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Assessment of *in vitro* Antacid Activity of Different Root Extracts of *Tephrosia purpurea* (L) Pers by Modified Artificial Stomach Model

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ABSTRACT

Objective: The purpose of the present investigation is to rule out the antacid activity of *Tephrosia purpurea* root by *in vitro* method using a self fabricated and modified artificial stomach model. The reason for selecting *in vitro* method was to minimize the usage of experimental animals. **Method:** In this work we have used air bubbles from an aerator to mimic the peristaltic movements of stomach. Sodium bicarbonate and water were used as reference and control respectively. The parameters evaluated were neutralizing effect, duration of neutralization effect and capacity and effect of temperature on pH. We evaluated the potency of the plant by extracting with three solvents in increasing order of polarity. **Results:** The neutralization effect, duration of neutralization effect and capacity were found to be higher for methanol root extract than sodium bicarbonate, whereas ethyl acetate and chloroform extract produced moderately good response but less when compared to standard drug but higher than water. **Conclusion:** Hence this plant can be an effective alternative for sodium bicarbonate which is reported to have side effects like edema in the feet, alteration in systemic pH, belching etc.

1. Introduction

Tephrosia purpurea (L) Pers. (Fabaceae) is a pan tropical coastal shrub and is also known as ahuhu auhola, or hola^[1, 2]. In Sanskrit it is known as ‘Sharpunkha’ and in Ayurvedic literature it is given the name ‘Sarwa wra n vishapaha’ which means it has the power to cure all kinds of wounds^[3–5]. The roots of the plant are used in the treatment of various ailments like dyspepsia, diabetes, rheumatism, asthma, diarrhea, urinary complaints and cough. The whole plant is used to treat ulcers, fever and liver disorders. The pods are used in vomiting and inflammation^[6]. The phytochemical investigations on this plant have shown the presence of coumarins, flavonoids and rotenoids, flavanones, isoflavanones and quercetin^[7–13]. The toxic properties of *T. purpurea* are due to the presence of flavonoids, rotenone and several of its isomers named deguelins. One of the deguelins, tephrosin, is poisonous to fish, but not to mammals. The leaves contain up to 2.5% rutin^[14].

Acidity is a common gastrointestinal disease which may

not be necessarily caused by a pathogenic infection. It is attributed to a functional disorder that can result due to a variety of reasons which is related to heartburn and gas formation in stomach. In acidity, gastro esophageal reflux disease which is commonly known as urdhva gata amalpitta in Ayurveda, there is a movement of gastric juices from the stomach into the lower esophagus^[15]. This is a condition which occurs when acidic contents in stomach move upward into the esophagus and make it dysfunctional. Gastric acid is a digestive fluid formed in the stomach having a pH of 1 to 2. It is a mixture of hydrochloric acid, large quantities of potassium chloride and sodium chloride. The acid in stomach plays a significant role in the digestion of proteins, by activating digestive enzymes and making ingested proteins unravel so that digestive enzymes can break down the long chain amino acids. General symptoms found in children are respiratory problems, inadequate weight, vomiting, coughing and turning down food. The symptoms shown by adults include long heartburn, chest and stomach pain, gas formation in stomach, inflammation in chest; gastro esophageal reflux, voice change and formation of ulcer in esophagus, pain during muscular contractions and pain in ears are some of the symptoms of acidity^[16–19]. Although there are a number of antacids and anti ulcer drugs, most of these have limitations, side effects and drug

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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 [20].

Tephrosia purpurea is used in various Ayurvedic preparations for its potent healing power. Deshpande.S.S et al has scientifically proved the anti ulcer property of the aqueous extract of root plant on rodents [3]. In our present investigation we have evaluated the antacid activity of the root by extracting with non polar solvents. The reason for selecting *T.purpurea* for the antacid activity is mainly the traditional claim/folklore claim of Andhra Pradesh more over the plant is widely available throughout the state. 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.

2. Materials and Methods

2.1. Plant materials

The plant specimen for the proposed study was collected from Talakona region, Thirupathy in Andhra Pradesh, South India. It was identified and authenticated by Dr.Madhava Chetty, Taxonomist, S.V University, Thirupathy. A herbarium specimen (NCOP–NLG/ph'cog/2009–10/011) was prepared and deposited in the college for further reference.

2.2. Chemicals and reagents

The standard drugs Quercetin and Rutin used for the thin layer chromatographic analysis were procured from Yucca Enterprises, 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.

2.3. Extraction and Thin Layer Chromatography Analysis

The dried root powder weighing about 500g were taken and subjected to soxhlet extraction using solvents with an increasing order of polarity. The selected solvents were chloroform, ethyl acetate and methanol. The temperature was set at 35–40°C and the extraction was performed for almost 50hrs. The different extracts were collected and concentrated under vacuum to obtain a dried extract and their percentage yields were calculated. The crude extracts were exposed to preliminary phytochemical screening to identify the primary and secondary metabolites present in the extract[21,22]. A thin layer chromatography was performed by using Quercetin and Rutin as the standard reference drug so as to identify the presence of these chemical constituents. The solvent system used was Toluene: ethyl acetate: acetic acid (30:40:5) and the detection system was U.V lamp. The TLC was performed on Pre-coated plates (silica gel 60 F254)

[23,24].

2.4. 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. The model consists of two separate units; reservoir unit [R] and artificial stomach unit [S] [25]. 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.

Validation of artificial stomach was done by comparing with USP dissolution test apparatus (paddle type, 50 rpm, phosphate buffer 5.8 for 30 mts)using paracetamol tablet as per USP/NF. 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 at λ max 243 nm. We then performed the experiment with artificial stomach by conducting several trials of various rate of air bubble per minute. This was to determine at what rate of air bubble per minute of artificial stomach gave matching results as that of dissolution apparatus. We found that 136 air bubbles/minute of the artificial stomach model gave matching results to 50 rpm of dissolution for a paracetamol tablet [26]. 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. Now the artificial stomach was ready for conducting antacid experiments.

2.5. 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.

2.6. 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 [25].

2.6.1/ 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 [25].

2.6.2. 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 [25].

2.6.3. Modified Vatier'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 [25].

2.6.4. 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 $0.06309 \text{ (m mol)} \times V \text{ (mL)}$ [25].

2.7. 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 $n=6$.

3. Results

3.1 Extraction and Thin Layer Chromatography Analysis

The % yield for successive solvent extraction from 70g of powder was found to be: TpC– 1.7 %w/w; TpE–0.795 % w/w; TpM– 5.4 %w/w. The preliminary chemical tests revealed the presence of the following phyto constituents for the different extracts:

TpC: Carbohydrates, Phenolic compounds, Glycosides, Flavonoids and Organic acids

TpE: Phenolic compounds, Flavonoids, Glycosides and Steroids.

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

From the TLC studies we could predict that rutin and

quercetin were present in the extracts. Among the three extracts Rf values of chloroform and methanolic extracts were complying with that of the standard drug quercetin (0.602). Further, one spot in the ethyl acetate extract has shown the same Rf value 0.58 of standard drug rutin. Additionally, colour of the spots obtained were found almost similar to that of the reference drugs. We observed slight changes in the colours and Rf values of spots obtained from all extracts when compared to the reference drugs. Hence our assumption is that the spots obtained may be the derivatives of rutin and quercetin (Table 1).

3.2. Determination of in vitro antacid activity by artificial stomach model

3.2.1. 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 TpC 100 and 250mg exhibited a pH range of 6.41 – 6.49 and 5.6 – 5.72, TpE 100 and 250mg showed 4.57 – 4.68 and 4.32 – 4.43, TpM 100 and 250mg gave 6.02 – 6.11 and 5.2 – 5.28, SB 100 and 250mg showed 7.93 – 8.07 and 8.25 – 8.36 and water showed a pH of 6.82 – 7.52 respectively.

3.2.2. 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 TpM at 100 and 250mg showed an excellent neutralizing effect by raising the pH of the artificial gastric juice from pH 1.2 to 1.73 ± 0.0126 and 1.95 ± 0.0114 , respectively. This outcome revealed that TpM has a profound neutralizing capacity than the standard SB at 100 and 250mg which showed a final pH of 1.65 ± 0.01 and 1.81 ± 0.01 , respectively. TpC and TpE at 250mg was able to increase the pH to 1.72 ± 0.01 and 1.79 ± 0.01 , respectively, which can also be considered as a better response since it was almost comparable with that of SB (Table 2).

3.2.3. Duration of neutralizing effect

The duration of neutralization was highest for TpM at 100 and 250mg which were found to be 90 ± 1.108 and 170 ± 1.447 minutes respectively. TpE at 100 and 250mg concentration also exhibited relatively similar duration of neutralizing effect of 80 ± 1.430 and 170 ± 1.333 minutes, whereas the standard SB at 100 and 250mg could give duration of only 65 ± 1.054 and 100 ± 1.506 minutes, respectively. These results revealed that TpM and TpE possess a substantial activity than the standard drug. The duration of neutralizing effect was found to be comparatively less for TpC at 100 and 250mg which could give 55 ± 1.483 and 125 ± 1.095 minutes, respectively. Hence it can be suggested that the possibility of a relapse in acidity will be delayed with TpM and TpE extracts (Table 2).

Table 1
TLC profile for the four different extracts of *T. purpurea* Pers.

S.NO.	Extract	no. of spots	Colour of the spots			Rf values
			D.L.	S.W.	L.W.	
1	Chloroform	7	Brown	Brown	Whitefluorescence	0.51
			Brown	Blue	Whitefluorescence	0.602
			LightBrown	Bluish brown	Whitefluorescence	0.66
			Yellow	Brownishblue	Whitefluorescence	0.75
			Brown	Blue	Whitefluorescence	0.82
			Brown	Blue	Whitefluorescence	0.87
			Light Brown	Blue	Whitefluorescence	0.92
2	Ethyl acetate	6	Brown	Brown	Bluefluorescence	0.3
			Yellow	Light blue	lightbluefluorescence	0.525
			Brown	Brownishblue	Whitefluorescence	0.58
			Yellow	Blue	Whitefluorescence	0.64
			Yellow	Blue	Whitefluorescence	0.74
			Yellow	Blue	Whitefluorescence	0.85
3	Methanol	6	Yellow	Brown	Bluefluorescence	0.3
			Brown	Brown	Whitefluorescence	0.54
			Yellow	Brownishblue	Whitefluorescence	0.602
			Lightbrown	Blue	Lightbluefluorescent	0.7
			Lightyellow	Lightblue	Lightfluorescence	0.77
			Lightyellow	Lightblue	Lightfluorence	0.83
			Lightyellow	Lightblue	Lightfluorence	0.83
4	Quercetin	1	Brown	Brown	Bluefluorescence	0.602
5	Rutin	1	Lightbrown	Lightbrown	Lightblue	0.58

Table 2
Invitro antacid evaluation of three extracts of *T.purpurea* Pers L.

S.No	Extract	Concentration mg/250ml	Initial Temperature effect on pH25°C – 37°C	Neutralisation Efficiency	Duration in minutes	Action efficiency(Amount of AGJ consumed)(mL)	No. ofH ⁺ ions consumedmmoles
1	Methanol (TpM)	100	6.02 – 6.11	1.73±0.0126**	90±1.108	19±0.365**	1.1987±0.023**
		250	5.2 – 5.28	1.95±0.0114**	170±1.447**	33.17±0.401**	2.0925±0.025**
2	Chloroform (TpC)	100	6.41 – 6.49	1.55±0.013**	5±1.483**	7.67±0.333**	0.5564±0.016**
		250	5.6 – 5.72	1.72±0.01**	125±1.095**	16.67±0.422**	1.0501±0.026**
3	Ethyl acetate (TpE)	100	4.57 – 4.68	1.58±0.01**	80±1.430**	9.33±0.211**	0.6016±.009**
		250	4.32 – 4.43	1.79±0.01**	170±1.333**	20.67±0.333**	1.3038±0.021**
4	NaHCO ₃ (SB)(Standard)	100	7.93 – 8.07	1.65±0.01**	65±1.054**	17.67±0.333**	1.1500±0.0463**
		250	8.25 – 8.36	1.81±0.01**	100±1.506**	21.33±0.333**	1.3454±0.020**
5	Water(Control)	250	6.82 – 7.52	1.38±0.01	92±1.317	1.33± 0.080	0.08412±0.005

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

3.2.4. Neutralizing capacity

The volume of artificial gastric juice consumed to reach pH 3 was noted for all the test compounds as well as control and standard. It was observed that TpM at 100 and 250mg was able to consume 19±0.365 and 33.17±0.401mL of the artificial gastric juice where as SB at 100 and 250mg was able to put away only 17.67±0.333 and 21.33±0.333mL, respectively. TpE and TpC at 250mg were able to consume 20.67±0.333 16.67±0.422mL of artificial gastric juice which was less in comparison. The number of H⁺ ions consumed by TpM at 100 and 250mg was determined to be 1.1987±0.023 and 2.0925±0.025 mmoles, whereas SB at the same concentration was found to be 1.1500±0.0463 and 1.3454±0.020 mmoles, respectively. In the case of TpE and TpC at 250mg the number of H⁺ ions consumed was observed to be 1.3038±0.021 and 1.0501±0.026 mmoles, respectively (Table 2).

4. Discussion

Stomach is an organ which undergoes propulsion, mixing of food, digestion and absorption of food along with the secretory functions. The parietal cells of the stomach secrete about 2500mL of the gastric juice daily. The acid in this gastric juice kills many bacteria and provide a low pH for pepsin to start protein digestion. Mucosal erosions or ulcerations take place when aggressive factors overwhelm the defensive factors of the gastrointestinal mucosa [27, 28]. This leads to the arrival of gastritis, peptic ulcer and gastro esophageal reflux disease [27]. The main aggressive factors well established for several decades are acid and pepsin. Hence peptic ulcer diseases are mostly treated with antacids, H₂ receptor antagonists and proton pump inhibitors. Amongst these, antacids have been widely used in the treatment of ulcer.

Antacids are generally inorganic salts which dissolve in acid gastric secretions which release anions that partially neutralize gastric hydrochloric acid. They generally react chemically to neutralize or buffer the existing quantities of stomach acid but do not have direct effect on its output. This action results in increased pH value of stomach contents and thus provide relief of hyperacidity symptoms. These medications also reduce acid concentration within the lumen of the esophagus which causes an increase in intra-esophageal pH and a decrease in pepsin activity [29]. These medicaments do not decrease the volume of gastric secretions. Most of the antacids available in the market are efficient but is often unacceptable because of the common side effects, especially altered bowel functions. Antacids that contain Aluminum contribute aluminum to the diet but may cause constipation or lead to phosphorous deficiency where as on long term or inappropriate use can lead to Aluminum toxicity. Calcium containing antacids contribute calcium to diet and may produce constipation. Magnesium containing antacids contribute magnesium to diet and may produce a side effect of diarrhea on prolonged use may even lead to magnesium toxicity [30]. It is reported that SB should be avoided even though it is a potent neutralizer of acid as it contains significant amounts of sodium and may alter the systemic pH. One major fact that should be considered while selecting an antacid for the treatment is its drug interactions. Significant interactions occur with quinolone antibiotics, tetracycline and iron sulfate [25]. Hence considering the side effects and drug interactions of antacids, the herbal drugs having fewer side effects should be identified as an alternative for the treatment of peptic ulcers. It is widely understood that herbal medicines have recently generated an increased interest in the treatment of gastritis. Hence in our present study we had applied the titration method of Fordtran's model and the modified model of Vatie's artificial stomach, which mimic the regular physiological functioning of a human stomach, to explore the antacid effects of the less polar extracts of *T. purpurea* which is well known for its potency to cure all types of wounds. In this investigation we have extracted the roots of *T. purpurea* using organic solvents and from the preliminary chemical tests we observed that all the three extracts contain phenolics and flavonoids as one of the major constituents along with other constituents. The TLC analysis has proved the presence of rutin and quercetin in these extract. The in vitro antacid activity screened here exhibits a potent activity for all the extracts. When compared with the water all the treatments including TpC, TpM, TpE 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 TpC, TpM, TpE were significantly longer than that of water. Also, SB, TpC, TpM, TpE exhibited significant antacid capacities compared to water. From our investigation we observed that TpM possessed the highest activity, which was found to be more profound than the standard SB. 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 our finding can be quoted as TpM >TpE >TpC.

Flavonoids comprise a vast array of biologically active compounds that are ubiquitous in plants, many of which have been used in traditional eastern medicine for thousands of years [31]. There is a growing belief that some of the flavonoids in particular quercetin, rutin hesperidin etc are claimed to benefit in treating as well as giving protection against various kinds of health problems. Substances such as flavonoids, aescin, aloe gel and many others, that possess antiulcer activity are of particular therapeutic importance as most of the anti-inflammatory drugs used in modern medicine are ulcerogenic [32,33]. Some recent reports showed that flavonoids have anti-ulcer activity. Oral treatment with flavonoid extract showed a good gastric protection. These compounds also increased mucous content, proteins and hexamines. It was reported that Quercetin and Rutin administered intraperitoneally inhibited dose dependent gastric damage in rodents. These bioflavonoids have an inhibitory effect on the intestinal secretions mediated through the alpha Adrenergic and Calcium system hence beneficial in the ulcer treatment [34].

In conclusion, the results obtained shows that the root extracts of *T. purpurea* are consistently active in the self fabricated and validated modified artificial stomach model. The TLC standardization confirmed the presence of Quercetin and Rutin in the extracts. Hence it can be assumed that these bioflavonoids may be responsible for significant antacid activity. The exact chemical reaction of flavonoids and hydrochloric acid is not yet understood, hence further studies is mandatory in these areas of research. TpM has exhibited a better antacid response than the standard SB which is reported to have side effects, thus it can be suggested that *T. purpurea* can be very well used as an alternative for the synthetic or commercially available antacids in the market. As the plant extracts contain other chemical entities further detailed research has to be performed to identify the exact chemical as well as the mechanism of antacid activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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