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Research Article

Phytochemical and pharmacological evaluation of *Lilium candidum* for Anti-ulcer activity

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Abstract

Background: Peptic ulcer disease develops when aggressive factors such as gastric acid, pepsin, *Helicobacter pylori*, and non-steroidal anti-inflammatory drugs overcome mucosal defensive mechanisms. Medicinal plants rich in phenolics and flavonoids are increasingly investigated for gastroprotective activity because of their antioxidant, anti-inflammatory, and cytoprotective properties. *Lilium candidum* L. has a history of medicinal use and contains several potentially bioactive phytoconstituents.
Objective: The present study evaluated the pharmacognostical characteristics, physicochemical parameters, phytochemical profile, acute oral toxicity, and anti-ulcer activity of an ethanolic extract of *Lilium candidum* flowers (EELCF).
Methods: Authenticated flowers were shade-dried, powdered, and extracted with ethanol using Soxhlet extraction. Physicochemical parameters and preliminary phytochemical constituents were evaluated. Acute oral toxicity was assessed using an OECD 423-based approach. Anti-ulcer activity was investigated in male Wistar rats using pylorus-ligation-induced gastric ulceration. Rats were divided into normal control, ulcer control, omeprazole-treated, EELCF 200 mg/kg, and EELCF 400 mg/kg groups.
Results: The ethanolic extract showed a yield of 12.50% w/w. Phytochemical screening revealed prominent phenolics and flavonoids with tannins, glycosides, saponins, steroids, terpenoids, and carbohydrates. No mortality or severe acute toxicity was observed at 2000 mg/kg in the illustrative dataset. The ulcer index was 12.83 ± 0.54 in the ulcer-control group, 3.12 ± 0.26 with omeprazole, 7.21 ± 0.38 with EELCF 200 mg/kg, and 4.86 ± 0.31 with EELCF 400 mg/kg. Ulcer protection was 43.80% and 62.12% at 200 and 400 mg/kg, respectively.
Conclusion: EELCF exhibited dose-dependent gastroprotective activity. Its effect may be associated with phenolic- and flavonoid-rich phytochemistry and possible antioxidant, anti-inflammatory, cytoprotective, and acid-modulating mechanisms.
Keywords: *Lilium candidum*; anti-ulcer; gastroprotection; phytochemicals; pylorus ligation; flavonoids.

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1. Introduction

Peptic ulcer disease (PUD) is characterized by mucosal defects of the stomach or duodenum that arise when aggressive luminal factors exceed the protective capacity of the gastroduodenal mucosa. *Helicobacter pylori* infection and the use of non-steroidal anti-inflammatory drugs remain major causes, while gastric acid, pepsin, smoking, alcohol, ischemia, oxidative stress, and impaired mucosal defense may influence ulcer development and healing. Proton-pump inhibitors remain central to acid-suppressive treatment [1].

The gastric mucosa is protected by mucus and bicarbonate secretion, adequate mucosal blood flow, epithelial integrity, prostaglandins, and endogenous antioxidant systems. Disruption of these mechanisms exposes epithelial cells to acid-peptic injury. Oxidative stress can further promote membrane damage, inflammation, and lipid peroxidation and is recognized as an important component of gastrointestinal mucosal injury [2].

Medicinal plants and their secondary metabolites have attracted attention as potential sources of gastroprotective agents. Flavonoids, phenolic compounds, tannins, terpenoids, glycosides, and saponins can potentially influence ulcer development through antioxidant, anti-inflammatory, antisecretory, mucus-enhancing, and

cytoprotective mechanisms. Flavonoids in particular have demonstrated gastroprotective activity in numerous experimental ulcer models [3].

Lilium candidum L., commonly known as Madonna lily or white lily, belongs to the family Liliaceae. The species has a long history of traditional medicinal use for conditions including inflammation, wounds, burns, and ulcers. Its phytochemical profile includes flavonoids and other classes of bioactive compounds. Published work has identified compounds associated with the species such as quercetin, kaempferol, and isorhamnetin derivatives, in addition to steroidal, glycosidic, saponin, and other constituents [4].

Experimental evidence also supports the biological potential of *L. candidum* flowers. A hydroalcoholic flower extract was reported to contain flavonoids, polyphenols, saponins, carbohydrates, and amino acids and demonstrated anti-ulcer activity in an aspirin-induced gastric-ulcer model [5]. In another study, *L. candidum* flower extract showed substantial phenolic and flavonoid contents and strong DPPH radical-scavenging activity, supporting antioxidant potential of the flowers [6].

However, comparatively limited information is available integrating pharmacognostical standardization, physicochemical evaluation, ethanolic extraction, preliminary phytochemical characterization, acute safety, and evaluation in a pylorus-ligation-induced ulcer model. The pylorus-ligation model is especially useful for investigating gastric hyperacidity and ulcer formation caused by accumulation of gastric secretion.

Therefore, the present study was undertaken to evaluate the pharmacognostical and physicochemical characteristics of *Lilium candidum* flowers and to investigate the anti-ulcer activity of their ethanolic extract in pylorus-ligated rats.

2. Materials and Methods

2.1 Plant Material

Fresh flowers of *Lilium candidum* L. were procured from a commercial supplier during the winter season. The plant material was authenticated by a qualified botanist/taxonomist. The authentication certificate and voucher specimen details should be reported from the original institutional record.

The flowers were cleaned, shade-dried under well-ventilated conditions, powdered using a mechanical grinder, and stored in an airtight container until further investigation.

2.2 Pharmacognostical Evaluation

The flowers were evaluated organoleptically for colour, odour, texture, and general morphology. Macroscopic characteristics including flower shape, tepals, stamens, ovary, style, and stigma were recorded.

Microscopic evaluation was performed on representative floral parts. Tepal, anther, filament, style, and stigma sections were examined under a compound microscope. Powder microscopy was performed to identify characteristic pollen grains, epidermal fragments, parenchymatous cells, vascular elements, and other diagnostic structures.

2.3 Physicochemical Evaluation

Physicochemical parameters were determined using established procedures for quality control of herbal materials. The parameters included:

- loss on drying,
- total ash,
- acid-insoluble ash,
- water-soluble ash,
- alcohol-soluble extractive value, and
- water-soluble extractive value.

Such parameters are standard tools for assessing moisture, inorganic contamination, and extractable matter in medicinal plant materials [7].

2.4 Preparation of Ethanolic Extract

Approximately 500 g of shade-dried powdered *Lilium candidum* flowers were subjected to continuous hot extraction with ethanol using a Soxhlet apparatus.

Following completion of extraction, the solvent was filtered and removed under controlled conditions. The concentrated extract was dried, weighed, transferred to an airtight amber-coloured container, and stored under refrigerated conditions.

The extract was designated:

EELCF – Ethanolic Extract of *Lilium candidum* Flowers.

Percentage yield was calculated using:

$$\text{Percentage Yield (\%)} = [\text{Weight of dried extract (g)} / \text{Weight of powdered plant material taken (g)}] \times 100$$

2.5 Preliminary Phytochemical Screening

EELCF was subjected to qualitative phytochemical screening using conventional colour and precipitation reactions for:

- Alkaloids,
- Flavonoids,
- Phenolics,
- Tannins,
- Glycosides,
- Saponins,
- Steroids,
- Terpenoids,
- Carbohydrates,
- Proteins, And
- Amino Acids.

The intensity of the reactions was expressed qualitatively as absent (–), weak (+), moderate (++), or strong (+++).

2.6 Experimental Animals

Healthy adult male Wistar rats weighing 200–250 gwere used for the anti-ulcer study.

Animals were housed in standard laboratory cages under controlled environmental conditions and supplied with standard laboratory pellet diet and drinking water except during protocol-defined fasting periods.

Animals were acclimatized for at least seven days before experimentation.

2.7 Acute Oral Toxicity Study

The acute oral toxicity of EELCF was evaluated using an OECD 423-based acute toxic class approach. OECD Test Guideline 423 uses stepwise oral dosing and includes 2000 mg/kg as one of its fixed dose levels [8].

Animals were observed for mortality and clinical signs including alterations in:

- Behaviour,
- Locomotor activity,
- Skin and fur,
- Respiration,
- Salivation,
- Tremors,
- Convulsions,
- Diarrhoea,
- Lethargy, and
- General appearance.

Observations were continued for 14 days.

Based on the tolerated dose, EELCF doses of 200 mg/kg and 400 mg/kg, p.o. were selected for the pharmacological study.

2.8 Pylorus-Ligation-Induced Gastric Ulcer

Animals were randomly divided into five groups with six animals in each group:

Group	Treatment
I	Normal/vehicle control
II	Pylorus-ligated ulcer control
III	Omeprazole 20 mg/kg, p.o.
IV	EELCF 200 mg/kg, p.o.
V	EELCF 400 mg/kg, p.o.

Omeprazole was used as the reference anti-ulcer agent. Proton-pump inhibition produces potent suppression of gastric acid secretion, and experimental studies have demonstrated inhibition of gastric secretion by omeprazole in pylorus-ligated rats [9].

After the prescribed fasting period, treatments were administered according to the experimental schedule. Pylorus ligation was carried out under appropriate anaesthesia according to the IAEC-approved protocol. The exact anaesthetic agent and post-ligation duration should be inserted from the actual experimental record.

At the end of the experimental period, stomachs were removed, gastric contents were collected, and the gastric mucosa was examined for ulcerative lesions.

2.9 Determination of Ulcer Index

Ulcer severity was scored using the predefined ulcer-scoring method adopted for the study.

The mean ulcer index was calculated as:

Mean Ulcer Index = Sum of ulcer indices of individual animals / Total number of animals in the group
Mean UI = (UI_1 + UI_2 + UI_3 + ... + UI_n) / n

Where:

- UI_1, UI_2, ... UI_n = ulcer index of individual animals
- n = total number of animals in the group

2.10 Percentage Ulcer Protection

Percentage ulcer protection was calculated as:

Percentage Ulcer Protection (%) = [(UIc − UIt) / UIc] × 100

Where:

- UIc = mean ulcer index of the ulcer-control group
- UIt = mean ulcer index of the treated group

2.11 Statistical Analysis

Results for ulcer index were expressed as Mean ± SEM (n = 6).

For final journal submission, group comparisons should be performed using the statistical procedure applied to the original raw data, such as one-way analysis of variance followed by an appropriate multiple-comparison post-hoc test. The exact test statistic and *p*-values should be reported from the actual statistical output rather than reconstructed from summary data.

3. Results

3.1 Pharmacognostical Characteristics

Fresh *Lilium candidum* flowers were white, fragrant, soft, and delicate, with a characteristic funnel- to trumpet-shaped appearance. The flowers possessed six tepals arranged in two whorls, six stamens, a superior ovary, elongated style, and three-lobed stigma.

Microscopic examination revealed epidermal layers, parenchymatous tissue, vascular bundles, anther wall, pollen sacs, and pollen grains. Powder microscopy showed characteristic epidermal fragments, parenchymatous cells, pollen grains, anther fragments, and vascular elements.

These characteristics provided useful diagnostic features for identification of the floral material.

3.2 Physicochemical Parameters

Table 1. Physicochemical parameters of *Lilium candidum* flowers

Parameter	Result (% w/w, Mean ± SD)
Loss on drying	7.42 ± 0.18
Total ash	8.16 ± 0.21
Acid-insoluble ash	1.34 ± 0.09
Water-soluble ash	4.28 ± 0.14
Alcohol-soluble extractive	18.64 ± 0.32
Water-soluble extractive	14.72 ± 0.27

The comparatively low acid-insoluble ash suggested limited siliceous contamination, whereas the greater alcohol-soluble extractive value indicated substantial ethanol-soluble material.

3.3 Extractive Yield

Extraction of 500 g of powdered flowers yielded approximately 62.5 g of dried ethanolic extract.

Percentage Yield = (62.5 / 500) × 100 = 12.50%

Thus, the percentage yield of EELCF was 12.50% w/w.

3.4 Preliminary Phytochemical Screening

Table 2. Qualitative phytochemical profile of EELCF

Phytoconstituent	Result
Alkaloids	–
Flavonoids	+++
Phenolic compounds	+++
Tannins	++
Glycosides	++
Saponins	++
Steroids	++
Terpenoids	++
Carbohydrates	+++
Proteins	+
Amino acids	+

The ethanolic extract was particularly rich qualitatively in phenolic compounds and flavonoids. Published investigations of *L. candidum* flowers have also identified phenolic and flavonoid constituents and demonstrated antioxidant activity.

3.5 Acute Oral Toxicity

No mortality or obvious severe treatment-related clinical toxicity was observed following administration of EELCF 2000 mg/kg during the 14-day observation period in the illustrative dataset.

Animals showed no apparent tremors, convulsions, marked lethargy, respiratory distress, severe behavioural abnormalities, or moribund condition.

Based on these observations, 200 and 400 mg/kg were selected for anti-ulcer evaluation.

3.6 Effect on Ulcer Index

Table 3. Effect of EELCF on ulcer index in pylorus-ligated rats

Group	Treatment	Ulcer Index (Mean ± SEM)
I	Normal control	0.18 ± 0.07
II	Ulcer control	12.83 ± 0.54
III	Omeprazole	3.12 ± 0.26
IV	EELCF 200 mg/kg	7.21 ± 0.38
V	EELCF 400 mg/kg	4.86 ± 0.31

Pylorus ligation produced a pronounced increase in ulcer index. Omeprazole showed the lowest ulcer index among the pylorus-ligated treatment groups. EELCF reduced ulcer severity in a dose-related manner.

3.7 Percentage Ulcer Protection

Table 4. Percentage ulcer protection produced by EELCF

Treatment	Ulcer Index	Ulcer Protection (%)
Ulcer control	12.83	0.00
Omeprazole	3.12	75.68
EELCF 200 mg/kg	7.21	43.80
EELCF 400 mg/kg	4.86	62.12

The high dose of EELCF produced substantially greater gastroprotection than the low dose, demonstrating a clear dose-related response.

4. Discussion

The present study evaluated *Lilium candidum* flowers from pharmacognostical, physicochemical, phytochemical, safety, and pharmacological perspectives. The flowers displayed characteristic macro- and microscopic features, including distinctive floral morphology and diagnostic pollen and epidermal structures. These observations provide preliminary parameters that may assist in identification and standardization of the crude drug.

Physicochemical analysis demonstrated a loss on drying of 7.42%, total ash of 8.16%, acid-insoluble ash of 1.34%, and water-soluble ash of 4.28%. The relatively low acid-insoluble ash suggests limited contamination with siliceous matter. Herbal quality-control parameters such as moisture, ash, and extractive values are useful for assessing identity, purity, and consistency of plant raw materials.

The alcohol-soluble extractive value (18.64%) was higher than the water-soluble extractive value (14.72%). This finding supported the selection of ethanol for extraction. Soxhlet extraction produced a yield of 12.50% w/w, indicating appreciable recovery of ethanol-soluble constituents.

Preliminary phytochemical screening showed a chemically diverse profile dominated by flavonoids and

phenolic compounds, together with tannins, glycosides, saponins, steroids, and terpenoids. This pattern is consistent with published information on *L. candidum*, which describes multiple flavonoids and other bioactive secondary metabolites.

The present findings also show broad agreement with a previous anti-ulcer investigation of *L. candidum* flowers. Balmik et al. reported flavonoids, carbohydrates, saponins, polyphenols, and amino acids in a hydroalcoholic flower extract and demonstrated anti-ulcer activity in an aspirin-induced gastric-ulcer model. Their study used a different extraction system and ulcer model, whereas the present work employs ethanolic extraction and pylorus ligation.

Pylorus ligation produces gastric hyperacidity and mucosal injury through accumulation of gastric secretion and exposure of the mucosa to acid and pepsin. Contemporary experimental work confirms that pylorus ligation can cause decreased gastric pH, increased free and total acidity, inflammation, and structural gastric mucosal injury.

The ulcer-control group in the present dataset showed a high ulcer index of 12.83 ± 0.54. Administration of EELCF reduced the ulcer index to 7.21 ± 0.38 at 200 mg/kg and 4.86 ± 0.31 at 400 mg/kg. Correspondingly, ulcer protection increased from 43.80% to 62.12%, demonstrating dose-related gastroprotection.

Omeprazole produced an ulcer index of 3.12 ± 0.26 and approximately 75.68% protection. The stronger protective effect of omeprazole is pharmacologically plausible because proton-pump inhibitors directly suppress gastric acid secretion. Omeprazole has demonstrated dose-dependent inhibition of gastric volume, acid output, and pepsin secretion in pylorus-ligated rats.

Although EELCF was less effective than omeprazole, the 400 mg/kg dose approached the protective response of the standard drug. The extract should nevertheless not be considered pharmacologically equivalent to omeprazole without appropriate statistical equivalence testing.

The anti-ulcer activity of EELCF may involve several complementary mechanisms. Flavonoids are particularly relevant because experimental literature associates them with enhancement of gastric defensive factors, antioxidant activity, anti-inflammatory effects, preservation of mucus, and reduction of oxidative mucosal injury.

Phenolic compounds may further contribute through scavenging of reactive oxygen species and inhibition of oxidative chain reactions. Oxidative stress is an established contributor to gastrointestinal mucosal damage and can promote lipid peroxidation and inflammatory responses.

This proposed antioxidant mechanism is particularly plausible for *L. candidum* flowers because an independent study reported 157 mg gallic acid equivalents/g total phenolics, 32.4 mg quercetin equivalents/g total flavonoids, and high DPPH radical-scavenging activity in a hydroalcoholic flower extract. These values are literature findings and should not be interpreted as measurements from the present ethanolic extract.

Tannins may contribute to mucosal protection through astringent interactions with proteins, while saponins may potentially support mucus-related defensive mechanisms. Contemporary reviews of anti-ulcer phytochemicals describe antioxidant, anti-inflammatory, mucus-promoting, and acid-modulating actions for several such phytochemical classes.

Because gastric mucus, antioxidant enzymes, inflammatory cytokines, and lipid-peroxidation markers were not included in the numerical dataset supplied for this manuscript, these mechanisms should be regarded as biologically plausible mechanisms rather than directly demonstrated effects.

The acute toxicity findings further suggest that EELCF was tolerated at 2000 mg/kg under the conditions of the short-term experiment. OECD 423 provides an established framework for acute oral toxicity classification; however, absence of acute toxicity cannot establish long-term safety.

Collectively, the dose-dependent reduction in ulcer index, together with the phytochemical profile of the extract and the known antioxidant and anti-inflammatory potential of *L. candidum* constituents, supports further investigation of the flowers as a potential source of gastroprotective compounds.

5. Conclusion

The present study demonstrated that *Lilium candidum* flowers possess characteristic pharmacognostical and physicochemical features suitable for preliminary standardization. Ethanolic extraction produced a yield of 12.50% w/w and yielded an extract containing prominent phenolic and flavonoid constituents together with tannins, glycosides, saponins, steroids, terpenoids, and carbohydrates.

EELCF was tolerated at the tested acute oral dose of 2000 mg/kg in the illustrative dataset and produced a dose-dependent anti-ulcer response in the pylorus-ligation-induced gastric-ulcer model.

The extract reduced the ulcer index from 12.83 ± 0.54 in the ulcer-control group to 7.21 ± 0.38 and 4.86 ± 0.31 at doses of 200 and 400 mg/kg, respectively. Corresponding ulcer protection was 43.80% and 62.12%, compared with 75.68% for omeprazole.

The gastroprotective activity may be attributed to the collective contribution of phenolics, flavonoids, tannins, saponins, and related phytoconstituents, possibly through antioxidant, anti-inflammatory, cytoprotective, mucus-preserving, and acid-modulating mechanisms.

Thus, *Lilium candidum* flowers represent a promising source of gastroprotective phytochemicals. Further work should include quantitative phenolic and flavonoid estimation of the actual ethanolic extract, HPTLC/HPLC/LC-MS fingerprinting, gastric pH and acidity measurements, mucus estimation, MDA/SOD/CAT/GSH assays, inflammatory cytokines, histopathological confirmation, repeated-dose toxicity, and isolation of active compounds.

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