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EVALUATION OF IN VITRO ANTI-ULCER ACTIVITY OF HIBISCUS MUTABILIS LINN. ROOT EXTRACT BY ACID NEUTRALIZING CAPACITY ASSAY

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ABSTRACT

Peptic ulcer disease is a common gastrointestinal disorder resulting from an imbalance between aggressive factors such as gastric acid and protective mechanisms of the gastric mucosa. Although conventional anti-ulcer drugs are effective, their long-term use may be associated with adverse effects. Therefore, medicinal plants are being extensively investigated as safer alternatives. *Hibiscus mutabilis Linn.* is a medicinal plant traditionally used for various therapeutic purposes owing to its rich phytochemical composition; however, its anti-ulcer potential has been inadequately explored, the present study was undertaken to evaluate the in vitro anti-ulcer activity of the root extract of *Hibiscus mutabilis Linn.* by determining its Acid Neutralizing Capacity (ANC) and comparing its activity with the standard antacid, sodium bicarbonate. The Acid Neutralizing Capacity assay was performed by reacting different concentrations (0.1–0.5 g) of the plant extract with a fixed volume of 0.1 N hydrochloric acid (HCl). After allowing sufficient time for neutralization, the excess acid was back-titrated using 0.1 N sodium hydroxide (NaOH) with phenolphthalein as the indicator. Sodium bicarbonate was used as the standard. The amount of acid neutralized by the extract was calculated and expressed as Acid Neutralizing Capacity (ANC).

KEYWORDS: *Hibiscus mutabilis Linn.*, Anti-ulcer activity Acid Neutralizing Capacity, (In vitro) Sodium bicarbonate, Hydrochloric acid, Gastric acidity.

1. INTRODUCTION

Disruption of the gastrointestinal (GI) mucosa due to gastric acid secretion or pepsin causes peptic ulcer disease (PUD). It penetrates the gastric epithelium all the way down to the muscularis propria layer. It typically affects the stomach and the first part of the duodenum.[1] The jejunum, distal duodenum, and lower oesophagus could be affected. In individuals with a stomach ulcer, epigastric pain typically develops within 15-30 minutes after a meal, but in patients with a duodenal ulcer, the pain typically occurs 2-3 hours after a meal. These days, everybody who suspects they may have peptic ulcer illness should get tested for Helicobacter pylori.[2] Some patients, especially those with concerning symptoms, may need an endoscopy for diagnosis. Triple-drug therapy based on proton pump inhibitors (PPIs) is now effective in treating the vast majority of patients. When a region of skin is subjected to continuous pressure for an extended period of time, it can cause tissue ischaemia, the stoppage of nutrition and oxygen supply to the tissues, and ultimately tissue necrosis.

The term "distortion or deformation damage" best describes the constant pressure that causes a pressure ulcer. If an external force (shear, compression, or both) is applied to any tissue, the tissue will suffer from localised, acute ischemic damage.[3] Despite the fact that pressure injuries that do not result in open wounds (such as blisters and non-blanching erythema) are not real sores but just "pressure damage" and nevertheless belong

to this family of pressure ulcers, the phrase "pressure sores" is often used in the UK. The European Pressure Ulcer Advisory Panel (EPUAP) has adopted the term "pressure ulcers" as the official European term despite its widespread use outside of Europe. Bedsores and decubitus ulcers are two other names for this condition; however, these terms are rarely used anymore because it is understood that the ulcers are not brought on by prolonged bed rest.[4] Occipital, trochanteric, sacral, malleolar, and heel skin are the most vulnerable to developing pressure sores. The ulcerogenic process occurs as a result of damage to the protective mucosal lining of the stomach and duodenum. Pylori infections and the use of NSAIDs and low-dose aspirin are known to damage the mucosal lining. The cost to the mucosal lining in the setting of an H. pylori infection is the result of both bacterial factors and the host's inflammatory response. In the case of NSAID (and aspirin) use, mucosal damage is secondary to inhibition of cyclooxygenase 1 (COX-1) derived prostaglandins which are important in maintaining mucosal integrity.[5] Once the mucosal layer is disrupted, the gastric epithelium is exposed to acid, and the ulcerative process ensues. If the process continues, the ulcer deepens reaching the serosal layer. A perforation occurs once the serosal layer is breached at which point the gastric contents are released into the abdominal cavity.[6] Gastric ulcers are classified into four types based on location.[7]

- **Type 1:** in the antrum, near the lesser curvature
- **Type 2:** combined gastric and duodenal ulcer
- **Type 3:** Prepyloric ulcer
- **Type 4:** ulcer in the proximal stomach or cardia

Gastric ulcers are most commonly found in the lesser curvature (55%), followed by a combination of duodenal and gastric ulcers. Duodenal ulcers are most commonly located in the first part of the duodenum.[8] Gastric ulcers have malignant potential compared to duodenal ulcers that do not have cancerous risk. A gastric ulcer greater than 3 cm is called a giant gastric ulcer which has a 6%-23% chance to turn into malignancy. In previous literature, the cancer rate in endoscopically diagnosed gastric ulcers ranges from 2.4%–21%.[9]

Hibiscus is a genus of flowering plants with numerous medicinal properties belongs to mallow family, Malvaceae. The genus is quite large, comprising several hundred species that is native to warm-temperate, sub-tropical and tropical regions throughout the world. There are about 275 species of *Hibiscus* in the tropical and sub-tropical regions.[10] Out of them *H. rosa sinensis, H. syriacus, H. cannabinus, H. radiatus, H. vitifolius, H. sabdariffa, H. schizopetalus* etc. are commonly found in India. Along with a flowering plant it also has various medicinal properties.[11] More than 7500 species out of 17000 species of higher plants are used in the various traditional systems of medicine like Ayurveda, Siddha and Unani.[12] The genus includes both annual and perennial herbaceous plants, as well as woody shrubs and small trees. The leaves are alternate, ovate to lanceolate, often with a toothed or lobed margin. The flowers are complete, large, conspicuous, and trumpet-shaped, with five or more petals, colour from white to pink, red, orange, peach, yellow or purple and from 4 to 18 cm broad. Flower colour in certain species, such as *H. mutabilis and H. tiliaceus* changes with age. The fruit is a dry five-lobed capsule, containing several seeds in each lobe, which are released when the capsule dehisces (splits open) at maturity.[13] The various parts of this plant have been known to contain numerous medicinal properties like anti-hypolipidemic, anti-proliferative, antioxidant, anti- microbial, -anti-inflammatory and other pharmacological properties.[14]

The genus of shrubs, subshrubs, trees or herbs of *hibiscus* in the Malvaceae family. Its several species are widely distributed in different regions of Asian continent for horticultural and ornamental purposes because of its showy and colorful flowers.[15] One of such plant is *Hibiscus mutabilis L.* (confederate rose) is an inland woody shrub (1.5−4.0 m tall) that is native to China and widely cultivated in Southeast Asia. Leaves are broadly ovate with mostly five triangular lobes. Although *H. mutabilis* produces large and beautiful flowers, its constraint as ornamental plants is the frequent and unsightly infestation of whiteflies. Flower colour change in *H. mutabilis* is most spectacular. Flowers are white in the morning, pink in the afternoon, and red in the evening. Temperature may be an important factor affecting the rate of color change as white flowers kept in the refriagerator remain white until they are taken out to warm, where up on they slowly turn pink.[16] The roots, leaves, and flowers of *Hibiscus mutabilis L* can be used as medicine. It is also included in the 2020 edition of Chinese Pharmacopoeia, which has the effects of cooling blood, detoxification, detumescence, and relieving pain. It mainly treats carbuncle and swelling, scald, eye red swelling and pain, and falling injury.[17] *Hibiscus mutabilis L* contains a variety of active ingredients, including flavonoids, organic acids, volatile components, and other components such as stigma, anthraquinone, coumarin, triterpenes, lignans, and inorganic elements. Flavonoids are the most important active components.[18] *Hibiscus mutabilis* has a useful ecological function, playing a very significant role in slope protection. This tree provides very good garden greenery, and is often grown in parks and gardens.[19]

MATERIALS AND METHODS

The plant material was collected from the nearby surroundings of Mandya district, Karnataka, India, in December 2025. The collected plant was identified and authenticated by Dr. Thejash Kumar M.P., Coordinator, Department of Botany, Postgraduate Research Centre, Bharathi College (Autonomous), Bharathinagara. The roots of *Hibiscus mutabilis* were thoroughly washed to remove adhering soil and other impurities, shade-dried at room temperature, and powdered into a fine coarse powder using a mechanical grinder. The powdered material was stored in an airtight container and

subsequently subjected to successive Soxhlet extraction using solvents of increasing polarity to obtain the crude extracts for further phytochemical and pharmacological studies.

IN-VITRO Acti-ulcer Activity

Acid Neutralizing Capacity

The acid neutralizing capacity (ANC) value for methanolic extract of root of Hibiscus *mutabilis* in different concentration((100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml**,** 500mg/ml) were compared with the standard Sodium Bicarbonate, after adding 5ml of the mixture and adding the remaining 70ml of water, the volume was 70ml. This was blended for one minute. About 30ml of 1.0 N HCl was added to the standard & test preparation and swirled for 15 minutes. Then, phenolphthalein was added & combined. The surplus HCl was promptly titrated with 0.5N sodium hydroxide until the pink colour reached 15. Calculating the moles of acid neutralised involves dividing the volume of HCl by its normality and the volume of NaOH by its normality. Moles of HCl neutralised divided by grams of antacid/extract equals acid neutralising capability (ANC) per gram of antacid.[20]

RESULTS AND DISSCUSSION

Standard Sample	Weight(g)	HCL Added (mL)	NaOH Consumed	ANC (mEq/g)
Sodium Bicarbonate	0.1g	10	9.1	0.9
Sodium Bicarbonate	0.2g	10	8.6	1.4
Sodium Bicarbonate	0.3g	10	8.2	1.8
Sodium Bicarbonate	0.4g	10	7.8	2.2
Sodium Bicarbonate	0.5g	10	7.5	2.5

Test Sample	Weight(g)	HCL Added (mL)	NaOH Consumed	ANC (mEq/g)
Hibiscus mutabilis	0.1g	10	9.5	0.6
Hibiscus mutabilis	0.2g	10	9.0	1.2
Hibiscus mutabilis	0.3g	10	8.5	1.5
Hibiscus mutabilis	0.4g	10	7.0	2.0
Hibiscus mutabilis	0.5g	10	7.5	2.3

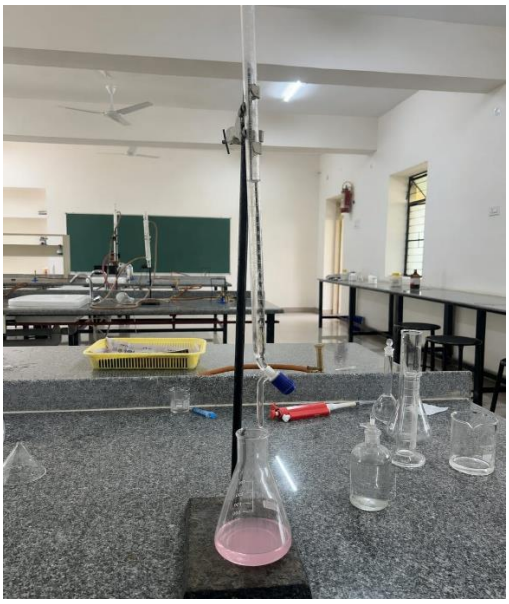

FIG. NO. 1 - Experimental Set-up.

FIG. NO. 2 – Indication of pale pink color.

CONCLUSION

The in vitro anti-ulcer activity of the *Hibiscus mutabilis Linn.* root extract was evaluated using the Acid Neutralizing Capacity (ANC) method and compared with the standard sodium bicarbonate. The extract exhibited dose-dependent acid-neutralizing activity, with the ANC increasing as the concentration of the extract increased. Although the standard showed superior acid-neutralizing capacity, the plant extract demonstrated appreciable neutralization of hydrochloric acid, indicating the presence of bioactive phytoconstituents with potential antacid properties. These findings suggest that the root extract of *Hibiscus mutabilis Linn.* possesses promising in-vitro anti-ulcer activity and may serve as a potential natural agent for reducing gastric acidity.

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