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ASSESSMENT OF *IN VITRO* ANTACID ACTIVITY OF DIFFERENT EXTRACTS OF *ABRUS PRECATORIUS* (L.) BY MODIFIED ARTIFICIAL STOMACH MODEL

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ABSTRACT

Objective: The purpose of present investigation is to rule out the antacid activity of *Abrus precatorius* leaves by *in vitro* method using a self fabricated modified artificial stomach model. The reason of selecting *in vitro* methods is to minimize the usage of Experimental animals. *Abrus precatorius* is a medicinal plant prevalent to found all throughout the plains of India, from Himalaya down to Southern India. This plant is used widely in folklore medicine for their antiulcer activity. **Material & Methods:** The preliminary chemical tests performed for the extracts showed the presence of alkaloids, tannins, flavonoids, rutin, glycosides, saponins, Steroides, carbohydrates and Glycyrrhizin as major Phytoconstituents. Then they were evaluated for the antacid potencies by working on the parameters like determination of pH of the prepared extracts, neutralizing effects on artificial gastric acids, duration of consistent neutralization effect on artificial gastric acids and neutralization capacity. Sodium bicarbonate and water were used as reference standard and control respectively. **Outcome:** The methanolic extract *A.precatorius* showed potent antacid activity when compared to standards. Hence *A. precatorius* can be considered as a potent substitute for the synthetic antacids that are well reputed for unwanted side effects like edema in the feet, alteration in systemic PH, bleaching etc. Further investigations on *in vivo* preclinical and clinical studies are yet to be performed.

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INTRODUCTION

The name *Abrus*, meaning beautiful or graceful, is used to describe the appearance of the seed [1]. Other names include prayer bean, Crab's eye, Indian liquorice, Ratti, jequerity pea, precatory beans, jumble beans, saga-saga, and lucky beans [2]. It belongs to the family Fabaceae. The Leaves resemble tamarind leaves having 20-40 leaflets and the plant is described as beautiful, much branched, slender, perennial, deciduous, woody, prickly twinning or climbing herb [3]. In the Ayurvedic medicine leaves of *A. precatorius* are laxative, expectorant and aphrodisiac medicines and are used in urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas/ leukemia and dysmenorrhoea[4]. *A. precatorius* leaves have also been used in Nigeria for the treatment of many diseases including malaria, typhoid, cough, respiratory tract infections and hepatitis [5]. The leaves are used as nerve tonic, applied on cuts and swellings and mouth ulcer [6] The phytoconstituents reported in leaves which are sweet in taste contain up to 10% Glycyrrhizin, triterpene glycosides, pinitol and alkaloids such as abrine, hepaphotine, choline and precatorine. The triterpene glycosides are abusosides A, B, and C, (which are highly sweet) and tree glycosides based on cycloartane-type aglycone, abrutigenin. Other compounds of the leaves are triterpenes abrusgenic acid, abruslactone A and methyl abrusgenate and flavonoids vitexin, liquiritigenin-7-mono- and diglycosides and toxifolin-3-glucosides [7].

Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. Several factors are implicated in the pathogenesis of gastric ulcer. These include increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer[8].

Acidity is a common GIT problem which is attributed to a functional disorder that can result due to a variety of reasons. Acidity is interconnected to heartburn and gas formation in stomach[9]. Many people use antacids for the relief of heartburns but these in turn can lead to discomforts. Most of the antacids are generally OTC drugs which are easily available. These medicaments directly neutralize the acid and raise the gastric pH. As commercially available antacids are reported with lot of side effects, therefore herbal drugs with potent antacid activity would be a better choice. Although there are a number of antacids and anti ulcer drugs, most of these have limitations, side effects and drug interactions. Herbal medicines generally deal with plants and its extracts for treatment of various ailments. These are usually considered to be safer and with no side effects.

The reason for selecting *Abrus precatorius* for the antacid activity is mainly the traditional claim/folklore claim of Gujarat more over the plant is widely available throughout the state and eastern India. The experimentation was carried out by using a self fabricated modified artificial stomach model. Using this method we have screened the effect of temperature on pH, neutralization effect, duration of neutralization and neutralization capacity. The present investigation results that Antacid activity of *Abrus precatorius* in methanolic extract is more potent than ethyl acetate and chloroform extract.

MATERIALS AND METHODS

Plant materials

The plant specimen for the proposed study was collected from Maligoan region, Guwahati India. It was identified and authenticated by Department of Botany, Guwahati university, Assam. A herbarium specimen (RO/PH-2017-Abrus) was prepared and deposited in the ROFEL, Shri G.M.B college of Pharmacy for further reference.

Extraction and preliminary chemical tests

The dried leaf powder of the plant weighing about 500g were taken and extracted with chloroform, ethyl acetate and methanol at 40-45°C 50hrs. The extracts were then collected and concentrated under vacuum and their percentage yields were calculated. They were then subjected to preliminary phytochemical screening. The 100mg and 250mg of all the above extracts were weighed and dissolved in 5ml of methanol and made upto 250 ml with distilled water.

Chemicals and reagents

The standard drugs Glycyrrhizin and Rutin, used for the thin layer chromatographic analysis were procured from Ozone^R International, Mumbai, India. Pepsin was purchased from HI Media Laboratories PVT. LTD, Hyderabad, India. The active control Sodium bicarbonate was purchased from Virat Labs, Mumbai, India. All the other chemicals and reagents used in this study were of analytical grades.

Thin Layer Chromatography Analysis

A thin layer chromatography was performed by using Glycyrrhizin and Rutin as the standard reference drug so as to identify the presence of these chemical constituents. The solvent system used was Petroleum ether: Methanol: Benzene and the detection system was U.V lamp. The TLC was performed on Pre-coated plates (silica gel 60 F254)[10]

Fabrication and Validation of artificial stomach model

To simulate the gastric conditions for conducting antacid studies, we have indigenously designed, fabricated and validated an artificial stomach model[11]. The model consists of two separate units; reservoir unit [R] and artificial stomach unit [S]. R consists of a temporary reservoir for the simulated gastric juice and unit S maintains to 90 ml of gastric juice. Separate provisions were made for checking the pH and for the aerator. Influxes to S and out flux from S were maintained by the valves provided. Gastric juice of pH 1.2 was prepared and reserved in the artificial stomach model for 24 hrs to check the variation in the pH. The same gastric juice was kept in a beaker under the same conditions as control.

Aerator by peristaltic pump was used to mimic the peristaltic movement. To determine the rate of air bubbles, first dissolution of paracetamol tablets were conducted by USP dissolution apparatus paddle type as per Pharmacopoeia. We then performed the experiment with artificial stomach by conducting several trials of various rate of air bubble per minute. This was to determine the rate of air bubble per minute of artificial stomach which gave matching results as that of dissolution apparatus. Aliquot volume of drug sample was withdrawn and fresh quantity of gastric juice was simultaneously replaced to maintain the sink conditions. Drug concentration was determined spectrophotometrically. We found that 136 air bubbles/ minute of the artificial stomach model gave matching results to 50 rpm of dissolution for a paracetamol tablet[12]. Further we have proceeded to maintain the contents of artificial stomach at 37°C. To achieve this, the level of reservoir was kept constant and was maintained at 38°C. This was done as a part of validation of artificial stomach. Now the artificial stomach was ready for conducting antacid experiments.

Preparation of extracts

The 100mg and 250mg of chloroform, ethyl acetate and methanol extracts were weighed and dissolved in 5 ml methanol and then made up to 250ml with distilled water.

Preparation of artificial gastric juice

Two grams of salt and 3.2 mg of pepsin enzymes were dissolved in 500 ml of water. Then 7.0ml hydrochloric acid and adequate water were added to make a 1000ml solution of the artificial gastric acid at pH 1.20. Sodium chloride weighing 9g was dissolved in adequate water to make 1000ml of saline [11].

Determination of pH of the prepared extracts

About 90ml of the prepared extracts were used for the pH determination at temperatures ranging from 25°C -37°C. The pH values of the control solution were also determined.

Determination of the neutralizing effects on artificial gastric acids

Test solution of 90 ml each were added to 100ml artificial gastric juices at pH 1.2. The pH values were determined to examine the neutralizing effect[11].

Modified Vatie's artificial stomach model for determination of the duration of consistent neutralization effect on artificial gastric acids

Test sample of 90 ml each was added to 100ml of artificial gastric juice at pH 1.2 maintained at 37°C. Aeration was given at 136 air bubbles per minute. Artificial juice was pumped at 3ml per minute into the stomach model and drained at the same rate. A pH meter was attached to monitor the pH changes. The duration of neutralization effects was determined when the pH was returned to 1.2 which was the initial value [11].

In vitro titration method of Fordtran's model for determination of the neutralization capacity

About 90 ml of test sample was placed in a 250ml beaker and warmed to 37°C and aeration was given at 136 air bubbles per minute to imitate the peristaltic movements. The test samples were titrated with the artificial gastric juice to obtain an end point of pH 3. The consumed volume (V) of artificial gastric juice was noted. The total consumed hydrogen ion (m mol) was measured as 0.06309 (m mol) xV (mL) [11].

Statistical Analysis

The statistical calculations were performed using the software Graph Pad InStat, version 3.05. The experimental data obtained were expressed as mean $\pm$ SEM where SEM=Standard error of Mean; Comparison between the groups were analyzed by One-way Analysis of Variance (ANOVA) using Dunnett Multiple Comparisons Test by considering Test Vs control. The differences were considered to be statistically significant when $**P<0.01$ where $n=6$.

RESULTS

Extraction and Thin Layer Chromatography Analysis

The % yield for successive solvent extraction from 100g of powder was found to be: ApC- 1.7 %w/w; ApE-0.795 % w/w; ApM- 5.4 %w/w. The preliminary chemical tests revealed the presence of the following phyto constituents for the different extracts:

ApC: Carbohydrates, Phenolic compounds, Glycosides, Flavonoids and Organic acids.

ApE: Phenolic compounds, Flavonoids, Glycosides and Steroids.

ApM: Carbohydrates, Alkaloids, Phenolic compounds, Glycosides, Flavonoids and Organic acids.

Determination of *in vitro* antacid activity by artificial stomach model

Effect of temperature on pH of the prepared extracts

The effect of temperature on pH showed that there was no much variation in the pH at temperature ranging from 25-37 °C (Table 2). We could observe only decimal point changes in the pH, whereby ApC 100 and 250mg exhibited a pH range of 6.21 – 6.40 and 6.45 – 6.55, ApE 100 and 250mg showed 5.57 – 5.68 and 5.32 – 5.44, ApM 100 and 250mg gave 6.02 – 6.0 and 6.9 – 6.8, SB 100 and 250mg showed 7.9 – 8.07 and 8.30 – 8.43 and water showed a pH of 6.8 – 7.5 respectively.

Neutralizing effects

The neutralizing effects of all the three extracts were studied for the two concentrations of each extract and standard sodium bicarbonate. All the values obtained were compared with that of the standard and the control. The results obtained envisage that the ApM at 100 and 250mg showed an excellent neutralizing effect by raising the pH of the artificial gastric juice from pH 1.2 to 1.72 ± 0.019 and 1.86 ± 0.014 , respectively. This outcome revealed that ApM has a profound neutralizing capacity than the standard SB at 100 and 250mg which showed a final pH of 1.78 ± 0.01 and 1.87 ± 0.01 , respectively. ApC and ApE at 250mg was able to increase the pH to 1.72 ± 0.01 and 1.78 ± 0.01 , respectively, which can also be considered as a better response since it was almost comparable with that of Standard.

Duration of neutralizing effect

The duration of neutralization was highest for ApM at 100 and 250mg which were found to be 88 ± 1.101 and 166 ± 1.21 minutes respectively. ApE at 100 and 250mg concentration also exhibited relatively similar duration of neutralizing effect of 78 ± 1.143 and 165 ± 1.22 minutes, whereas the standard SB at 100 and 250mg could give duration of only 75 ± 1.506 and 99 ± 1.504 minutes, respectively. These results revealed that ApM and ApE possess a substantial activity than the standard drug. The duration of neutralizing effect was found to be comparatively less for ApC at 100 and 250mg which could give 45 ± 1.148 and 120 ± 1.093 minutes, respectively. Hence it can be suggested that the possibility of a relapse in acidity will be delayed with ApM and ApE extracts (Table 2).

Neutralizing capacity

The volume of artificial gastric juice consumed to reach P^H 3 was noted for all the test compounds as well as control and standard. It was observed that ApM at 100 and 250mg was able to consume 19 ± 0.365 and 34.12 ± 0.41 mL of the artificial gastric juice where as SB at 100 and 250mg was able to put away only 11.45 ± 0.333 and 21.12 ± 0.322 mL, respectively. ApE and ApC at 250mg were able to consume 23.55 ± 0.333 , 24.55 ± 0.432 mL of artificial gastric juice which was less in comparison. The number of H^+ ions consumed by ApM at 100 and 250mg was determined to be 1.197 ± 0.021 and 2.142 ± 0.022 mmoles, whereas SB at the same concentration was found to be 0.723 ± 0.451 and 1.332 ± 0.022 mmoles, respectively. In the case of ApE and ApC at 250mg the number of H^+ ions consumed was observed to be 1.481 ± 0.021 and 1.542 ± 0.032 mmoles, respectively. (Table 2)

TABLE1: TLC profile for the four different extracts of *A. precatorius*.

SL.NO	EXTRACTS	No. of spots	Rf values	Colour of spots(day light)
1	Chloroform	10	0.96,0.95,0.85,0.81,0.76,0.64,0.52,0.32,0.24,0.15	Light brown
2	Ethyl acetate	11	0.85,0.80,0.78,0.68,0.62,0.50,0.42,0.33,0.25,0.20	Yellow to brown
3	Methanol	12	0.82,0.79,0.72,0.62,0.59,0.52,0.38,0.30,0.27,0.13	Light yellow
4	Glycyrrhizin	1	0.28	Light yellow
5	Rutin	1	0.58	Light brown

TABLE:2 *In vitro* antacid evaluation of different extracts of *A. precatorius*.

Sl.	Extract	P^H	Concentration In mg/250ml	Neutrlizing Effect	duration of neutrali tralization	Volume	H^+ ions Consumed
1	Methanol (ApM)	6.02-6.0 6.9-6.8	100 250	$1.72 \pm 0.019^{**}$ $1.86 \pm 0.014^{**}$	$88 \pm 1.101^{**}$ $166 \pm 0.21^{**}$	$19 \pm 0.365^{**}$ $34.12 \pm 0.41^{**}$	$1.197 \pm 0.021^{**}$ $2.142 \pm 0.022^{**}$
2	Chloroform (ApC)	6.21 - 6.40 6.45-6.55	100 250	$1.62 \pm 0.014^{**}$ $1.72 \pm 0.012^{**}$	$45 \pm 1.148^{**}$ $120 \pm 1.093^{**}$	$5.65 \pm 0.301^{**}$ $24.55 \pm 0.432^{**}$	$0.356 \pm 0.023^{**}$ $1.542 \pm 0.032^{**}$
3	Ethyl acetate (ApE)	5.57-5.68 5.32-5.44	100 250	$1.64 \pm 0.014^{**}$ $1.78 \pm 0.012^{**}$	$78 \pm 1.143^{**}$ $165 \pm 1.222^{**}$	$9.23 \pm 0.211^{**}$ $23.55 \pm 0.333^{**}$	$0.582 \pm 0.009^{**}$ $1.481 \pm 0.021^{**}$
4	NaHCO ₃ (SB) (Standard)	7.9-8.07 8.3-8.43	100 250	$1.78 \pm 0.01^{**}$ $1.87 \pm 0.01^{**}$	$75 \pm 1.506^{**}$ $99 \pm 1.504^{**}$	$11.45 \pm 0.333^{**}$ $21.12 \pm 0.322^{**}$	$0.723 \pm 0.451^{**}$ $1.332 \pm 0.022^{**}$
5	Water (Control)	6.8-7.5	250	$1.32 \pm 0.012^{**}$	$93 \pm 1.13^{**}$	$1.23 \pm 0.0801^{**}$	$0.077 \pm 0.004^{**}$

Results are expressed as mean SEM; Dunnett Multiple Comparisons Test shows that differences were considered to be statistically significant when $P \leq 0.01$

DISCUSSION

The antiulcer activity of the drug can be attributed to free-radical scavenging property, inhibition of acid secretory parameters, strengthening of gastric mucosal barrier, diminishing in vascular permeability and MDA content, improving the cytoprotective mechanisms of the gastric mucosa, elevating the level of glutathione etc. The different way of mechanism of ulcer inhibition may be occurred due to presence of important phytoconstituents in respective plant extracts. A variety of botanical substances have been reported to possess antiulcer activity.[12]

Current stalemates of modern medicine in the management of various ailments incline research tendencies to traditional medicine. In this respect, traditional medicine has introduced good protocols for treatment of various gastrointestinal disorders. Chemical substances derived from plants have been used to treat human diseases since the dawn of medicine. Roughly 50% of new chemical entities introduced during the past two decades are from natural products. Recent technological advances have renewed interest in natural products in drug discovery. Therefore, efforts should be directed towards isolation and characterization of the active principles and elucidation of the relationship between structure and activity. There are various medicinal plants and their extracts (containing active chemical constituents, e.g., tannins and flavonoids) that have significant antiulcer activity *in vivo* experiments on animal models.

In the present research investigation we have conducted a comparative evaluation of the preliminary study *in vitro* antacid activity of *Abrus Precatorius*, traditionally used in ulcer. The work was performed by using a self-fabricated artificial stomach model. In this research we have extracted the leaf of the plants with methanol and then subjected to preliminary chemical tests which revealed the presence of chemical constituents including alkaloids, steroids and other triterpenoids, isoflavanoquinones, anthocyanins, starch, tannin, protein, flavanoids, phenolic compound, fixed oil, amino acid and the flavones luteolin, abrectorin, orientin, isoorientin and desmethoxycentaureidin 7-O-rutinoside.[13]

It is well documented that while synthetic antacids have a lot of side effects and drug interactions, the herbal drugs have fewer side effects which has to be identified as an alternative for the treatment of hyperacidity. We had used synthetic antacid sodium bicarbonate as standard. It was observed that, all three solvent extracts and standard reported to show a stable pH at altered temperatures, which confirms that temperature does not affect the pH. The *in vitro* antacid activity screened here exhibits a potent activity for all the extracts. When compared with the water all the treatments including ApC, ApM, ApE and SB were shown to possess significant gastric acid neutralizing effects. With regard to the duration for consistent neutralization of gastric acid, the neutralization duration of ApC, ApM, ApE were significantly longer than that of water. Also, SB, ApC, ApM, ApE exhibited significant antacid capacities compared to water. From our investigation we observed that ApM possessed the highest activity.

Apparently it was observed that the antacid activity was attributed by the polar solvent extracts than the non polar solvent extracts, which can be justified by pointing out that the bioactive compounds are present in the polar solvents than in the non polar solvents. Hence pattern of antacid potential can be quoted as ApM > ApE > ApC.

The titration methods involved in this work were the Fordtran's model and the modified model of Vatie's artificial stomach, which mimic the regular physiological functioning of human stomach. The duration of neutralization was found to be highest for ApM-250mg, when compared with the standard as well as ApE and ApC. This reveals that ApM is able to neutralize the acid secreted for longer time than the synthetic antacid used in this evaluation.

Sodium bicarbonate acts instantaneously but the duration is short. It is a potent neutralizer but has several disadvantages like larger doses will induce alkalosis, produces carbon dioxide in stomach which lead to distension, discomfort, belching and risk of ulcer perforation. It may increase Na⁺ ions level which may worsen edema and CHF. Na citrate too has similar side effects like NaHCO₃. But this does not evolve CO₂.

In conclusion, the results obtained shows that the leaf extracts of *Abrus precatorius* are consistently active in the self fabricated and validated modified artificial stomach model. The TLC standardization confirmed the presence of Glycyrrhizin and Rutin in the extracts. Hence it can be assumed that these bioflavonoids may be responsible for significant antacid activity. As the plant extracts contain other chemical entities too. Hence, further research is needed to identify the exact chemical constituents as well as the mechanism of antacid activity in different models.

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Conflicts of interest statement

We herewith declare that we have no conflict of interest.

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