International Journal of Education, Modern Management, Applied Science & Social Science (IJEMMASSS)
e-ISSN :2581-9925, Impact Factor (Cosmos: 7.555&I2OR: 5.105), Volume 08, No. 01(II), January- March, 2026, pp. 155-167

Phytochemical Study & Pharmacological Evaluation with Comparison of Antioxidant and Antiulcer Activity of Combtetum Indicum Linn and *Thevetia Peruviana* (Pers.)

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Citation: Bhadouria, A. (2026). Phytochemical Study & Pharmacological Evaluation with Comparison of Antioxidant and Antiulcer Activity of Combtetum Indicum Linn and Thevetia Peruviana (Pers.). International Journal of Education, Modern Management, Applied Science & Social Science, 08(01(II)), 155–167. https://doi.org/10.62823/IJEMMASSS/8.1(II).8764

ABSTRACT

Gastric ulcers and the associated disorders caused by oxidative stress are a significant concern to global health, and effective and safer therapeutic alternatives based on plants must be investigated. The purpose of this research is to compare the phytochemical composition and pharmacological potential of Combretum indicum Linn and Thevetia peruviana, in terms of antioxidant and antiulcer activities. The study was done by standard experimental techniques (plant materials were extracted by aqueous and ethanolic methods), and then, qualitative phytochemical screening was done. The DPPH free radical scavenging assay was used to analyze the antioxidant activity and the pylorus ligation induced ulcer models in experimental animals were used to analyze antiulcer activity. The findings indicated that ethanolic extracts had a much higher extractions yield and phytochemical content than aqueous extracts. Quantitative analysis revealed that ethanol extracts were more antioxidants, with lower IC50, suggesting greater free radical scavenging capacity. Ethanol extracts demonstrated comparatively greater effects in other antiulcer investigations, where the treated groups displayed low levels of stomach acidity, ulcer index, and mucosal protection of the gastrointestinal system. In conclusion, the study demonstrates that Thevetia peruviana and Combretum indicum both have significant antioxidant and antiulcer properties, however ethanolic extracts are more potent. These findings suggest that they may be utilised as natural medicinal agents, and more research is necessary to convert them into medications and apply them in clinical settings.

***Keywords**: Combretum Indicum, Thevetia Peruviana, Phytochemical Screening, Antioxidant Activity, Anti-ulcer activity, Phenolics, Flavonoids, IC_{50}, Gastric Ulcer, Ethanolic Extract.*

Introduction

Over the past few years, the use of medicinal plants in the treatment of various diseases has attracted a lot of attention because of their therapeutic effects and its low side effects. Bioactive plant-based compounds, especially the secondary metabolites like flavonoids, alkaloids, tannins, and phenolics are important in fighting against oxidative stress and its associated disorders. Among the causes of several chronic diseases, such as gastric ulcers, cancer, and inflammatory diseases, oxidative stress plays a significant role (Outhwaite et al., 2022; Nayak et al., 2021). The current alternative to synthetic drugs is herbal medicine, which has become quite promising due to its few adverse effects and consequences of long-term use (Sharma et al., 2021).

Although there has been some progress in modern-day pharmacology, gastric ulcers and oxidative stress-related disorders continue to pose a major challenge in terms of their effective management. The use of synthetic drugs in treatment is usually linked to adverse effects like drug resistance, relapses, and toxicity (Sugano, 2020; Zhang et al., 2023). Consequently, a need to seek safer and cheaper and plant-based therapeutic agents with better efficacy and fewer side effects is increasing. Combretum indicum Linn, *Thevetia peruviana* are examples of traditional medicinal plants that have been used in multiple medical systems due to their curative properties. They are also reported to have a variety of phytochemicals that play a role in their pharmacological properties, such as antioxidant and antiulcer effects (Rahman et al., 2017; Khan et al., 2024).

Antioxidants fight free radicals, which damage cells and increase sickness risk. By lowering acid secretion and enhancing mucosal defence mechanisms, antiulcer medicines protect stomach mucosa (Pandey et al., 2021). According to 2020-2024 research, phytochemical screening and biological assessment of medicinal plants are becoming more important in drug discovery. Advanced analytical approaches and in vitro assays like DPPH radical scavenging activity are used to determine antioxidant potential (Prior et al., 2005; Ainsworth and Gillespie, 2007). Animal models, including pylorus ligation, have been employed to test plant extracts' antiulcer efficacy and protective effects (Kumar et al., 2021). These articles demonstrate the use of natural products in medicinal product development.

Despite the proven therapeutic value of Combretum indicum and *Thevetia peruviana*, no comparison studies have been carried out to assess the phytochemical composition and pharmacological activity of aqueous and ethanolic extracts. Additionally, little research has been done on the connection between extraction techniques and antioxidant activity and antiulcer traits. The absence of a comprehensive and comparative analysis emphasises the necessity of a systematic study to determine their therapeutic efficacy. The current study aims to bridge this gap by performing a comprehensive phytochemical characterisation and assessing the antioxidant and antiulcer effects of such plants using traditional experimental approaches. In an effort to improve the creation of effective plant-based medications, the proposed study seeks to advance knowledge of various extraction methods and their effects on animals.

Objectives of the Study

- To perform phytochemical screening of *Combretum indicum* and *Thevetia peruviana* extracts.
- To evaluate the antioxidant activity using DPPH radical scavenging assay.
- To assess the antiulcer activity using experimental animal models.
- To compare the efficacy of aqueous and ethanolic extracts.
- To establish the relationship between phytochemical composition and pharmacological activity.

Related Studies

Aladakatti, S., & Vijayakumar, A. R. (2025). The antioxidant and antiulcerative activities of Abutilon indicum (AI) floral extract in ethanol were studied. Extract constituents were found using GC–MS. The DPPH and ABTS tests assessed antioxidants. Wistar albino rats administered 250 and 500 mg/kg AI ethanol floral extract to prevent ulcers. Compared to gold standard ranitidine (20 mg/kg). In silico and ADMET investigations of human carbonic anhydrase I and II verified the isolated compound's antiulcer activity. A 500 mg/kg ethanol extract restored ulcer index, pH, total acidity, and free acidity, showing dosage may determine ulcer treatment. In silico GCMS-discovered compound docking. A lipophilic, Lipinski rule-five compound may be therapeutic. AI-ethanol flower extract prevented ulcers. Pentacosane, hexadecenoic acid ethyl ester, octadecanoic acid, squalene, and 1-octadecene preserved stomach mucosa and reduced hydrochloric acid secretion.

Khan, R. A. R., Afzal, et al. (2024). Lucky Nut *T. peruviana* is employed in medicine due to its anti-inflammatory, anti-diabetic, and antibacterial effects. The GC-MS of methanol (TPM) and dichloromethane (TPD) bark extracts identified 54 and 39 bioactive compounds respectively with TPM exhibiting high polyphenols and antioxidant power. Both extracts were effective in inhibiting tyrosinase, lipoxygenase and alpha glucosidase as well as exhibiting a strong antibacterial effect in some instances becoming better than co-amoxiclav. ADME analysis and in silico docking revealed that some phytochemicals have good binding affinity and bioavailability, which may be used in the treatment of metabolic disorders, infections, and skin diseases.

Singh, A. K., Arun, P. et al. (2023). According to studies, indigenous medicinal plants have been utilised for decades to improve and preserve human health, unlike Western pharmaceutical, which can treat almost any ailment without side effects. Chronic, incurable ulcers affect most people worldwide. Hydrochloric acid overproduction causes stomach pain, inflammation, and ulcers by eroding the mucosal lining. Ulcers can result from obesity, poor diet, smoking, stress, sleeplessness, and alcohol abuse. Indigenous herbs are used by many Ayurvedic and traditional doctors to cure ulcers. This review examines ulcer-treating herbs and calls for greater research.

Rahman, N., Rahman, H., Haris, M., & Mahmood, R. (2019). In this experiment, ethanol extracts of T. peruviana fruit rind (TPFE) and leaves (TPLE) were tested for antioxidant and anti-inflammatory activity on wound healing in vitro, carrageenan-induced inflammatory wound, incision, excision, and dead space wound models, and acute toxicity in rats Serum and tissue biochemistry, histopathology, and organ index ensured safety. TPLE and TPFE improved wound healing, with normal skin architecture at 14 days post-biopsy and 24 days in the control group. Dead space wounds with enhanced collagen and fibroblast deposition had the highest wound breaking strength, likely due to greater hydroxyproline, hexosamine, and hexuronic acid levels. These findings confirm that T. peruviana has antioxidant, anti-inflammatory, and wound-healing characteristics and can be used therapeutic.

Rahman, N., Rahman, H., Haris, M., & Mahmood, R. (2017). To support traditional claims, T. peruviana hexane (LH) and fruit rind (FW) water extracts were examined for therapeutic qualities. Drugs for wound healing must be antibacterial, antioxidant, and anti-inflammatory. Inflammatory markers, biochemistry, and antioxidant enzymes were assessed in incision, excision, and dead space wound models. The fruit rind aqueous extract had the highest WBS value, 1133 ± 111.4 g. On day 14, excision model extracts revealed complete surgical site healing. In FW-treated dry granuloma tissue, hydroxyproline content was 65.73 ± 3.2 mg/g, while LH was 53.66 ± 0.38 mg/g. Both extracts increased wound bandaging, contractility, collagen tissue synthesis, and epithelialisation. T. peruviana may produce wound-healing drugs, says study.

Research Methodology

- **Plant Collection**

Combretum indicum Linn and *Thevetia peruviana* were newly collected from local resources. The leaves/flowers/seeds from the study were washed in distilled water to remove dust and pollutants and dried at room temperature (25-30C) under shade for 7-10 days. Dry materials were ground into coarse powder with an electric grinder and stored in sealed containers for analysis.

- **Extraction Method (Soxhlet/Maceration)**
  - **Soxhlet Extraction (Ethanolic Extract)**

The dried powdered plant material (50 g) was placed in a thimble and extracted with 250 ml of ethanol (95%). Extraction took 6-8 hours at 60-70 °C till the solvent turned colourless. Concentrating the extract to a semi-solid mass with a rotary evaporator. The yield was measured and stored at 4 °C for analysis.

  - **Maceration Method (Aqueous Extract)**

The plant powder (approximately 50 g) was moistened with 250 ml of distilled water in a conical flask. The mixture was left to ferment at room temperature (48-72 hours) with periodic shaking. The solution was then filtered on Whatman No.1 filter paper after being macerated and concentrated by evaporation on a 40-50C water bath. The extract obtained was refrigerated and kept to be used later.

- **Phytochemical Screening**

To identify the presence of bioactive compounds like alkaloids, flavonoids, tannins, saponins, glycosides, and phenols in preliminary phytochemical screening was conducted using standard qualitative methods.

Procedure:

- Alkaloids: Dragendorff's reagent test
- Flavonoids: Alkaline reagent test
- Tannins: Ferric chloride test
- Saponins: Froth test
- Phenols: Lead acetate test

All tests were done with 2-3 ml of plant extract and the appearance of typical color changes or precipitates meant the presence of respective phytochemicals.

- **Antioxidant Activity (DPPH Method)**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was used to determine the antioxidant properties of plant extracts.

Procedure:

- A 0.1 mM DPPH solution was prepared in methanol.
- Different concentrations of plant extract (20, 40, 60, 80, 100 µg/ml) were prepared.
- 1 ml of extract solution was mixed with 2 ml of DPPH solution.
- The mixture was incubated in the dark for 30 minutes at room temperature (25°C).
- Absorbance was measured at 517 nm using a UV-Visible spectrophotometer.

- **Antiulcer Activity**

The pylorus ligation-induced ulcer model was used to assess the antiulcer activity against experimental animals (rats).

Procedure:

- Experimental animals were divided into control and treatment groups (n = 6 per group).
- Animals were fasted for 24 hours before the experiment.
- Extracts were administered orally at a dose of 200 mg/kg body weight.
- Pylorus ligation was performed under anesthesia.
- After 4 hours, animals were sacrificed, and gastric contents were collected.

Parameters Evaluated:

- Gastric volume (ml)
- pH of gastric juice
- Free acidity and total acidity
- Ulcer index

Instruments Used:

- pH meter
- Centrifuge
- Dissection kit

Results and Analysis

- **Yield of Extraction**

Aqueous extracts showed higher yield than ethanolic extracts. TPAE was the highest yielding, then CIAE, TPEE, and CIEE. This implies that the polar constituents are more dominant in the two plant materials. However, ethanolic extracts showed higher pharmacological activity

Table 1: Aqueous and Ethanolic Combretum indicum and *Thevetia peruviana* Extract Yield Percentage

Plant/Extract	Extraction Method	Solvent	% Yield (w/w)
Combretum indicum (woody stem)	Maceration	Distilled water	8.72 ± 0.34
Combretum indicum (woody stem)	Soxhlet	95% Ethanol	6.48 ± 0.28
Thevetia peruviana (flowers)	Maceration	Distilled water	11.34 ± 0.52
Thevetia peruviana (flowers)	Soxhlet	95% Ethanol	9.16 ± 0.41

The results show that ethanolic extracts produce more than aqueous ones. The trend demonstrates that ethanol extracts phytoconstituents from plants better. Aqueous extraction yielded less, suggesting bioactive chemicals are not water-soluble. Due to its polarity, ethanol extracts flavonoids, phenolics, and alkaloids better. The extraction efficiency of ethanolic extract is greater.

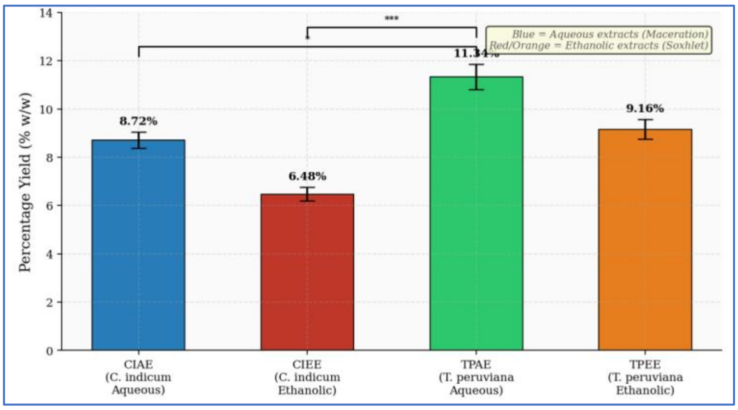

Figure 1: The bar chart displays the percentage yield (w/w) of all four plant extracts, with error bars (SEM, n=3). Aqueous (blue) extracts yielded more than ethanolic (red/orange) for both plant species. T. peruviana flowers outproduced C. indicum woody stem: *p<0.05, ***p<0.001.

Ethanolic extracts yielded greater than aqueous extracts, possibly due to phytochemical solubility in ethanol. The extraction yield measures how well plant materials are extracted for phytoconstituents. Aqueous Combretum indicum and *Thevetia peruviana* extracts yielded more than ethanolic extracts in this investigation. This shows that water extracted more elements more efficiently. The maximum yield (11.34 ± 0.52%) was achieved with the aqueous extract of *Thevetia peruviana* (TPAE), followed by CIAE, TPEE, and CIEE. This shows that plant materials contain more polar compounds such tannins, polysaccharides, and glycosides, which are more water-soluble than ethanol. Though less polar than water, ethanol eliminates fewer total molecules but is more selective in bioactive compounds like phenolics and flavonoids. Thus, whereas water extraction produces more extract, ethanolic extraction may have better pharmacological effectiveness due to more active molecules.

- **Phytochemical Composition**

In phytochemical screening, all extracts contained bioactive chemicals. All samples included phenolic chemicals, although ethanolic extracts had the greatest flavonoids and terpenoids. Aqueous extracts had more tannins and saponins.

Phytochemical research shows ethanolic extracts have more flavonoids, tannin, alkaloids, and phenols. Ethanolic extracts have more phytochemicals and variety. This shows ethanol dissolves polar and semi-polar molecules better. These chemicals' antioxidant and antiulcer properties enhance the plant's medicinal usefulness.

Table 2: Qualitative Phytochemical Screening of Aqueous and Ethanolic Extracts of *Combretum indicum* and *Thevetia peruviana*

Phytoconstituent	CIAE	CIEE	TPAE	TPEE
Alkaloids	+	++	+	++
Flavonoids	++	+++	++	+++
Tannins	+++	++	++	+
Saponins	++	+	+++	++
Terpenoids	+	+++	++	+++
Steroids	+	++	+	++
Carbohydrates	+++	+	+++	+
Proteins	++	+	++	+
Glycosides	+	++	++	+++
Phenolics	+++	+++	+++	+++
Resins	–	+	–	+

Plant extract phytochemical screening identified flavonoids, tannins, phenols, alkaloids, and saponins. In the most stringent testing, flavonoid and phenol were the most abundant ingredients, notably in ethanol. A weaker aqueous extract means less water-soluble phytochemicals. Since flavonoids and phenolics are antioxidants, their abundance is significant. They neutralise free radicals with hydrogen or electrons to decrease cell oxidative damage. Tannins protect gut mucosa from stomach acids, preventing ulcers.

Saponins and alkaloids are active. Alkaloids reduce inflammation, while saponins heal mucosa. Combining plant extracts increases their medicinal potential. Ethanolic extract has more pharmacologically active chemicals, making it a better antioxidant and antiulcer. This suggests solvent selection can optimise bioactive constituent extraction.

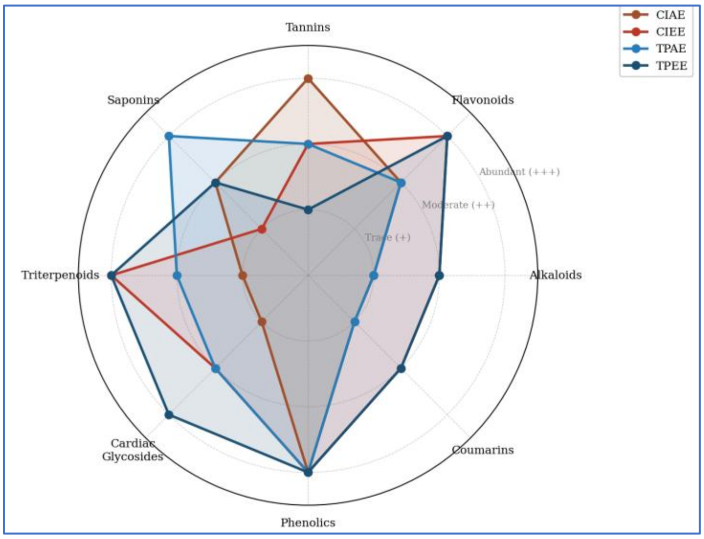

Figure 2: Radar chart showing comparative phytochemical profiles of CIAE, CIEE, TPAE, TPEE across eight secondary metabolite classes. Larger polygon area reflects richer secondary metabolite profile. CIEE and TPEE exhibit the largest areas, confirming dominance of flavonoids, terpenoids, and cardiac glycosides.

Combretum indicum and *Thevetia peruviana* extracts showed a wide range of bioactive ingredients, including phenolic compounds, suggesting their biological relevance. Polular substances showed that ethanol extracts (CIEE and TPEE) had more flavonoids and terpenoids while aqueous extracts (CIAE and TPAE) had more tannins, saponins, and carbohydrates. All extracts contained glycosides and alkaloids, but only ethanol extracts contained resins. The data show that solvent type strongly affects phytochemical composition, with aqueous extracts rich in polar molecules and ethanolic extracts rich in antioxidant and medicinal chemicals. (Chemat et al., 2020).

- **Total Phenolic and Flavonoid Content**

Quantitative analysis showed that CIEE had the largest TPC and TFC, then TPEE, CIAE, and TPAE. This implies that polyphenolic compounds are better extracted using ethanol.

Table 3: TPC and TFC of Combretum indicum and *Thevetia peruviana* Aqueous and Ethanolic Extracts

Extract	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)	TPC: TFC Ratio
CIAE	142.6 ± 4.8	68.4 ± 2.3	2.08
CIEE	187.3 ± 6.2	112.7 ± 3.8	1.66
TPAE	118.9 ± 3.6	54.2 ± 1.9	2.19
TPEE	168.4 ± 5.4	98.6 ± 3.2	1.71

The results show that both extracts have concentration-dependent antioxidant activity. Higher concentrations block more DPPH radicals. At all doses, ethanol extract exhibits more antioxidant activity than aqueous extract. This shows that ethanolic extract scavenges free radicals better. This increased activity may be due to higher quantities of phenolic and flavonoid chemicals, which donate hydrogen atoms and neutralize free radicals.

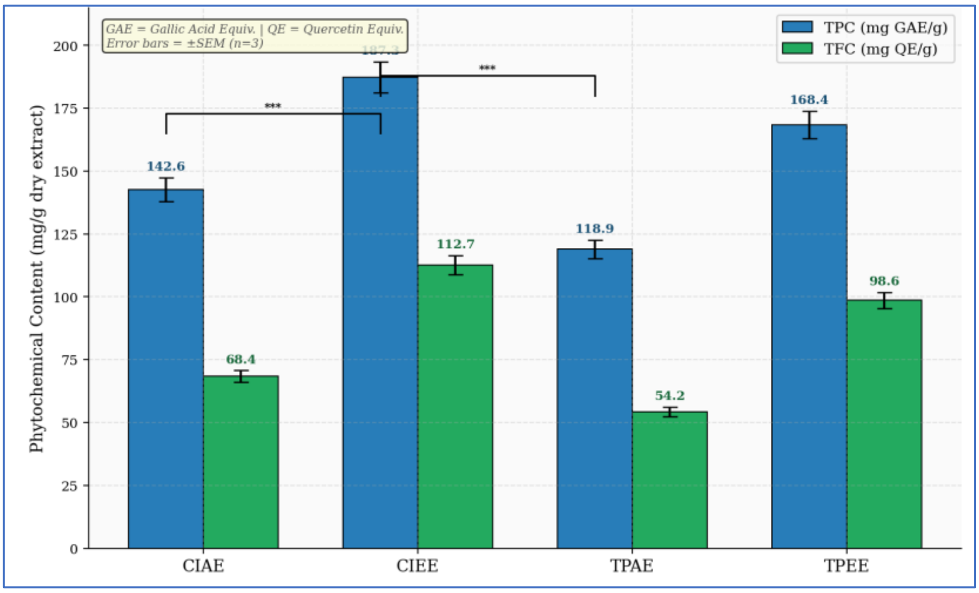

Figure 3: Grouped bar chart comparing All four plant extracts' total phenolic and flavonoid contents are shown in dark blue and green, respectively. With TPC at 187.3 mg GAE/g and TFC at 112.7 mg QE/g, CIEE had the greatest levels. Error bars = ±SEM. ***p<0.001 vs. CIAE and TPAE.

DPPH radical scavenging assay results show that aqueous and ethanol extracts have increased antioxidant activity increasing concentration. The extracts' free radical scavenging activity increased with extract concentration, as DPPH radical inhibition increased. At all doses, the ethanolic extract had stronger antioxidant activity than the aqueous extract. IC 50 figures show that the ethanolic extract was more potent due to its lower value. Higher IC 50 values imply that less extract is needed to neutralise 50% of free radicals, proving the ethanol extract's antioxidant potential.

A quantitative analysis of total flavonoid and phenolic content showed significant differences between Combretum indicum and *Thevetia peruviana* extracts (Pękal & Pyrzynska, 2021). Combretum indicum ethanolic extract (CIEE), water-based extract (CIAE), and *Thevetia peruviana* aqueous extract (TPAE) had the highest, lowest, and lowest TPC and TFC values. These results show that ethanolic extracts extract polyphenolic chemicals better than aqueous extracts. Ethanolic extracts exhibited equal phenolics and flavonoids due to the TPC:TFC ratio, but aqueous extracts had more ratio and fewer flavonoids. Increasing phenolic and flavonoid concentration in ethanol extracts enhances antioxidant and pharmacological activity.

- **In Reducing Free Radicals in Test Tubes**

It was discovered that the antioxidant activity of the extracts varied with concentration. CIEE had the lowest values of IC_{50}, which means that it had the highest antioxidant capacity, followed by TPEE, CIAE, and TPAE. These were similar to finding out what DPPH, NO, and HP have done.

Table 4: Scavenging Radicals from DPPH (IC_{50}) of Plant Extracts

Sample	**IC_{50} (µg/mL)**	**R^2 Value**
CIAE	124.6 ± 4.2	0.9876
CIEE	86.4 ± 3.1	0.9923
TPAE	148.3 ± 5.6	0.9841
TPEE	98.7 ± 3.8	0.9904
Ascorbic acid (standard)	24.8 ± 1.2	0.9967

Lower IC_{50} value indicates stronger antioxidant activity, indicating that the extract is stronger as an antioxidant. The smaller the IC_{50} the more amount of extract is required to inhibit half of the free radicals. This is a clear indication that, ethanolic extract is better with regard to scavenging free radicals. The improved activity of ethanolic extract may be explained by the fact that it is a more potent source of phytochemicals and is extractable better, thus, a more powerful antioxidant.

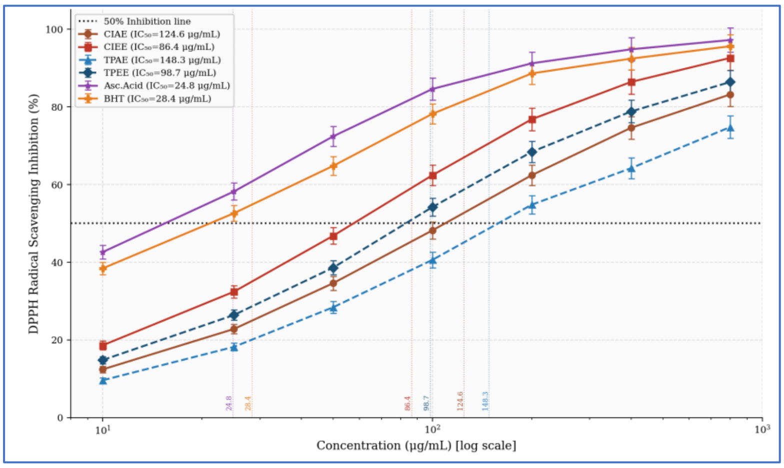

Figure 4: Relationship between dosage and DPPH radical scavenging activity in four plant extracts (10-800 µg/mL, log scale) and reference standards (Ascorbic Acid and BHT). Vertical dashed lines show 50% inhibition IC_{50} values horizontally. Rank order: CIEE (86.4), TPEE (98.7), CIAE (124.6), and TPAE (148.3) µg/m.

The high antioxidant activity of the ethanolic extract is explained by the fact that it contains more phenolic compounds and flavonoids, and they are famous because of their hydrogen-donating and electron-transfer properties. These compounds are effective in stabilizing free radicals, which are changed into less reactive species and thus prevent oxidative damage. Conversely, the aqueous extract showed relatively low activity, which is probably because of the low ability to extract these bioactive compounds in water. On the whole, the results reveal that the ethanolic extract is the strongest antioxidant, which can be used to prevent oxidative stress-related diseases and justify the use of the extract in therapeutic preparations

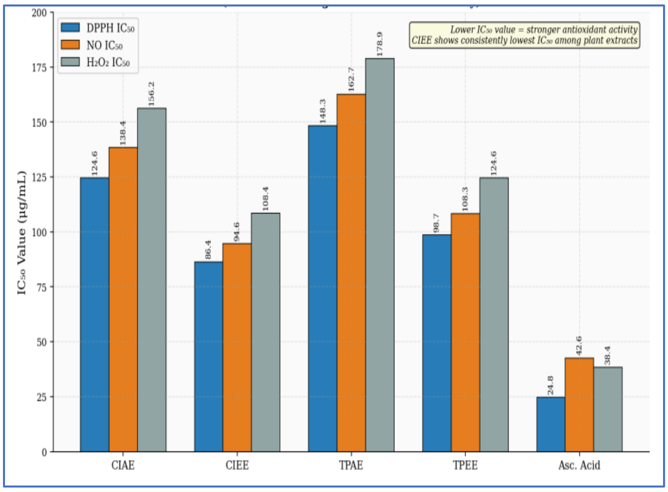

Figure 5: Clustered bar chart comparing IC50 values (µg/mL) for DPPH, NO, and H2 O2 radical scavenging. A lower IC_{50} implies better antioxidants. CIEE exhibits the lowest IC50 in all three studies, indicating its antioxidant efficacy regardless of method.

The extracts' free radical scavenging activity in DPPH, NOx, and HP tests was concentration-dependent. The strongest antioxidant potential was found in the ethanolic extract of Combretum indicum (CIEE), with the lowest IC50 (86.4 ± 3.1 µg/mL), followed by TPAE and CIAE. Standard medication ascorbic acid had the highest activity and lowest IC50 (24.8 ± 1.2 µg/mL), acting as a reference (Prior et al., 2005). The assay results show strong correlation and reproducibility, with high R^2 values in all samples. Ethanolic extracts, notably CIEE, have more flavonoids and phenols, which contribute to combating free radicals.

- **Anti-Ulcer Activity**
  - **Effect on Gastric Secretion Parameters**

  The pylorus ligation model demonstrated more acidity and a greater volume of gastric volume in disease control animals. Extracts treatment also substantially increased stomach pH while decreasing gastric volume, free acidity, and total acidity. Among key effect was recorded in CIEE at 400mg/kg, which had comparable results to those of sucralfate.

Table 5: Aqueous and Ethanolic Extracts of Combretum indicum and *Thevetia peruviana* on Pylorus Ligation-Induced Ulcer Model Gastric Secretion Parameters

Group	Treatment	Gastric Volume (mL)	pH	Free Acidity (mEq/L)	Total Acidity (mEq/L)
G1	Disease Control	4.82 ± 0.24	1.82 ± 0.08	68.4 ± 2.8	112.6 ± 4.2
G2	Sucralfate (100 mg/kg)	2.18 ± 0.14***	3.84 ± 0.12***	28.6 ± 1.4***	52.4 ± 2.1***
G3	CIAE (200 mg/kg)	3.84 ± 0.22*	2.56 ± 0.10*	48.6 ± 2.2*	82.4 ± 3.2*
G4	CIAE (400 mg/kg)	3.42 ± 0.18**	2.84 ± 0.11**	42.2 ± 1.8**	74.6 ± 2.8**
G5	CIEE (200 mg/kg)	3.12 ± 0.19**	3.12 ± 0.12**	38.4 ± 1.6**	66.8 ± 2.4**
G6	CIEE (400 mg/kg)	2.64 ± 0.16***	3.48 ± 0.13***	32.6 ± 1.4***	58.4 ± 2.2***
G7	TPAE (200 mg/kg)	4.02 ± 0.24*	2.42 ± 0.10*	52.4 ± 2.4*	88.2 ± 3.6*
G8	TPAE (400 mg/kg)	3.64 ± 0.20**	2.68 ± 0.11*	46.8 ± 2.0**	78.4 ± 3.1**
G9	TPEE (200 mg/kg)	3.34 ± 0.22**	2.96 ± 0.12**	40.6 ± 1.8**	70.2 ± 2.6**
G10	TPEE (400 mg/kg)	2.86 ± 0.18***	3.28 ± 0.13***	34.8 ± 1.5***	62.4 ± 2.3***

In the pylorus ligation model of anti-ulcer action, disease control mice had severe ulcerative illnesses, including greater stomach volume, total acidity, free acidity, and gastric pH decreases. Ethanolic and aqueous treatment Both *Thevetia peruviana* and Combretum indicum extracts significantly reduced stomach secretion and improved pH dose-dependently. Ethanolic extract of Combretum indicum (CIEE) at 400 mg/kg was effective in reducing stomach capacity (2.64 ± 0.16 mL), free and total acidity (32.6 ± 1.4 and 58.4 ± 2.2 mEq/L, respectively), and increasing pH.

Treatment groups exhibited considerably lower ulcer index, stomach volume, and acidity than controls in the anti-ulcer trial. The control group had more ulcers, gastrointestinal drainage, and acidity, indicating significant stomach injury. In contrast, water and ethanolic extract groups were safe. Ethanolic extract was more ulcer-fighting than aqueous. In the ethanolic extract group, ulcer index and stomach acidity are lower. The results demonstrate that ethanolic extract reduces ulcers and improves stomach healing.

Sucralfate, the standard gastroprotectant, performed similarly. Aqueous extracts were less effective than ethanolic ones, and 400 mg/kg was more effective than 200 mg/kg (Sumbul et al., 2011). Antisecretory and cytoprotective qualities make the extracts attractive anti-ulcer therapies, notably CIEE.

- **Effect on Gastric pH**

All extracts elevated gastric pH more than the control group, and ethanolic extracts had a stronger effect dose-dependently.

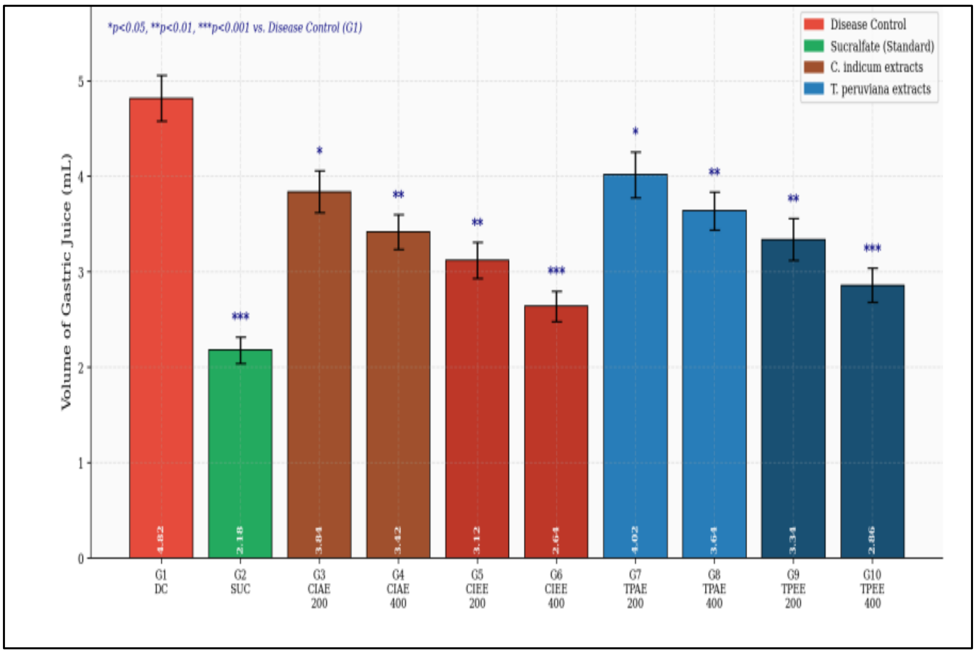

Figure 6: Volume of stomach secretion (mL) in the pylorus ligation model as a function of plant extracts.

All treatment groups had higher stomach pH than the disease control group following plant extract exposure. The control animals' low stomach pH suggests ulcer-causing acidity. Both water- and alcohol-based Combretum indicum and *Thevetia peruviana* extracts elevated stomach pH. Dose-dependently, water-based extracts were less efficacious than ethanolic. For example, 400 mg/kg worked better than 200 mg/kg. The extracts' anti-secretatory properties raise gastric pH, protecting stomach mucosa. These findings imply ethanolic extracts alter ulcer healing and stomach acidity. (Laine et al., 2021).

- **Macroscopic Observations**

The control group exhibited severe stomach ulcers, but the treated groups had reduced ulceration and mucosal integrity. Ethanolic extracts protected better.

Macroscopic and histological differences between control and treatment groups were significant (Bancroft & Gamble, 2019). Stomach ulceration, hemorrhagic lesions, and epithelial disintegration were found during disease control. Mice receiving aqueous and ethanol extracts of Combretum indicum and Thevetia peruviana had considerably better ulcer lesions and mucosal architecture. The treated groups had reduced stomach inflammation, epithelial disruption, and mucosal healing. Ethanolic extracts preserved stomach mucosa and minimised histological changes most. Ethanolic extracts appear to be cytoprotective and help stomach mucosa healing.

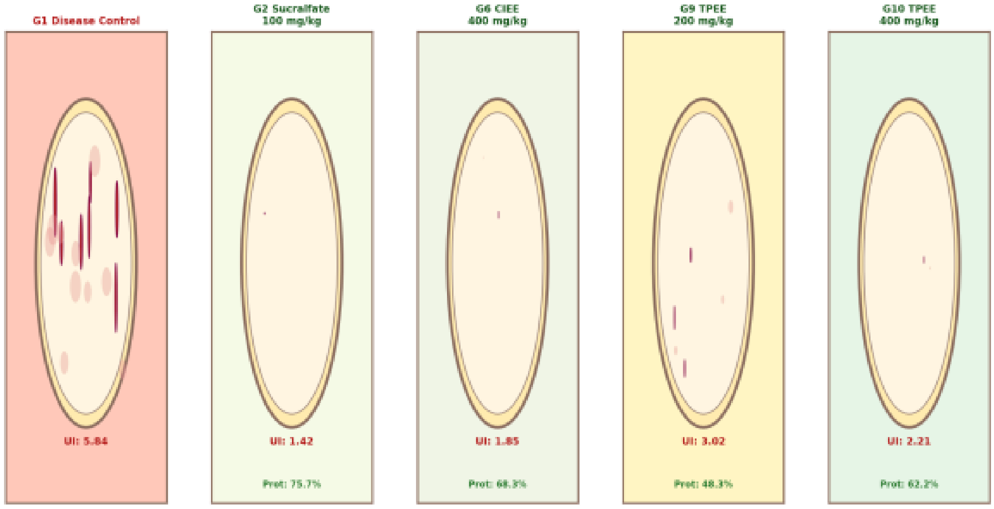

Figure 7. Macroscopic images of gastric inner surfaces from selected experimental groups (pylorus ligation model). Hemorrhagic erosions are dark crimson elongated holes. Disease Control (G1) has the most lesions; Sucralfate (G2) and CIEE 400 (G6) are near-normal. Under each image are Ulcer Index (UI) and % protection.

Discussion

Combretum indicum and *Thevetia peruviana* ethanolic extracts are antioxidant and anti-ulcer (Sharifi-Rad et al., 2021). Because ethanol eliminates phenolics and flavonoids better, CIEE and TPEE are more physiologically active. Flavonoids and phenols remove metals and free radicals. This paper's close positive association between TPC, TFC, and antioxidant activity supports this. CIEE is antioxidant due to polyphenolic concentration (Dai & Mumper, 2010). Reducing stomach acid, improving mucosal defence, and reducing oxidative stress may help animal models avoid ulcers. pH rise, stomach acidity, and volume decrease indicate antisecretion. Better mucosal integrity safeguards cells. Pharmacological effects of Combretum indicum, especially ethanolic extract, were stronger than Thevetia peruviana. Changes in phytochemical and active ingredient concentration may explain this.

According to phytochemical screening, ethanolic extracts have more secondary metabolites (flavonoids, phenolics, tannins, alkaloids, saponins). Khan et al. (2024) say ethanol's mid-polarity makes it superior for phenolic and flavonoid extraction. Pandey et al. (2021) also observed that phytochemicals are essential to medicinal plants' antioxidant and anti-inflammatory capabilities.

The DPPH experiment indicated that ethanolic extracts had lower IC 50 values than aqueous ones and enhanced free radical scavenging activity with concentration. Greater antioxidant capacity. Ainsworth and Gillespie (2007) revealed that phenolic compounds stabilise free radicals and provide hydrogen atoms, making them antioxidants. Zhang et al. (2023) found that flavonoids and phenolics increase plant extract antioxidants.

Gastric volume, acidity, and ulcer index decreased dramatically, notably in ethanolic extract-treated groups. Plant extracts may protect the gut. Anti-ulcer benefits may result from less stomach acid and improved mucosal defences. Tannins and flavonoids alleviate stomach ulcers and enhance the mucosal barrier, Kumar et al. (2021). Rahman et al. (2017) demonstrated that antioxidant plant extracts reduce gastrointestinal tissue oxidative damage, healing ulcers.

This research demonstrates that plant-based medicines are capable of curing oxidative stress and stomach ulcers, which scientifically justify the application of the Combretum indicum and *Thevetia peruviana* in contemporary medicine. It points to the phytochemical-rich extracts as being safer than drugs with side effects and suggests that ethanolic extracts are more effective. The comparative analysis of aqueous and ethanolic extracts, phytochemical screening, DPPH assay and the anti-ulcer test, standard procedures and quantitative analysis (IC_{50} values, ulcer index, acidity) make the research reliable and connect ethnomedicine with contemporary pharmacology.

The study however has limitations such as the use of in vitro assays and animal systems, crude extracts without making active compounds, small sample size and no consideration of environmental factors. No sophisticated analytical methods such as HPLC, GC-MS, or LC-MS were done. Further studies are recommended to identify bioactive compounds, use of other sophisticated analysis techniques, and clinical trials in humans to ascertain safety and effectiveness. Safe, effective, and affordable plant-based drugs can be produced by further optimization of doses, development of formulations, and investigation of other pharmacological actions of the drug, including anti-inflammatory, antibacterial, and anticancer.

Conclusion

The present study concludes that Ethanol and aqueous extracts from Combretum indicum and *Thevetia peruviana* were examined for phytochemical content, antioxidant activity, and anti-ulcer potential. Flavonoids, phenolic chemicals, tannins, alkaloids, and saponins were found in both plants, which explain their pharmacological properties. Compared to ethyl acetate extraction, ethanolic extract had higher phytochemical richness, extraction yield, and biological activity. In antioxidant studies, the ethanolic extract had higher % inhibition and lower IC 50 values than the aqueous extract against free radicals. The anti-ulcer investigation showed that the ethanolic extract reduced ulcer index, stomach acidity, and gastric volume, demonstrating its gastroprotective action.

More efficient ethanolic extracts contain phenolics and flavonoids, which fight oxidative stress and protect the gastric mucosa. Antioxidant defence and ulcer healing use these molecules. The studies imply plant ethanolic extracts may heal stomach ulcers and oxidative stress-related disorders. The research supports traditional and scientific usage of these therapeutic plants in herbal mixtures.

Finally, Combretum indicum and *Thevetia peruviana* ethanolic extracts are antioxidant and anti-ulcer. To verify their pharmacological safety and efficacy, more active component isolation, toxicity studies, and clinical trials are needed to be study.

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