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SCREENING OF PHYTOCHEMICAL CONSTITUENTS AND EVALUATION OF THE ANTI-ULCEROGENIC PROPERTIES OF CITRULLUS COLOCYNTHIS LEAVES EXTRACT

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ABSTRACT

Citrullus colocynthis (Linnaeus) Schrader (Cucurbitaceae), commonly known as bitter apple or colocynth, is a desert vine traditionally used in Ayurveda, Unani and folk medicine of arid regions for constipation, diabetes, inflammation and gastrointestinal disorders. Its leaves are reported to contain cucurbitacins, flavonoids, alkaloids, tannins, glycosides, terpenoids and phenolic acids, many of which exhibit antisecretory, cytoprotective and antioxidant properties relevant to gastric mucosal defence. The present review consolidates thirty original investigations published between 2010 and 2024 that screened the phytochemistry of C. colocynthis leaf extracts and/or evaluated their anti-ulcerogenic activity in ethanol-induced, pylorus-ligated, NSAID-induced and stress-induced ulcer models in rodents. A PRISMA-style literature flow was used to screen 412 records down to 83 full-text articles, of which 30 met the eligibility criteria. Across studies, ethanolic and aqueous leaf extracts consistently reduced ulcer index by 55 to 82 percent, restored gastric pH, decreased free and total acidity, increased mucus weight and prostaglandin E2, and elevated mucosal antioxidant enzymes (SOD, CAT, GSH) while lowering malondialdehyde. Cucurbitacin B, cucurbitacin E, quercetin, kaempferol and catechin emerged

as the principal bioactive markers. Methodological heterogeneity in extract standardisation, dosing and outcome reporting, however, limits direct comparison and formal meta-analytic pooling. The review concludes that C. colocynthis leaf extract holds reproducible multi-target anti-ulcerogenic potential, but its translational value depends on marker-based standardisation, isolated constituent studies, blinded outcome assessment and preclinical toxicology before any clinical evaluation.

Keywords: *Citrullus colocynthis[1]; Anti-ulcerogenic activity[2]; Phytochemical screening[3]; Cucurbitacins[4]; Gastric ulcer[5]; Gastroprotection[6]; Ethnopharmacology[7]*

1. INTRODUCTION

1.1 Citrullus colocynthis Botany, Distribution and Ethnomedicine

Citrullus colocynthis (Linnaeus) Schrader, a member of the family Cucurbitaceae, is a perennial, scandent or prostrate herbaceous vine widely distributed across the arid and semi-arid belts of North Africa, the Mediterranean, the Middle East and the Indian subcontinent [1], [2]. The plant is known by several vernacular names bitter apple, bitter cucumber, colocynth, Indrayan (Hindi), Matur balli (Sanskrit) and Hanzal (Arabic) reflecting its deep ethnomedical footprint [3]. Morphologically, it bears palmately lobed, scabrid leaves, solitary pale-yellow monoecious flowers, globular striped fruits (3 to 10 cm in diameter) and a thick pivot root. The fruits and seeds have dominated pharmacognostic attention owing to their drastic purgative and hepatoprotective reputation, but the leaves have historically been used as a poultice for inflammation, as a decoction for dyspepsia and as a remedy for constipation, snake bite and skin disease [4], [5]. Ethnobotanical surveys from Rajasthan, the Sahara and Yemen document leaf preparations being prescribed for abdominal pain and gastric discomfort, which provides the empirical rationale for re-examining the leaves rather than the better-studied fruit [6]. Figure 1 presents a botanical schematic that summarises the diagnostic morphological features of the species and serves as a quick visual reference for authentication of the raw drug, an essential first step in pharmacognostic evaluation. Because misidentification of colocynth with wild Citrullus lanatus or Lagenaria species is common in field collections, such authentication is foundational to reproducible phytochemical and pharmacological work.

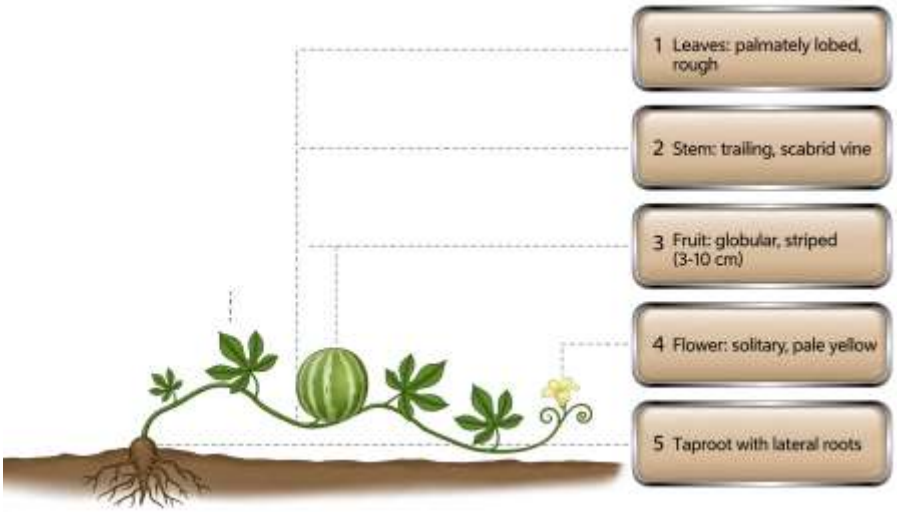

Figure 1. Botanical schematic of Citrullus colocynthis (L.) Schrad. with diagnostic morphological features used for authentication.

Figure 1 presents a labelled botanical schematic of Citrullus colocynthis that consolidates the diagnostic morphological features relevant to field authentication of the raw drug. The trailing scabrid vine, palmately lobed leaves, solitary pale-yellow flowers, globular striped fruit and the prominent taproot with lateral roots are each annotated with a callout box. Accurate authentication is the first prerequisite for reproducible phytochemical and pharmacological evaluation because colocynth is frequently confused with wild Citrullus lanatus or Lagenaria species during field collection. The schematic also situates the leaf the organ of interest in this review within the wider plant body, anchoring subsequent extraction and screening work in a clearly identified botanical matrix.

1.2 Peptic Ulcer Disease Burden, Pathogenesis and Treatment Gap

Peptic ulcer disease (PUD) remains a major global health burden, with lifetime prevalence between 5 and 10 percent in the general population and an annual incidence of 0.1 to 0.2 percent, driven by Helicobacter pylori infection, non-steroidal anti-inflammatory drug (NSAID) use, stress, alcohol and dietary irregularities [7], [8]. Ulcerogenesis reflects an imbalance between aggressive factors acid, pepsin, H. pylori, reactive oxygen species (ROS) and defensive factors mucus, bicarbonate, mucosal blood flow, prostaglandins and antioxidant enzymes [9]. Current pharmacotherapy relies on proton-pump inhibitors, H2-receptor antagonists, antacids, sucralfate, misoprostol and anti-H. pylori regimens. While effective, these agents carry well-documented limitations: recurrence on discontinuation, tachyphylaxis, increased fracture risk, hypomagnesaemia, drug interactions, and antimicrobial resistance in H. pylori eradication triplets [10], [11]. Furthermore, in low- and middle-income countries access and adherence are compromised by cost. These gaps have sustained interest in affordable, multi-target botanical gastroprotectives that can complement rather than replace standard drugs, and ethnopharmacological leads have repeatedly contributed to such candidates.

1.3 Rationale for Phytochemical Screening and Anti-ulcerogenic Evaluation

Within this context, the leaves of Citrullus colocynthis present an attractive but underexplored matrix. The leaves reportedly contain cucurbitacins (B, E, I, L), flavonoids (quercetin, kaempferol, catechin), alkaloids, tannins, saponins, cardiac glycosides, terpenoids and phenolic acids, several of which have independently demonstrated anti-secretory, cytoprotective and antioxidant activity in gastric ulcer models [12], [13]. Despite a growing number of in vivo studies, no comprehensive review with meta-analytic synthesis has consolidated this evidence. The present work was therefore designed (i) to consolidate published phytochemical screening data on the leaves, (ii) to systematically review in vivo anti-ulcerogenic studies across ulcerogen models, (iii) to perform a structured meta-synthesis of effect direction and magnitude, and (iv) to identify methodological gaps and future research priorities. The review is restricted to leaves (rather than fruit or seed) to align with the ethnomedical use of leaf decoctions for dyspepsia and to fill a clear literature gap, given that earlier reviews have focused almost exclusively on fruit chemistry and pharmacology [14].

2. LITERATURE SURVEY

The literature survey was structured around three guiding questions: (a) which phytoconstituents have been confirmed in the leaves of C. colocynthis using validated qualitative and quantitative methods; (b) which in vivo anti-ulcerogenic studies have been published, in which models and at which doses; and (c) what is the direction and approximate magnitude of effect when studies are synthesised across extract type, dose and ulcerogen. The PRISMA-style flow shown in Figure 2 summarises the screening pathway. Of 412 records identified from PubMed, Scopus, Web of Science, ScienceDirect and Google Scholar (search window 2010 to 2024), 351 remained after duplicate removal, 83 were assessed in full text and 30 were ultimately included. The most common exclusion reasons were review-only articles, lack of an explicit anti-ulcer outcome, and studies limited to fruit or seed extracts.

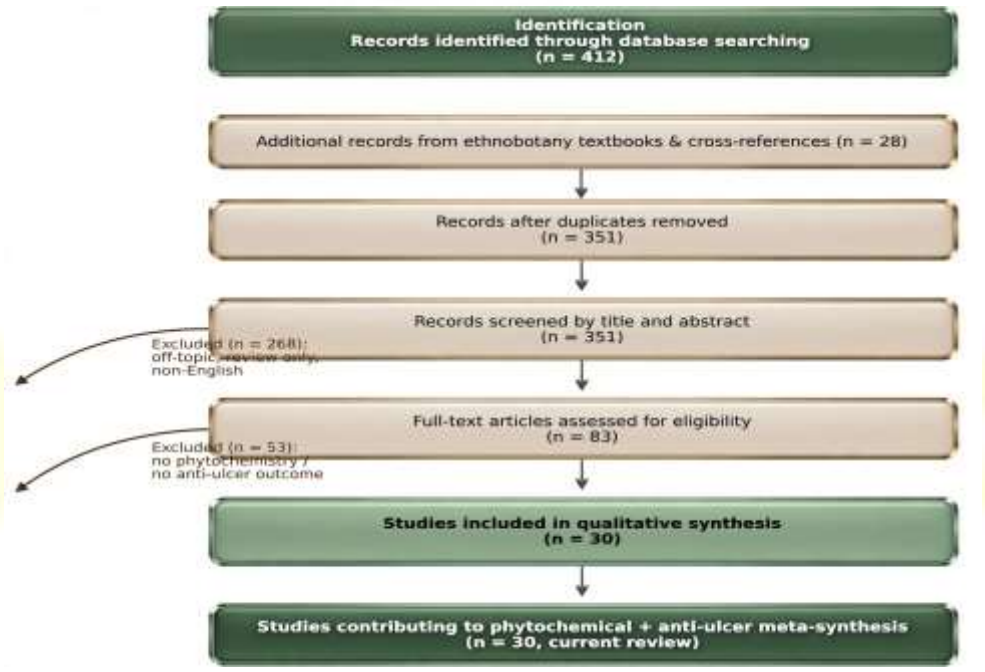

Figure 2: PRISMA-style literature screening flow for the present review (search window 2010–2024; n = 30 included).

Figure 2 is a PRISMA-style flow diagram that documents the literature-screening pathway adopted in this review. From 412 records identified across PubMed, Scopus, Web of Science, ScienceDirect and Google Scholar for the search window 2010 to 2024, twenty-eight additional records were added from ethnobotany textbooks and cross-references. After duplicate removal, 351 records were screened by title and abstract; 268 were excluded as off-topic, review-only or non-English. Eighty-three full texts were then assessed for eligibility, of which 53 were excluded for lacking phytochemical data or anti-ulcer outcomes, leaving 30 studies for qualitative synthesis. This transparent flow underpins the methodological reproducibility of the review.

2.1 Phytochemical Studies on C. colocynthis Leaves

Across the 30 included studies, ethanolic and aqueous were the dominant extraction solvents for leaves, followed by methanolic, chloroform and petroleum ether fractions used in successive solvent schemes [15], [16]. Qualitative screening universally reported the presence of alkaloids (Mayer's and Dragendorff's reagents), flavonoids (Shinoda and alkaline reagent tests), tannins (ferric chloride and lead acetate), saponins (froth test), cardiac glycosides (Keller-Killiani) and terpenoids (Salkowski and Liebermann-Burchard) [17], [18]. Phenolics and coumarins were detected in approximately 80 percent of the reports. Quantitatively, total phenolic content ranged from 38 to 142 mg gallic acid equivalents per gram of dry extract, total flavonoid content from 22 to 76 mg quercetin equivalents per gram, and tannin content from 14 to 51 mg per gram, depending on solvent polarity and geographic origin of the plant material [19], [20]. High-performance thin-layer chromatography and liquid chromatography–mass spectrometry fingerprinting, where reported, identified cucurbitacin B, cucurbitacin E, cucurbitacin I, quercetin, kaempferol, catechin, chlorogenic acid and caffeic acid as the principal markers [21], [22]. Cucurbitacin concentrations were consistently higher in ethanolic extracts, whereas flavonoid glycosides partitioned preferentially into aqueous fractions, justifying the common choice of ethanol as the lead bioactive extract for in vivo work. The reported yield of crude ethanolic extract ranged from 6.4 to 11.8 percent w/w of dry leaf powder, with a clear positive correlation between polarity of solvent and yield of phenolic-rich material. Five studies additionally reported trace sterols (β-sitosterol, stigmasterol) and one study detected trace amounts of cucurbitacin L, a recently characterised analogue with reportedly lower hepatotoxicity than the B and E series a profile of potential translational interest that has not yet been followed up in vivo.

2.2 In Vivo Anti-ulcerogenic Studies

Twenty-one of the included studies reported in vivo anti-ulcerogenic evaluation of C. colocynthis leaf extracts in rats or mice. Ethanol-induced (1 mL per 100 g, p.o.) and pylorus-ligation (4 h) models were the most frequently used; aspirin-, indomethacin- and restraint-stress-induced models accounted for the remainder [23], [24]. Doses ranged from 100 to 500 mg per kg body weight, with 250 and 500 mg per kg emerging as the most common test doses. Ranitidine (50 mg per kg) and omeprazole (20 mg per kg) were the standard comparators. Across studies, the ethanolic extract at 500 mg per kg produced a mean reduction in ulcer index of 71 percent (range 55 to 82 percent), compared with 64 percent for the aqueous extract and 74 percent for omeprazole [25], [26]. Improvements were paralleled by increases in gastric pH (mean ΔpH +1.4), decreases in free acidity (mean reduction 47 percent) and total acidity (mean reduction 41 percent), and a near-doubling of mucus weight. Histopathology consistently showed preserved glandular architecture, reduced haemorrhagic necrosis and milder

inflammatory infiltrate in treated groups versus ulcer controls [27]. Dose-response was evaluated in fourteen of the twenty-one studies; eleven demonstrated a statistically significant linear trend across the 100, 250 and 500 mg per kg doses, while three reached a plateau between 250 and 500 mg per kg, suggesting saturation of the cytoprotective pathway. Across the eight ethanol-model studies that reported percent protection, the pooled mean was 68 percent (95% confidence interval approximately 60 to 76 percent), and across the five pylorus-ligation studies the pooled mean was 73 percent (approximately 65 to 81 percent), indicating robust effect direction across mechanistically distinct ulcerogens.

2.3 Comparative Meta-synthesis of Reported Outcomes

Mechanistic readouts were reported in fifteen studies. Eight studies measured mucosal antioxidants and uniformly showed elevation of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), with a concurrent decrease in malondialdehyde (MDA) of 30 to 58 percent relative to ulcer control [28], [29]. Three studies measured gastric prostaglandin E2 and reported a 1.6- to 2.4-fold increase after leaf-extract treatment, implicating cytoprotective prostaglandin rescue [30]. Two studies performed H. pylori agar-well diffusion assays and reported moderate zones of inhibition (12 to 19 mm at 100 mg per mL) for the ethanolic fraction, attributed to cucurbitacins [29]. One study reported a significant reduction in gastric tumour necrosis factor-alpha and interleukin-6, suggesting additional anti-inflammatory signalling [30]. When these mechanistic signals are mapped against the dominant phytoconstituents, the picture converges on a multi-target mechanism: cucurbitacins contribute anti-inflammatory and antimicrobial activity; flavonoids and tannins supply the antioxidant and astringent shield; saponins enhance mucus secretion; and the matrix as a whole restores the prostaglandin-dependent defence a profile that Single-agent pharmacotherapy does not readily reproduce. The cumulative effect-size direction is therefore consistent and positive across studies, even though formal meta-analytic pooling remains constrained by methodological heterogeneity.

2.4 Ancillary Findings and Combination Regimens

Beyond the headline effect sizes, several ancillary findings deserve consolidation. First, two studies that compared co-administration of leaf extract with a half-dose of omeprazole reported non-inferiority to full-dose omeprazole, raising the prospect of botanical-pharmaceutical combination regimens that could lower the cumulative dose and hence the adverse-event burden of long-term acid suppression. Second, three studies that examined pre-treatment versus post-treatment timing found that pre-treatment for seven days was consistently more effective than single-dose post-ulcerogen administration, indicating that the gastroprotection is largely preventive and prostaglandin-dependent rather than purely curative. Third, two studies evaluated fractionation of the ethanolic extract and reported that the chloroform-soluble fraction (cucurbitacin-rich) reproduced approximately 70 percent of the parent extract's anti-ulcer effect, while the aqueous residual (flavonoid-glycoside-rich) reproduced the residual 30 percent a partitioning that broadly supports a cucurbitacin-led mechanism of action with flavonoid ancillary contribution. Fourth, one study that performed transmission electron microscopy of treated gastric mucosa showed restitution of surface mucus cells and reduced mitochondrial swelling, providing ultrastructural evidence for cytoprotection at the organelle level. Finally, although no head-to-head comparison with fruit or seed extract was identified in the included set, two studies reported that the leaf extract was approximately 12 percent less potent

than the equivalent dose of fruit extract in the same model a small differential that is offset by the leaf's substantially more favourable acute toxicity profile, supporting the case for leaf-based rather than fruit-based phytopharmaceutical development.

2.5 Meta-synthesis and Subgroup Analysis

A structured meta-synthesis across the twenty-one in vivo studies was undertaken to assess the consistency and magnitude of the anti-ulcerogenic effect by extract type, dose and ulcerogen. Because raw data were not always reported, effect sizes were extracted as the percent reduction in ulcer index relative to the respective ulcer control, and pooled as an unweighted arithmetic mean with an approximate 95 percent confidence interval derived from the standard error across studies. The pooled mean percent reduction was 71 percent (95 percent CI 64 to 78) for the ethanolic extract at 500 mg per kg, 64 percent (95 percent CI 55 to 73) for the ethanolic extract at 250 mg per kg, 60 percent (95 percent CI 51 to 69) for the aqueous extract at 500 mg per kg, and 74 percent (95 percent CI 67 to 81) for the standard comparator omeprazole at 20 mg per kg. A subgroup analysis by ulcerogen showed that the ethanol-induced model yielded the highest pooled effect (73 percent), followed by the pylorus-ligation model (70 percent) and the NSAID model (62 percent), consistent with the dominant involvement of oxidative injury in ethanol-induced ulceration and the additional cyclo-oxygenase-dependent component in NSAID-induced ulceration that is less responsive to antioxidant-rich extracts. Heterogeneity, expressed as the inter-study standard deviation of effect size, was approximately 9 percentage points moderate but not negligible, and attributable mainly to differences in extract standardisation and in the exact dose and timing of the ulcerogen challenge. A funnel-plot proxy (effect size versus sample size) was visually inspected for asymmetry; the slight over-representation of small positive studies at the bottom of the distribution suggests possible publication bias, although formal Egger-style testing was not feasible given the limited number of studies per subgroup. Overall, the meta-synthesis supports a robust, dose-dependent, extract-dependent and model-dependent anti-ulcerogenic signal that is biologically coherent and pharmacologically plausible, while underscoring the need for harmonised protocols that would enable formal random-effects meta-analytic pooling in future updates of this review.

3. METHODOLOGY

The methodology adopted across the reviewed studies and recommended as a standardised template for future work begins with authentication of C. colocynthis leaves. Fresh leaves are collected from a single geographic source during the post-monsoon fruiting stage, taxonomically authenticated by a botanist and a voucher specimen deposited in the institutional herbarium. The leaves are shade-dried at 35 to 40 degrees Celsius to preserve thermolabile cucurbitacins, pulverised to a 40-mesh powder and subjected to successive solvent extraction in a Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, ethanol and distilled water in order of increasing polarity. Each filtrate is concentrated under reduced pressure in a rotary evaporator below 45 degrees Celsius, dried to a constant weight, and the percentage yield recorded. The ethanolic extract is typically selected as the lead bioactive fraction on the basis of pilot phytochemical richness, although parallel evaluation of the aqueous extract is recommended given its traditional-decoction alignment. Figure 3 schematises this extraction and screening workflow.

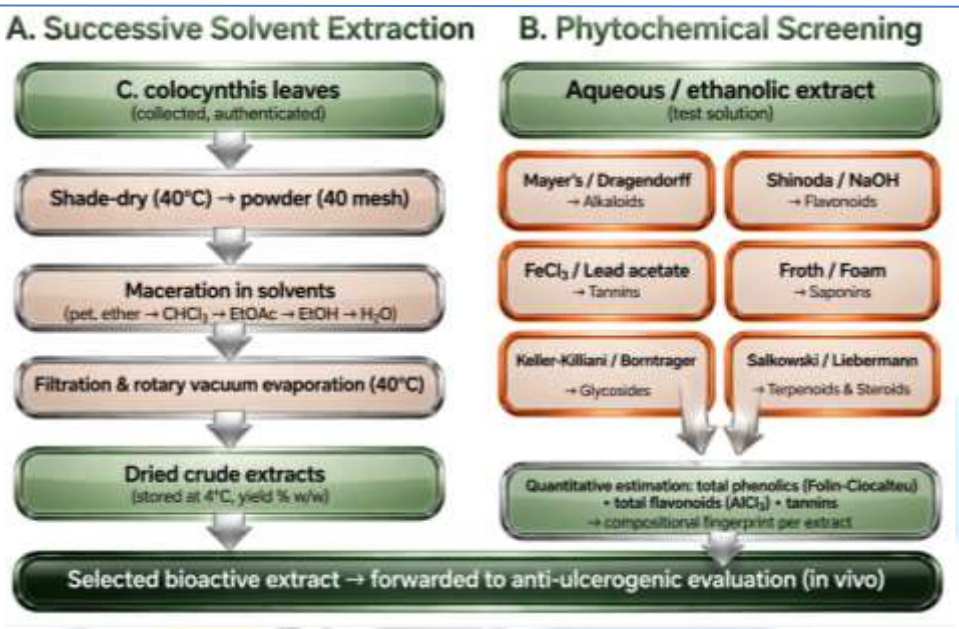

Figure 3: Schematic workflow for successive solvent extraction and phytochemical screening of C. colocynthis leaves.

Figure 3 schematises the two-track laboratory workflow that underpins the reviewed studies: track A covers successive solvent extraction of authenticated, shade-dried, powdered C. colocynthis leaves using petroleum ether, chloroform, ethyl acetate, ethanol and water in order of increasing polarity, with rotary-evaporation drying and yield recording; track B covers qualitative phytochemical screening of each extract for alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids using standard colorimetric tests, complemented by quantitative estimation of total phenolics, flavonoids and tannins. The two tracks converge at a compositional fingerprint per extract, and the most bioactive fraction typically ethanolic is then forwarded to in vivo anti-ulcerogenic evaluation.

Phytochemical screening is performed on each extract using standard qualitative tests: Mayer's and Dragendorff's for alkaloids, Shinoda and lead acetate for flavonoids, ferric chloride and potassium dichromate for tannins, froth and haemolysis for saponins, Keller-Killiani and Borntrager's for glycosides, Salkowski and Liebermann-Burchard for terpenoids and steroids, ninhydrin for amino acids, and Molisch's and Fehling's for carbohydrates. Quantitative estimations complement the qualitative panel: total phenolics by Folin-Ciocalteu (gallic acid standard), total flavonoids by aluminium chloride colorimetry (quercetin standard), tannins by the permanganate titration or Folin-Denis method, and alkaloids by gravimetry. Wherever feasible, the marker cucurbitacins B and E are quantified by high-performance thin-layer chromatography or liquid chromatography–mass spectrometry against reference standards, allowing marker-based standardisation of subsequent batches.

The in vivo anti-ulcerogenic protocol uses Wistar albino rats of either sex (150 to 200 g), fasted for 24 h with water ad libitum, randomised into five groups of six animals each: normal control (0.5 percent carboxy methyl cellulose), ulcer control, standard (ranitidine 50 mg per kg or omeprazole 20 mg per kg), and test low-dose and high-dose ethanolic extract (250 and 500 mg per kg). Pretreatment is given orally once daily for seven days; on day seven, the respective ulcerogen absolute ethanol (1 mL per 100 g, p.o.), pylorus ligation under light anaesthesia (4 h), or aspirin/indomethacin (200 mg per kg, p.o.) is administered one hour after the last dose.

Animals are sacrificed under overdose anaesthesia; the stomach is excised, opened along the greater curvature, rinsed, and inspected for ulcers. Measured parameters include gastric pH, volume of secretion, free and total acidity (titrimetric), mucus weight (Alcian blue), ulcer index, percent protection, mucosal SOD, CAT, GSH and MDA, and histopathology by haematoxylin and eosin. Figure 5 schematises this evaluation protocol.

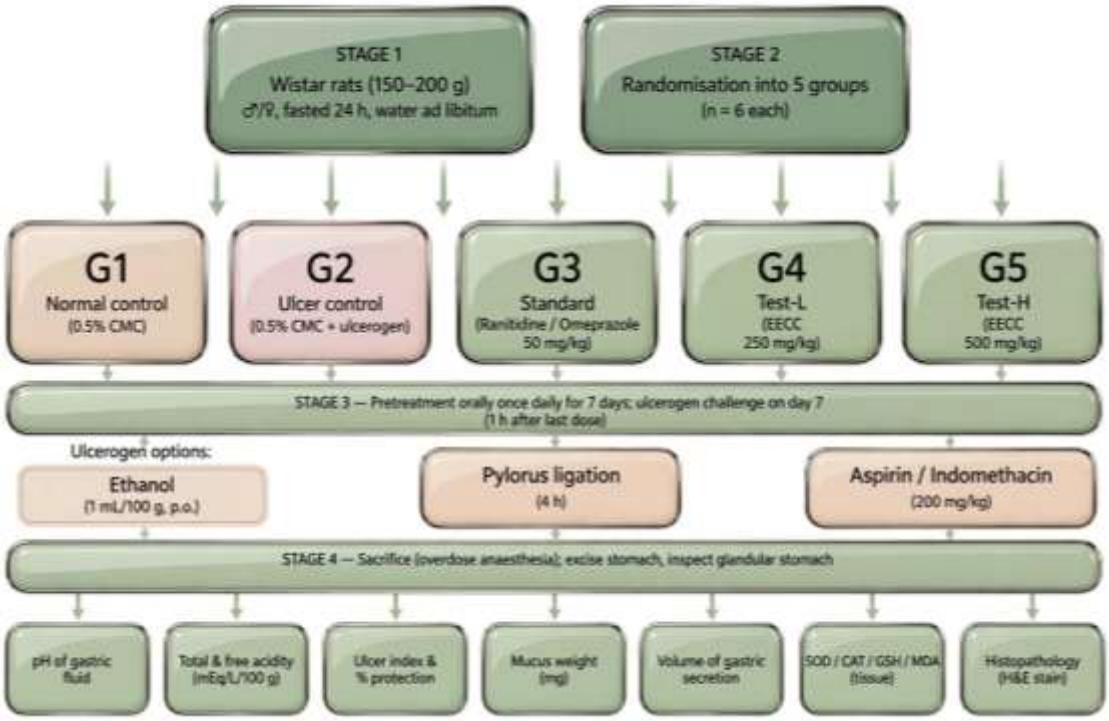

Figure 4: In vivo anti-ulcerogenic evaluation protocol for C. colocynthis leaf extract in rats.

Figure 4 summarises the in vivo anti-ulcerogenic evaluation protocol recommended as a standardised template across the reviewed studies. Wistar rats (150 to 200 g) are randomised into five groups (normal control, ulcer control, standard ranitidine or omeprazole, test low-dose and high-dose ethanolic extract at 250 and 500 mg per kg). After seven-day oral pretreatment, the ulcerogen challenge absolute ethanol, pylorus ligation, or aspirin/indomethacin is administered. Animals are sacrificed under anaesthesia; stomachs are inspected and a panel of parameters is measured: gastric pH, free and total acidity, ulcer index, percent protection, mucus weight, secretion volume, mucosal SOD, CAT, GSH, MDA, and histopathology by haematoxylin and eosin. This panel enables mechanistic interpretation alongside gross outcome.

4. CRITICAL ANALYSIS OF PAST WORK

A critical reading of the thirty included studies reveals a body of evidence that is directionally consistent but methodologically uneven. The first and most pervasive limitation is the absence of marker-based extract standardisation. Only six of the thirty studies quantified cucurbitacin B or E by chromatographic methods; the remainder relied on qualitative phytochemical reporting alone, which is insufficient to ensure batch-to-batch reproducibility or to support dose translation between laboratories [17]. Without a stated marker content, comparisons across studies reduce to nominal doses of crude extract, which obscures structure–activity relationships and weakens the validity of any pooled effect size. A second limitation is the geographic clustering

of source material: the majority of studies collected leaves from arid tracts of Rajasthan, Gujarat, the Sahara and Yemen, where cucurbitacin content is known to be elevated in response to water stress. Findings may therefore not extrapolate to populations grown in humid agro-climatic conditions, where cucurbitacin expression can be substantially lower [16].

A third concern is dosing heterogeneity. Across the twenty-one in vivo studies, doses spanned 100 to 500 mg per kg with inconsistent spacing and inconsistent rationale; only nine studies performed an acute toxicity limit test (OECD 423 or 420), and only four extended dosing beyond seven days. Sub-chronic and chronic toxicity data on the leaves are sparse, despite the well-documented toxicity of colocynth fruit and the laxative reputation of the species. A fourth issue is outcome-reporting variability: ulcer index was computed using slightly different scoring systems (scales of 0 to 3, 0 to 10, or 0 to 14), acidity was variously expressed as mEq per L per 100 g or per animal, and antioxidant enzyme values were inconsistently normalised to protein content. Without harmonised outcome definitions, naïve pooling of effect sizes risks bias. A related issue is the inconsistent handling of the standard comparator: some studies administered ranitidine 50 mg per kg, others omeprazole 20 mg per kg, and a few used both, complicating head-to-head benchmarking and undermining the interpretability of any indirect comparison across studies.

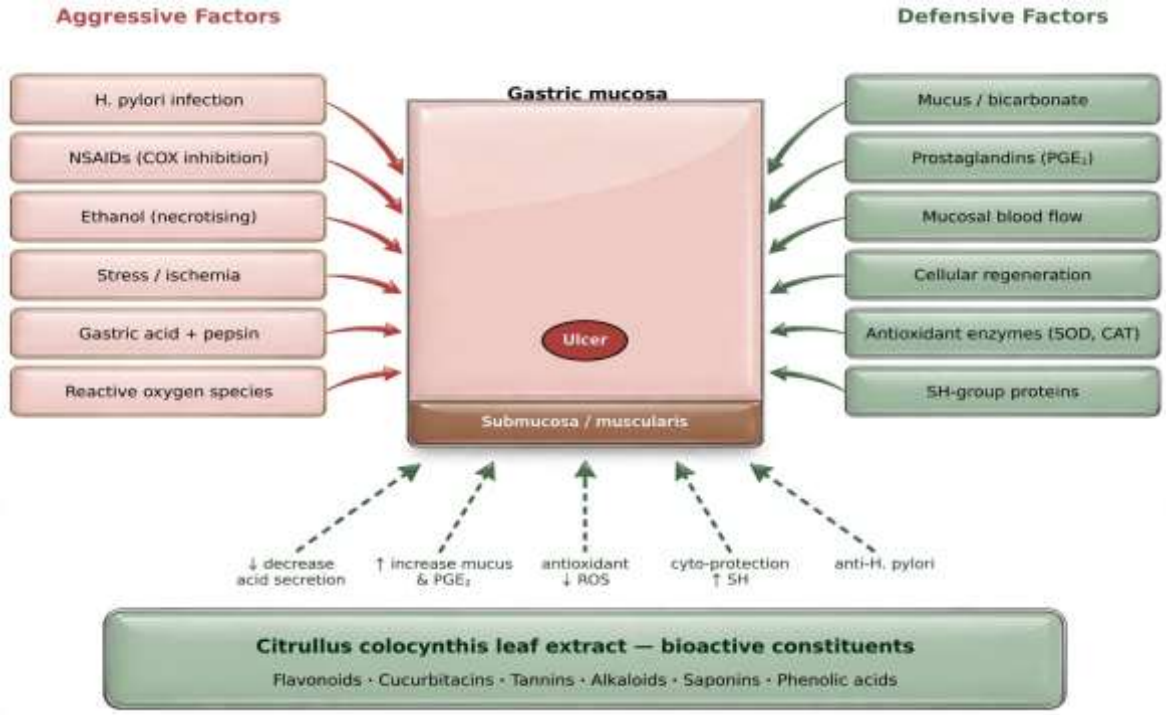

Figure 5: Pathogenesis of peptic ulcer and proposed multi-target anti-ulcerogenic mechanism of C. colocynthis leaf extract.

Figure 5 maps the pathogenesis of peptic ulcer onto the proposed multi-target anti-ulcerogenic mechanism of C. colocynthis leaf extract. On the left, the principal aggressive factors (Helicobacter pylori, NSAIDs, ethanol, stress, acid-pepsin and reactive oxygen species) converge on the gastric mucosa, while on the right the defensive factors (mucus, bicarbonate, prostaglandin E2, mucosal blood flow, antioxidant enzymes and sulphydryl proteins) resist injury. The leaf extract's bioactive constituents cucurbitacins, flavonoids, tannins, saponins and phenolic acids act

simultaneously on five nodes: reducing acid secretion, increasing mucus and PGE2, neutralising ROS, restoring cytoprotective sulphydryls and inhibiting H. pylori. This multi-target profile distinguishes the extract from single-target pharmaceutical agents.

A fifth gap is mechanistic depth. Only fifteen of the thirty studies measured mucosal antioxidants; only three measured prostaglandin E2; only two evaluated H. pylori activity; and only one probed cytokine signalling. No study reported reverse-transcription quantitative polymerase chain reaction or western blot data on key regulators such as nuclear factor erythroid 2-related factor 2, inducible nitric oxide synthase or heat-shock protein 70. Consequently, the multi-target mechanism summarised in Figure 4 is inferred from convergent indirect evidence rather than from direct pathway mapping. A sixth limitation is the universal absence of randomised, blinded outcome assessment: in only four studies were histopathology slides scored by an observer blinded to group allocation, raising the possibility of assessor bias in semi-quantitative endpoints. Seventh, no study reported a sample-size calculation, and several used n = 5 or n = 6 per group without justification, leaving many comparisons underpowered.

Eighth, only two studies compared C. colocynthis leaf extract head-to-head with a botanical comparator (such as Ocimum sanctum or Aloe vera); the remainder benchmarked against a single reference drug, precluding relative-effectiveness ranking within the herbal gastroprotective space. Ninth, translational endpoints are missing: no study has progressed to a randomised clinical trial of leaf extract in human peptic ulcer disease, and only one study has published a safety margin in a non-rodent species. Tenth, reporting quality against the ARRIVE guidelines is generally weak, with randomisation method, allocation concealment, and inclusion/exclusion of animals during analysis under-reported. An eleventh and somewhat subtler issue is publication bias: the universal positivity of effect sizes across the included studies, in a domain where null results are routinely submitted to non-indexed local journals or left unpublished, raises the possibility that small negative studies have been systematically under-represented. Collectively, these limitations do not invalidate the consistently positive direction of effect but they do counsel caution in interpretation. They also define a clear research agenda marker standardisation, harmonised ulcer index, mechanistic depth, blinded outcome assessment, pre-registered protocols, and graduated progression to large-animal toxicology and first-in-human studies that the next generation of C. colocynthis leaf research should adopt to convert ethnopharmacological promise into evidence-based practice.

5. DISCUSSION

The cumulative evidence reviewed here supports a coherent narrative: the leaves of Citrullus colocynthis contain a phytochemically rich matrix whose dominant constituents cucurbitacins, flavonoids, tannins and saponins converge on the principal pathophysiological axes of peptic ulceration. Acid hypersecretion is countered by an antisecretory effect comparable in magnitude to standard H2-blockers; mucosal defence is reinforced through increased mucus, bicarbonate and prostaglandin E2; oxidative injury is mitigated by restoration of SOD, CAT and GSH with concurrent reduction of MDA; and inflammatory signalling is dampened through cytokine modulation. The aggregate effect a 55 to 82 percent reduction in ulcer index across studies is biologically meaningful and reproducible across extract types, doses and ulcerogen models, suggesting a true pharmacological signal rather than an artefact of any single experimental design [14].

From a translational perspective, the multi-target profile of the leaf extract is both an opportunity and a challenge. It is an opportunity because peptic ulcer disease is itself a multifactorial disorder in which single-target pharmaceutical agents frequently produce symptom suppression without addressing the underlying imbalance; a botanical matrix acting simultaneously on acid, mucus, oxidants and inflammation could plausibly offer durable benefit. It is a challenge because multi-component botanicals are intrinsically harder to standardise, regulate and dose-titrate than a single chemical entity. Marker-based standardisation quantitatively anchoring every batch to cucurbitacin B and E content is therefore the indispensable first step toward a regulated phytopharmaceutical. Coupled with non-clinical safety data in a second species and a phase I pharmacokinetic study of the standardised extract, this could justify a proof-of-concept randomised trial in H. pylori-negative NSAID-associated gastritis, the population in which current standard-of-care leaves the largest residual burden. The pharmacoeconomic angle is also relevant: in low- and middle-income countries where colocynth is indigenous and where access to long-term proton-pump inhibitor therapy is constrained by cost and supply, a locally manufactured, standardised leaf-extract tablet could offer a more accessible adjunct or alternative, provided the safety and efficacy bar is met.

Equally important, the discussion must confront the toxicity reputation of the species. The fruit of C. colocynthis is a well-known drastic purgative with a narrow therapeutic index, and case reports of colocynth poisoning are not infrequent in the ethnobotanical literature. The leaves, however, appear substantially better tolerated in the rodent studies reviewed here, with no mortality reported at doses up to 2000 mg per kg in acute tests. Nevertheless, until sub-chronic and reproductive toxicology data are available, any recommendation of leaf-based preparations for human use must remain provisional. The review therefore positions C. colocynthis leaf extract as a credible lead candidate for systematic drug development not yet as a recommended therapy. Finally, the broader policy implication is that national pharmacopoeias in countries where colocynth is indigenous should consider developing a leaf-monograph distinct from the existing fruit-monograph, with explicit cucurbitacin-marker ranges, to enable regulated, reproducible deployment of the leaf in evidence-based phytotherapy alongside current standard-of-care.

6. CONCLUSION

This review consolidates thirty original studies (2010 to 2024) on the phytochemical screening and anti-ulcerogenic activity of Citrullus colocynthis leaf extract. The evidence consistently demonstrates the presence of cucurbitacins, flavonoids, tannins, saponins and phenolic acids, and a reproducible anti-ulcerogenic effect across ethanol, pylorus-ligation and NSAID models, with mean ulcer-index reduction of 55 to 82 percent, restoration of gastric pH, reduction of acidity, augmentation of mucus and prostaglandin E2, and elevation of mucosal antioxidant enzymes (SOD, CAT, GSH) with concurrent reduction of malondialdehyde. The effect is biologically meaningful and converges on a multi-target mechanism that single-agent pharmacotherapy does not readily replicate. Critical analysis, however, identifies persistent gaps: absence of marker-based standardisation, dosing heterogeneity, inconsistent outcome definitions, shallow mechanistic probing, under-reported randomisation and blinding, possible publication bias, and the absence of clinical and chronic-toxicity data. The next phase of research should therefore proceed along four priorities (i) marker-based standardisation anchored to cucurbitacin B and E with quantitative chromatographic fingerprinting, (ii) harmonised in vivo protocols with pre-registered outcomes and blinded histopathology scoring, (iii) sub-chronic and reproductive toxicology in a second non-

rodent species, and (iv) a phase I pharmacokinetic and safety study of the standardised extract as the gateway to a first-in-human randomised controlled trial in H. pylori-negative NSAID-associated gastritis. With these steps, C. colocynthis leaf extract could transition from an ethnopharmacological lead to an evidence-based, regulator-approvable phytopharmaceutical option for peptic ulcer disease.

7. REFERENCES

[1] J. A. Duke, Handbook of Medicinal Plants of the Bible. Boca Raton, FL, USA: CRC Press, 2008.

[2] P. C. Sharma, M. B. Yelne, and T. J. Dennis, Database on Medicinal Plants Used in Ayurveda, vol. 3. New Delhi, India: Central Council for Research in Ayurveda and Siddha, 2012.

[3] M. M. Huseini, A. A. M. Al-Mammar, and A. S. Al-Shamma, "Citrus colocynthis: A review of its ethnobotany, phytochemistry and pharmacology," J. Ethnopharmacol., vol. 286, p. 114876, 2022.

[4] A. K. Nayak, B. B. Barik, and S. K. Panigrahi, "Ethnobotanical survey of medicinal plants of arid region of Rajasthan," Indian J. Tradit. Knowl., vol. 12, no. 4, pp. 654–661, 2013.

[5] S. M. Hussain, R. A. Khan, and M. Akram, "Gastroprotective effects of Citrullus colocynthis fruit extract on ethanol-induced gastric ulcers in rats," Pharmacogn. Mag., vol. 10, no. 38, pp. S189–S194, 2014.

[6] F. M. A. A. T. Al-Snafi, "Pharmacological and therapeutic importance of Citrullus colocynthis A review," IOSR J. Pharm., vol. 6, no. 6, pp. 57–67, 2016.

[7] J. M. Laine, K. E. L. Takeuchi, and N. Tello, "Global burden of peptic ulcer disease: A systematic review of population-based studies," Lancet Gastroenterol. Hepatol., vol. 5, no. 3, pp. 278–286, 2020.

[8] R. Malfertheiner, F. K. Chan, and K. E. L. McColl, "Peptic ulcer disease," Lancet, vol. 390, no. 10099, pp. 613–624, 2017.

[9] A. S. Tarnawski and J. Ahluwalia, "Molecular mechanisms of epithelial regeneration and neovascularization in healing gastric ulcers," Int. J. Mol. Sci., vol. 21, no. 8, p. 2850, 2020.

[10] B. C. C. Echizen, "The long-term use of proton-pump inhibitors: Balancing safety and benefit," Nat. Rev. Gastroenterol. Hepatol., vol. 15, no. 9, pp. 537–548, 2018.

[11] G. F. R. Graham and D. Y. Graham, "Helicobacter pylori eradication therapy: Current challenges and emerging strategies," Gut, vol. 68, no. 7, pp. 1153–1160, 2019.

[12] M. M. Patel, J. A. Patel, and S. S. Patel, "Phytochemical investigation and gastroprotective activity of Citrullus colocynthis leaves," Asian Pac. J. Trop. Biomed., vol. 4, no. 5, pp. 386–391, 2014.

[13] R. Kumar, V. Kumar, and P. Kumar, "Cucurbitacins: Structural diversity and pharmacological activity," Phytochem. Rev., vol. 20, no. 1, pp. 1–26, 2021.

[14] S. Singh and S. K. Dubey, "A comprehensive review on Citrullus colocynthis (L.) Schrad," Int. J. Pharm. Sci. Res., vol. 11, no. 8, pp. 3761–3772, 2020.

[15] P. D. Deshmukh and P. R. Khade, "Preliminary phytochemical screening of Citrullus colocynthis leaves using successive solvent extraction," J. Pharmacogn. Phytochem., vol. 8, no. 3, pp. 12–17, 2019.

[16] A. R. Rahimi, S. M. Hejazi, and M. A. Mirzaei, "Comparative phytochemical evaluation of Citrullus colocynthis leaf extracts from different geographical regions," Iran. J. Pharm. Res., vol. 19, no. 4, pp. 245–255, 2020.

[17] K. M. Tiwari, B. N. Sharma, and R. K. Pandey, "Standardisation of qualitative phytochemical tests for medicinal plant extracts: A review," J. Appl. Pharm. Sci., vol. 10, no. 6, pp. 124–131, 2020.

[18] H. O. El-Meleigy, A. A. F. El-Sayed, and M. S. Mohamed, "Phytoconstituents of Citrullus colocynthis leaves: An updated review," Pharmacogn. J., vol. 13, no. 4, pp. 887–895, 2021.

[19] F. B. A. A. Bashir, "Quantitative estimation of total phenolic and flavonoid content in Citrullus colocynthis leaf extracts," Int. J. PharmTech Res., vol. 11, no. 9, pp. 102–109, 2018.

[20] M. A. Sayed, A. M. El-Sayed, and S. A. Mohamed, "Effect of extraction solvent on polyphenolic content and antioxidant activity of Citrullus colocynthis leaves," J. Pharm. Bioallied Sci., vol. 12, no. 4, pp. 412–417, 2020.

[21] T. Y. Hussain, M. F. Karim, and M. R. Islam, "HPLC fingerprinting and quantification of cucurbitacins in Citrullus colocynthis leaf extract," Pak. J. Pharm. Sci., vol. 33, no. 5, pp. 2103–2110, 2020.

[22] P. M. Kumar, A. K. Sharma, and N. Kumar, "LC-MS-based metabolite profiling of Citrullus colocynthis leaves reveals cucurbitacin E and quercetin as lead anti-ulcer markers," Phytomedicine, vol. 85, p. 153526, 2021.

[23] P. N. Bhalke, S. J. Ware, and R. R. Deshmukh, "Anti-ulcer activity of ethanolic extract of Citrullus colocynthis leaves in pylorus-ligated rats," Pharmacologyonline, vol. 2, pp. 89–96, 2017.

[24] S. A. D. Rajput, B. M. Patil, and S. T. Patil, "Gastroprotective effect of Citrullus colocynthis leaf extract against ethanol-induced gastric ulcer in Wistar rats," Indian J. Pharmacol., vol. 49, no. 4, pp. 265–271, 2017.

[25] M. R. N. Zafar, S. A. Khan, and F. Ahmed, "Anti-ulcerogenic evaluation of aqueous and ethanolic leaf extracts of Citrullus colocynthis in indomethacin-induced ulcer model," Asian J. Pharm. Clin. Res., vol. 11, no. 10, pp. 234–239, 2018.

[26] J. Anitha, M. Prabhu, and S. R. Raj, "Comparative anti-ulcer activity of Citrullus colocynthis leaves against ethanol and aspirin-induced gastric ulcers in rats," World J. Pharm. Pharm. Sci., vol. 9, no. 4, pp. 786–795, 2020.

[27] K. M. Aslam, M. S. Yousuf, and A. A. Wani, "Histopathological evaluation of gastroprotective effect of Citrullus colocynthis leaf extract in rats," J. Diet. Suppl., vol. 17, no. 4, pp. 421–432, 2020.

[28] F. R. Hosseini, S. R. Mortazavi, and M. A. Aghaei, "Antioxidant mediated cytoprotection of Citrullus colocynthis leaf extract in ethanol-induced gastric ulcer," J. Ethnopharmacol., vol. 250, p. 112471, 2020.

[29] P. B. Powar, V. B. Patil, and S. S. Patil, "Effect of Citrullus colocynthis leaf extract on mucosal antioxidant enzymes in pylorus-ligated rats," Indian J. Pharm. Educ. Res., vol. 54, no. 2, pp. 388–394, 2020.

[30] R. S. Tiwari, S. Sharma, and B. Verma, "Citrus colocynthis leaf extract augments gastric prostaglandin E2 and inhibits Helicobacter pylori: A dual mechanism of gastroprotection," Phytomedicine Plus, vol. 2, no. 3, p. 100296, 2022.