

# ANTI-ULCER ACTIVITY OF STEM EXTRACT OF SMILAX CHINA BY USING IN-VITRO STUDIES

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## ***Abstract:***

*The stem of smilax china is traditionally used to treat various diseases like gout, paralysis, urinary tract infections, syphilis and ulcers. The aim of present study was to evaluate the in-vitro anti-ulcer activity of smilax china. It can be determined by estimating the acid neutralizing capacity of both allopathic and natural drug (smilax china). The results obtained are compared with each other. After comparison the results reveals that smilax china (natural medication) possess high potent anti-ulcer activity than allopathic medication (aluminium hydroxide + magnesium hydroxide).*

## Introduction :

Ulcers are mainly caused when there is an imbalance between the digestive juices produced by the stomach and the various factors that protect the lining of the stomach. Most ulcers occur in the first layer of the inner lining. A hole in the stomach or duodenum is called a perforation. This is a medical emergency<sup>1</sup>.

An ulcer is defined as a discontinuity or break in a bodily<sup>2</sup> membrane that impedes normal function of the affected organ. According to Robbins's pathology, "ulcer is the breach of the continuity of skin, epithelium or mucous membrane caused by sloughing out of inflamed necrotic tissue"<sup>2</sup>.

The etiologic of peptic ulcer is not clearly known, because of disproportion between aggressive (acid, pepsin, bile, and H.pylori) and defensive (gastric mucus, bicarbonate secretion, prostaglandins, nitric oxide, innate resistance of mucosal cells) factors<sup>3</sup>.

Occurrence and prevalence in a systematic review of 31 published studies, the pooled incidence of uncomplicated peptic ulcer disease (PUD) was approximately one case per 1000 person-years in the general population, and the incidence of ulcer complications was approximately 0.7 cases per 1000 person in these present years<sup>4</sup>.

In accordance with the latest WHO data published in 2020 Peptic Ulcer Disease Deaths in India reached 68,108 or 0.80% of total deaths<sup>5</sup>. The age adjusted Death Rate is 6.24 per 100,000 of population ranks India in the world.

Presently, a desperate need of most effective and safer anti-ulcer agents aiming to relieve pain, heal the ulcer and delay ulcer recurrence. Therefore herbal medicines are considered safer alternatives because of natural ingredients with<sup>6</sup> no side effects. In present study used plant *Smilax china* L., belongs to the Smilacaceae family<sup>7,8</sup>. It is a climbing plant species and a well-known Chinese traditional medicine in china as genus *Smilax*. It usually grows in the southern parts of China, Korea, Japan, Philippines, Vietnam, Thailand, Myanmar, Assam and tropical countries<sup>8</sup>.

*Smilax china* has many different names in various languages<sup>9</sup>, China root in English, Chopchini, Chobchini and Toupchini in Hindi, Ayadi in Tamil, Kaltamara in Malayalam, Ghotvel in Marathi, Kondadantena in Telugu.

On their own, *Smilax* plants will grow as shrubs, forming dense impenetrable thickets. They will also grow over trees and other plants up to 10 m high, their hooked thorns allowing them to hang onto and scramble over branches. The genus includes both deciduous and evergreen species. The leaves are heart shaped and vary from 4–30 cm long in different species.

Greenbrier is dioecious. Although, only about one in three colonies have plants of both genders. Plants flower in May and June with white/green clustered flowers. If pollination occurs, the plant will produce a bright red to blue-black spherical berry fruit about 5–10 mm in diameter that matures in the fall<sup>10</sup>.

It is showing <sup>11</sup>carminative, diaphoretic, antiepileptic, antirheumatic, antigout, <sup>12</sup>antisiphilitic, <sup>13</sup>anti-inflammatory, <sup>14</sup>prevents obesity, <sup>15</sup>anti-nociceptive, <sup>16</sup>anti-diabetic, <sup>17</sup>diuretic, <sup>18</sup>antioxidant, <sup>19</sup>anticonvulsant, <sup>11</sup>and aphrodisiac activities in scientific studies. It is proved that *Smilax china* is used as tonic for male sexual energy and it also

improves muscles mass and body strength. By doing large survey It is confirmed that this plant is used to treat urinary tract infection,<sup>20</sup> paralysis, stone and ulcers in bladder, many skin infections which produces ulcers. Basing on literature survey it is used for anti-ulcer.<sup>21</sup> Smilax china also found to have antimicrobial property that acts against acne-causing bacterium.

## **Materials and method :**

**Chemicals :** The chemicals aluminium hydroxide and magnesium hydroxide were purchased from Mahalasa pharma ,goa, india . hydrochloric acid , sodium hydroxide were purchased from Surya Fine Chem , pune , india. And phenolphthalein indicator was purchased from Suvidhinath Laboratories , Gujarat , india .

**Processing of Plant Material:** The collected plant material has authenticated by botany S. Prasad rao , Research Officer , Survey of Medicinal Plants Unit, Sir C. R. Reddy autonomous College, Eluru. A voucher specimen (Voucher No. ATC27/08/2022) has been deposited at the herbarium unit of the Department of Botany, Sir C. R. Reddy autonomous College, Eluru , Eluru (District), Andhra Pradesh, India. The plant was washed with tap water 3 times and sterilized by spraying with 70% alcohol.

The purified plant material was shade dried at room temperature mainly to avoid chemical changes. When the plant material was dried entirely, then it is subjected to prepare fine powder with the help of a motor and pestle. The fine material powder is collected and used for extraction of the crude drug in alcoholic solvents by maceration method<sup>22</sup>.

**Extraction by maceration:** 100gms of the plant powder of smilax china is weighed and extracted using water by maceration method in the drug:solvent proportion of 1:5. This process is done for 10 days and the extract is concentrated to dryness under controlled temperature in hot air circulated oven. Then the extract is subjected to qualitative preliminary phytochemical screening.

## **Preliminary phytochemical tests for identification of plant constituents**

The aqueous extract of Stem of *Smilax china* was subjected to preliminary phytochemical screening for detection of various plant constituents.

### **Tests for carbohydrates :**

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

To<sup>23</sup> the filtrate solution add few drops of alcoholic alpha-naphthol solution, shaken and add concentrated sulphuric acid from sides of test tube formation of violet ring at the junction of two liquids indicates the presence of carbohydrates.

**Benedict's test :**

Equal volume of benedicts reagent and filtrate solution in test tube were mixed. Then heated in boiling water for 5mins. Appearance of yellow, green, or red solution may indicate the presence of reducing sugars.

**Tests for proteins :****Biuret test :**

To the 3ml of filtrate solution add 4% of sodium hydroxide and add few drops of 1% copper sulphate solution. Formation of violet or pink colour indicates the presence of proteins.

**Xantho proteins test :**

To 3ml of filtrate solution add 1ml of concentrated sulphuric acid. formation of white precipitate indicates the presence of proteins.

**Tests for amino acids :****Ninhydrins test :**

Heat 3ml of filtrate solution and add 3 drops of 5% ninhydrin's solution in boiling water bath for 10 mins. Formation of purple or bluish colour indicates the presence of aminoacids.

**Millons test :**

Heat 3ml of filtrate solution with millons reagent. formation of dark red colour indicates the presence of amino acids.

**Test for fats and oils :****Sudan iii test :**

Place a drop of filtrate solution on glass slide. Add sudan red iii reagent. After 2 min wash with 50 % alcohol. Mount in glycerine and observe under microscope. Formation of red oil globules indicates the presence of fats and oils.

**Tests for alkaloids :**

To the filtrate solution add dil, hcl shake well and filter with the filtrate perform the following.

**Dragendroff's test:**

To 2-3ml of filtrate add few drops of dragendroff reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

**Hagers test :**

To 2-3ml of filtrate add few drops of hagers reagent . formation of yellow precipitate indicates the presence of alkaloids.

**Tests for steroid :****Salkowski reaction test :**

To the filtrate add 2ml of chloroform and 2ml of concentrated sulphuric acid and shake well. Chloroform layer doesn't appear as red and acid layer shows greenish yellow fluorescence indicates the presence of absence of steroids.

**Liebermanns test :**

Mix 3ml of filtrate solution with 3ml of acetic anhydride. Blue colour doesn't appear indicates the absence of steroids.

**Test for flavonoids :****Shinoda test :**

To the filtrate add 5ml of 95% ethanol, few drops of concentrated HCl and 0.5g of magnesium turnings. Pink colour is not observed. indicates the absence of flavonoids.

**Lead acetate test :**

To the small quantity of filtrate add lead acetate solution. Yellow colour precipitate is not formed indicates the absence of flavonoids.

**Test for glycosides :****Keller killani test :**

To the 2ml of filtrate solution add glacial acetic acid, one drop of 5% ferric chloride and concentrated sulphuric acid. Appearance of reddish brown colour at the junction of two liquid layers and upper layer appears as bluish green. Indicates the presence of glycosides.

**Legal test :**

To the filtrate solution add 1ml of pyridine ,1ml of sodium nitroprusside. Formation of pink to red colour indicates the presence of glycosides.

**Tests for tannins and phenolic compounds :****Ferric chloride test :**

To the filtrate add 5% ferric chloride solution . formation of deep blue-black colour indicates the presence of tannins/phenolic compounds.

**Bromine water test :**

To the filtrate solution add bromine water. Discolouration of bromine water indicates the presence of tannins/phenolic compounds<sup>23</sup>.

Table no. 1

S.no	Phytochemical constituents	Smilax china
1	Carbohydrates	+ve
2	Proteins	+ve
3	Aminoacids	+ve
4	Steroids	-ve
5	Tannins/phenol	+ve
6	Fats and oils	+ve
7	Flavonoids	-ve
8	Glycosides	+ve
9	Saponins	+ve
10	Alkaloids	+ve

### In-vitro Evaluation of Antiulcer Activity: Acid Neutralizing Capacity:

#### In vitro antacid activity :

The 5ml of standard antacid mixture containing magnesium hydroxide and aluminium hydroxide in the ratio of 1:1, 250mg each/ml. to this water is added and makeup to the volume of 70ml and it is mixed for 1 min. then 30ml of 1.0 N HCL was added to the both standard and test preparations<sup>24</sup> ( aqueous extracts 500mg, 1000mg, 1500mg, and 2000mg/5ml individually are stirred for 15min. the excess HCL was titrated against 0.5N Sodium hydroxide (NaOH) Solution to obtain a stable PH of 3.5 (for 10 to 15 sec). the temperature of this mixture was maintained at 37+ or -0.5 c throughout the experiment.

Total mEq of acid consumed =  $(30 \times N \text{ HCl}) - (V \text{ NaOH} \times N \text{ NaOH})$

Where N in the above formula denotes the normality of HCl and NaOH, and V denotes the volume of NaOH which is used for titration. Here the ANC values are reported as mEq of acid consumed by 5 ml of standard as well as test preparation and also the test preparation obtained values are compared with the standard<sup>25</sup>.

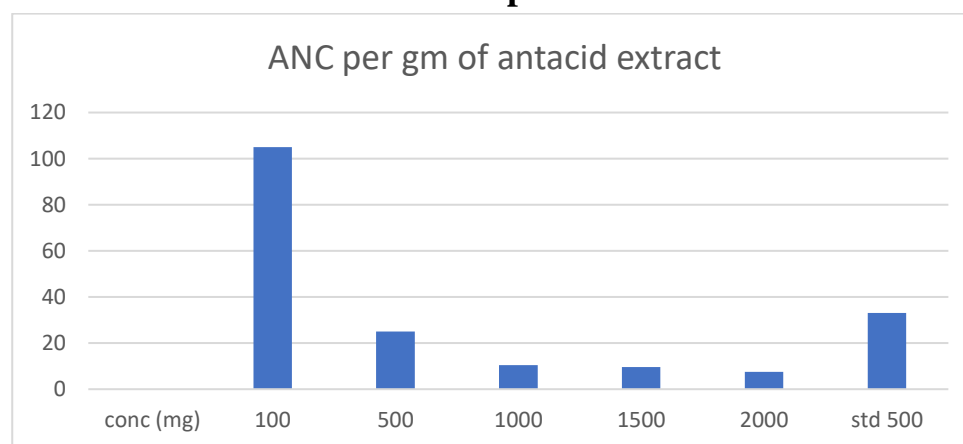
#### Results and discussion :

To determine the acid neutralizing capacity of smilax china (test preparation) these are taken in different concentrations and obtained values are compared with the standard (aluminium hydroxide and magnesium hydroxide 250mg per ml. The ANC values obtained for smilax china are 105, 25, 10.5, 9.6 and 7.5 and standard ANC was 33 in table no.2. The test value at 2000mg was found to neutralize acid more significantly than standard. It clearly mentioned that there is a concentration dependent reduction in acid neutralizing capacity per gm of antacid in graph no.1. Therefore the results are tabulated in the following table no.2 and<sup>25</sup>graph no.1.

**Table no. 2**  
**Acid Neutralizing Capacity of SMILAX CHINA**

S.no	Concentration (mg)	Concentration (gm)	Volume of NaoH	Moles of acid consumed	ANC per gram of antacid extract
1	100	0.1	39	10.5	105
2	500	0.5	36	12	25
3	1000	1	39	10.5	10.5
4	1500	1.5	31	14.5	9.6
5	2000	2	30	15	7.5
<b>STANDARD</b>	<b>500</b>	<b>0.5</b>	<b>27</b>	<b>16.5</b>	<b>33</b>

**Graph**



### **Conclusion :**

The botanical name of china root is smilax china which belongs to the family smilacaceae. We considered smilax china is a natural medicine which is having anti-ulcer activity. The anti-ulcer activity of smilax china is due to the presence of alkaloids, glycosides, saponins, aminoacids, carbohydrates, proteins, tannins, fats and oils. So that's why we selected this natural medicine for comparing with the allopathic medicine.

The stomach is one of the organ in digestive system where food travels from the esophagus and is further broken down before its nutrients are absorbed in the small intestine. It produces acid and various enzymes that break down food into simple substances. The inside wall of the stomach is protected from the acid and enzymes by a mucous lining.

Acidity is a common gastrointestinal problem that can be caused due to excessive secretion of gastric acid or stomach acid inflames the stomach lining mucosa and produces ulceration. Antacids are the medications that can act by neutralizing gastric acid and thereby reduce the acid that it can neutralize. The results clearly states that the ANC is purely based on concentration dependent reduction. Therefore we can conclude that natural medications (smilax china) have high anti ulcer activity than allopathic medications.

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