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EXPLORING THE ANTI-ULCER POTENTIAL OF ETHANOLIC EXTRACT FROM INDIGOFERA ASPALATHOIDES: COMPARATIVE EVALUATION AND SPECTROSCOPIC INSIGHTS

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ABSTRACT

Various areas of the body can be affected by ulcers, characterized by open sores or lesions of the skin or mucous membranes. A number of factors can lead to ulcers, such as infections, poor blood circulation, and autoimmune disorders. There are various medicinal systems that use *Indigofera aspalathoides* (Fabaceae), a plant that grows in diverse geographical regions and is used for treating a wide range of ailments. It is possible to shed light on Indigofera aspalathoides' anti-ulcer potential through extracts like ethanolic extracts. Compounds such as flavonoids, tannins, and other phytochemicals found in this family have displayed various health-promoting effects, including anti-inflammatory and wound-healing properties. The Rf value for the ethanolic extract of *Indigofera aspalathoides* (Specifically targeting flavonoids) was determined to be 1.11. An FT/IR-4600 type A spectrophotometer with a scanning range of 7800 to 350 cm^{-1}, a resolution of 4 cm^{-1}, and a scanning rate of 10 frames/sec was used to acquire the IR spectra of ethanolic extract of *Indigofera aspalathoides*. Interestingly, the spectral patterns observed in the stretching vibrations of Omeprazole closely resemble those found in our ethanolic extract of *Indigofera aspalathoides*. In this study, our objective is to investigate the anti-ulcer activity of *Indigofera aspalathoides* using the ethanolic extraction method. To induce gastric lesions, an oral dose of 200 mg/kg of acetyl salicylic acid was administered to the rats. Notably, pre-administration of EEIA orally, at doses of both 100 and 200 mg/kg, exhibited significant prevention of gastric ulcer formation. However, the efficacy of EEIA in this regard appeared to be comparatively lower when contrasted with the performance of the standard drug, omeprazole.

KEYWORDS: *Indigofera aspalathoides,* Anti-ulcer, Aspirin, Pyloric ligation, H.pylori.

INTRODUCTION

An ulcer is a break or discontinuity in a bodily membrane that impairs the normal functioning of the affected organ. Essentially, an ulcer occurs when the sloughing of inflamed necrotic tissue causes a breach in skin, epithelium, or mucous membrane.[1] It is estimated that over 5,00,000 new cases of peptic ulcer disease occur each year in the US, affecting 5 million people. Interesting enough, the disease is most likely to affect generations born in the mid-20th century.

With the peak incidence occurring between 50-65 years of age, ulcer disease is primarily a disease affecting the elderly. Gastric ulcers were less common in men than duodenal ulcers. At the margin of a gastroenterostomy in the jejunum, ulcers can form in the oesophagus, stomach, or duodenum. Gastric acid causes at least some symptoms of peptic ulcer disease, a disorder of the upper gastrointestinal tract. It is possible to experience mild abdominal discomfort to catastrophic bleeding and perforation in a patient with peptic ulcer disease.

As a result of gastric acid secretion or pepsin, a peptic ulcer causes discontinuity in the lining of the gastrointestinal tract. Gastric epithelium is covered by it in the muscularis propria layer. Most of the time, it occurs in the stomach and proximal duodenum. Symptoms may include lower esophageal obstruction, distal duodenal obstruction, or jejunal obstruction.

Gastritis is caused by *H.pylori*, a gram-negative bacillus in the gastric epithelium. Gastric ulcers are caused by 90% of this bacterium. Low socioeconomic status is more likely to contract *H.pylori* infection during childhood, and infection is more prevalent in those with low educational status. Gastric mucosal adhesion and inflammation are possible due to virulence factors within the organism.[2] The result is hypochlorhydria or achlorhydria, which causes ulceration of the stomach.

On the other hand, epigastric pain usually occurs between 15-30 minutes following a meal in people who suffer from gastric ulcers. The pain associated with duodenal ulcers occurs two to three hours after a meal. Patients with peptic ulcer disease are now recommended to take tentung for Helicobacter pylori. Some patients may require endoscopy to confirm their diagnosis, especially if they are showing sinister symptoms. A proton pump inhibitor can be used to treat most patients if they are on triple drug therapy.[3]

Peptic ulcer disease is most commonly caused by NSAIDS, followed by infections with H. pylori. Reduces gastric mucus and bicarbonate production by inhibiting the cox-1 enzyme, leading to a decrease in mucosal blood flow and prostoglandin synthesis. *Indigofera aspalathoides* (Fabaceae), a perennial shrub found in diverse geographical regions, has been traditionally used in various medicinal systems to treat a multitude of ailments. For local use and trade, it is harvested from the wild.

The ingredient was found in a variety of proprietary preparations. Among its potential benefits, this plant has shown promise in possessing anti-ulcer properties. There are several compounds in this family that promote health, including tannins, flavonoids, and other phytochemicals. As well as promoting tissue repair and accelerating wound healing, these compounds may also reduce inflammation.

It is believed that leaves, flowers and tender shoots are cooling and demulcent in the traditional medicinal system. *Indigofera aspalathoides* decoction is used for leprosy and cancer.[4] Oedematous tumours are treated with the whole plant, and dandruff is treated with ashes. Joint pains can be treated with the powder of the whole plant. Itching, scabies, and kuddam are among the skin disorders traditionally treated with the stem.

A mixture of powdered bark and coconut oil is applied continuously for six months to cure leprosy. It has also been shown that methanol extractions of *indigofera aspalathoides* are hepatoprotective. In the treatment of chemically induced fibrosarcomas, *Indigofera aspalathoides* can be used.

An extract of *Indigofera aspalathoides* is used as an antifungal agent[5]. In this study, our objective is to investigate the anti-ulcer activity of *Indigofera aspalathoides* using the ethanolic extraction method. Through the exploration of potential mechanisms, such as antioxidant and anti-inflammatory pathways, we aim to elucidate the ways in which *Indigofera aspalathoides* exerts its therapeutic effects. Ultimately, this research contributes to expanding our understanding of plant-based interventions for ulcers and provides a foundation for further investigations into their therapeutic applications.

METHODOLOGY

Plant Collection and Authentication

Indigofera aspalathoides whole plant was collected from local area in Madurai, Tamilnadu and it was authenticated by Dr. D. Stephen, Professor, Department of Botany, American college.

Plant extraction

The entire plant has been shade-dried for one week. Mechanical grinders were used to grind them. A hot continuous percolation method was used to extract Ethanol from powdered material for 16 hours. Distillation was done for 2 hours to remove the solvent completely.

Preliminary phytochemical analysis

Extracts are analyzed for the presence of alkaloids, saponins, carbohydrate, flavonoids, glycosides, protein, phenolics, steroids, and tannins.[6-10] A table 1 shows the phytochemical composition of *Indigofera aspalathoides*.

Thin layer chromatography

The process began by dissolving silica in water and applying it to a glass slide. A mixture of Toluene, Ethyl acetate, Acetic acid, and Methanol was chosen as the mobile phase (2.5:7.0:0.25:0.25). A specific standard sample, like Quercetin, was selected. The dry plate was labeled for standards and tests. The sample was added using a capillary tube, and the slide was placed in the mobile phase and sealed. After solvent migration, Ninhydrin reagent was sprayed to reveal spots. Heating the plate in a hot air oven enhanced visibility, aiding in spot detection and analysis.

Spectral analysis

A spectroscopic measurement of infrared radiation is infrared spectroscopy, which measures the absorption, emission, and reflection of infrared radiation with matter. A chemical substance or functional group can be studied and identified in solid, liquid, or gaseous form using it. Using mid-infrared energy, the fundamental vibrations of phytochemical structures (Functional group) can be studied. The frequency ranges from about 4000cm^{-1} to 400cm^{-1}. Many medicinal plants use Fourier Transform Infra-Red (FTIR) spectroscopy to study their main constituents integrally, since their functional groups absorb at different IR regions. An FT/IR-4600 type A spectrophotometer with a scanning range of 7800 to 350 cm^{-1}, a resolution of 4 cm^{-1}, and a scanning rate of 10

frames/sec was used to acquire the IR spectra of ethanolic extract of *Indigofera aspalathoides*.

Evaluation of anti-ulcer activity by using ethanolic extract of *Indigofera aspalathoides*[11]

Study protocol

Animal selection

During the course of the experiment, albino rats were maintained on a standard diet weighing between 180 to 220 grams. These rats were also provided unrestricted access to water throughout the duration of the study. To induce gastric lesions, an oral dose of 200 mg/kg of acetyl salicylic acid was administered to the rats[12]. Before the commencement of the experiments, the rats were grouped into sets of five, with each group consisting of six animals. They were allowed to acclimate to the laboratory environment for a minimum of two days. During this acclimatization period, the rats had free access to both food and water.

Group design

S. No.	Group	Dose/Treatment
1	G-1	10ml per kg of normal saline as normal control
2	G-2	200mg per Kg of ASA as Ulcer control
3	G-3	2mg per kg (1/2 hr prior ASA administration with Omeprazole) as standard control
4	G-4	100mg per kg of EEIA extract (1/2 hr prior ASA administration) as Treatment control
5	G-5	200mg per kg of EEIA extract (1/2 hr prior ASA administration) as Treatment control

*EEIA= Ethanolic extract of *Indigofera aspalathoides*

Ulcer Model induced by Aspirin and Pyloric ligation[13]

Each group contains six albino rats. This study lasted for four days.[14] The rats were treated with aspirin 200mg/kg 30 minutes after receiving the treated dose. The rats were subjected to fasting on the third day after being administered the drug. Pyloric ligation was performed the next day. It was done four hours later by cervical dislocation and clamping the esophagi. After carefully exposing the stomach along the greater curvature, the luminal contents were removed, and the total volume of gastric secretion, the total acidity and the free acidity were measured using a titration method. According to Gangly and Bhatnagar,[15] the ulcer index was calculated. Hand lenses (10x) were used to count the lesions, which are rated based on severity.

An ulcer index was calculated based on the mean ulcer score for each animal. Accordingly, ulcer inhibition was measured as follows:

$$\text{Ulcer protection}(\%) = \frac{\text{Mean ulcer index (Control)} - \text{Mean ulcer index (test)}}{\text{Mean ulcer index (Control)}} \times 100$$

Statistics

The data were analyzed with a one-way ANOVA followed by Newman Keul's multiple range test, and probability values were considered significant at $p<.0.01$.

RESULTS

Phytochemical screening

In this study, the extracts were analyzed for the presence of alkaloid, saponins, carbohydrate, flavonoids, glycosides, proteins, phenols, and steroid and the result were observed in the table 1.

Table 1: Preliminary phytochemical screening.

S. No.	Phytochemical constituents	Present/Absent
1	Alkaloids	Present
2	Carbohydrates	Absent
3	Flavanoids	Present
4	Glycosides	Present
5	Proteins	Present
6	Phenols	Present
7	Steroids	Present
8	Triple sugar test	Absent
9	Tannins	Present

TLC profile

Ethanolic Extraction of *Indigofera aspalathoides* showed phytochemicals on TLC plates. Using the formula below, we calculated the retardation factor for eluted samples

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

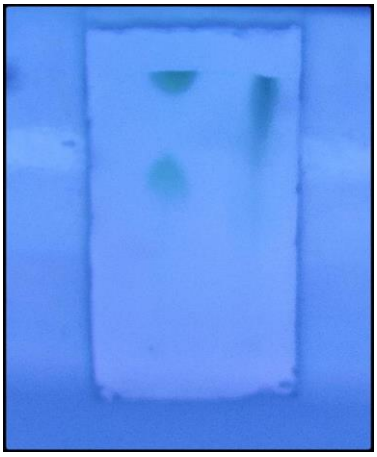
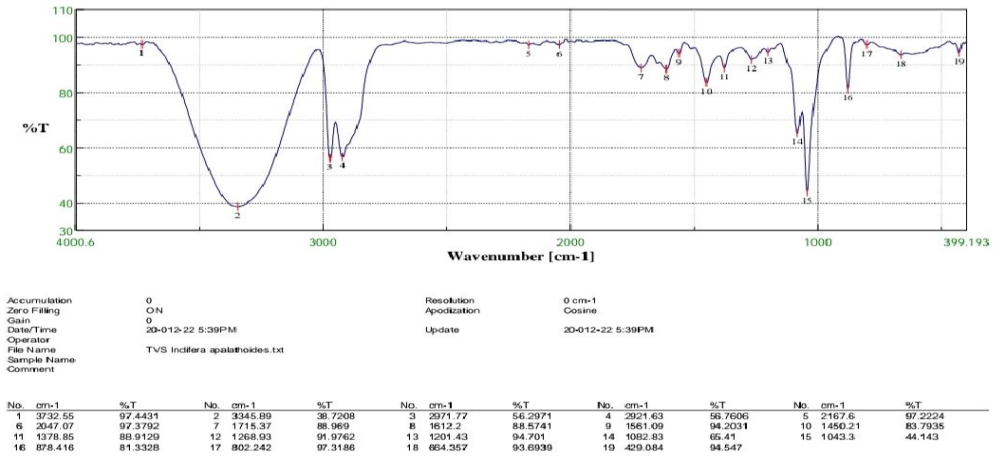


Fig. 1: TLC plate under UV light at 254nm.

Fig. 2: TLC plate.

The Rf value for the ethanolic extract of *Indigofera aspalathoides* (specifically targeting flavonoids) was determined to be 1.11. This value signifies the distance traveled by the compound during chromatographic separation relative to the distance covered by the solvent front. The Rf value serves as a crucial parameter in identifying and characterizing compounds within the extract, aiding in the assessment of their interaction with the chromatographic stationary phase and mobile phase.

IR-Spectral Analysis



No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3732.55	97.4431	2	3345.89	38.7208	3	2971.77	56.2971	4	2921.63	56.7606	5	2167.6	97.2224
6	2047.07	97.3792	7	1715.37	88.969	8	1612.2	88.5741	9	1561.09	94.2031	10	1450.21	83.7935
11	1378.85	88.9129	12	1268.93	91.9762	13	1201.43	94.701	14	1082.83	65.41	15	1043.3	44.143
16	878.416	81.3328	17	802.242	97.3186	18	664.357	93.6939	19	429.084	94.547			

Fig. 3: IR spectra of *Indigofera aspalathoides.*

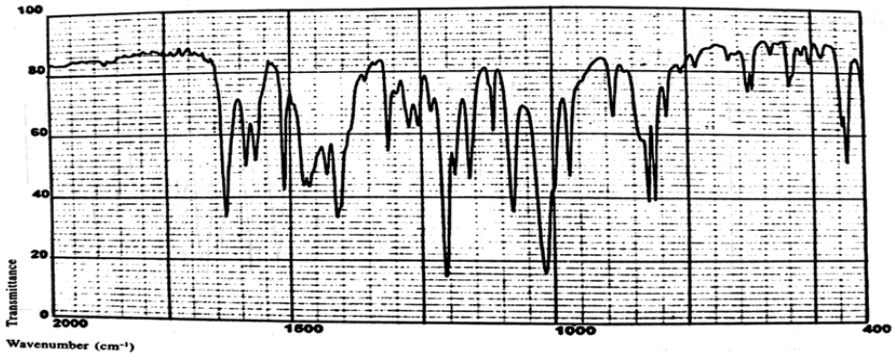


Fig. 4: IR spectra of omeprazole.[16]

The extraction process of *Indigofera aspalathoides* has yielded a diverse range of compounds, including Alkynes, Ketones, Aromatic rings, Alkanes, Aromatic Amines, Isocyanates, primary and secondary amines, Aryl alkyl ethers, and Esters, among others. Interestingly, the spectral patterns observed in the stretching vibrations of Omeprazole closely resemble those found in our ethanolic extract of *Indigofera aspalathoides*. Notably, four specific stretching vibrations align exceptionally well with the characteristics of our plant species. This observation has led to the selection of Omeprazole as a preferred standard drug for our forthcoming pharmacological investigations. This choice is grounded in the remarkable similarity between the spectral features of Omeprazole and the compounds present in our extract, supporting its suitability for further comparative studies and analysis.

Anti-ulcer activity of ethanolic extract of *Indigofera aspalathoides*

Group	Treatment	Dose mg/kg	Total volume of gastric secretion (ml/100 gm)	Total acidity (meq/l/100g)	PH	Ulcer score	% protection
G-1	Normal control	10 ml/kg of normal saline	3.75± 0.55	424.20± 21.25	2.3± 0.15	0.3± 0.01	0.000
G-2	Ulcer control	200mg/kg ASA	5.2± 0.82^{*a}	485.30±^{*a} 23.40	1.5±^{*a} 0.18	2.0±^{*a} 0.14	0.000
G-3	Standard control	2mg/kg omeprazole	2.60± 0.34	335.80± 15.20	4.2± 0.65	0.5± 0.04	75.00
G-4	Treatment control	EEIA 100mg/kg	3.05± 0.40^{*b}	378.40±^{*b} 17.15	3.2±^{*b} 0.30	0.8±^{*b} 0.10	60.00
G-5	Treatment control	EEIA 200mg/kg	3.18± 0.45^{*b}	362.35±^{*b} 16.25	2.7±^{*b} 0.27	0.7±^{*b} 0.08	65.00

*Value expressed in as Mean±S.E.M; *a= Significant difference of normal control ($p<0.01$); *b= Significant difference of ulcer control group ($p<0.01$)

The intragastric administration of ASA suspension at a dose of 200 mg/kg consistently induced hemorrhagic lesions in the glandular stomach mucosa, confirming the presence of actual ulceration, as corroborated by the histological analyses. Notably, pre-administration of EEIA orally, at doses of both 100 and 200 mg/kg, exhibited significant prevention of gastric ulcer formation. However, the efficacy of EEIA in this regard appeared to be comparatively lower when contrasted with the performance of the standard drug, omeprazole.

DISCUSSION

The Indian system of medicine places a high value on *Indigofera aspalathoides* as a result of its well-established medicinal properties. In traditional medicine, the entire plant has been used to treat disorders such as leprosy, cancer, syphilis, edema, abscesses, and various skin conditions. Scientific exploration is needed to validate its potential therapeutic value in light of its strong historical use. According to preliminary phytochemical analysis of *Indigofera aspalathoides*, ethanolic extraction contained a number of significant phytochemical compounds.

Compounds such as alkaloids, flavonoids, glycosides, proteins, phenols, steroids, and tannins belong to this class. As a result of these findings, *Indigofera aspalathoides* may owe its medicinal attributes to its diverse phytochemical content. In the ethanolic extraction of *Indigofera aspalathoides*, thin-layer chromatography revealed the possibility of separation of phytochemical components. By separating them, specific detection methods can be used to identify them. Previously, complex plant extracts were characterized and validated using chromatographic techniques. IR spectra interpretation further confirmed the presence of

group compounds in *Indigofera aspalathoides* ethanolic extractions. In similar studies, IR spectroscopy has been widely used to identify potential bioactive molecules by revealing the functional groups present in plant extracts. *Indigofera aspalathoides* extract was evaluated for its antiulcer activity.

The extraction demonstrated protection against antiulcer activity at concentrations of 100mg/kg and 200mg/kg, respectively, of 60% and 65%. Researchers[17] studied the antiulcerogenic properties of *I. truxillensis* aerial parts in rats and mice. In all models, the methanol extract significantly inhibited ($p< 0.05$) gastric lesions at 250, 500, and 1000 mg/kg doses, as well as displaying antisecretory and cytoprotective properties, thus providing gastroprotection. In a study,[18] an assessment was made regarding the protective effects of *I. truxillensis*. As outlined by the authors in 2012, the methanol extract from the leaves, administered at a dosage of 50 mg/kg, demonstrated notable properties. This included a substantial inhibition of gastric mucosal damage, enhancement in mucus production, and elevated antioxidant enzyme activity in rats. These effects were particularly highlighted due to their significant impact in curbing gastric mucosal damage. The leaves of *I. suffruticosa* are also reported to be gastroprotective in vivo.[19]

Plant extracts may have gastroprotective effects, based on this observation. Based on these findings, *Indigofera aspalathoides* appears to be a pharmacologically relevant plant extract with antiulcer potential. In contrast, *Indigofera aspalathoides* ethanolic extract was found to possess favorable antiulcer activity. In spite of this, omeprazole, the standard drug, has shown superior effectiveness in preventing ulcers. In comparison to plant-based remedies, synthetic drugs are more effective based on existing literature. A study shows remarkable reduction of degree of symptoms with highly significant improvement of the selected symptoms such as heart burn, epigastric pain, indigestion, nausea & vomiting and eructation.[20]

CONCLUSION

Based on the analysis of hematological parameters, *Indigofera aspalathoides'* ethanolic extraction exhibited significant antiulcer activity. Based on the findings, the plant extract has significant positive effects on these parameters, suggesting it may have gastroprotective properties. Even so, our extract appears to be relatively less effective than Omeprazole, despite its pronounced effects when compared to its well-established standard. Based on this comparison, it appears that our extract has significant therapeutic potential but needs further exploration and refinement. The results suggest that *Indigofera aspalathoides* is effective in treating ulcer-related conditions and adds to the growing body of evidence supporting its medicinal value. In order to enhance the extract's effectiveness for enhanced therapeutic outcomes, further studies are needed to elucidate the underlying mechanisms.

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