



## In vitro evaluation of anti-ulcer activity of ethyl acetate extract of *Strychnos wallichiana* roots

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### Abstract



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*Strychnos wallichiana* is a native plant that has been used extensively in traditional medicine to cure a variety of ailments. The current study aimed to show the ethyl acetate extract of *Strychnos wallichiana*'s *in-vitro* anticancer activity. The inhibitory activity of *in-vitro* H<sup>+</sup>-K<sup>+</sup> ATPase was assessed. The spectrophotometric analysis of H<sup>+</sup>-K<sup>+</sup> ATPase inhibitory activity. The outcome was expressed as an ethyl acetate extract of *Strychnos wallichiana* that contained alkaloids, steroids, tannins, and phenolic compounds and that, when used at a concentration of 100 g/ml, had a proton pump inhibitory action comparable to that of omeprazole on goat stomach mucosal homogenate. The results of the observations suggested that *Strychnos wallichiana* might be helpful in the treatment of stomach ulcers.

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### INTRODUCTION

One chronic illness that affects up to 10% of people worldwide is peptic ulcers. Peptic ulcer frequency rose with age, reaching a maximum in the fifth decade of life at 28.8% [1]. Peptic ulcers formed depend upon the presence of gastric juice PH and the decrease in mucosal defenses [2]. The exact cause of peptic ulcers is unknown; however, in the majority of cases, there is an imbalance between defensive and aggressive forces that lead

to ulcers (e.g., stomach mucus, bicarbonate secretion, prostaglandins, nitric oxide, and innate resistance of mucosal cells). The mucosal layer damage is an initial step in the generation of ulcers, primarily due to the hypersecretion of HCl from parietal cells of gastric mucosa through the proton pump and oxidative stress [3]. *Helicobacter pylori* (*H. pylori*) infection and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) are the two main factors impacting mucosal resistance to injury [4]. Antagonists of the histamine-2 (H<sub>2</sub>) receptor and proton pump inhibitors (PPIs), which are common peptic ulcer treatments, have been shown to have adverse side effects, relapses, and a variety of pharmacological interactions [5]. Due to lesser side effects compared to synthetic drugs, presently, 80% of the world's population depends on natural products for the first line of primary health care. Decoction of *Strychnos wallichiana* roots is used in rheumatism, ulcers, elephantiasis, fever, and epilepsy [6].

## **MATERIALS AND METHOD:**

### **Collection and extraction process [7]**

20gm of root powdered collected and extracted with 250ml of ethyl acetate with the help of a Soxhlet device. The extraction process was continued until the extractive was colorless.

The solvent was removed entirely from Marc. The solvent was evaporated from their extract using a heating mantle at 37 degrees centigrade. The extract was kept in a desiccator for further experiments to identify the chemical groups present.

### **Preliminary phytochemical screening [8]**

#### **Preliminary phytochemical analysis**

The following initial phytochemical analyses were carried out on each extract:

#### **Test for alkaloids**

Dilute hydrochloric acid was applied to the extracts and filtered. Many alkaloidal reagents were applied to the filtrate.

#### **Mayer's test**

A cream-colored precipitate appeared when the filtrate was treated with Mayer's reagent, potassium mercury iodine solution, indicating the presence of an alkaloid.

#### **Dragendorff's test**

The presence of alkaloids was detected by forming an orange-brown precipitate in the Potassium Bismuth Iodine Solution after the filtrate was treated with Dragendorff's reagent.

#### **Hager's test**

The presence of alkaloids was shown by forming a yellow-colored precipitate when the filtrate was treated with picric acid, Hager's reagent.

### **Test for reduced sugars and carbs [9]**

300 mg of the extracts were dissolved in 4 milliliters of filtered distilled water.

#### **Molisch's test**

Sulfuric acid and Molisch's reagent were used to treat a tiny amount of the filtrate. The formation of a violet indicates carbohydrate content.

#### **Fehling's test**

Fehling's solutions A and B were applied to the extracts. The sediment's reddish-brown hue suggested the presence of reducing carbohydrates.

#### **Benedict's test**

After adding Benedict's reagent to the extracts, reducing sugars show up as a reddish-orange hue.

#### **Barfoed's test:**

Heat and Barfoed's reagent treatment were applied to the extracts. Reducing sugars were present because of the appearance of the reddish-orange precipitate.

#### **Test for steroids**

##### **Liebermann Burchard test**

To do the Liebermann-Burchard test, the extracts were exposed to three milliliters of acetic anhydride, a few drops of glacial acetic acid, and a drop of strong sulfuric acid. Steroids were present because of their bluish-green appearance.

##### **Salkowski test**

Three milliliters of concentrated sulfuric acid drops containing acetic anhydride were applied to the extracts. Steroids were evident when the color turned yellow [10].

#### **Protein tests**

##### **The Biuret test**

The extracts were exposed to a solution of copper sulfate, and then a solution of sodium hydroxide was added; the emergence of a violet color indicated the presence of proteins.

##### **Millon's test**

After the extracts were treated with Millon's reagent, proteins were present because a pink hue appeared.

##### **Tannin test**

After treating the extracts with a 10% lead acetate solution, the presence of tannins was revealed by the appearance of a white residue. A white precipitate appeared after treating the extract with an aqueous bromine solution.

### **Test for flavonoids [11]**

a) With 10% v/v sulfuric acid, 5 milliliters of the extracts were hydrolyzed and cooled. Next, it was

separated into three pieces in three test tubes and extracted using diethyl ether. Three different amounts were introduced to the test tubes: one milliliter each of 0.1N sodium hydroxide, diluted sodium carbonate, and robust ammonia solution. Flavonoids were present in each test tube, as evidenced by the yellow color that developed.

#### Shinoda test

The extracts were dissolved in alcohol, to which a small amount of magnesium chloride was added, and then the mixture was heated, and the HCL was added dropwise. The magenta color appearance indicates the presence of flavonoids.

#### Flavanone Test

10% sodium hydroxide was added to the extracts. A yellow-to-orange appearance indicates the presence of flavanones. Cone sulfuric acid was applied to the extract. An orange to crimson red appearance indicates the presence of flavanones.

#### Test for gums and mucilage

Filter the extract after treating it with 25 milliliters of pure alcohol. We looked at the filtrate's swelling index.

#### Test for glycosides

Glycosides are present when a crimson ring forms at the intersection of two liquids after a small amount of the extracts is treated with glacial acetic acid, a few drops of ferric chloride solution, and intense sulfuric acid.

#### Saponin test

Shake well in a test tube after diluting 1 ml of the extracts to 20 ml with distilled water. Saponins were present because foam formed in the test tube's upper section.

#### Triterpenoids test

#### Salkowski's test

Tin and thionyl chloride were added to the material to warm it. Triterpenoids are indicated by the color pink.

#### Hirschsohn's response

Red to purple coloring is seen when the material is heated with trichloroacetic acid.

#### In vitro Antiulcer activity

#### Assay of H<sup>+</sup>-K<sup>+</sup> ATPase activity [12]

H<sup>+</sup>- K<sup>+</sup> ATPase was prepared from mucosal scrapings of goats. Take a newly butchered goat's stomach gently rinsed with tap water. The mucosal layer scraped from the goat fundus. The scrapped mucosal layer was homogenized in an ice-cold phosphate buffer, PH-7.4. The obtained homogenate was centrifuged for 20 min at 15000rpm. After centrifuge, the supernatant was received and centrifuged for 60 min at 15000 rpm. The pellets were obtained after recentrifugation. The formed pellets were resuspended in a homogenization buffer. Different concentrations of the extract 20-100 micrograms were grown to a volume of 1 milliliter by incubating them in the reaction mixture (40 milliliters of Tris Hcl buffer, PH-7.4, 2 milliliters of mgcl<sub>2</sub>, and ten micrograms of membrane protein). The reaction was then started with 2 milligrams of ATP Tris salt. This mixture was incubated at 37°C for 20 minutes. Adding 1 milliliter of ice-cold trichloroacetic acid (10%v/v) will terminate the process. The H<sup>+</sup>-K<sup>+</sup> ATPase activity was measured in the presence and absence of various extract dosages and the common medication omeprazole. The UV spectrophotometer measured the amount of inorganic phosphate produced from ATP within the 400 nm wavelength range.

Enzyme inhibition = [activity (control) - (activity test)/Activity (control)]×100

## RESULTS

### Phytochemical Screening

The ethyl extract of *strychnos wallichina* showed the presence of Alkaloids, Steroids, Tannins, and Phenolic compounds (Table 1)

**Table 1 Phytochemical Screening of ethyl extract of *strychnos Wallachia***

Test	Ethyl acetate extract
Alkaloids	+
Carbohydrates	-
Steroids	+
Proteins	+
Tannins	+
Phenolic compounds	+
Test for gums and mucilage	-
Test for glycosides	-
Test for saponins	-
Test for triterpenoids	-

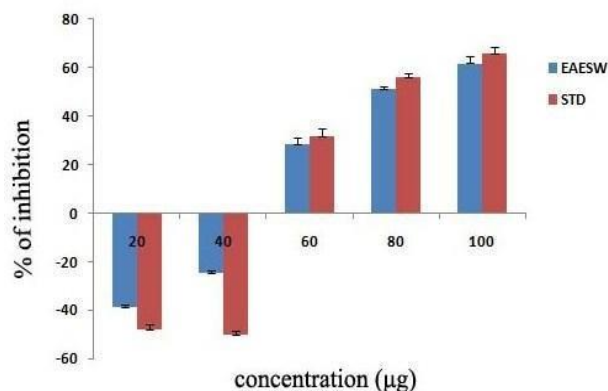
Note: + = Present , - = absent

**H<sup>+</sup>-K<sup>+</sup> ATPase inhibition activity:**

Omeprazole has been used as the standard to examine the H<sup>+</sup>-K<sup>+</sup> ATPase inhibitory activity of ethyl acetate extract at different concentrations (20µg, 40µg, 60µg, 80µg, and 100µg). A dose-dependent pattern of activity was observed in the extract. At 100µg of extract, the maximum % inhibition was found to be 61.79±1.51, while standard omeprazole showed 66.05±0.67. Table 2 and Figure 1 represent the results.

**Table 2 Effect of Ethyl acetate extract of SW on in vitro H<sup>+</sup>-K<sup>+</sup> ATPase inhibition activity**

Concentration (µG)	Percentage of Inhibition (%) (MEAN±SEM)	
	Extract	Standard (Omeprazole)
20	-38.59±0.7	-47.92±1.40
40	-24.9±1.32	-50.01±0.59
60	28.50±1.06	31.74±0.73
80	51.06±0.98	55.93±1.9
100	61.79±1.51	66.05±0.67



**Figure 1 Effect of Ethyl Acetate Extract of SW on in vitro H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition activity**

**DISCUSSION:**

Peptic ulcer results from an imbalance between aggressive and defensive factors. Acidity is a common gastrointestinal problem that can result for various reasons. High amounts of Hydrochloric acid secretion inflame the stomach lining and produce ulcers. Hypochlorhydria is a problem caused by the uncontrolled hyper secretion of hydrochloric acid from the parietal cells of gastric mucosa through a proton pump. One of the main enzymes that produce acidity is H-K ATPase. It is situated on the parietal cells' apical secretory membrane. The extract exhibited a maximum

percentage inhibition of 61.79% in H-K ATPase inhibitory activity at a dose of 100µg.

**CONCLUSION**

Nowadays, everyone in the world is suffering from ulcers due to many reasons. According to the above results, the ethyl acetate extract of *Strychnos wallichiana* may be a source of novel antiulcer drugs. However, a detailed study on isolating active constituents from the plant and its antiulcer effect will be conducted in the future.

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