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Citrullus colocynthis (Colocynth) Extracts in Therapy: Extraction, Standardization, Antidiabetic and Anticancer Mechanisms, and Toxicity Management - A Comprehensive Review

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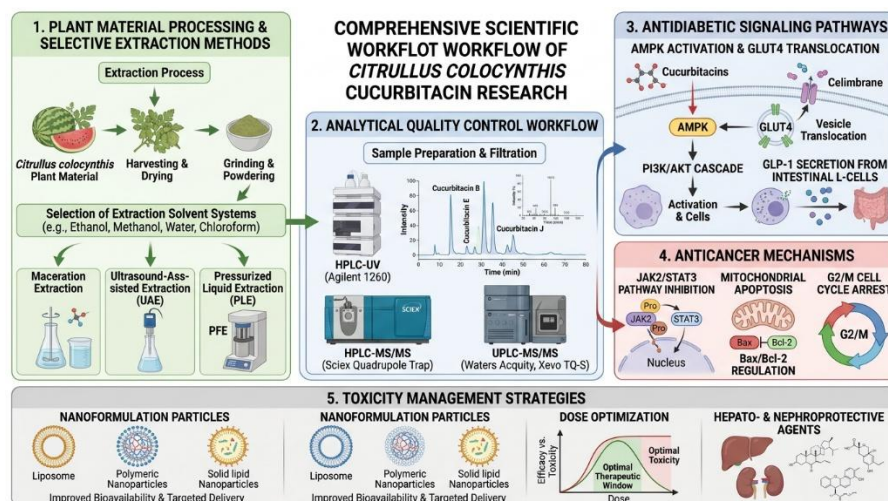
ABSTRACT

Citrullus colocynthis (L.) Schrad, known as colocynth or bitter apple, is a perennial species of cucurbit native to dry zones of Africa, Asia and the Mediterranean basin. Bitter gourd is rich in cucurbitacins (highly oxygenated tetracyclic triterpenoids) and its fruit pulp, seeds and leaves have been a subject of continuous study interest due to their marked antidiabetic and anticancer activities. However, the clinical translation of colocynth-derived preparations is hampered by the absence of standardised extraction techniques, the variation in phytochemical profiles between regional accessions, and the short therapeutic window due to cucurbitacin-associated toxicity. This review offers a comprehensive evaluation of selective extraction procedures for the enrichment of cucurbitacins (mainly types B, E, I and their glycosides) from *C. colocynthis* plant material and evaluation of contemporary analytical quality control platforms like HPLC-UV, HPLC-MS/MS, UPLC-MS/MS and HPTLC techniques which are important for batch-to-batch standardisation. The molecular mechanisms of the antidiabetic action of cucurbitacins are discussed, focusing on the modulation of the AMPK/PI3K/Akt cascade, GLUT4 translocation and GLP-1 stimulation, along with the anticancer pathways including the inhibition of JAK2/STAT3 and the induction of apoptosis by regulation of Bax/Bcl-2 and cell cycle arrest. Finally, the toxicological profile of colocynth preparations is critically discussed and practical options for the mitigation of side effects are given, including dose optimisation, nanoformulation, prodrug derivatisation and co-administration with hepatic-protective drugs. This review intends to connect ethnopharmacological knowledge with evidence-based medicinal development of cucurbitacin-rich colocynth extracts.

Highlights

- Selective extraction protocols for cucurbitacins B, E, and I from *C. colocynthis* are systematically compared.
- Validated HPLC-MS/MS and UPLC-MS/MS methods enable batch standardization with LODs in the picogram range.
- Antidiabetic activity of cucurbitacins is mediated through AMPK activation, GLUT4 translocation, and GLP-1 release.
- JAK2/STAT3 pathway inhibition and Bax/Bcl-2-mediated apoptosis constitute the principal anticancer mechanisms.
- Nanoformulation, dose titration, and hepatoprotective co-administration are proposed to manage cucurbitacin toxicity

Graphical Abstract



1. Introduction

The increasing prevalence of metabolic and neoplastic illnesses has stimulated the quest for bioactive natural items that could supplement or, in some circumstances, replace conventional therapies. One of the plant species that has garnered increased interest in this respect is *Citrullus colocynthis* (L.) Schrad (family Cucurbitaceae), a herbaceous vine native to the deserts of North Africa, the Middle East and South Asia [1,2]. Known as “bitter apple” or colocynth, this plant has been used in traditional medicine systems for millennia, mentioned in ancient Egyptian, Greco-Roman, Ayurvedic and Unani pharmacopoeias for the treatment of constipation, diabetes, jaundice and numerous inflammatory disorders [3,4]. Most of its pharmacological efficacy is conferred by the major bioactive ingredients, cucurbitacins which are a structurally heterogeneous group of highly oxygenated tetracyclic triterpenoids, intensely bitter and cytotoxic [5,6]. For several decades the ethnopharmacological uses have been impressive, but the discovery of medicinal medicines from colocynth has been delayed by a number of inter-related issues. The most important of them is the lack of standardised extraction and purification processes that could lead to repeatable cucurbitacin profiles from diverse plant accessions and geographical sources [7]. The cucurbitacin concentration of *C. colocynthis* changes substantially with genotype, soil type, environment and stage of fruit development so that crude extracts may vary considerably from batch to batch [8]. This variability is compounded by the extremely narrow therapeutic index of cucurbitacins, where dosages that provide the desired pharmacological effects are uncomfortably close to doses that cause gastrointestinal, liver and renal damage [9,10]. Thus, thorough analytical quality control is required for colocynth preparations to move from the pre-clinical domain to regulated therapeutic products. Here, we present a systematic and critical review of the current literature dealing with (i) selective extraction strategies for the enrichment of cucurbitacins from *C. colocynthis* tissues, (ii) state-

of-the-art analytical techniques for their identification and quantification, (iii) the molecular mechanisms underlying the antidiabetic and anticancer activities of cucurbitacins, and (iv) evidence-based approaches for toxicity management. The goal of this synthesis of these four categories into a single cohesive story is to give academics and pharmaceutical developers a practical blueprint for turning the ethnopharmacological promise of colocynth into standardised, clinically viable formulations.

2. BOTANICAL DESCRIPTION AND ETHNOBOTANICAL BACKGROUND

Citrullus colocynthis is a monoecious perennial trailing vine with deeply lobed leaves and spherical fruits about 7–10 cm in diameter that change from green to pale yellow when ripe [1]. The species is found in sandy, dry soils over the Saharan region, Arabian Peninsula, Iran, Pakistan and north-west India and is highly drought tolerant by virtue of a deep taproot system [2,4]. In traditional treatments, the fruit pulp has traditionally been the main part of the plant that is employed, however seeds, leaves and roots have also been used in medical applications by other civilisations [3]. In traditional Arabian and Persian medicine, decoctions of the dried fruit were administered orally as a purgative and anthelmintic, and topically for joint pain and skin infections [4,11]. Ayurvedic practitioners used colocynth preparations to manage liver disorders and fever, while North African communities applied the seed oil as an emollient and the fruit paste as an anti-inflammatory poultice [3,12]. Perhaps the most persistent traditional claim, and the one that has attracted the most modern scientific validation, is the antidiabetic use: several ethnobotanical surveys from Iran, Saudi Arabia, and Morocco have independently documented the consumption of dried colocynth fruit by type 2 diabetic patients seeking glycemic control [13,14]. These converging ethnopharmacological observations provided the initial impetus for the pharmacological and phytochemical investigations

that would eventually identify cucurbitacins as the primary therapeutic agents [5,6].

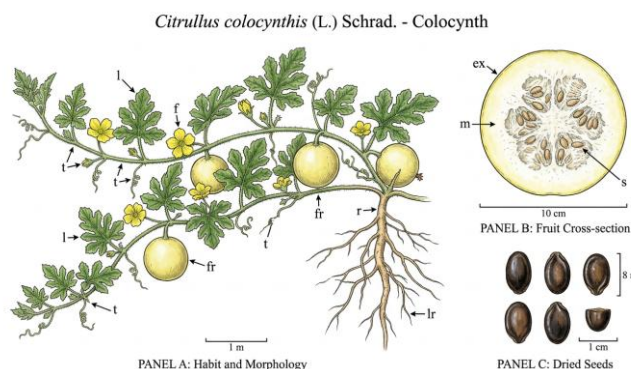


Figure 1. (A) Whole plant morphology of *C. colocynthis* showing trailing vine, lobed leaves, fruit, and taproot system. (B) Cross-section of the mature fruit displaying the spongy mesocarp and seeds. (C) Dried seeds (~7–10 mm).

3. PHYTOCHEMISTRY OF *C. colocynthis*: CUCURBITACINS AS PRINCIPAL BIOACTIVES

The phytochemical profile of *C. colocynthis* comprises of glycosides, flavonoids, alkaloids, phenolic acids, saponins, fatty acids, essential oils and most importantly cucurbitacins [5,6,15]. Cucurbitacins are tetracyclic triterpenes which are officially generated from the cucurbitane skeleton (19-(10 $\rightarrow$ 10 β)-abeo-10 α -lanost-5-ene) and at least 20 different cucurbitacins and their glycosides have been isolated from colocynth fruit pulp and seeds up to now [6,16]. The most pharmacologically relevant among these are cucurbitacin B (CuB), cucurbitacin E (CuE), cucurbitacin I (CuI), cucurbitacin E 2-O- β -D-glucopyranoside (CuE-glu), and cucurbitacin L, along with colocynthosides A and B [6,17,18]. Structurally, they share a common triterpenoid backbone but differ in their oxidation pattern, degree of saturation and glycosylation state, which together influence bioactivity and toxicity [5,19]. For example, cucurbitacin B and E are powerful cytotoxic aglycones, and their glucosides, though less poisonous, are nevertheless biologically active [17,18]. The current SAR investigations reveal that the pharmacophoric characteristics essential for the anticancer action are the C-2 hydroxyl, the C-3 carbonyl, and the Δ 23-double bond in the side chain, whereas the C-2 glycosylation regulates solubility and bioavailability [5,19,20]. These structural differences influence directly the extraction strategy and quality control, defining the analytical markers to target and the chemical features to take advantage of throughout the purifying process. It should be noted that the cucurbitacin profile is different amongst the plant organs. Cucurbitacins B and E are generally accumulated in the fruit pulp in higher proportions, while cucurbitacins I and L are preferentially accumulated in leaves [6,16]. In contrast, seeds possess a relatively low cucurbitacin content but are rich in fixed oils, proteins, and phenolics, which provide particular nutritional and antioxidant qualities [12,21]. This organ-specific distribution has ramifications for extraction strategy, since the choice of starting material directly determines both the cucurbitacin profile

and the impurity matrix of the final extract. Furthermore, environmental stressors such UV exposure, water deficiency and herbivore pressure have been demonstrated to upregulate cucurbitacin production as a chemical defence mechanism, indicating that plants cultivated in more stressful environments may produce larger cucurbitacin titers [8,31]. Such biological diversity indicates the need for proven analytical procedures to allow the assessment of each batch on its own merits and not just on generic assumptions of composition.

Table 1. Major cucurbitacins identified in *C. colocynthis*: molecular data and reported biological activities. This table was compiled and prepared by the authors; the molecular data (molecular formula, molecular weight and CAS number) were obtained from public chemical databases and the reported biological activities were summarised from the cited literature. It is not reproduced from any single previously published table.

Compound	Mol. Formula	MW	CAS No.	Key Activities	Biological
Cucurbitacin B	C ₃₂ H ₄₆ O ₈	558.70	6199-67-3	Anticancer (STAT3 inhibition), anti-inflammatory, hypoglycemic, hepatoprotective	
Cucurbitacin E	C ₃₂ H ₄₄ O ₈	556.70	18444-66-1	Anticancer (apoptosis, actin disruption), antitumor, anti-allergic	
Cucurbitacin I (JSI-124)	C ₃₀ H ₄₂ O ₇	514.65	2222-07-3	Selective JAK2/STAT3 inhibitor, anticancer, anti-angiogenic	
Cucurbitacin E-glu	C ₃₈ H ₅₄ O ₁₃	718.83	65116-86-3	Anti-breast cancer, antioxidant (ROS scavenging), reduced toxicity vs. aglycone	
Cucurbitacin L	C ₃₀ H ₄₄ O ₇	516.67	595-89-1	Anti-allergic, anti-inflammatory, moderate cytotoxicity	
Colocynthoside A	C ₃₆ H ₅₂ O ₉	636.79	--	Antidiabetic, anti-inflammatory	

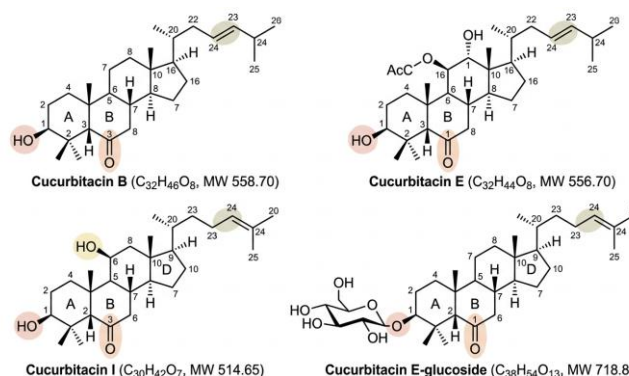


Figure 2. Representative chemical structures of cucurbitacins B, E, I, and E-glucoside isolated from *C. colocynthis*. The common cucurbitane tetracyclic skeleton is shown with key functional group annotations.

4. SELECTIVE EXTRACTION TECHNIQUES FOR CUCURBITACINS

4.1. Conventional Solvent Extraction

The earliest and still most widely adopted approach to extracting cucurbitacins from colocynth tissue involves sequential solvent extraction based on polarity gradients. In a typical protocol, dried and powdered fruit pulp is first defatted with *n*-hexane, then extracted with chloroform or dichloromethane to recover cucurbitacin aglycones, followed by ethyl acetate and methanol fractions that capture more polar glycosylated forms [6,15,21]. Tannin-Spitz et al. [17] isolated cucurbitacin glucosides from colocynth fruit that inhibited human breast cancer cell growth using this simple partitioning method. Cucurbitacin B and I aglycones were highest in chloroform, while CuE-glucoside was highest in ethyl acetate [21,22].

Optimising temperature is crucial but often overlooked. The thermolabile side-chain activities of cucurbitacin degrade at above 80 °C, although Cucurbitaceae species yielded the most phenolic and terpenoid compounds at 60 °C with low thermal degradation [23,24]. Too long an extraction time can co-extract unwanted matrix components, complicating downstream purification, and too short a contact time will leave a significant portion of the cucurbitacins trapped in the plant cell walls [24].

4.2. Advanced and Green Extraction Approaches

Due to the advantages of the traditional maceration method, various research groups have investigated the faster extraction methods. The pressurised liquid extraction (PLE) presents the benefit to work at high temperature and pressure that increases the solvent penetration in the plant matrix and reduces the time of the extraction from hours to minutes [25]. Another potential approach is ultrasound-assisted extraction (UAE), where the cavitation forces created by the ultrasonic waves break down the cell walls and release the intracellular cucurbitacins more efficiently than by passive diffusion alone [26]. The use of supercritical CO₂ extraction (SFE) on related Cucurbitaceae species has been investigated and it is a green

technology per se, avoiding residues from organic solvents. However, the application of SFE to cucurbitacins from *C. colocynthis* has not been reported and needs systematic investigation [27]. Another improvement is enzyme-assisted extraction (EAE) in which cellulases or pectinases are used to breakdown cell-wall polysaccharides prior to solvent extraction, thus enhancing the accessibility of intracellular cucurbitacins [26]. More recently, response surface methodology with a Box–Behnken design has been applied to systematically optimise the solvent extraction of bioactive compounds from *C. colocynthis*, identifying ethanol concentration and temperature as the most influential parameters while improving extraction efficiency [63].

Irrespective of the extraction procedure, a flash chromatography stage and subsequent semi-preparative HPLC are usually necessary to attain the purity levels (>95%) required for pharmacological testing and creation of reference standards [28,29]. The US patent by Lavie et al. [29] described a practical protocol involving sequential hexane–chloroform extraction of pressed fruit juice, flash column chromatography and semi-preparative reverse-phase HPLC with acetonitrile–water gradients for the isolation of cucurbitacin B and D at >97% purity from *C. colocynthis* and *Cucurbita andreana*.

Table 2. Comparison of extraction techniques for cucurbitacins from *C. colocynthis*.

Method	Solvent System	Conditions	Target Analytes	Advantages / Limitations
Maceration / Soxhlet	CHCl ₃ , EtOAc, MeOH (sequential)	60 °C, 6–24 h	CuB, CuE, CuI, CuE-glu	Simple setup / Long time, large solvent volumes
PLE (ASE)	MeOH or EtOH	80–120 °C, 100–200 bar, 10–20 min	Total cucurbitacins	Fast, low solvent use / Expensive equipment
UAE	EtOH:H ₂ O (70:30)	40 kHz, 40–60 °C, 30–60 min	Cucurbitacins + phenolics	Enhanced yield, scalable / Heat control needed
Supercritical CO₂	sc-CO ₂ + EtOH modifier	40–60 °C, 150–350 bar	Nonpolar cucurbitacins	Green, no residue / Low polarity limits glycosides
Enzyme-Assisted	Cellulase/pectinase + MeOH	50 °C, pH 4.5–5.5, 2–4 h	Intracellular cucurbitacins	Higher accessibility / Extra enzyme cost

Flash semi-prep HPLC	+	ACN:H ₂ O gradient	C18 column, UV 229– 254 nm	CuB, CuD (>97% purity)	High purity for standards / Low throughput
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5. ANALYTICAL QUALITY CONTROL OF CUCURBITACIN-RICH EXTRACTS

5.1. Chromatographic Methods

Reliable quantification of cucurbitacins is the backbone of extract standardisation and is critical for dose repeatability in preclinical and clinical research. The most accessible platform is HPLC with ultraviolet detection (HPLC-UV), which has been frequently used for cucurbitacin profiling. Typical detection wavelengths are 229–254 nm, which exploit the UV-absorbing conjugated enone system present in the cucurbitacin core [30,31]. Still the gold standard for analytical separation are reverse phase C18 columns with acetonitrile–water mobile phases, which have validated procedures reaching limits of quantification (LOQs) in the low microgram-per-milliliter range for plant extracts [30].

HPLC-MS and HPLC-MS/MS have become essential for applications needing increased sensitivity and specificity such as pharmacokinetic research and trace level impurity screening. Bajcsik et al. [32] developed a validated HPLC-MS method for the simultaneous detection of cucurbitacins B, E, I, and E-glucoside in plant material and human bodily fluids. A pentafluorophenyl column and negative electrospray ionisation were used. LOQs of 0.05–0.42 ng/mL were obtained. Recently, UPLC-MS/MS platforms have further improved sensitivity. For example, Zhong et al. [33] reported a UPLC-MS/MS method to quantify cucurbitacin B in rat plasma with a LOQ of 0.05 ng/mL and an outstanding linearity range of 0.05–50 ng/mL. These advancements in detection sensitivity are not only academic, but are crucial to enabling meaningful pharmacokinetic modelling, which influences dose selection for clinical studies [33,34].

5.2. HPTLC and Complementary Techniques

High-performance thin-layer chromatography (HPTLC) is a fast and inexpensive alternative for semi-quantitative quality control and preliminary screening [35], particularly in resource-limited settings. Debnath et al. [35] validated an HPTLC approach for quantitative determination of cucurbitacin E in cucumber cultivars with good linearity in the range of 2–10 µg/spot. HPTLC, while not as sensitive or as resolving as HPLC-MS, provides high throughput (many samples analysed in a single run) and minimal solvent consumption, which are desirable for routine batch-release testing of standardised extracts. Nuclear magnetic resonance (NMR) spectroscopy and infrared (FT-IR) spectroscopy are valuable orthogonal approaches for structural confirmation of isolated cucurbitacins, but are less suited for quantitative investigation of complicated extracts [31,36].

Table 3. Summary of validated analytical methods for cucurbitacin quantification.

Technique	Analytes	Matrix	LOQ	Linear Range	Reference
HPLC-UV	CuB, CuD	Plant extract	~1 µg/mL	1–100 µg/mL	Potterat & Hamburger, 2014 [30]
HPLC-MS (Q1)	CuB, CuE, CuI, CuE-glu	Plant + serum	0.05–0.42 ng/mL	1–50 ng/mL	Bajcsik et al., 2017 [32]
UPLC-MS/MS	CuB	Rat plasma	0.05 ng/mL	0.05–50 ng/mL	Zhong et al., 2022 [33]
UHPLC-MS/MS	CuB, CuE	Rat plasma	1.60 ng/mL	1.6–800 ng/mL	Wang et al., 2015 [34]
LC-MS/MS	CuE, CuI	Rat plasma	0.5 ng/mL	0.5–200 ng/mL	Oliveira et al., 2019 [34]
HPTLC	CuE	Plant extract	~2 µg/spot	2–10 µg/spot	Debnath et al., 2021 [35]

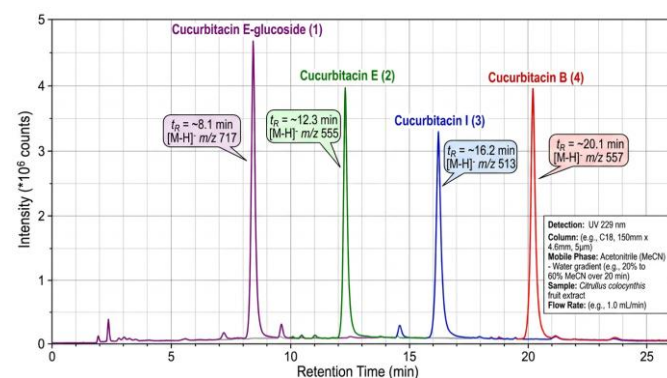


Figure 3. Representative HPLC-MS chromatogram showing separation and detection of cucurbitacins B, E, I, and E-glucoside in a *C. colocynthis* fruit extract. Peaks are color-coded: CuE-glu (purple), CuE (green), CuI (blue), CuB (red). This figure is an original schematic illustration prepared by the authors to depict the typical chromatographic profile of the major cucurbitacins; it is not reproduced or adapted from any previously published chromatogram.

6. ANTIDIABETIC MECHANISMS OF ACTION

6.1. In Vivo and Clinical Evidence

There is a large amount of preclinical evidence and a modest number of human studies that support the antidiabetic potential of *C. colocynthis*. Oral administration of colocynth fruit extracts (50–300 mg/kg body weight) has consistently shown significant reduction of fasting blood glucose, HbA1c, and serum lipid profiles in animal experiments on the streptozotocin-induced and alloxan-induced diabetic rodent models [13,37,38]. Interestingly, a pilot clinical experiment on 36 type 2 diabetes patients showed that topical administration of a *C. colocynthis* extract to the feet lowered blood glucose by 46–65 mg/dL per application, although the open-label form of the study limits the validity of the conclusions [39]. Randomised, double-blind, placebo-controlled trials are still lacking and constitute an important gap in the clinical evidence base [39,40]. In a recent (2026) study, oral administration of *C. colocynthis* extract

to streptozotocin-induced diabetic rats lowered blood glucose and glycated haemoglobin, raised serum insulin and restored the normal localisation of GLUT2 in pancreatic β -cells, further supporting its antidiabetic potential [65].

6.2. Molecular Mechanisms

At the molecular level, the hypoglycemic effects of cucurbitacins seem to be mediated by various convergent mechanisms. Perhaps the best characterised mechanism is the activation of 5'-adenosine monophosphate-activated protein kinase (AMPK), a master metabolic sensor that when phosphorylated, promotes the translocation of glucose transporter type 4 (GLUT4) from intracellular vesicles to the plasma membrane, thereby enhancing glucose uptake into skeletal muscle and adipose tissue [41,42]. Kim et al. [43] have shown that cucurbitacin B activated duodenal AMPK in db/db mice and promoted glucagon-like peptide-1 (GLP-1) secretion from enteroendocrine L-cells via bitter taste receptor signalling, resulting in hypoglycaemic effects comparable to metformin. In a similar investigation, cucurbitacin administration in insulin-resistant rat models enhanced PI3K/Akt signalling and increased phosphorylation of the insulin receptor substrate (IRS-1) leading to the upregulation of GLUT4 membrane translocation [42,44]. Additional data indicates regulation of peroxisome proliferator-activated receptor gamma (PPAR- γ), a transcription factor that, when activated, enhances insulin sensitivity in peripheral tissues [42]. In vitro, cucurbitacins have shown α -amylase and α -glucosidase inhibitory action as well as glucose metabolism, hinting at a possible role in slowing down postprandial carbohydrate digestion and blunting glycaemic spikes [45]. More recent in silico and in vitro work has additionally identified inhibition of aldose reductase, the rate-limiting enzyme of the polyol pathway implicated in diabetic complications, as a further mechanism by which *C. colocynthis* constituents may act [66].

7. ANTICANCER MECHANISMS

7.1. JAK2/STAT3 Pathway Inhibition

Cucurbitacins have been shown to be powerful inhibitors of Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signalling axis that is constitutively activated in a broad array of human malignancies including breast, lung, pancreatic and hepatocellular carcinomas [5,19,46]. It has been established that cucurbitacin B and I inhibit STAT3 tyrosine phosphorylation (Tyr705) and nuclear translocation, resulting in the downregulation of the downstream target genes involved in proliferation (cyclin D1, c-Myc), survival (Bcl-2, Bcl-xL, survivin) and angiogenesis (VEGF) [46,47]. Seminal data by Blaskovich et al. [47] indicated that cucurbitacin I specifically inhibited STAT3 activation of human cancer cell lines at nanomolar doses, but not STAT3-negative cells. This finding indicated the pathway specificity of these drugs. Specifically, the fractions of ethyl acetate enhanced with cucurbitacins in *C. colocynthis* displayed preferential cytotoxicity towards pancreatic (MIA PaCa-2) and skin (A431) cancer cell lines with minimal damage to normal cells [22,48].

7.2. Apoptosis Induction and Cell Cycle Arrest

In addition to JAK/STAT3, cucurbitacins induce apoptosis via the intrinsic (mitochondrial) route. Upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2 results in an increased Bax/Bcl-2 ratio favouring mitochondrial outer membrane permeabilization, release of cytochrome c and activation of the caspase cascade [17,49]. Ayyildiz et al. [49] observed that *C. colocynthis* extract dramatically increased the production of Bax mRNA in human melanoma A375 cells, and the Bcl-2/Bax ratio was decreased concurrently. Furthermore, Cucurbitacin B causes cell cycle arrest at the G2/M phase by disturbing the dynamics of the actin cytoskeleton and inhibiting tubulin polymerisation, which prevents mitotic spindle formation [5,50]. The formation of reactive oxygen species (ROS) that increase oxidative stress in tumour cells and activate p53-dependent and -independent apoptotic pathways is also a complimentary mechanism [19,20]. These multimodal anticancer properties provide cucurbitacin-enriched colocynthis extracts ideal candidates for combination therapy where synergism with traditional chemotherapeutics could potentially reduce the required doses as well as the associated toxicities of both drugs [46,50]. Recent (2026) studies have reinforced these anticancer mechanisms: *C. colocynthis* extract downregulated PI3K and AKT gene expression in human lung cancer (A549) cells in vitro [67], and an ethanolic seed extract significantly suppressed tumour growth in an Ehrlich ascites carcinoma solid-tumour model in mice [68].

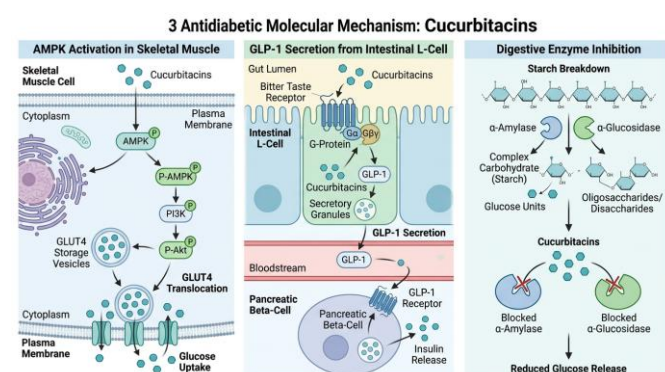


Figure 4. Proposed molecular mechanisms of antidiabetic action of cucurbitacins. Three convergent pathways are depicted: AMPK/PI3K/Akt/GLUT4 translocation (left), bitter taste receptor/GLP-1/insulin release (center), and α -amylase/ α -glucosidase inhibition (right).

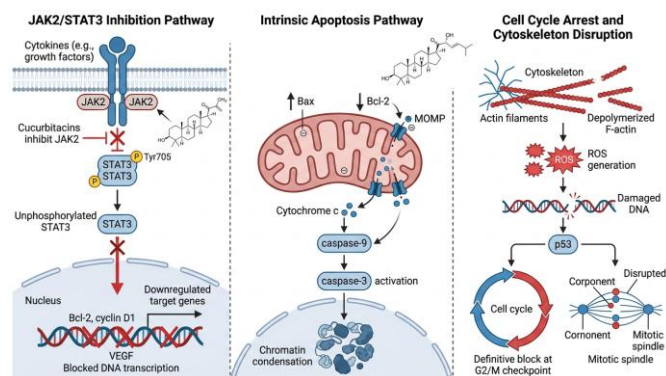


Figure 5. Anticancer signaling pathways modulated by cucurbitacins: JAK2/STAT3 inhibition (left), Bax/Bcl-2 intrinsic apoptosis (center), and ROS-mediated G₂/M cell cycle arrest (right).

Table 4. In vitro and in vivo anticancer activities of cucurbitacins and *C. colocynthis* extracts.

Compound / Extract	Cancer Type	Cell Line	IC ₅₀	Mechanism	Reference
CuB	Pancreatic	Panc-1	~0.3 μM	JAK/STAT3 inhibition, caspase activation	Thoenissen et al., 2009 [49]
CuI (JSI-124)	Multiple	Various	nM range	Selective STAT3 inhibition	Blaskovich et al., 2003 [46]
CuE-glucoside	Breast	MCF-7, MDA-MB-231	~40 μM	Bax/Bcl-2, growth inhibition	Tannin-Spitz et al., 2007 [17]
EtOAc extract	Pancreatic / Skin	MIA PaCa-2, A431	Dose-dependent	Antioxidant, cytotoxic, minimal normal cell harm	Shawkey et al., 2023 [22]
Fruit extract	Melanoma	A375	400–500 μg/mL	Bax ↑, Bcl-2 modulation	Ayyildiz et al., 2022 [49]
CuB	Lung (NSCLC)	A549, H1299	~50 nM	ROS ↑, intracellular thiol depletion	Kausar et al., 2013 [59]

8. TOXICITY PROFILE AND STRATEGIES TO MANAGE TOXICITY

8.1. Toxicological Concerns

The therapeutic use of cucurbitacins is limited by a toxicity profile that should not be ignored. Acute intake of colocynthis fruit, particularly the pulp, is linked with severe gastrointestinal discomfort, including profuse watery or bloody diarrhoea, abdominal cramps, and haematemesis, due to cucurbitacins irritating the

intestinal mucosa [9,10,51]. Hepatotoxicity (elevated ALT, AST), nephrotoxicity (elevated serum creatinine and blood urea nitrogen), electrolyte abnormalities, hypotension and death in uncontrolled ingesting cases have been reported in case reports at larger doses [9,10,52]. A recent case from Eastern Africa revealed multiorgan toxicity in a patient who ingested colocynthis to relieve constipation, with rapid onset of bloody diarrhoea, hepatic injury, and acute kidney impairment requiring intense supportive treatment [52]. Subacute toxicity tests in rats on methanol extract of *C. colocynthis* fruit at doses higher than 131 mg/kg for 28 days demonstrated substantial changes in hepatic and renal biomarkers suggesting hepato-nephrotoxic potential at supratherapeutic dosages [53]. However, it is noteworthy that doses in the range of 50–100 mg/kg seem to be relatively well tolerated in rodent models, and a complete assessment determined that the plant is safe for usage at doses up to around 400 mg/kg when taken within prescribed limits [10,40,54].

8.2. Strategies for Toxicity Mitigation

Several evidence-based methods have been proposed to extend cucurbitacin's therapeutic window. First, dose titration must be based on verified analytical quantification of each batch's cucurbitacin content. Similar to Section 5's quality control techniques [32,33]. Second, nanoformulation methods like encapsulation in polymeric nanoparticles, liposomes, solid lipid nanoparticles, or cyclodextrin complexes can increase cucurbitacin bioavailability and control release kinetics to lower peak plasma concentrations and acute toxicity [55,56]. In preclinical models, nanoencapsulated cucurbitacin B showed better anticancer efficacy at lower dosages than the free drug, showing that formulation technology can shift the risk–benefit balance [56]. Consistent with this, a recent (2026) study showed that green-synthesised, *C. colocynthis*-derived iron oxide nanostructures functionalised with 8-hydroxyquinoline exhibited markedly enhanced anticancer activity against MCF-7 and HepG2 cells, illustrating how nanoformulation of colocynthis-derived material can improve efficacy while controlling the dose [64].

Third, the co-administration of hepato- and nephroprotective drugs is a pharmacological approach to combat organ-specific toxicity. Silymarin, N-acetylcysteine and vitamin E have all been explored as preventive adjuncts in situations of oxidative hepatocellular damage induced by cucurbitacin with promising findings in mouse models [57]. Interestingly, *C. colocynthis* extract per se has been shown to have hepatoprotective effects against cisplatin-induced liver injury at moderate dosages indicating a biphasic dose-response relationship [58]. Fourth, semisynthetic derivatisation of the cucurbitacin scaffold, e.g., prodrug strategies that mask the reactive enone moiety until enzymatic activation at the tumour site, could decouple therapeutic activity from systemic toxicity, although this approach is still largely at the proof-of-concept stage [19,59]. Finally, a simpler method could be the use of glycosylated cucurbitacin forms (e.g., cucurbitacin E-glucoside) instead of free aglycones, as glucosides often show lower toxicity and nevertheless have a considerable bioactivity [17,18].

Table 5. Reported toxicological findings for *C. colocynthis* and proposed mitigation strategies.

Toxic Effect	Dose / Context	Model	Proposed Strategy	Mitigation
GI irritation (diarrhea, cramps)	Crude fruit ingestion (uncontrolled dose)	Human case reports	Dose standardization; enteric coating; glycoside formulations	
Hepatotoxicity (↑ALT, AST)	MeOH extract >131 mg/kg, 28 d	Wistar rats	Co-administration of silymarin or NAC; dose reduction	
Nephrotoxicity (↑creatinine, BUN)	MeOH extract >131 mg/kg, 28 d	Wistar rats	Hydration; dose titration; nanoencapsulation for sustained release	
Multiorgan failure	Massive acute ingestion	Human (case report)	Public awareness; extract standardization; prescription-level dosing	
Hematological changes	50–100 mg/kg, 28 d	Wistar rats	Periodic blood monitoring; use of purified glycoside fractions	
Systemic cytotoxicity (CuB)	Parenteral high-dose CuB	In vivo/in vitro	Lipid nanoparticles; tumor-targeting prodrugs; combination chemotherapy at reduced doses	

9. FUTURE PERSPECTIVES AND CLINICAL TRANSLATION CHALLENGES

Although the scope of pharmacological data is significant, the pathway from ethnomedicine to licensed medical product is still riddled with difficulties. Foremost is the requirement for internationally harmonised monographs, which establish permissible values of cucurbitacin content, as well as heavy metals, microbial contamination, and pesticide residues in colocynth raw material and standardised extracts [7]. Formal monographs for *C. colocynthis* have not yet been published by organisations such as the WHO, European Pharmacopoeia and national regulatory agencies and this gap must be filled to achieve Good Manufacturing Practice (GMP)-compliant commercial production [7,60]. Equally pressing are well-designed, adequately powered randomised controlled trials (RCTs) using particular cucurbitacin formulations at defined doses in diabetic and oncology patient populations with meticulous monitoring of safety endpoints [40].

In terms of formulation, the low water solubility of cucurbitacin aglycones continues to be a hurdle for oral bioavailability and new nanodelivery technologies should be evaluated for efficacy, long-term safety and scalability [55,56]. Further insights into the pleiotropic cucurbitacin processes and the identification of patient subgroups that are most likely to benefit might be gained by multi-omics approaches including metabolomics, transcriptomics and proteomics [61]. Moreover, the network pharmacology analysis can also predict herb-drug interactions, which is of great interest for diabetic and cancer patients with polypharmacy regimens [62]. Finally, agronomic research for the development of high-cucurbitacin

cultivars through selective breeding or metabolic engineering of the cucurbitacin biosynthetic pathway could assure a sustainable and uniform supply of raw material, reducing one of the most fundamental sources of variability in extract quality [2,8].

Another not yet much exploited option is the use of computational chemistry and molecular docking to speed up structure-activity relationship investigations. In silico screening of cucurbitacin derivatives against proven therapeutic targets such as STAT3, AMPK and α -amylase could prioritise candidates for synthesis and biological evaluation, hence reducing the cost and time associated with traditional drug discovery pipelines [22, 36]. Recently, Shawkey et al. [22] performed molecular dynamics simulations and explained the anti-pancreatic cancer activity of cucurbitacin derivatives found by LC–MS in ethyl acetate fractions of *C. colocynthis*. Furthermore, the application of artificial intelligence and machine learning models that are trained on datasets of cucurbitacin bioactivity can accelerate the discovery of structural analogues that have optimal activity and low toxicity. Such multidisciplinary approaches will be essential to overcome the long-standing translational gap between the ethnopharmacological heritage of colocynth and the rigorous evidence required for regulatory approval of cucurbitacin-based medicines.

10. CONCLUSION

Citrullus colocynthis remains one of the most pharmacologically fascinating species of the Cucurbitaceae family, with cucurbitacins being the chemical engines of its antidiabetic and anticancer activities. This research has demonstrated that selective extraction, in particular polarity-directed solvent partitioning, coupled with sophisticated techniques such as PLE and UAE can yield cucurbitacin-enriched fractions for pharmaceutical development. Today, chromatographic techniques, especially HPLC-MS/MS and UPLC-MS/MS, provide the analytical sensitivity and specificity required for effective batch standardisation, with detection limits in the sub-nanogram range. Mechanistically, cucurbitacins exert antidiabetic activities mainly through AMPK activation, PI3K/Akt-mediated GLUT4 translocation and GLP-1 stimulation, whereas their anticancer activity converges on JAK2/STAT3 inhibition, Bax/Bcl-2 regulated apoptosis and cell cycle arrest at the G2/M checkpoint. The narrow therapeutic window due to gastrointestinal, hepatic and renal toxicity necessitates a multi-pronged management strategy that combines precision dosing, nanoformulation, co-administration of hepatoprotective agents and derivatisation of prodrugs. Addressing the current limitations, especially the lack of regulatory monographs and phase II/III clinical trials, will be crucial to the use of standardised colocynth extracts in the mainstream medical system to tap the therapeutic potential.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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