + 143 (100, base peak), 135 (40), 105 (39), 107 (40), 79 (46), 51 (13); ¹H NMR (CD_{3}OD, 600 MHz) δ 2.61 (s), 7.26−7.27 (d, J = 6.7 Hz), 7.42–7.43 (t, J = 6.9 Hz), 7.63–7.75 (t, J = 83.1 Hz), 8.00–8.05 (d, J = 35.7 Hz), 8.09–8.11 (d, J = 7.3 Hz), 8.42– 8.43 (d, J = 1.6 Hz); ¹³C NMR (CD_{3}OD, 150 MHz) δ 150.6, 148.2, 135.5, 130.0, 128.5, 128.1, 126.5, 124.4, 122.9, 19.6 [13].

Amid the examination of *Citrullus colocynthis* in vivo (leaf, stem, fruit and root) and in vitro callus a flavonoid quercetin was obtained. Rf value (0.82) of quercetin separated from extract samples resembles the Rf value of standard quercetin and in addition characteristic infrared spectral peaks were superimposable with individual standard reference mixes of quercetin. The HPLC parameter showed retention time of 3.475 min which matched with that of standard quercetin. Quercetin was present both in vivo and in vitro samples of *Citrullus colocynthis* [14].

In an investigation the reversed-phase preparative investigation of the butanol portion of the methanol concentrate of *C. colocynthis* fruits gave three flavonoid glycosides, isosaponarin, isovitexin and isoorientin 3′-O-methyl ether and two cucurbitacin glucosides, 2-O-β-D-glucopyranosylcucurbitacin L and 2-O-β-D-gluco-pyranosylcucurbitacin I. An ESIMS mass spectrum of isosaponarin showed [M+H]+ (positive ion mode) ion peak at m/z 595, M_r = 594 and obtained molecular formula as C_{27}H_{30}O_{15}. An ESIMS mass spectrum of isovitexin obtained as [M+H]+ ion peak at m/z 433, M_r = 432 and C_{21}H_{20}O_{10}. An ESIMS mass range of isoorientin 3′-O-methyl ether demonstrated [M+H]+ ion peak at m/z Figure 1: *Citrullus colocynthis* plant with leaves and fruits in the desert region.

Figure 2: Dried fruits and seeds of *Citrullus colocynthis* plant.
of 2-O-β-D-gluco-pyranosylcucurbitacin I gave [M+H]+ (positive ion mode) particle peak at m/z 677, suggesting M+ = 676 and understanding for C22H22O11, and ESIMS mass spectrum of 2-O-β-D-glucopyranosyl-cucurbitacin I demonstrated [M+H]+ (positive ion mode) proton peak at m/z 679, Mr = 676 and C22H22O11 [15].

The chloroform concentrate of the defatted Citrullus colocynthis plant was fractionated to result four glycosides as, 2-O-β-D-glucopyranosyl-cucurbitacin E, 2-O-β-D-glucopyranosyl-cucurbitacin I as the major product, 2-O-β-D-glucopyranosyl-cucurbitacin L and a novel glycoside 2-O-β-D-glucopyranosyl-(22-β-D-glucopyranosyl)-(22-β-D-glucopyranosyl)carbonyl absorption (1690 cm⁻¹) in the infrared spectrum showed observable parent ions either as [M +1]+ or [M + Na]+ ions. The spectral evidence lead to the structural assignment of 2-O-β-D-glucopyranosyl-(22-β-D-glucopyranosyl)-(22-7-hexanor-cucurbitacin I, which was the only degraded cucurbitacin glycoside reported till 1988. This compound was isolated as an amorphous powder, and investigation demonstrated a sub-atomic particle crest at m/z 585 [M (C22H22O11) + Na]+ in the FAB mass spectrum, and ketonic carbonyl absorption (1690 cm⁻¹) in the infrared spectrum [16].

In this study alcoholic concentrate of Citrullus colocynthis was extracted with chloroform and the product of this extraction after maintaining the pH 5.2–5.4 and addition of elaterase enzyme showed the formation of elaterin in the sediment. Further experimental studies segregated a white crystalline substance distinguished as Elaterin B (II) (cucurbitacin I). Thereafter two more compounds were isolated as dihydroelaterin B (III) and tetrahydroelatericin B (IV) with petroleum ether and the compounds were purified by utilizing Florisil® column section stepwise eluted with various blends of methanol: chloroform. Mass examination of the compound demonstrated the atomic particle peak at m/z 719. The elemental analysis demonstrated molecular recipe to be C38 H55 O13 and I.R., 308 H55 O13 + Na]+ in the FAB mass spectrum, and ketonic carbonyl absorption (1690 cm⁻¹) in the infrared spectrum [16].

During the gas chromatography-mass spectrometry spectral investigation of methanolic extract of Citrullus colocynthis 33 bioactive phytochemical compounds were obtained by investigating the retention time molecular weight, peak area and molecular formula. Spectral analysis of C. colocynthis revealed the existence of the methyl 6-oxoheptanoate, hexanoic acid, 2-isopropyl-2-methyl-5-oxo-, methyl ester, dodecanoic acid, 3-hydroxy, benzoferan,2,3-dihydro, 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl, 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl, 12,15-octadecadiynoic acid, methyl ester, (β)-pregnan-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutan, 3-N,Ndimethyllaurylammonio) propanesulfonate, 2H-1-benzopyran-3,4-diol, 2-(3,4-dimethoxyphenyl)-3,4-dihydro-6-met, 11,13-dihydroxy-tetradec-5-ynoic acid, methyl ester, cyclopenta [1,2] cycloheptan-3(3aH)-one, 1,2,3,6,7, 4-(2,4,4-trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one,

1-tetradecamine,N,N-dimethyl, α-D-glucopyranoside, O-α-D-glucopyranosyl-(1,6-fwarw. 3)-β-D-fructo, acetamide, N-methyl-N-[4-(3-hydroxypropyridinilidinyl)-2-buty nyl] -9-octadecenamide, (z) -butyrophenone, 2,3,4,6-tetramethyl-ethyl, 5,8,11,14-eicosatetraenoate, 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,ZZ), 1H-cyclopropa [3,4] benz [1,2-e] ezulene-5,7-b,9,9a 476.241018tetrol,1a,1b,4,4a, 9,12,15-octadecatrienoic acid, 9,10-Seccocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5,7Z,7E)-9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,ZZ), triazido-(1,2,3,4,5-pentamethycyclopenta-2,4-dienyl)-german, ethyl iso-allocholate, α-N-Normethadol, octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, phthalic acid, decyl oct-3-yl ester, 1,2-benzenedicarboxylic acid, bis-(8-methylthionyl)ester, phthalic acid, di(6-ethyl-3-octyl) ester, y-tocopheryl, 1,4-ethanophthalene -6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,7,10 and vitamin E [6].

In this study the chromatographic purification obtained from C. colocynthis fruits extract resulted in cucurbitacin E and cucurbitacin I glycosides. These compounds demonstrated promising outcomes against in vitro cytotoxic action against hepatoma cell line (HepG2) and mice-bearing tumor of Ehrlich’s ascites carcinoma (EAC). The in vivo study showed the tendency of both compounds prolonging the survival time, life span and normalize the biochemical parameters of the infected mice with EAC. The two compounds had strong inhibitory effect on HepG2 with IC50 3.5 and 2.8 nmol/ml individually [18].

During investigation of Citrullus colocynthis fruits using different solvents (n-hexane, methylene chloride, chloroform and ethanol) showed the presence of six compounds, and the most bottomless of them had retention time (Rt.) 4.8 min. (69.3%). This compound was purified by utilizing Florisil® column section stepwise eluted with various blends of methanol: chloroform. Mass examination of the compound demonstrated the atomic particle peak at m/z 719. The elemental analysis demonstrated molecular recipe to be C38 H55 O13 and I.R., proton and 3C NMR analysis recognized the compound as 2-O-β-D-glucopyranosylcucurbitacin E [19].

PHARMACOLOGICAL ACTIVITIES

Traditional uses

Citrullus colocynthis (Linn.) Schrad is an imperative therapeutic plant of Cucurbitaceae family. It is recognized plant in the ethical medicine and was utilized by individuals in country zones as a laxative, anti-diabetic and bug spray. Citrullus colocynthis has a valuable impact in sciatica and gout. It is valuable as douche during colic, sciatica, spinal pain, and loss of motion distresses. C. colocynthis oil obtained by boiling pulp with sesame or olive oil is externally used for ear pains, tinnitus, toothache, and male pattern baldness. The leaf of C.
**Antioxidant activity**

Methanolic fruit extract of *Citrullus colocynthis* showed the total phenolic content as 0.74% of gallic acid equivalents of phenolic and the total flavonoid content as 0.13% of catechin equivalents. The free radical scavenging effect of fruit extract of this plant on the 2,2-diphenyl-1-picrylhydrazyl radical found to be 88.0±2.7% (p < 0.005), at concentration of 2500 mg/mL while scavenging effects of ascorbic acid, BHA and α-tocopherol found to be 50 mg/mL of 89.5±1.1, 83.2±1.1 and 67.5±0.8% (p < 0.05) respectively with concentration 50 mg mL⁻¹. The most astounding antioxidative and free radical scavenging capacity of the fruit extract was seen at 2500 mg mL⁻¹ concentration [21].

**Antihyperlipidemic effect**

*Citrullus colocynthis* pulp and the seeds were investigated for the antihyperlipidemic effects on New Zealand rabbits. The hypercholesterolemic regimen of *Citrullus colocynthis* essentially expanded the measure of LDL-C, blood cholesterol, triglyceride, HDL-C and glucose (p<0.05). The reduction of low density lipoprotein-cholesterol in the groups administered with pulp extracts and 100 mg/kg of seed extract found significant (p<0.05). High density lipoprotein-cholesterol decrease was found in the groups administered with diet containing the standard regimen, along with cholesterol (0.5%) and 100 mg/kg of *Citrullus* pulp extract as well as with diet having standard treatment, with cholesterol (0.5%) and 100 mg/kg of *Citrullus* seed extract. The impact of *C. colocynthis* on the blood lipid profile in rabbits might be because of high measures of saponins in *C. colocynthis* which diminished cholesterol levels by lessening the ingestion of cholesterol, expanding the repulse of feces estriol, and looseness of the bowels because of expanded peristalsis. In this trial, the utilization of *C. colocynthis* came about huge diminishment of total serum cholesterol and LDL-C in groups administered with extracts [22].

**Antifertility effects**

The present investigation of *C. colocynthis* 50% ethanolic extract suppresses sperm density and motility of rats. But fruit extract administration showed a serious and reversible restraint of sperm fertility and density. The sperm density approached to around 10 million/mm³ in all treatment groups as compared to 46.5 million/mm³ in the vehicle-treated group. The weights of testicles, epididymis, original vesicles, and ventral prostate extraordinarily decreased after *C. colocynthis* treatment in the different groups due to the antiandrogenic nature of the drug recommending androgen imbalance and inhibition of the androgen generation by the testicles. Hence 50% ethanol extract of *C. colocynthis* fruit actuated reversible antifertility activity in male rats because of antiandrogenic nature [23].

**Antiulcer activity**

The present examination explored the antiulcer capability of ethanolic and aqueous extracts of *Citrullus colocynthis* plant. Ethanolic and aqueous extracts at 400 mg/kg indicated noteworthy (p<0.001) diminish in the total acidity, free acidity and gastric volume. The pH of the gastric juice significantly (p<0.001) ascended at the dose of 400 mg/kg. It indicated additionally significant (p<0.001) diminish in number of ulcer score index & ulcers using pylorus ligation ulceration model. *Citrullus colocynthis* fruit extracts exhibited a significant antiulcer activity in experimental male Wistar rats. Ethanolic extract indicated preferable hostile than aqueous extract [24].

**Anticonvulsant activity**

This examination researched the *Citrullus colocynthis* fruit extract as anticonvulsive in the treatment of seizures. Pentylenetetrazole induced convulsions were made in albino mice pretreated with fruits extract of 10, 25, 50, and 100 mg/kg dose. 25 and 50 mg/kg of hydroalcoholic extract delayed the beginning of seizures and diminished the duration in comparison to control group. *Citrullus colocynthis* pulp extract demonstrated a measurable significant reduction in the seizures term and increment in latency period of seizures instigated by pentylenetetrazole in mice. Anticonvulsive effect increased dose dependently with following doses 10, 25, and 50 mg/kg. The primary activity of the pentylenetetrazole-instigated seizure is diminishing Y-aminobutyric acid level in the cortex [8].

**Antimicrobial effect**

Antimicrobial effect of aqueous extract of the *Citrullus colocynthis* demonstrated high antibacterial action against *Staphylococcus aureus* and *E. coli* and significantly less impact against *Klebsiella pneumoniae* and *Bacillus subtilis*. While, methanolic extracts of this plant showed significant antibacterial action against *Bacillus subtilis*, *Streptococcus pyogenes*, *Salmonella typhi*, considerably less activity against *Streptococcus faecalis* and there was no impact against *Proteus vulgaris*, *Vibrio cholera* and *Proteus mirabilis*. The methanolic extract also indicated high antifungal activity against...
Aspergillus fumigatus, Mucor sp., and Aspergillus flavus, Candida albicans, Penicillium sp., and Rhizopus sp. did not demonstrated any antifungal action. The outcomes acquired in the investigation propose the antimicrobial role of Citrullus colocynthis in treating diseases caused by the test organisms [25].

Antifungal activity

The study assessed the antifungal action of hydroalcoholic extract of Citrullus colocynthis fruits against various Aspergillus and Candida strains. Activity was determined utilizing broth of macrodilution and disc diffusion methods. All tested parasitic strains indicated sensitivity to the extract. The growth restraint value of the fruits extract indicated high antifungal action against A. niger and A. fumigatus and a lesser impact against C. kruisi and C. guilliermondii. The minimum fungicidal concentration (MFC) and minimal inhibitory concentration (MIC) values ranged from 3.125–25 mg/ml and 1.56–12.5 mg/ml respectively [26].

Antibacterial and anticandidal activity

Citrullus colocynthis aqueous and diluted acetone extracts of stems, roots, leaves and maturation stages of its seeds and fruit) demonstrated activity against every single microbial strain such as Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis and Escherichia coli and different Candida spp. i.e., Candida kruzi, Candida parapsilosis, Candida albicans and Candida glabrata. The most elevated minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) were obtained from the fruit aqueous extracts (MIC 0.20 mg/ml against Escherichia coli and Pseudomonas aeruginosa and 0.10 mg/ml against Candida albicans and Candida glabrata) and obtained lowest activity from the plant root extracts [28].

Hypoglycemic activity

A study showed presence of saponin glycosides, triterpenoids, alkaloids, flavonoids and resins in aqueous extract of roots of Citrullus colocynthis which lessened the glucose level (58.70%) when compared with ethanolic (36.60%) and chloroform (34.72%) extracts (p< 0.01). Assumed mechanism behind the lessening in the blood glucose levels of diabetic rats treated with the extracts is due to stimulation of residual pancreatic mechanism or by increment in fringe use of glucose. The water extracts of Citrullus colocynthis enhanced the parameters like serum urea, body weight, serum creatinine and serum protein additionally lipid profile and furthermore reestablished the serum level of bilirubin add up to serum glutamate oxaloacetate transaminase (SGOT), conjugated bilirubin, serum glutamate pyruvate transaminase (SGPT) and antacid phosphatase [29].

Antihyperglycaemic effect

Oral dosage of aqueous extract of Citrullus colocynthis (300 mg/kg) in normal rabbits produced noteworthy decrease in plasma glucose after 1 h and exceptionally huge after 2, 3 and 6 h. The hypoglycemic impacts of tertiary and quaternary alkaloids, glycoside and saponin segments introduce in this plant at a measurements (50 mg/kg p.o) were studied in normoglycemic rabbits. The alkaloidal extract did not essentially bring down the blood glucose levels while the glycosidic extract fundamentally brought down the fasting glucose levels after 2 and 3 h and exceptionally huge after 6 h. The action was more articulated with saponin extract which decreased the glucose levels (fasting) after 1 and 2 h and significantly (p<0.001) after 3 and 6 h [30].

Antidiabetic activity

Antidiabetic action of petroleum ether fruits extract of Citrullus colocynthis against Streptozotocin initiated hyperglycemic rats was assessed after oral administration of two distinct doses (300 and 500 mg/
kg) of *Citrullus colocynthis*. Additionally subacute impact i.e., antihyperglycemic effect was seen on seventh and in addition day-14 of the analysis. Administration of petroleum ether extract of *Citrullus colocynthis* fundamentally enhanced body weight of diabetic rats in a dose and time dependent manner. The total hemoglobin and glycosylated hemoglobin levels (p < 0.01) was also restored by administration of extracts. The investigation reported that petroleum ether extract of *Citrullus colocynthis* demonstrated critical pharmacological action towards bringing down blood glucose in diabetes [31].

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**Keywords:** Cucurbitaceae family, Cucurbitacins, Phytochemistry, Pharmacological activites

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**Guarantor**

The corresponding author is the guarantor of submission.

**Conflict of Interest**

Authors declare no conflict of interest.

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**REFERENCES**


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