Evaluation of Geranium wallichianum D. Don Ex Sweet's Ethanolic Extract's Anti-Ulcer Activity

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Abstract
The current investigation gives proof that the ulcer-fighting properties of Geranium wallichianum D. Don Ex Sweet exist. The plant generated a large amount of antiulcer activity, which was marked by anti-secretory, cytoprotective, and proton pump inhibitory qualities. More investigation is needed to determine the chemical element of this extract that gives rise to its antiulcer activity. One of the most common conditions that affect the digestive tract is the peptic ulcer. Although the precise causes of peptic ulcer illness are not entirely understood, experts think that an imbalance in the secretion of the digestive acids, pepsin, and acid, as well as a lack of protective mechanisms in the mucosa, may contribute to the disease. Nonsteroidal anti-inflammatory drug use, H. pylori infection, stress, or pathological conditions like Zollinger-Ellison Syndrome are the most frequent causes of peptic ulcer disease. The family Geraniaceae includes the plant species Geranium wallichianum D. Don ex Sweet. It has numerous potential applications in medicine. It is commonly found on the Himalayan mountain sides, in Assam, and in Nepal. The rhizomes of Geranium wallichianum D. Don Ex Sweet have been found to contain volatile oil, gycozoline, xanthotoxin, and alkaloids, according to the results of a phytochemical investigation. Researchers have found that the leaf extract contains hepatoprotective, antibacterial, anti-dysentery, and anti-oxidant properties. It also has hypoglycemic and antibacterial properties. The leaf extract of this plant are loaded with these kinds of antiulcer capabilities.

Keywords: Ethanolic extract, Aspirin-induced ulcer model, NSAIDs, Ulcerative Model, Antiulcer.

Introduction

A peptic ulcer is essentially a ruptured area of the mucous membrane lining the digestive tract that is inflamed. It is a portion of the stomach that has become excoriated, primarily as a result of the digesting activity of gastric juice and upper small intestinal secretions. Non-steroidal anti-inflammatory drug (NSAID) use over an extended period of time and Helicobacter pylori infection are two key variables that can impair mucosal resistance. While the frequency of stomach ulcers has slightly increased recently due primarily to the widespread use of NSAIDs, the frequency of duodenal ulcers has decreased dramatically over the past few decades (Muhammad, et al., 2012, Jabeen, et al., 2022). The identification of H. pylori as the primary causal agent and the discovery of effective and potent acid suppressants are the two most significant advances connected to the overall decline in rat cases of peptic ulcer disease. In essence, as the infectious cause of stomach ulceration is successfully treated, a greater proportion of Americans are developing gastritis and developing ulcers as a result of prolonged pharmaceutical use, especially NSAIDs. In response to a disturbance in the body's internal equilibrium, stress is an adaptive physiological reaction (Ismail, M., 2007, Jan, et al., 2021). Excessive stress can harm organs or exacerbate conditions including cancer, hypertension, diabetes, and gastric ulcers. One common example of the organ damage caused by stress is stomach ulcers. Globally, acute stomach mucosal damage is a significant clinical issue. Since exposure to water immersion greatly accelerated the development of stomach lesions during restraint stress. The progress of ulceration during immersion in water may be significantly aggravated by the increase in acid secretion (Abbasi, et al., 2019). The use of herbal medicine has grown in popularity across the globe, and it is thought that medicinal plants represent a significant source of novel chemicals with potential therapeutic benefits. Additionally, numerous traditional Asian herbal medicines have been found to treat gastrointestinal conditions, according to recent reports. Aspirin and other nonsteroidal anti-inflammatory medicines (NSAIDs) are now used to prevent cardiovascular and cerebrovascular disorders. Low dose aspirin therapy, however, might be harmful to the digestive system. Aspirin and other NSAIDs are known to cause stomach mucosal damage and slow ulcer healing. This is most likely because they impede the activity of the enzyme cyclooxygenase and lower gastric PG levels. Angiogenesis, a phenomena that involves the creation of new blood vessels from the pre-existing microvasculature, is primarily controlled by angiogenic growth factors like vascular endothelial growth factor -A. It is a crucial part of the recovery from

ischemia situations. Angiogenesis is crucial for the repair of stomach ulcers. It has been hypothesised that VEGF is crucial for the healing of stomach ulcers (Badoni, et al., 2019). To get a positive result, numerous researchers have examined a vast range of spices and herbs for their antiulcer properties. Numerous medical plants and food ingredients, including Aloe, Terminalia chebula, Vetiveria ziziinoides, ginseng, and capsicum, among others, have been found to have gastro-protective properties. Even though it is one of the well-known medicinal plants used in Indian traditional medicine to treat a variety of illnesses, there are very few studies on the pharmacological characteristics of some medicinal plants (Ismail, et al., 2009).

In folklores all throughout the world, plants and items produced from them have long been utilised to treat a variety of maladies and disorders. Nowadays, herbal remedies are gaining popularity as effective alternatives to synthetic medications sold commercially for the management and treatment of peptic ulcers. This is supported by its reduced price, perceived effectiveness, accessibility, and lack of or little side effects (Abbasi, et al., 2019). Numerous of these herbal treatments have established gastro protective characteristics and have been used for many years to treat digestive disorders, peptic ulcers, and other associated conditions. One of the most prevalent illnesses that affects people all around the world is ulcer (Jan, et al., 2021, Tewari, K. 2019). The allopathic ulcer treatment has a negative impact on health since it has negative side effects. Many herbal plants and secondary metabolites are being used to cure ulcers. Many herbal plants are utilised in the current evaluation to treat ulcers, and their root, rhizome, bark, leaves, and fruits are listed in the table. Ineffective, secure, and generally accessible alternative remedies for peptic ulcer illness have recently been replaced by medicinal plants (Chand, T.R., 1948). This research has objective was to examine the medicinal plants and phytochemicals that have been used to treat peptic ulcer disease in order to assess the potential contribution of natural substances to the development of herbal treatments for the condition. GWDS has a wide range of therapeutic benefits (Shaheen, et al., 2017 and Iqbal, et al., 2019, Ashfaq and Khan, 1978). We therefore think that GWDS may have antiulcer activity based on its documented other activities and chemical makeup. Therefore, we examined GWDS's antiulcer activity in the current study.

Material and Method

The specimen of the plant G. wallichianum was procured from the neighborhood market. The plant's identification was confirmed, and a voucher specimen was left in the herbarium of the CT Institute of Pharmaceutical Sciences, Jalandhar, Punjab, India. so that it would be easily accessible in the future. In order to do research on the powder form of the medication, the leaf extract were sun-dried and the rhizomes were dried in the shade. After that, each of these portions were ground into a powder using an electric grinder so that they could be analysed using microscopic and physicochemical techniques. In preparation for future research, the leaf extract of the plant that was obtained were sun-dried before being ground into a powder.

Preliminary phytochemical screening

The ethanolic extract of GWDS underwent a preliminary phytochemical analysis with the purpose of identifying distinct phytoconstituents. In order to conduct tests for the presence of typical phytochemicals, K.R. Khandelwal's practical pharmacognosy standard procedures were followed.

Preparation of plant extract

The plant material was shade dried and coarsely powdered. About 500 g of plant material was soaked in ethanol for 48hrs. After 48 hrs of soaking the solvent wasdistilled off under reduced pressure at 50°C and dried in vacuum. The process began with the gathering of fresh flowers and barks, which was followed by drying them at room temperature in the shade. After the bark had been allowed to air-dry, it was ground into a coarse powder before being placed on a Soxhlet column and subjected to an extraction process that involved 70% ethanol and 60^0 C. In order to concentrate the extracts, they were put through a rotary flash evaporator while operating at a pressure that was lower than normal. The dehydrated extracts were stored in a container that prevented air from escaping inside of a refrigerator at a temperature that was less than one hundred degrees Celsius. In the process, distilled water was utilised so that the solutions of the ethanolic extract could be manufactured (Tewari, et al., 2015 and Badoni, et al., 2019). Following these experiments, the ethanolic extract of GWDS rhizome was put through its paces, which included screening for phytochemicals in their raw form. An aspirin-induced ulcer model was utilised to establish the presence of antiulcer action.

Experimental animals

CT Institute of Pharmaceutical Sciences, Jalandhar, Punjab, India, provided healthy adult male Swiss albino rats, weighing 120–150g. Prior to the trial, the animals were given a five-day acclimatization period in a lab setting. The animals were kept in conventional polypropylene cages. In order to give the animals enough room and prevent needless sickness and mortality, six animals were kept in each cage. The typical conditions for animal care were a 12:12 hr light/dark cycle, a temperature of 25°C, and a humidity of 65.5%. All research were carried out in accordance with CPCSEA ethical principles after receiving the required approval from the committee.

Experimental design for animal studies Common control groups

Group 1 Normal male albino wistar rats.
Group 2 Diseased animals received sub-plantar injection of

0.1 ml/kg bw. Of formalin.

Group 3 Pretreated with ethanolic rhizome extract (100mg/kg bw.) (Single dosage of formalin on 30th day).

Group 4 Pretreated with ethanolic rhizome extract (200mg/kg bw.) (Single dosage of formalin on 30th day).

Group 5 Pretreated with ethanolic rhizome extract (300mg/kg bw.) (Single dosage of formalin on 30th day).

Group 6 Animals treated with formalin + standard drug indomethacin

Experimental design for acute toxicity studies

The ethanolic rhizome extract extract of G. Wallichianum was made for the acute toxicity experiments and then exposed to toxicity studies in 5 separate groups, with each group consisting of 6 animals. These investigations were conducted on G. Wallichianum. Five distinct groups of animals were each given a single dose of 1, 2, 3, 4, or 5 gms per kg of body weight, and they were monitored closely for a period of 21 days.

Figure 1. Morphology of the Geranium Wallichianum, G. plant

Anti-ulcer activity:

Model of ulcer induced and we choose five groups of albino rats of either sex that weighed between 150 and 200 g. Group I: Vehicle management Group II: As a negative control Group IV: Aspirin and 200 mg/kg of GWDS ethanolic extract Group V: 400 mg/kg of GWDS ethanolic extract and standard in Group III. In order to create ulcers before to the studies, participants were allowed to drink just water for 24 hours. The test drugs were administered orally 30 min before the rantidine challenge. After 4 hours, the animals were put to sleep, and the stomach was removed for histopathology-based ulcer index evaluation (Table 1).

Table 1. Ulcer scoring done

Sr. No.	Ulcer Score	Description
1	0	Normal colour
2	0.5	Red Colour
3	1	Spot Ulcers
4	1.5 2	Haemorrhagic Streak Ulcers >3mm
5	3	Ulcers >5mm

Mean ulcer score for each animal is expressed as ulcer index.

The percentage protection was calculated using the formula

Percentage protection = 1 Ut Uc x 100

Where, Ut = ulcer index of treated group

Uc = ulcer index of control group.

The result was expressed as mean SEM and subjected to statistical analysis Using ANOVA followed by Turkey Kramer test.

Results and discussion

Preliminary phytochemical screening of various rhizome extracts of geranium

There is presentation of information regarding the data of the results of the preliminary phytochemical screening of various leaf extract extracts. Tannins were present in hexane, ethyl acetate, ethanol, and water extracts; terpenes were present in hexane, chloroform, and ethanol extracts; coumarins were present in chloroform, ethyl acetate, ethanol, and water extracts; sterols were present in all extracts except for the water extract; flavonoids, alkaloids, and proteins were present. Up to a level of 5 gram per kilogram of body weight, the plant extract did not show any signs of toxicity or

mortality in the experimental animals. Additional pharmacological and biochemical research was conducted using three sub maximal dosages in rats (100, 200, and 300 mg/kg.bw), all of which were proven to be safe for the animals. (Table 2).

Table 2. Effectiveness of extracts for Antiulcer activity

Groups	**Dose(mg/kg)**	**Observations**
Group –I Normal saline	2ml/kg	Normal
Group –II Standard	20 mg/kg	Better
Group –III Aqueous rhizome extract	175 mg/kg	Moderate
Group –IV Ethanolic leaf extract	250 mg/kg	Good
Group –V Aqueous leaf extract	175 mg/kg	Moderate
Group –VI Ethanolic leaf extract	250 mg/kg	Good

Antiulcer Activity

Effect of ethanolic leaf extract of geranium **on paw thickness**

When compared with normal control groups, the sub-cutaneous injection of diluted formalin into a hind paw in this study elicited a biphasic pattern of pain-related behaviour. This included an early phase with a shorter duration associated with neurogenic activity, followed by a second phase that was associated with inflammation that lasted for a longer period of time. Pretreatment with the extracts, on the other hand, demonstrated a considerable inhibition in the late phase of formalin-induced pain in a dose-dependent way, which was well equivalent to the effect that the standard indomethacin. (Table 3).

Table 3. Effectiveness of extracts for indomethacin induced Antiulcer activity

Groups	**Dose(mg/kg)**	**Observations**
Group –I Normal saline	2mL/kg	Normal
Group –II Standard	20 mg/kg	Better
Group –III Aqueous rhizome extract of geranium L.	175 mg/kg	Moderate
Group –IV Ethanolic rhizome extract of geranium L.	250 mg/kg	Good

Group –V Aqueous rhizome extract of geranium L.	175 mg/kg	Moderate
Group –VI Ethanolic rhizome extract of geranium L.	250 mg/kg	Good

Anti-ulcer studies

Effect of ethanolic leaf extract of geranium **on ulcer index, total acidity and pepsin levels**

Gastric ulcer created a typical mucosal lesion when compared with the normal control group as demonstrated by the number of lesions in the stomach. The ulcer was induced by the oral administration of an ethanol-HCl solution to control groups. Pretreatment with ethanolic extract had a protective effect against ethanol-HCl induced gastric lesion, as shown by the results of the current investigation (Table 4), and these results were well equivalent to those shown in groups that had been treated with the standard medicine. When contrasted with the normal control groups. On the other hand, animals that had been pretreated with ethanolic extract exhibited a considerable reduction in the levels of stomach acid and pepsin, which was on par with that seen in groups that had been given the usual medication.

Table 4. The results of anti-ulcer activity

Groups	**Dose**	**Volume of gastric juice ml/4hr**	**pH**	**Free acidity mEq/L**	**Total acidity mEq/L**	**Ulcer index**	**Percentage Inhibition of ulcer**
Group –I Normal saline	2 mg/kg	4.48 ±0.117	1.74 ±0.2	25.34 ±0.08	71.16 ±0.20	29.6 ±1.5	------
Group –II Standard	20 mg/kg	2.68 ±0.18	4.86 ±0.1	10.42 ±0.02	22.24 ±0.1	11.6 ±0.8	76.61
Group –III Aqueous rhizome extract of Geranium L.	175 mg/kg	4.28 ±0.098	3.11 ±0.14	20.18 ±0.05	51.41 ±0.38	17.88 ±1.5	40.41
Group –III Ethanolic rhizome extract of Geranium L.	250 mg/kg	3.58 ±0.075	3.76 ±0.1	15.24 ±0.04	42.71 ±0.38	14.77 ±1.2	60.25
Group –IV Aqueous rhizome extract of Geranium L.	175 mg/kg	4.05 ±0.163	4.36 ±0.16	10.76 ±0.06	30.62 ±0.26	18.16 ±3.1	45.21
Group –V Ethanolic rhizome extract of Geranium L.	250 mg/kg	3.05 ±0.147	3.36 ±0.16	13.76 ±0.02	32.32 ±0.14	8.11 ±2.1	65.21

Conclusion

The medical community continues to struggle with the problem of gastric ulcers, which are a major cause of morbidity. Asthma, acne vulgaris, autoimmune illnesses, atherosclerosis, inflammatory bowel diseases, pelvic inflammatory diseases, and rheumatoid arthritis are only a few of the many human diseases that are characterised by abnormalities linked with inflammation. For the treatment of inflammation, many steroids, including glucocorticoids, mineralocorticoids, and NSAIDs, are frequently recommended. A peptic ulcer is a digestive tract condition marked by mucosal damage brought on by pepsin and gastric acid production. Usually, the stomach and proximal duodenum are affected. Serious consequences include GI bleeding, haemorrhage, perforation, or obstruction of the stomach outlet affect about 25% of people with peptic ulcer disease. Patients who are older and who are using NSAIDs are more likely to develop silent ulcers and problems. Numerous studies have shown that different pharmaceutical therapies, such as antacids, anticholinergics, and H2 receptor antagonists, either

alone or in combination, are effective in treating peptic ulcer disease, although they are not without side effects. The efficacy and safety of these medications are still in question, despite the fact that they have significantly altered the way that ulcer and inflammation are treated. Clinical reviews of these medications have revealed instances of relapse, negative effects, and the risk of drug interactions. In order to find fresh and novel molecules that provide superior protection and lower the likelihood of relapse, the hunt for the optimal anti-inflammatory and anti-ulcer medicine has thus been expanded to include herbal drugs. This led to the selection, an Asteraceae plant, for the current study's evaluation of its potential to reduce inflammation and prevent ulceration.

The evaluation of a plant sample's ash and moisture contents, extractive values, and determination of foreign matter are only a few of the physicochemical factors that are crucial for figuring out the quality and purity of a plant sample. On an air-dried basis, the amount of chemically active components in crude leaf extract powder is often determined. To avoid the crude medicine breaking down owing to a chemical change or microbial contamination, the leaf powder's moisture level should be kept to a minimum. Ash levels are utilised to find any siliceous pollution and salts that are water soluble. These values are significant quantitative standards because they can be used to assess the legitimacy and purity of medications. In the current study, displayed the lowest moisture content (4.25%), the smallest amount of foreign material (2.7%), the highest percentage of total ash (6.85%), and the lowest percentages of water soluble ash (4.92%) and acid insoluble ash (1.64%), indicating that the plant material was pure and free of silicaceous contamination.

Traditional medicine is still widely used, and plants continue to be a significant source of natural antioxidants that might be used as a starting point for the creation of new medications. Plant extracts' antioxidant activity does, however, vary depending on the assay technique. As a result, three different techniques were used to evaluate the antioxidant activity of aqueous and ethanolic rhizome extracts. Nitric oxide, DPPH, and reducing power assays were used to assess the extract with the best anti-oxidant activity. The findings indicated that the antioxidant activity of the ethanolic rhizome extract was more pronounced than that of the aqueous extract, making ethanol a superior solvent for further research.

Living tissue's pathophysiological reaction to damage is inflammation, which causes a localised buildup of plasmatic fluid and blood cells. Although it is a defence mechanism, many diseases can be created, perpetuated, or made worse by the complex events and mediators involved in the inflammatory reaction, necessitating the use of anti-inflammatory medicines. Anti-inflammatory medications like steroids or NSAIDs, however, have a number of negative side effects, including gastrointestinal pain, a reduction in platelet aggregation, and toxicity of the liver and kidneys. A large number of people around the world suffer from gastric ulcers. Although a large variety of medications are available for the treatment of ulcers, many of them fall short of the standards and have been linked to unpleasant side effects such drowsiness, indigestion, headaches, upset stomachs, and an anti-androgenic effect. These worries about the therapeutic inadequacies and side effects of contemporary medications have led to a rise in the use of herbal treatments for treating inflammation and ulcer in recent years.

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