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A COMPARATIVE EVALUATION OF *IN VITRO* ANTACID ACTIVITY OF TWO *Tephrosia* SPECIES USING MODIFIED ARTIFICIAL STOMACH MODEL.

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Key words: Artificial gastric juice, Antacid, Flavonoids, Fordtran's model, Vatier's artificial stomach model.

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ABSTRACT

Plan: *The methanol extracts of Tephrosia calophylla and Tephrosia maxima (Fabaceae) roots were assessed for the antacid activity by using self-fabricated modified artificial stomach model.*

Preface*: Tephrosia calophylla and Tephrosia maxima are two medicinal plants prevalent to Andhra Pradesh, Karnataka and Tamil Nadu. These two plants are used widely in folklore medicine for their antiulcer activity.*

Methodology*: The preliminary chemical tests performed for the extracts showed the presence of tannins, flavonoids, glycosides, saponins and carbohydrates. 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. Two commercially available antacids were used as the standard.*

Outcome: *The methanolic extract T.maxima showed potent antacid activity when compared to T.calophylla and the two standards. Hence T.maxima can be considered as a potent substitute for the synthetic antacids that are well reputed for unwanted side effects. Further investigations on in vivo preclinical and clinical studies are yet to be performed.*

1. INTRODUCTION

Gastro intestinal tract is one of the major endocrine systems in the human body and is also the site of many common pathologies including simple dyspepsia to Crohn's disease. Medicines used for the treatment of GIT disorders comprise some 8% of total prescriptions.

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The stomach secretes about 2.5 L of gastric juice per day. The mucus secreting cells secrete bicarbonate ions which are trapped in the mucus and form a gel like protective barrier that maintains the mucosal surface at pH of 6-7. Disturbances in these secretary and protective mechanisms usually end up in pathogenesis. 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[1]. 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. *Tephrosia calophylla* and *Tephrosia maxima* are two important medicinal plants which are endemic to Andhra Pradesh, Karnataka and Tamil Nadu. *Tephrosia calophylla* BEDD is a perennial under shrub with pink flowers in terminal racemes[2]. Phytoconstituents reported to be isolated from the whole plant of *Tephrosia calophylla* are tephcalostan, 7-*O*- methylglabranin and kaempferol-3-*O*-b -D-glucopyranoside .Two flavonones namely (2S)-5-hydroxy-7,4'-di-O-(gamma,gamma-dimethylallyl)flavanone and 6-hydroxy-E-3-(2,5-dimethoxybenzylidine)-2',5'-dimethoxyflavanone; tephrowatsin C, afrormosin , a benzil, calophione. A and three coumestan derivatives like tephcalostan B, C and D were also reported to be isolated from the roots of *Tephrosia calophylla*[3-5].

Tephrosia maxima or *Galega maxima* L is a prostate herb with pink flowers arranged in leaf opposed pseudoracemes. The plant is distributed in wastelands and dry deciduous forests[2]. Maxima isoflavone H and 7, 8-methylenedioxyisoflavone were isolated from *Tephrosia maxima* along with the known isoflavone, maxima isoflavone B[28]. Another flavonone, maxima flavonone A was also isolated from the chloroform extract of the roots of *Tephrosia maxima* along with maxima isoflavone J and T [6, 7].

In the current research investigation we have screened the antacid activity of these two medicinally important herbs which are generally used in the treatment of ulcer by the folklore of Andhra Pradesh of south India by comparing with commercially available antacids.

2. MATERIALS AND METHODS

Plant material: The plant specimens for the proposed study were collected from Talakona region of Andhra Pradesh, South India. It was identified and authenticated by Dr. Madhava Chetty, Taxonomist, S.V University, Thirupathy, Andhra Pradesh, India. Herbarium specimens were prepared in deposited in the Department of Pharmacognosy, Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh for future references. The voucher number obtained for the plant species were: *T. calophylla*- NCOP-NLG/ph'cog/2009-10/010); *T.maxima*-NCOP-NLG/ph'cog/2009-10/020)

2.1. Extraction and preliminary chemical tests

The dried root powder of both the plants weighing about 500g were taken and defatted with petroleum ether. The defatted root powder was then extracted with methanol at 40-45ºC 50hrs. The extracts were then collected and concentrated under vacuum and their percentage yields were calculated. They were then subjected to preliminary phytochemical screening[8-10].

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. 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.

2.2. Preparation of extracts

The 100mg and 250mg of methanol extracts of both the plants were weighed and dissolved in 250ml of distilled water. Two standards were used in the proposed study, in which the antacid-1 was sodium bicarbonate and antacid -2 was a combination of aluminium hydroxide and magnesium hydroxide.

2.3. 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].

2.4. 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[11].

2.5. 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[11,12].

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 37ºC. 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].

2.6. 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 37ºC 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 (mmol) was measured as 0.06309 (mmol) × V (ml)[11].

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 ± SEM. 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 $^{**}P<0.01$, $^{*}P<0.05$ and ns when $P> 0.05$.

3. RESULTS

3.1. Extraction and preliminary chemical tests

The preliminary chemical screening of the methanolic extract of both the plants revealed the presence of carbohydrate, tannins and flavonoids in common, whereas *T.maxima* showed the presence of saponins and glycosides in addition.

3.2. Determination of pH of the prepared extracts

The effect of temperature on pH was determined to rule out whether any changes in pH has occurred when the artificial gastric juice was subjected to room temperature and human body temperature. The results obtained showed that pH of all the extracts were stable (Table 1). The pH of TCM 100mg and 250mg at 25°C and 35°C was 5.02-5.09 and 6.1-6.2; TMM 100mg and 250mg was 5.7-5.85 and 6.01-6.12; A1 100 mg and 250mg was 7.93-8.07 and 8.25-8.36; A2 100 mg and 250mg was 7.2-7.35 and 7.51-7.86 and control showed a pH of 6.9-7.2.

3.3. Determination of the neutralizing effects on artificial gastric acids

TMM 100mg, 250mg and A1 250showed the best neutralizing effect which was found to be 1.828±0.014, 1.822± 0.014 and 1.811±0.01. It was observed that A2 100mg showed a poor neutralizing which was found to be statistically non-significant (Table 1& Figure 1).

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

The duration of consistent neutralization effect on artificial gastric acids performed by using modified Vatier's stomach model revealed that TMM- 250mg was the most potent antacid when compared to the TCM and both the standards A1 and A2, as it showed an excellent duration of neutralizing effect which was found to be 254.33±5.46 minutes (Table 1). A2 at 250mg showed the second best activity with duration of neutralization of 206.33±3.91minutes. TCM -100mg and A1- 100mg showed any significant activity. TMM-100mg, TCM-250mg, A1-250mg and A2-100mg showed moderate period of neutralization (Table 1& Figure 2).

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

The neutralization capacity performed by Fordtran's *in vitro* titration model revealed that TMM-250mg to possess a potent activity as it could neutralize 94.17±1.6mL of gastric acid where by 6.0124 ±0.1233 number of H+ ions were consumed. TMM-100mg was found to be the second best as it was able to neutralize 47.33±0.9098 mL of gastric juice and consume 3.3135±0.1468 number of H+ ions. The neutralization capacity of both the standard drug used were found to be less when compared to the test compounds (Table 1, Figure 3 & 4).

Table 1: Effect of various concentrations of TCM, TCC, TMM and TMC on invitro antacid activity by artificial gastric stomach model

Drug concentration	*pH*	*Neutralizing effect*	*Duration of neutralizing effect*	*volume*	*H+ ions consumed*
TCM-100mg	5.02-5.09	1.615±0.019**	60.67±2.5^{ns}	13.33±0.9545*	0.79367±0.0153*
TCM-250mg	6.1-6.2	1.665±0.014**	134.17± 2.70**	29.5±0.6191**	1.847 ±0.0388**
TCC 100mg	5.1-5.3	1.45	40	5	0.3155
TCC 250mg	5.9-6.1	1.6	100	12	0.7571
TMM-100mg	5.7-5.85	1.828±0.014**	189.67±3.66**	47.33±0.9098**	3.3135±0.1468**
TMM-250mg	6.01-6.12	1.822± 0.014 **	254.33±5.46 **	94.17±1.600 **	6.0124 ±0.1233**
TMC 100mg	6.15-6.20	1.6	95	16	1.009
TMC 250mg	5.9-5.82	1.65	160	45	2.839
TPM 100mg	6.02-6.11	1.73	90	18	1.136
TPM 250mg	5.2-5.8	1.95	170	34	2.145
TPC 100mg	6.41-6.4	1.55	55	7	0.442
TPC 250mg	5.6-5.72	1.72	125	17	1.07
A1-100mg	7.93-8.07	1.65±0.01**	65±1.054 ^{ns}	17.67±0.333**	1.0924±0.0602**
A1-250mg	8.25-8.36	1.811±0.01**	100±1.506**	21.33±0.333**	1.331±0.0761**
A2-100mg	7.2-7.35	1.457±0.015^{ns}	152.83±2.34**	3.780±0.6420^{ns}	0.1419±0.0073^{ns}
A2-250mg	7.51-7.86	1.543±0.015**	206.33±3.91**	10.00±0.577*	0.6444±0.00721*
Control (water)	6.9-7.2	1.468 ± 0.005	66.098±1.317	7.667±0.6146	0.5082±0.0128

***P*<0.01and ns when *P*> 0.05**,** **P*<0.05 , ANOVA

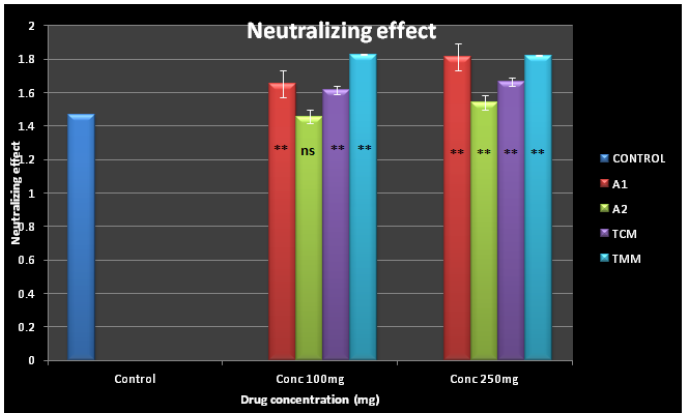

Figure 1: *T.maxima* and A1 at 250mg had shown the potent neutralizing effect when compared to all other samples. Control- water, A1- sodium bicarbonate, A2- combination of aluminium hydroxide and magnesium hydroxide, TCM-*Tephrosia calophylla* methanol extract and TMM-*Tephrosia maxima* methanol extract. Results were expressed as mean ± SEM. 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*<0.01and ns when *P*> 0.05.

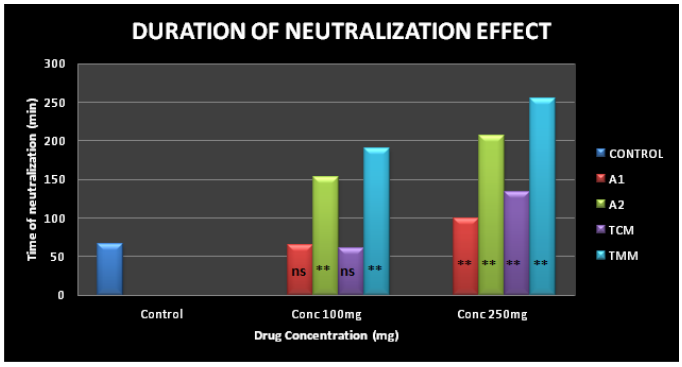

Figure 2: Duration of neutralization effect. *T.maxima* has exhibited more time in neutralizing the flow of gastric acid, which proved that it was able to neutralize more amount of the acid. Control- water, A1- sodium bicarbonate, A2- combination of aluminium hydroxide and magnesium hydroxide, TCM-*Tephrosia calophylla* methanol extract and TMM-*Tephrosia maxima* methanol extract. Results were expressed as mean ± SEM. 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 and ns when *P*> 0.05.

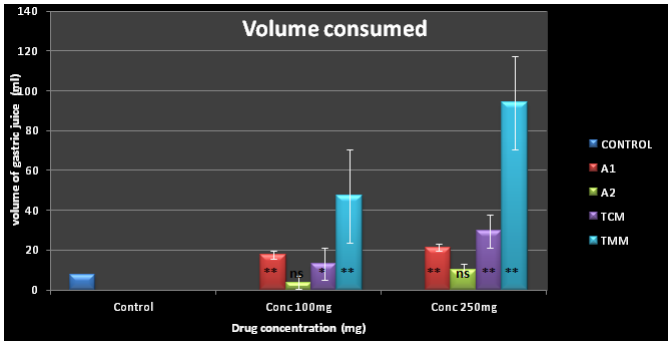

Figure 3: *T.maxima* was able to consume more volume of artificial gastric juice when compared to all other samples. Control- water, A1- sodium bicarbonate, A2- combination of aluminium hydroxide and magnesium hydroxide, TCM-*Tephrosia calophylla* methanol extract and TMM-*Tephrosia maxima* methanol extract. Results were expressed as mean ± SEM. 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, **P*<0.05 and ns when *P*> 0.05.

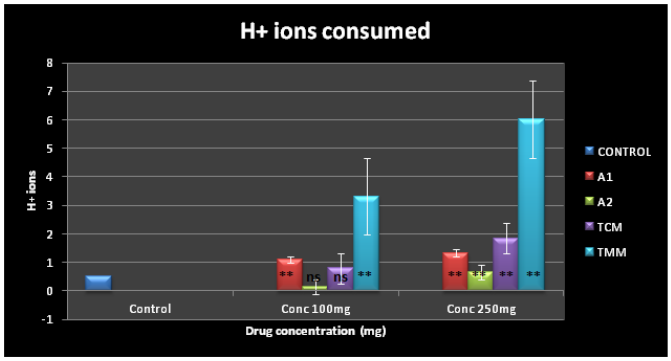

Figure 4: *T.maxima* was able to consume maximum number of H+ ions which confirms it to be a potent antacid than the rest of the samples tested. Control- water, A1- sodium bicarbonate, A2- combination of aluminium hydroxide and magnesium hydroxide, TCM-*Tephrosia calophylla* methanol extract and TMM-*Tephrosia maxima* methanol extract. Results were expressed as mean ± SEM. 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*<0.01and ns when *P*> 0.05.

4. DISCUSSION

Gastric hyperacidity and gastro duodenal ulcer are common problems and are usually developed when the balance between the gastro protective and aggressive factors are lost. The major aggressive factors involve acid, pepsin, *H.pylori* and bile salts. The defence factors involved are mucus bicarbonate secretion and prostaglandins[14]. Hyper secretion of acid is a condition in which uncontrolled release of hydrochloric acid from the parietal cells of the mucosa take place through the proton pumping.

In the present research investigation we have conducted a comparative evaluation of the preliminary study *in vitro* antacid activity of *Tephrosia calophylla* and *Tephrosia maxima* which are traditionally used in dyspepsia and ulcer. The work was performed by using a self-fabricated artificial stomach model. In this research we have extracted the root of both the plants with methanol and then subjected to preliminary chemical tests which revealed the presence of chemical constituents like carbohydrate, tannins and flavonoids in common, whereas *T.maxima* showed the presence of saponins and glycosides in addition. 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 two synthetic antacids like sodium bicarbonate and a combination of aluminium hydroxide and magnesium hydroxide as standards. It was observed that, both the test extracts and standards reported to show a stable pH at altered temperatures, which confirms that temperature does not affect the pH. It was observed that TMM in both the concentration revealed a potent neutralizing effect as that of sodium bicarbonate at 250mg.

The titration methods involved in this work were the Fordtran's model and the modified model of Vatier's artificial stomach, which mimic the regular physiological functioning of human stomach. The duration of neutralization was found to be highest for TMM-250mg, when compared to both the standards as well as TCM. This reveals that TMM is able to neutralize the acid secreted for longer time than any other synthetic antacids used in this evaluation.

Systemic antacids like sodium bicarbonate acts instantaneously but the duration is short. It is a potent neutralizer but has several demerits 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 oedema and CHF. Na citrate too has similar side effects like $NaHCO_3$. But this does not evolve CO_2.

TMM 250mg was found to neutralize 94.17±1.600ml of artificial gastric juice, which depicted its potent antacid nature. TMM 100mg was also identified as a potent antacid when compared to TCM, control and both the standards used. TCM 250mg was found to be a better consumer of gastric juice when compared to the standards used. TMM 250mg was able to consume 6.0124 ±0.1233 number of H+ ions followed by TMM 100mg and TCM 250mg. This revealed the antacid potency of the test extracts. The non systemic antacids like magnesium hydroxide, magnesium trisilicate, aluminium hydroxide gel, magaldrate and Calcium carbonate are insoluble and poorly absorbed basic compounds. They react in the stomach to form the corresponding chloride salts. These compounds rebound acidity is mild and brief. All Mg salts have laxative action by generating osmotically active $MgCl_2$ in the stomach and through Mg^{2+} ion induced cholecystokinin release. Aluminium hydroxide gel is a weak and slowly reacting antacid.

It causes constipation due to its smooth muscle relaxant and mucosal astringent action. It binds phosphate in the intestine and produce hypophosphatemia on prolonged use. This may in turn lead to osteomalacia. This medicament cannot be used in renal failure as small amount of Al^{3+} that is absorbed is excreted by the kidney. Calcium carbonate is a potent and rapidly acting acid neutralizer but the greatest draw back acid rebound is marked and is constipating to most individuals but in some it causes loose motions[15]. Antacids are the simplest of all the therapies for treating the symptoms of excessive gastric secretion. They directly neutralize acid, thus raising the gastric pH; this also has the effect of inhibiting the activity of peptic enzymes which practically ceases at pH 5. Given in sufficient quantity for long enough they can produce healing of duodenal ulcers but are less effective for gastric ulcers. There was a suggestion-no longer believed that if aluminium was absorbed it could trigger Alzheimer's disease[16].

Since *T.maxima* and *T.calophylla* had proved to be potent antacids, it can be considered to be an efficient substitute for the synthetic antacids. Further investigations are warranted on the preclinical and clinical studies in order to substantiate the results obtained.

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