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PHYTOCHEMICAL SCREENING AND EVALUATION OF *IN VITRO* ANTIULCER ACTIVITY OF *TYLOPHORA INDICA* (BURM.F.) MERRILL

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ABSTRACT

The *in vitro* antiulcer activity of various extracts of *Tylophora indica* was examined for acid neutralization method and H^+ K^+ ATPase inhibitory activity method**.** Ethyl acetate extract of T.indica showed significant proton pump inhibition than that of various extracts in comparison with standard lansoprazole. The percentage inhibition was found to be concentration dependent manner. And in acid neutralization capacity method ethyl acetate and alcoholic extract showed good acid neutralising capacity when compared to standard. These *in vitro* methods indicated that this plant extract is a better source of natural antiulcer agent.

INTRODUCTION

Peptic ulcer disease (PUD) refers to a group of ulcerative disorders of the upper gastrointestinal tract that require acid and pepsin for their formation[1]. Peptic ulcer is a kind of heterogeneous disorder causing due to imbalance between defensive and aggressive factors such as stress,[2] exposure to bacterial infection[3] and use of the non-steroidal anti-inflammatory drugs [4]. Gastroduodenal ulcers are one of the most common problems faced by populace worldwide. Hyperchlorhydria is a condition characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump[5]. In conventional drug therapy there were many limitations like increased risk of recurrence, arrythmia, haematopoetic disorders, adverse reactions like hypersensitivity etc. Herbal medicine is used as fast emerging alternative treatment of ulcers[6]. Thus the main concern of the current study is to introduce a safe remedy of natural origin for the treatment of peptic ulcer with less side effects. *Tylophora indica* (Burm.f.) Merrill belonging to family Asclepiadaceae is a twining perennial plant distributed throughout southern and eastern part of India in plains, forests, and hilly places[7]. The literature survey showed that Tylophora indica is folkloriclly used for ulcer and there is no scientific support for this.

MATERIALS AND METHODS

2.1 Plant materials

Fresh aerial parts of *Tylophora indica* were collected from Ettumanoor, kottayam district, kerala during February 2013. It was further identified and authenticated by Dr.N.Sasidharan, Scientist, Kerala Forest Research Institute, Peechi, Trissur. These were cut, shade dried and ground into powder.

2.2 Preparation of extract

The dried, powdered aerial parts of *T. indica* were extracted in Soxhlet extractor using petroleum ether and the marc was collected and subjected to successive extraction using various solvents such as chloroform, ethyl acetate and alcohol.

2.3 Preliminary phytochemical screening

Qualitative phytochemical analysis of aerial parts of *T.indica* was carried out for the presence of phenolics, saponins, alkaloids, flavonoids, steroid, terpenoids, carbohydrate.

2.4 Evaluation of *invitro* antiulcer activity

Acid neutralising capacity

The acid neutralizing capacity was carried out as per USP[8]. The acid nuetralising capacity was carried out at a temperature 37±3ºC. A pH meter was standardised using 0.05 M potassium

biphthalate and 0.05 M potassium tetraoxalate standardized buffers. Magnetic stirrer was used to produce stirring rate 300±30 rpm. 0.5 g of extracts or standard were transferred to 250ml beaker and 70ml distilled water was added. It was mixed with magnetic stirrer for 1min. Then 30ml 1N HCl was added to the test solution with continous stirring for 15min. Excess HCl was titrated with 0.5N NaOH to attain stable pH of 3.5.

2.5 H^+ K^+ ATPase inhibitory activity method

Preparation of Parietal cells

Gastric membrane containing H^+ K^+ ATPase was prepared from mucosal stomach scrapings of sheep[9] and was homogenized in 20 mM Tris–HCl buffer (pH 7.4). The homogenate was centrifuged for 20 min at 15,000 rpm and the resulting supernatant was used to determine the H^+ K^+ ATPase activity and its inhibition[10]. The protein content of the supernatant was determined by Bradford's method using bovine serum albumin as a standard[11].

H^+K^+ ATPase Assay

The enzyme extract containing 100 µl (300 µg) proteins was taken for testing the activity of H^+ K^+ -ATPase. Reaction was carried out in 16mM Tris buffer (pH 6.5). The reaction was initiated by adding substrate (2mM ATP, 2mM $MgCl_2$ and 10mM KCl), made up to 2 ml and incubated for 30 min at 37°C. The reaction was stopped by the addition (1 ml) of an assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid. Phosphomolybdate formed was measured spectrophotometrically at 400 nm[12].

Inhibition of H^+ K^+ ATPase *in vitro*.

The enzyme extract containing 100 µl of protein was taken for testing the activity of H^+ K^+ ATPase in the presence of different concentrations (50–200 µl) of plant extracts. Plant extracts were incubated with H^+ K^+ ATPase for 30 min. Subsequently, reaction was carried out as described above. The results were expressed as percent inhibition of enzymatic activity at each concentration.

RESULTS ND DISSCUSSION

3.1 Qualitative phytochemical analysis

The preliminary qualitative phytochemical analysis revealed that the aerial parts of *T.indica* extracts showed the presence of carbohydrates, proteins, steroids, alkaloids, flavonoids, carbohydrates, tannins and phenols.

3.2 Acid neutralising capacity

Stomach's acidic interior is generated by stomach acid (especially 0.1M HCl). This acid is necessary for digestion but too much stomach acid can cause discomfort. One way of relieving

excess acidity in the stomach is to neutralise some of the acid with a weak base or antacid. Acid neutralising capacity (ANC) is a ability to neutralise acid inputs. The present study aimed at comparing ANC of various extracts of *T.indica* with that of standard ($Al(OH)_3$ and $Mg(OH)_2$). In this ethyl acetate and alcoholic extract showed good ANC when compared to standard.

Table 1: Acid neutralisation capacity of standard and various extracts

Sample	Acid neutralizing capacity (mEq. HCl)
$Al(OH)_3$ and $Mg(OH)_2$ (std)	25.4 ± 0.30
Petroleum ether extract	15.2 ± 0.31
Chloroform extract	18.7 ± 0.12
Ethyl acetate extract	23.6 ± 0.31
Alcoholic extract	22.1 ± 0.06

3.3 H^+ -K^+ ATPase inhibitory activity method

The human stomach is found with the numerous gastric pits from which acid get secrete. One of the cells, which lining the gastric pits is parietal cell, which is responsible for the acidification of stomach. The proton pump present in parietal cell is responsible for acid, which locates in the gastric membrane vesicle and actively transports protons into the lumen of stomach with the hydrolysis of the cytoplasmic ATP. Hyper secretion of this enzyme leads to acidity and ulcer. Therefore, this regulatory enzyme has found to be a pharmacological target to treat ulcer. In this study *in vitro* proton pump inhibition activity of various extracts of *T. indica* was carried out and it was evaluated by using sheep parietal cells where lansoprazole was used as the standard. IC50 values for different extracts were petroleum ether extract (194.8129µg/ml), chloroform extract (158.0429µg/ml), ethyl acetate extract (105.0729µg/ml), alcoholic extract (133.4629µg/ml). In this the ethyl acetate extract showed a high degree of proton pump inhibition in comparison with the standard (IC_{50} 84.29µg/ml).

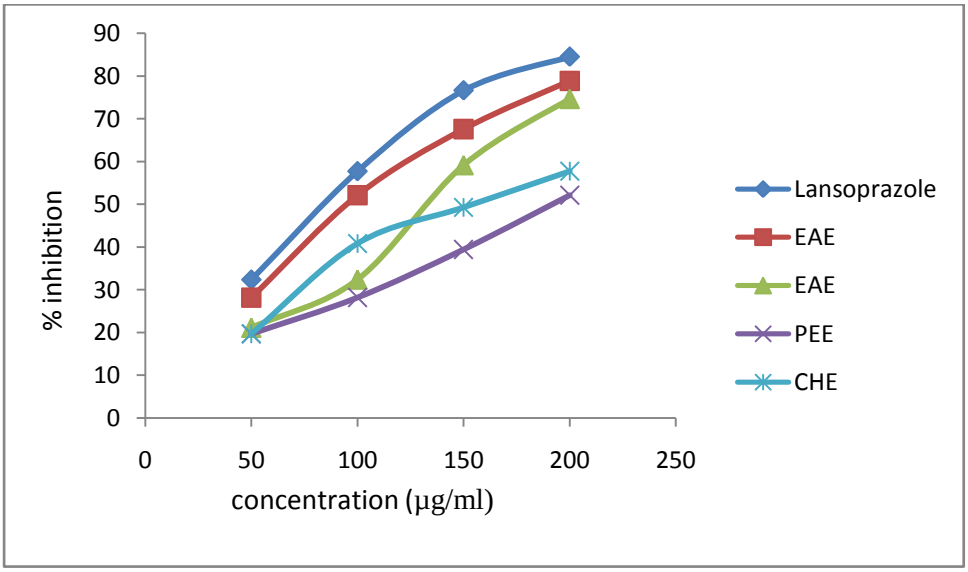

Figure 1: H+ K+ ATPase inhibitory activity of standard and various extracts

CONCLUSION

From the results obtained in the present study, it is concluded that the ethyl acetate extract of *Tylophora indica*, exhibited high antiulcer activitiy,due to the presence of phyotoconstituents like alkaloids, flavanoids, tannins, phenolics, carbohydrates. These *In-vitro* methods indicated that this plant extract is a significant source of natural antiulcer agent.

Furthermore, investigations are needed to provide some additional insight into the *in vivo* antiulcer activity of the plant with a view to obtaining useful antiulcer agents.

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