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ANTI-ULCEROGENIC ACTIVITY OF A COMBINATION OF *CLITORIA TERNATEA AND CASSIA TORA* IN INDOMETHACIN INDUCED GASTRIC ULCER

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ABSTRACT

Aim: The present study aimed to evaluate the phytochemical composition, antioxidant activity, and anti-ulcer potential of ethanol extracts of *Clitoria ternatea* and *Cassia tora*, individually and in polyherbal formulation, using an indomethacin-induced gastric ulcer model in rats. **Materials and Methods:** Leaves of both plants were extracted using Soxhlet apparatus with ethanol. Qualitative phytochemical screening and quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) were performed. Antioxidant activity was assessed by DPPH radical scavenging assay. Herbal suspensions were formulated and evaluated for physicochemical parameters. Anti-ulcer activity was investigated in Wistar rats using indomethacin (25 mg/kg, p.o.)-induced gastric ulcer model, followed by assessment of ulcer index, gastric volume, pH, and free acidity. **Results:** *Clitoria ternatea* exhibited higher phenolic (48.1 mg GAE/g) and flavonoid content (60.1 mg RE/g) compared to *Cassia tora*. It also demonstrated stronger antioxidant activity (IC_{50} = 24.06 µg/mL) than *Cassia tora* (IC_{50} = 30.59 µg/mL). In vivo studies showed significant reduction in ulcer index, gastric volume, and free acidity in treated groups compared to ulcer control. The polyherbal formulation showed the most pronounced gastroprotective effect, with gastric parameters approaching those of the standard drug-treated group. **Conclusion:** The findings indicate that both plants possess significant antioxidant and anti-ulcer activity, with enhanced efficacy observed in combination, supporting their potential as natural gastroprotective agents.

KEYWORDS: *Clitoria ternatea*, Gastroprotective, antioxidant, anti-ulcer and *Cassia tora*.

1. INTRODUCTION

Medicinal plants continue to serve as a prolific source of bioactive compounds with significant therapeutic potential, contributing to the development of novel pharmacological agents. In recent years, there has been a growing interest in plant-derived formulations as safer and more cost-effective alternatives to synthetic drugs, particularly for the management of gastrointestinal disorders such as gastric ulcers **(Anoop&Jegadeesan, 2003).** Gastric ulceration is a multifactorial condition primarily resulting from an imbalance between aggressive factors, including gastric acid and pepsin secretion, and protective mechanisms such as mucin-bicarbonate barrier, prostaglandin synthesis, and mucosal blood flow**(Sairam *et al.*, 2003).** Disruption of this equilibrium leads to mucosal damage and ulcer formation. Current therapeutic strategies focus on reducing gastric acid secretion and enhancing mucosal defense; however, long-term use of conventional anti-ulcer drugs is often associated with adverse effects and recurrence**(Ellison *et al.*, 2002).** *Clitoria ternatea* (Fabaceae), commonly known as Aparajita, is widely distributed across India and has been extensively utilized in traditional Ayurvedic medicine. The plant is reported to possess diverse pharmacological activities, including hepatoprotective, antidiabetic, neuroprotective, anxiolytic, antidepressant, antimicrobial, and anti-inflammatory effects**(Meena *et al.*, 2010).** Its rich phytochemical profile, particularly the presence of flavonoids and phenolic compounds, contributes to its strong antioxidant potential. Similarly, *Cassia tora* Linn., a well-recognized medicinal herb found in India and

other tropical regions, has been traditionally employed for the treatment of various ailments**(Pawar&D'mello, 2011).** The plant contains bioactive constituents such as anthraquinones, flavonoids, and glycosides, which are associated with its laxative, anti-inflammatory, hepatoprotective, and dermatological applications**(Sarwa *et al.*, 2014).** Previous studies have also highlighted its potential role in mitigating oxidative stress and inflammation. Despite the individual therapeutic significance of these plants, their combined effects in a polyherbal formulation, particularly in the context of anti-ulcer activity, remain inadequately explored**(Dev *et al.*, 2019).** Polyherbal approaches are often preferred in traditional systems of medicine due to their synergistic effects, enhanced efficacy, and reduced toxicity. Therefore, the present study was designed to systematically evaluate the phytochemical composition, antioxidant activity, formulation and gastroprotective potential of ethanol extracts of *Clitoriaternatea* and *Cassia tora*, both individually and in combination. The study aims to provide scientific validation for their traditional use and to explore their potential as effective natural anti-ulcer agents.

2. MATERIALS AND METHODS

2.1 Plant collection

The leaves of *Clitoria ternatea* (400 g) and *Cassia tora* (350 g) were collected from the localregion of Bhopal, Madhya Pradesh, India. The plant materials were authenticated by aqualified botanist to ensure taxonomic accuracy.

2.2 Extraction process

The extracts were prepared using a Soxhlet extraction apparatus. Leaf powders of *Clitoria tematea*(400 g) and *Cassia tora*(350 g) were loaded into a thimble, which was then placed in a distillation flask containing ethanol as the solvent and maintained at 60–80 °C. The extraction cycle was repeated continuously until completion. The resulting filtrate was concentrated using a rotary vacuum evaporator, dried, and the percentage yield of the extract was subsequently calculated **(Borodulin *et al.*, 2020).**

2.3 Solubility testing of the extract

The solubility of the test compound was evaluated in five different solvents: distilled water, dimethyl sulfoxide (DMSO), chloroform, acetone, and methanol. Solubility was categorised qualitatively as soluble (clear solution), partially soluble (cloudy solution or partial precipitation), or insoluble (undissolved solid visible). All tests were performed in triplicate to ensure reproducibility **(Hussain, 2017)**.

2.4 Phytochemical investigation

A comprehensive qualitative phytochemical screening was carried out to evaluate the presence or absence of various bioactive constituents in the plant extract. The analysis focused on major classes of phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, and steroids. These tests provide preliminary insight into the chemical composition of the extract and help in identifying potential pharmacologically active compounds (**Yun *et al.*, 2022**).

2.5 Quantitative Phytochemical Estimation

2.5.1 Determination of Total Phenolic Content (TPC)

The total phenolic content of the plant extracts was determined using the Folin–Ciocalteu colorimetric method with slight modifications. The total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE/g) of dry extract (**Orhan *et al.*, 2011)**.

2.5.2 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined using the aluminium chloride colorimetric method. The total flavonoid content was expressed as milligrams of rutin equivalents (mg RE/g) of dry extract(**Shraim *et al.*, 2021).**

2.6 DPPH Radical Scavenging Assay

The antioxidant activity of *Clitoria ternatea* and *Cassia tora* extracts was determined using the DPPH free radical scavenging assay. The absorbance was measured at 517 nm using a UV–visible spectrophotometer, with methanol as blank and DPPH solution as control. All measurements were performed in triplicate**(Suhartati *et al.*, 2021).**

Formulation of

2.6.1 Formulation F1: *Clitoria ternatea* Suspension

Sodium carboxymethyl cellulose (CMC, 2.0 g) was dispersed in ~50 mL warm distilled water with continuous stirring and allowed to hydrate for 1 h. Tween 80 (0.1% v/v), methyl paraben (0.2% w/v), sucrose (10 g), and sorbitol (5 g) were added and mixed to obtain a uniform base. *Clitoria ternatea* extract (2.0 g) was incorporated, and the volume was adjusted to 100 mL with distilled water under constant stirring.

2.6.2 Formulation F2: *Cassia tora* Suspension

The suspending base was prepared as described above using CMC (2.0 g). Tween 80 (0.1% v/v), methyl paraben (0.2% w/v), sucrose (10 g), and sorbitol (5 g) were added with continuous stirring. *Cassia tora* extract (2.0 g) was incorporated, and the final volume was adjusted to 100 mL with distilled water.

2.6.3 Formulation F3: Polyherbal Suspension

The suspending base was prepared using CMC (2.0 g) dispersed in warm distilled water and hydrated for 1 h. Tween 80 (0.1% v/v), methyl paraben (0.2% w/v), sucrose (10 g), and sorbitol (5 g) were added with continuous stirring. A combination of *Clitoria ternatea* extract (1.0 g) and *Cassia tora* extract (1.0 g) was incorporated, and the volume was adjusted to 100 mL with distilled water**(Roopa *et al.*, 2015).**

2.7 Quality control parameters of Herbal Suspensions

2.7.1 pH

The pH of the formulations was evaluated using a calibrated digital pH meter to ensure accuracy.

2.7.2 Redispersibility

The herbal suspension was placed between two clean glass slides and subjected to a specified load to form a uniform thin layer.

2.7.3 Viscosity

The viscosity of the prepared herbal suspension was evaluated using a Brookfield viscometer to determine its flow characteristics.

2.7.4 Sedimentation volume

The sedimentation volume of the herbal suspension was determined to evaluate the physical stability of the formulation. This parameter measures the ratio of the final, settled volume of the suspended particles to the total volume of the suspension**(Júnior *et al.*, 2011).**

2.8 Pharmacological Study

All *in vivo* experiments were conducted following approval from the Institutional Animal Ethics Committee (IAEC) and in accordance with CPCSEA guidelines. Adult male Wistar rats (200 ± 60 g) were used for the study. Animals were housed in polypropylene cages under controlled environmental conditions (22 ± 2 °C; 12 h light/dark cycle) and acclimatized prior to experimentation. Standard pellet diet (Golden Feed, New Delhi, India) and water were provided ad libitum throughout the study period.

2.8.1 *In-vivo* Anti-Ulcer Activity

Experimental Design

The anti-ulcer activity was evaluated using an indomethacin-induced gastric ulcer model. Animals were randomly divided into six groups (n = 6). All rats were fasted for 24 h prior to induction of ulcers, with free access to water.

- **Group I (Normal control):** Received saline orally without ulcer induction.
- **Group II (Ulcer control):** Received indomethacin (25 mg/kg, p.o.)
- **Group III (Standard):** Received indomethacin (25 mg/kg, p.o.) followed by ranitidine (25 mg/kg, p.o.)
- **Group IV:** *Clitoria ternatea* suspension (Formulation I), 200 mg/kg, p.o.
- **Group V:** *Cassia tora* suspension (Formulation II), 200 mg/kg, p.o.
- **Group VI:** Polyherbal suspension (Formulation III), 200 mg/kg, p.o.

Indomethacin (25 mg/kg, p.o.) was used for ulcer induction in all groups except the normal control. Test formulations were administered orally after ulcer induction **(Kumar *et al.*, 2022).**

2.8.2 Ulcer index

The occurrence and severity of gastric lesions were assessed using a standardized scoring system. Ulcers were counted and graded according to the Kulkarni method **(Samuel *et al.*, 2013).**

2.8.3 Volume of gastric juice

The volume of gastric juice for each animal was measured after centrifugation at 1000 rpm for 10 minutes. The centrifuged sample volume was then expressed as millilitres per 100 g of body weight.

2.8.4pH of gastric juice

The acidity of the gastric juice was determined using a digital pH meter. The pH of the diluted sample was then recorded using the pH meter, which was calibrated prior to measurement according to the manufacturer's instructions

28.5 Determination of free acidity

To determine the free acidity of gastric juice, 1 milliliter of the sample was first diluted with distilled water and transferred into a 50 milliliter conical flask. Two drops of phenolphthalein were added as an indicator. The solution was then titrated with 0.01 N sodium hydroxide (NaOH) until a permanent light pink color appeared, indicating the endpoint. The volume of NaOH used during titration was recorded, and the free acidity was calculated using the following formula.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 1: Percentage Yield.

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1.	*Clitoria ternatea*	Ethanol	400	24.28	6.07
2.	*Cassia tora*	Ethanol	350	16.02	4.57

3.3 Preliminary Phytochemical study

Table 2: Phytochemical testing of the extract of methanol.

Sr.no	Experiment	Presence or absence ofphytochemical test
1.	Alkaloids	Present
2.	Glycoside	Present
3.	Carbohydrates	Present
4.	Proteins and Amino Acids	Present

5.	Flavonoids	Present
6.	Tannin and Phenolic Compounds	Absent
7.	Saponin	Absent
8.	Test for Triterpenoids and Steroids	Absent

3.4 Total Phenolic content (TPC) estimation and Total Flavonoids content (TFC) estimation.

Table 3: Standard table for Gallic acid.

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.184
2.	40	0.215
3.	60	0.256
4.	80	0.297
5.	100	0.346

Table 4: Total Phenolic content.

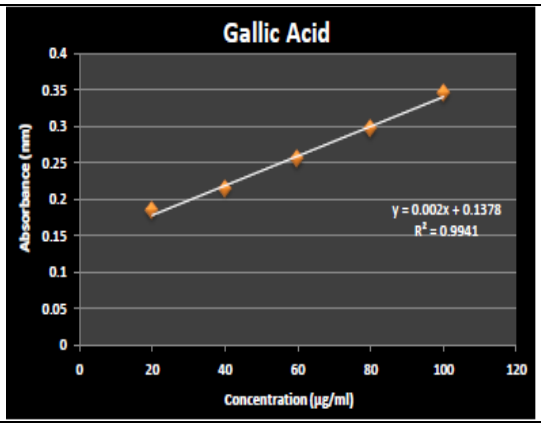


Table 5: Total Phenolic Content in *Clitoria ternateaand Cassia tora*extract.

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
Clitoriaternatea	0.193	48.1mg/gm
	0.236	
	0.275	
Cassia tora	0.171	25.1mg/gm
	0.185	
	0.209	

3.4.1 Total Flavonoids content (TFC) estimation

Table 6: Standard table for Rutin.

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.151
2.	40	0.178
3.	60	0.216
4.	80	0.263
5.	100	0.283

Table 7: Total Flavonoid Content.

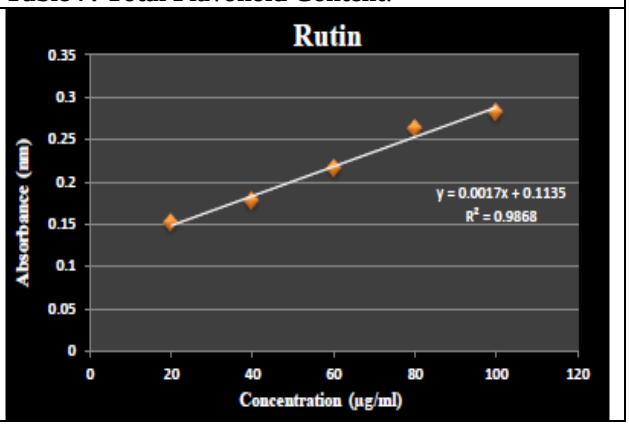


Table 8: Total Flavonoid Content in *Clitoria ternateaand Cassia tora* extract.

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
Clitoria ternatea	0.186	**60.1 mg/gm**
	0.257	
	0.305	
Cassia tora	0.134	43 mg/gm
	0.149	
	0.187	

7.4.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 9: DPPH radical scavenging activity of Std. Ascorbic acid.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.477	52.060
40	0.418	57.989
60	0.334	65.226
80	0.274	72.462
100	0.131	86.834
Control 0.995 IC5019.74		

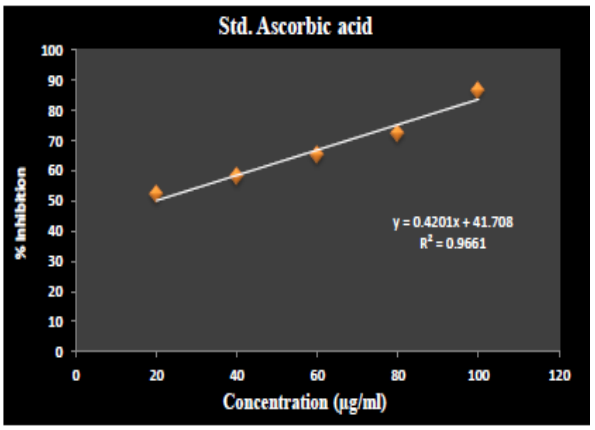


Table 10: DPPH radical scavenging activity of Ethanol extract of *Cassia tora*.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.522	47.537
40	0.470	52.710
60	0.457	54.070
80	0.418	57.989
100	0.369	60.201
Control 0.995		**IC5030.59**

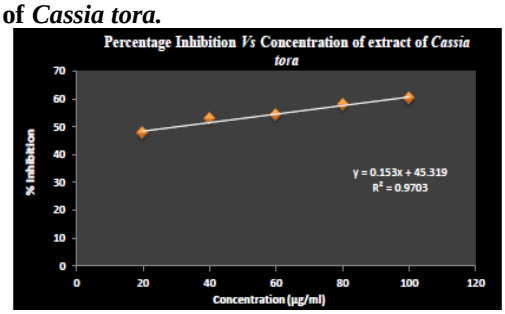


Table 11: DPPH radical scavenging activity of Ethanol extract of *Clitoria ternatea*.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.508	48.944
40	0.456	54.170
60	0.443	55.477
80	0.404	59.396
100	0.355	64.257
Control 0.995 IC50 24.06		

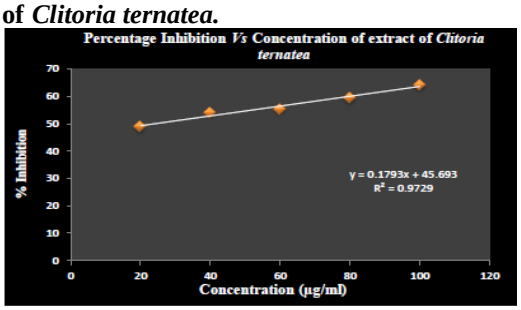


Figure 22: Represents the Inhibition*Vs*Concentration of extractof *Clitoria ternatea.*

7.4 Physicochemical parameters of formulated suspensions

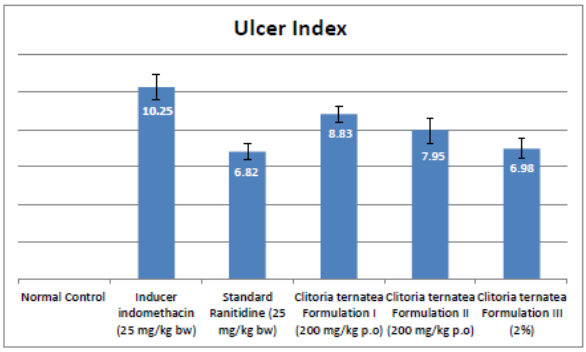
Table 12: pH, Viscosity, Redispersibility and Sedimentation volume.

S. No	Formulation	pH	Viscosity determination (cps)	Redispersibility	Sedimentation volume
1.	Formulation1.	6.15	52.9	Good	0.27
2.	Formulation 2	6.35	54.2	Good	0.24
3.	Formulation 3	6.53	55.4	Good	***0.19***

7.5 Determination of ulcer index

Table 13: Observation of Ulcer Index.

Groups	Ulcer Index
Group I- Normal Control	0
Group II Inducer indomethacin (25 mg/kg bw)	10.25±0.67
Group III Standard Ranitidine(25 mg/kg bw)	6.82 ±0.41
Group IV *Clitori aternatea* Formulation I (200 mg/kg p.o)	8.83±0.42
Group V *Cassia tora* Formulation II (200 mg/kg p.o)	7.95±0.67
Group VI Polyherbal Suspension Group Formulation III (2%)	6. 98± 0.52

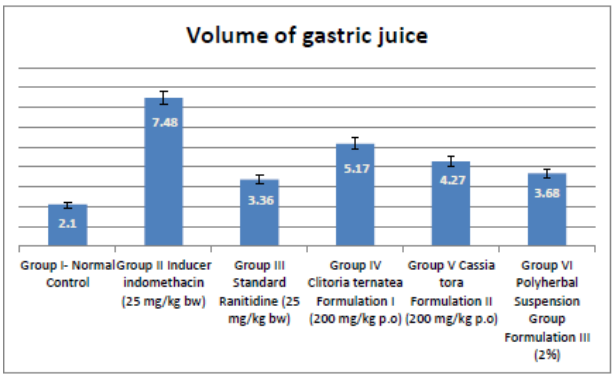


Graph 1: **Bar chart represents ulcer index in indomethacin induced ulcer in rats.**

7.6 Determination of Volume of gastric juice

Table 14: **Observation of volume of gastric juice.**

Groups	Volume of gastric juice
Group I- Normal Control	2.10 ± 0.15
Group II Inducer indomethacin (25 mg/kg bw)	7.48 ± 0.34
Group III Standard Ranitidine (25 mg/kg bw)	3.36 ± 0.23
Group IV *Clitoriaternatea*Formulation I (200 mg/kg p.o)	5.17 ± 0.28
Group V *Cassia tora* Formulation II (200 mg/kg p.o)	4.27 ± 0.26
Group VI Polyherbal Suspension Group Formulation III (2%)	3.68± 0.21

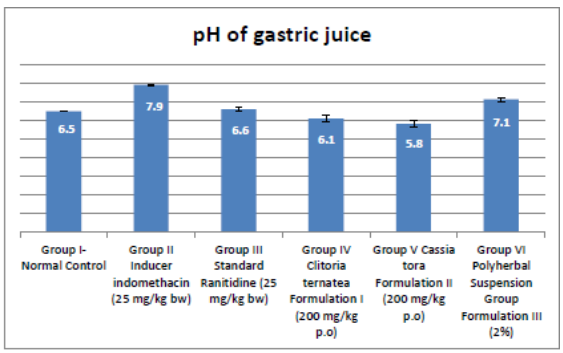


Graph 2: **Bar chart represents gastric volume in indomethacin induced ulcer in rats.**

7.7 Determination of pH of gastric juice

Table 15: **Observation of pH of gastric juice.**

Groups	pH of gastric juice
Group I- Normal Control	6.5 ± 0.0 8
Group II Inducer indomethacin (25 mg/kg bw)	7.9 ± 0.04
Group III Standard Ranitidine (25 mg/kg bw)	6.6 ± 0.08
Group IV *Clitoria ternatea* Formulation I (200 mg/kg p.o)	6.10± 0.19
Group V *Cassia tora*Formulation II (200 mg/kg p.o)	5.8 ± 0.16
Group VI Polyherbal Suspension Group Formulation III (2%)	7.1 ± 0.11

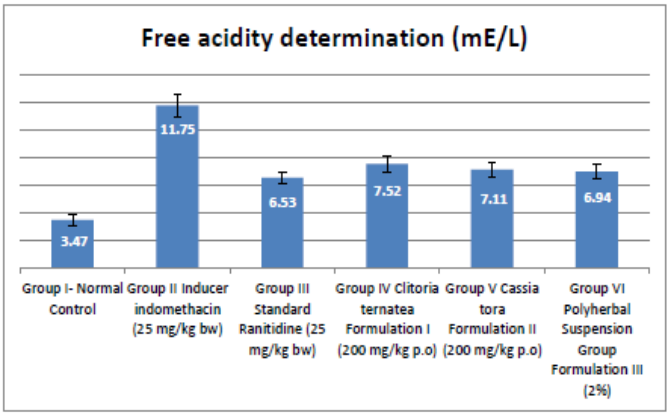


Graph 3: **Bar chart represents pH in indomethacin induced ulcer in rats.**

7.8 Free acidity determination

Table 16: **Observation of free acidity in indomethacin induced peptic ulcer in rats.**

Groups	Free acidity determination (mE/L)
Group I- Normal Control	3.47±0.42
Group II Inducer indomethacin (25 mg/kg bw)	11.75±0.82
Group III Standard Ranitidine (25 mg/kg bw)	6.53±0.41
Group IV *Clitoria ternatea* Formulation I (200 mg/kg p.o)	7.52±0.56
Group V *Cassia tora* Formulation II (200 mg/kg p.o)	7.11±0.50
Group VI Polyherbal Suspension Group Formulation III (2%)	6.94±0.54



Graph 4: Bar chart represents free acidity determination in indomethacin induced ulcer in rat.

4. DISCUSSION

The present study demonstrates that ethanol extracts of *Clitoria ternatea*and *Cassia tora* possess significant antioxidant and anti-ulcer activities, both individually and in combination. The higher extractive yield and greater phenolic (48.1 mg GAE/g) and flavonoid content (60.1 mg RE/g) observed in *Clitoria ternatea* correlated well with its superior antioxidant activity (IC_{50} = 24.06 µg/mL) compared to *Cassia tora*. This supports the established role of phenolic compounds in free radical scavenging and mucosal protection.

Increased stomach volume, acidity, and ulcer index were the outcomes of indomethacin-induced ulceration, indicating disturbance of mucosal defensive systems. Increased ulcer index (10.25 ± 0.67), elevated stomach volume (7.48 ± 0.34 mL), and greater free acidity (11.75 ± 0.82 mEq/L) were indicators of significant gastric mucosal damage in the ulcer control group in the in vivo anti-ulcer investigation employing the indomethacin-induced paradigm. These alterations are in line with the established mechanism of indomethacin, which causes ulceration by inhibiting prostaglandin synthesis, which results in increased secretion of stomach acid and weakened mucosal defense. These values were considerably lowered after treatment with plant extracts, suggesting successful gastroprotection. *Clitoria ternatea* shown comparatively stronger anti-ulcer effectiveness among individual treatments, probably because of its greater capacity as an antioxidant. Notably, the polyherbal formulation showed the strongest protective effect, improving gastric pH and significantly lowering ulcer index, gastric secretion, and acidity. This increased effectiveness points to a cooperative relationship between the two plants' bioactive components. Overall, antioxidant actions, decreased stomach acid output, and improved mucosal defense may be responsible for the anti-ulcer effectiveness. These results provide credence to the potential of *Cassia tora*and *Clitoria ternatea* as strong natural gastroprotective agents, especially when combined.

5. CONCLUSION

The present study demonstrates that *Clitoria ternatea*and *Cassia tora* possess significant anti-ulcer potential in the indomethacin-induced gastric ulcer model. Both extracts effectively reduced ulcer index, gastric volume, and free acidity while improving gastric pH, indicating their protective effect on gastric mucosa. Among the treatments, the polyherbal formulation exhibited superior activity, suggesting a synergistic interaction between the bioactive constituents of both plants.

The observed gastroprotective effect may be attributed to the presence of phenolics and flavonoids, which contribute to antioxidant and mucosal protective mechanisms. Overall, the findings support the therapeutic potential of these plants, particularly in combination, for the management of gastric ulcers and warrant further investigation for clinical applications.

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