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Research Article

Qualitative and Quantitative Determination of Secondary Metabolites and Evaluation of Antiulcer Activity of Hydroalcoholic Extract of *Buchanania lanzan* Leaf Extract

Deepa Shrivastava *[1], Surabhi Saksena [2]

[1] *Radharaman College of Pharmacy, Ratibad, Bhopal, MP, 462044*

[2] *Radharaman Institute of Pharmaceutical Sciences, Ratibad, Bhopal, MP, 462044*

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*Address for Correspondence:

Deepa Shrivastava, Radharaman College of Pharmacy, Ratibad, Bhopal, MP, 462044

Abstract

The most prevalent digestive condition in clinical practice is peptic ulcer. It should be regarded as a superior choice for the treatment of peptic ulcer given the numerous side effects of contemporary medicine, the initial acquisition of fewer side effects, and the therapy of indigenous substances. Throughout most of India's deciduous woods, *Buchanania lanzan* (*B. lanzan*, Chironji, Achar, Anacardiaceae) is a significant non-wood tree species. *B. lanzan* is a commonly used plant that has been used for many years in traditional medicine to treat a variety of illnesses. Thus, the goal of the current investigation was to assess the hydroalcoholic extract of *B. lanzan* leaves antiulcer properties in rats. The established test procedure described in the literature was used to determine the quantitative analysis of total phenolics and flavonoids as well as the qualitative analysis of different phytochemical constituents. Rats with pylorus-ligated peptic ulcer models were used to test the hydroalcoholic extract of *B. lanzan* leaves' in vivo anti-ulcer activity. The volume and pH of stomach fluid, free acidity, total acidity, ulcer index, and percent inhibition of ulcer index were among the outcome indicators that varied based on the model. An initial phytochemical examination indicated the existence of flavonoids, phenols, glycosides, alkaloids, and tannins. The leaves hydroalcoholic extract had a total phenolic content of 32.5 mg/gm, with flavonoids coming in second at 27.60 mg/gm. In rats with pylorus ligation-induced ulcer models, additional hydroalcoholic extract at 200 and 400 mg/kg/p.o. significantly ($p<0.01$) decreased the gastric volume, pH, ulcer number, ulcer index, free acidity, and total acidity. The results of this investigation verified that *B. lanzan* extract, because of one or more of its secondary metabolites, possesses pharmacologic activity that is anti-ulcer. This study thus supports the use of it in Indian traditional medicine as an anti-ulcer agent. It is necessary to conduct additional research to isolate particular phytochemicals and clarify their mechanisms of action.

Keywords: *Buchanania lanzan,* Phytochemical constituents, Antiulcer, Pylorus ligation, Peptic ulcer

INTRODUCTION

One of the most common GIT conditions, peptic ulcers have a higher morbidity rate and afflict 5-10% of the population at some point in their lives[1]. Although scientists and researchers have provided a common ground to comprehend the possible pathogenesis of peptic ulcer, the disease known as peptic ulcer disease has numerous etiologies. Research is ongoing to determine the precise pathogenesis of peptic ulcer. An imbalance between the stomach's defensive (gastric mucus and bicarbonate secretion, prostaglandins, intrinsic resistance of the mucosal cells) and aggressive (acid, pepsin, and Helicobacter pylori) components results in peptic ulcers[2]. These variables could include lifestyle choices, illnesses, and natural causes[3-5]. Peptic ulcers can be managed with a variety of therapy options, including prostaglandin analogs, histamine receptor antagonists, proton pump inhibitors, and cytoprotective medicines. However, the majority of these medications cause a number of unwanted side effects, such as headaches, upset stomachs, dizziness, constipation, and diarrhea. When used over an extended period of time, they may also change the body's normal biochemical homeostasis, resulting in elevated serum aluminum levels from antacids and sucralfate and decreased calcium absorption from proton pump inhibitors[6]. Much research has been done on natural medications in the past few years to see whether or not they could be useful in preventing stomach ulcers. Due to its availability, affordability, lack of side effects, and demonstrated efficacy, herbal therapy shows promise as an ulcer treatment alternative to currently available synthetic pharmaceuticals[7,8]. Pharmacological reports have indicated that a number of natural herbs have strong anti-ulcer properties[9-12]. India's hot and dry deciduous woodlands are home to *B. lanzan* (Chironji), an economically valuable tree in the Anacardiaceae family[13]. The leaves have historically been used as a purgative, expectorant, and digestive in addition to managing wounds[14]. According to certain studies, the herb is also used as an astringent and cardiotonic, and it is also used to cure skin conditions and glandular swelling[15]. There are reports that the root can treat blood disorders and be used as an expectorant[16]. Research on *B. lanzan* has shown that its leaves have anti-inflammatory properties[17], and its bark has been shown to shield mice from oxidative stress and genotoxicity caused by cyclophosphamide[15,16]. It has been

claimed that the dry fruits of *B. lanzan* possess astringent and immuno stimulant qualities[18]. The plant's kernel is recognized to have anti-inflammatory and antioxidant properties[19]. It has been reported that the plant's roots have astringent qualities and are used to treat diarrhea. Flavonoids, tannins, glycosides, phenols, steroids, saponin, gallic acid, and myricetin 3′-rhamnoside-3-galactoside have all been found in the plant's phytochemical study[20, 21]. Therefore, assessing the *B. lanzan* leaves extract's in vivo antiulcer activity was the goal of the current study.

MATERIALS AND METHODS

Plant material

In January 2024, leaves of *B. lanzan* were gathered from the surrounding area of Bhopal (M.P.).

Chemicals and reagents

Analytical grade chemicals, pharmaceuticals, and solvents were all used in the investigation. A free sample of omeprazole was acquired from the Musalgaon MIDC, Sinner, Nashik, India, Sci-tech laboratory. S. D. Fine Chemicals, Mumbai, India was the source of all other chemicals, such as methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, and ethylene diamine tetra acetic acid disodium salt. We bought phenolphthalein, Topfer's reagent, Folin's reagent, and Tris buffer from Hi-Media Pvt. Ltd. in Mumbai, India.

Extraction by maceration process

B. lanzan leaves were shade-dried and kept at room temperature. The plant material that had been shade-dried was ground into a coarse powder and extracted using petroleum ether. Up until the material's defatting, the extraction process was carried out. 50 grams of dried powdered *B. lanzan* leaves were extracted using a maceration procedure for 48 hours and a hydroalcoholic solvent (30:70). Before being used, the extracts were allowed to evaporate above their boiling temperatures and then sealed in an airtight container to prevent contamination. Lastly, the dried extracts' percentage yields were determined.

Phytochemical screening

In order to identify the various phytoconstituents using conventional tests and procedures, a qualitative phytochemical analysis was conducted on the hydroalcoholic extract of *B. lanzan* leaves[22,23].

Total phenol determination

Olufunmiso et al.'s technique[24] was used to calculate the total phenolic content. Two milliliters (ml) of *B. lanzan* extracts or standard leaves were combined with five milliliters (ml) of Folin Ciocalteau reagent (previously diluted 1:10 v/v) and four milliliters (75g/l) of sodium carbonate. The mixture was left to stand at room temperature for fifteen minutes. The UV/visible spectrophotometer were used to measure the blue color that had evolved at 765 nm. The gallic acid standard graph was used to compute the total phenolic content, and the findings were represented as gallic acid equivalent (mg/g).

Total flavonoids determination

The method of Olufunmiso et al[24] was used to calculate the total flavonoid content. After adding 1 ml of 2% $AlCl_3$ methanolic solution to 1 ml of extract or standard, the mixture was left to stand for 60 minutes at room temperature. A UV/visible spectrophotometer were used to measure the absorbance of the reaction mixture at 420 nm. Using a conventional quercetin graph, the content of flavonoids was determined. The results were represented as mg/g of quercetin equivalent.

Animals

In this study, six Wistar rats weighing between 180 and 200 grams were kept in groups with controlled humidity and temperature (25-65%) and a standard 12-hour light/dark cycle. Water was available at all times, along with conventional rat feed. Prior to doing the trials, the rats were given seven days to become used to the lab environment. Every experiment was conducted from 8:00 to 15:00 in a quiet room. Each series of trials had a different group of six rats. The Ministry of Environment and Forests, Government of India, New Delhi, India, established the Institutional Animal Ethics Committee (IAEC) to oversee and regulate the use of experimental animals. The IAEC granted approval for the animal experiments.

Acute toxicity test

The OECD 420 guideline's limit test dose of 2000 mg/kg was followed in the acute toxicity investigation. Three female albino rats were given a 24-hour fast but were still able to drink water at will. A hydroalcoholic extract of *B. lanzan* leaves containing 2000 mg/kg was given to each animal, and after dosing continuously for two hours, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days, the animals were observed for morbidity or mortality, autonomic profiles (defecation and urination), neurologic profiles (spontaneous activity, reactivity, touch response, pain response, and gait), and behavioral profiles (alertness, restlessness, irritability, and fearfulness) and for morbidity or mortality[25].

Pylorus ligation model

Five groups of six albino rats each were randomly assigned to the animals[26, 27].

Experimental design

- **Group I:** Normal control animals
- **Group II:** Disease control (pylorus ligated)
- **Group III:** Hydroalcoholic extract of *B. lanzan* (200 mg/kg p.o) suspended in 1% *w/v* CMC
- **Group IV:** Hydroalcoholic extract of *B. lanzan* (400mg/kg p.o) suspended in 1% *w/v* CMC
- **Group V:** Standard treated Omeprazole (20mg/kg) suspended in 1% *w/v* CMC

Procedure

Using an oral feeding tube, the animals in Groups III, IV, and V were administered low, high doses of *B. lanzan* extract and omeprazole, respectively, on the day of the experiment. The sole treatment given to the control group was regular saline. Following a one-hour medication treatment, the animals in Groups II, III, IV, and V were sedated with anaesthetic ether, and a minor midline incision was made to open their abdomens. In order to prevent traction to the pylorus or harm to its blood supply, the pyloric region of the stomach was gently elevated and ligated. Sutures were used to seal the abdominal wall after the stomach was carefully restored. Rats were pylorically ligated for six hours before being killed by an over abundance of anesthetic ether. The stomach's cardiac end was removed, the abdomen was opened, and the contents were drained into a glass tube. After measuring the gastric juice's volume, it was centrifuged for ten minutes at 2000 rpm. To measure the pH, total, and free acids, aliquots (1 ml of each) were collected from the supernatant. Every stomach was

checked for lesions in the part of the stomach that faces the front and ranked based on how severe they were.

Biochemical estimations

Determination of gastric volume

The stomach was removed when the rat was sacrificed. After being moved into the centrifuge tube, the stomach contents were filtered and centrifuged. After that, the liquid supernatant was moved to a measuring cylinder so that its volume could be determined.

Determination of pH of gastric content

Using a digital pH meter, the pH of one milliliter of the stomach liquid was measured directly.

Determination of ulcer index

The larger curvature of the stomachs was opened, and the number of ulcers was counted. The following scoring scheme was used to assign points for ulcers:

0=no ulcer,

1=superficial ulcer,

2=deep ulcer,

3=perforation.

Ulcer index was calculated by using following formula

$$UI=UN+US+UP\times10-1$$

Where,

UI=ulcer index,

UN=mean of ulcer number,

US=mean of ulcer score,

UP=ulcer probability for each group.

Determination of free acidity and total acidity

It was measured how much gastric content there was overall. Following a centrifugation, the stomach contents were filtered. Using two to three drops of Topfer's reagent as an indicator, one milliliter of the gastric juice was pipetted out, and the solution was titrated against 0.1N sodium hydroxide until the point at which the solution turned yellowish orange in color. This showed how much NaOH was needed to balance the gastric juice's free hydrochloric acid. After adding two to three drops of phenolphthalein solution, the titration was carried out until a distinct red color emerged. The amount of NaOH needed to neutralize the combined acid in the stomach juice was shown by the difference between the two values. The sum of the two titrations was the total acid present in the gastric juice.

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH}}{\text{Volume of gastric juice used}}$$

RESULTS AND DISCUSSIONS

Following the maceration process, the crude extracts were concentrated further on a water bath to completely evaporate the solvents and achieve the true extraction yield. 4.3%w/w was the yield of *B. lanzan* extracts. Table 1 displays the findings of the qualitative phytochemical analysis performed on the crude leaf powder of *B. lanzan*. *B. lanzan* leaf samples that were hydroalcoholically extracted revealed the presence of glycosides, phenols, alkaloids, flavonoids, and carbohydrates. Total flavonoid content (TFC) and total phenolic content (TPC) were determined for a quantitative phytochemical experiment. The TPC was computed using gallic acid as the standard, while the TFC was computed using quercetin as the standard. In the hydroalcoholic extract, the TPC and TFC were determined to be 32.5 mg/g and 27.60 mg/g, respectively, Table2&Fig. 1, 2. The OECD 425 standards were followed in conducting the acute oral toxicity investigation. When *B. lanzan* leaves were given orally at a dose of 2000 mg/kg, there were no negative effects or deaths noted in the animals. This suggests that the maximum safe dose is 2000 mg/kg. Therefore, 200 and 400 mg/kg of body weight, or 1/10th and 1/5thi of the maximum safe dose, were chosen to be studied for their *in vivo* anti-ulcer properties. The hydroalcoholic extract of *B. lanzan* leaves was used to investigate the effects on stomach acid and mucus secretion in ulcers created by Pylorus ligation. Gastric acid builds up in the stomach as a result of the pyloric end of the stomach being tied up. Stomach ulcers are brought on by this increase in the release of gastric acid. Rats are fasted for twenty-four hours, after which the pyloric end of their stomachs is clamped shut. Four hours later, the ulcer index is calculated. The stomach's lumen contains the lesions created by this technique. The hydroalcoholic extract of *B. lanzan* with omeprazole markedly improved pH and greatly reduced total and free acidity, indicating that it may have an anti-secretory action. Rats with control ulcers caused by Pylorus ligation displayed perforated ulcers, deep granular epithelium ulcerations, and nearly complete sub mucosal reduction. At 200 mg/kg, the hydroalcoholic extract of *B. lanzan* showed mucosal erosion and a partially healed ulcer with few inflammatory cells; at 400 mg/kg, the ulcer was healed, the mucosa was normal, and there were no inflammatory cells Table 3-5.

Table 1: Phytochemical evaluation of *B. lanzan*leaves

Tests	Pet. ether	Hydroalcoholic
Carbohydrates		
Molish	+ ve	+ ve
Fehlings	+ ve	+ ve
Benedit's	+ ve	+ ve
Protien& amino acids		
Biurets	- ve	- ve
Ninhydrin	- ve	- ve
Glycosides		
Borntrager	+ ve	+ ve
Killer killani	+ ve	+ ve
Alkaloids		
Mayers	+ ve	+ ve
Hagers	+ ve	+ ve
Wagners	+ ve	+ ve
Saponins		
Froth	- ve	+ ve
Flavonoids		
Lead acetate	+ ve	+ ve
Alkaline reagent test	+ ve	+ ve
Treterpenoids & Steroids		
Salwoski	- ve	- ve
Libberman Burchard	- ve	- ve
Tannin & Phenolics		
Ferric chloride	+ ve	+ ve
Lead acetate	+ ve	+ ve
Gelatin	+ ve	+ve

(+) Indicates Presence; (-) Indicates Absence

Table 2: Total phenolic and flavonoid content of extracts

S. No.	Solvents → Bioactive compound ↓	Hydroalcoholic extract
Leaves of *B. lanzan*		
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	32.5
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	27.60

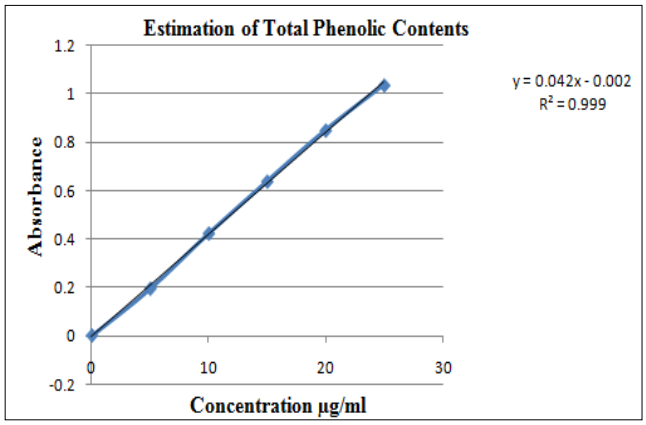

Figure 1: Graph of estimation of total phenolic content

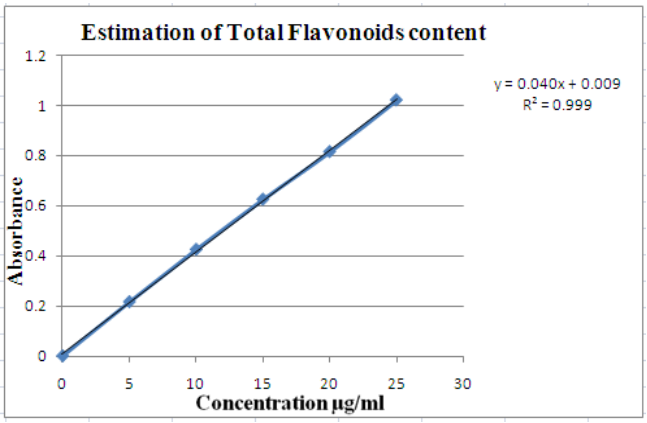

Figure 2: Graph of estimation of total flavonoid content

Table 3: Effect on gastric volume and pH in pylorus ligated gastric ulcer

Group	Gastric Volume	Gastric pH
Group I: Control (CMC)	2.41 ± 0.47	2.88±0.25
Group II: Pylorus ligated	7.45± 0.44**	2.21 ±0.23**
Group III: Treated with *B. lanzan*(200mg/kg)	6.41 ± 0.27**	2.58 ± 0.17*
Group IV: Treated with *B. lanzan*(400mg/kg)	4.88 ± 0.25**	3.27 ± 0.40**
Group V: Treated with *Omeprazole* (20 mg/kg)	3.73 ± 0.45***	3.71 ± 0.15***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

Table 4: Effect on number of ulcer and ulcer index in pylorus ligated gastric ulcer

Group	Number of Ulcer	Ulcer Index
Group I: Control (CMC)	0.21±0.17	2.79±0.20
Group II: Pylorus ligated	4.67±0.26**	26.25±0.25**
Group III: Treated with *B. lanzan*(200mg/kg)	3.61±0.13*	18.19±0.13*
Group IV: Treated with *B. lanzan*(400mg/kg)	2.29±0.47**	15.89±0.31**
Group V: Treated with *Omeprazole* (20 mg/kg)	1.61±0.46***	12.59±0.29***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

Table 5: Effect on free acidity and total acidity in pylorus ligated gastric ulcer

Group	Free acidity content	Total acidity content
Group I: Control (CMC)	4.61± 0.25	5.61 ± 0.22
Group II: Pylorus ligated	7.87 ±0.22**	8.47 ± 0.17**
Group III: Treated with *B. lanzan*(200mg/kg)	6.70±0.22**	7.62 ±0.20*
Group IV: Treated with *B. lanzan*(400mg/kg)	5.02± 0.18**	5.22 ± 0.18**
Group V: Treated with *Omeprazole* (20 mg/kg)	3.62±0.20***	3.98± 0.10***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

CONCLUSION

The hydroalcoholic extract of *B. lanzan* leaves revealed the presence of flavonoids, saponins, polyphenols, and other compounds according to a preliminary phytochemical analysis. After being tested for acute oral toxicity, hydroalcoholic extract was determined to be safe. *B. lanzan* leaf hydroalcoholic extract has strong antioxidant and oxidant properties as well as notable anti-ulcer properties. Our findings concluded that the extract's anti-ulcer activity was likely caused by the active phytoconstituents' anti-secretory, cytoprotective, and antioxidant qualities, which are known to be gastric ulcer healing mechanisms. These results imply that *B. lanzan* leaves may be used as an adjuvant in the management of stomach ulcers. To determine the precise mechanism of action in the healing of gastric ulcers and to isolate the active ingredients responsible for the anti-ulcer activity, more research is required.

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