IN-VITRO EVALUATION OF ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF STEMS OF *TEPHROSIA PURPUREA* (L.)

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Abstract

Tephrosia Purpurea (l.), an evergreen tree, is used by several folkloric practitioners to treat peptic ulcers in India. The present study was carried out to evaluate the antiulcer activity of the aqueous extract (AE) of the stems of Tephrosia Purpurea (l.) in-vitro method. ***Methods:*** *In this study, we performed , anti-ulcer activities using aqueous extract concentrations 100mg, 500mg, 1000mg, 1500mg in in-vitro method as the acid neutralizing capacity and H+/K+ - ATPase inhibition activity method and in acid-neutralizing capacity (ANC), the extract significantly reduced ANC to 17.05 at a concentration of 100 mg as compared to 18.07 with standard Aluminium hydroxide + Magnesium hydroxide (500mg).* ***Conclusion:*** *The stems of Tephrosia Purpurea (l.) possess significant antiulcer activity and a detailed study on the isolation of active constituents from this species and its underlying mechanism of action responsible for its antiulcer effect is to be studied in the future.*

INTRODUCTION:

Peptic ulcer diseases are referred as gastric and duodenal ulcers that are posing a major threat to the world's population over the past two centuries with a high pensiveness and ephemerality[1]**.** These ulcers may be induced by several factors like stress, smoke, alcoholism, Helicobacter pylori Infection is known to effect at least 40% men and 20% woman all over the world[2]. Achalasia can also be another reason for ulcers formation, this condition is due to hereditary, autoimmune and infections in oesophagus. Protective mechanism for gastro oesophagus reflux includes production of bicarbonate and saliva which neutralise refluxed acid[3]. The modern life style has gravely induced stress, smoking, alcoholism, and spicy food habits have disturbed the reflux condition leading to ulcer formation. Over use of non-steroidal anti-inflammatory drugs (NSAIDs) , hyper secretion of gastric acids, decreased mucous secretions , inhibition of Arachadonic acid pathway, mucosal blood flow, surface active phospholipids, prostaglandins (PG), nitric oxide (NO), as well as enzymatic and non-enzymatic antioxidant performance has also contributed to this condition[4].

H. pylori infection has go undetected for long as it is asymptomatic. Approximately 70% of the total population is colonized by H. Pylori, and has 10%-30% susceptibility of developing into peptic ulcer[5].

Tephrosia purpurea (L.) is a best folk medicinal value containing drug which grows in saline-sodic soil conditions. The Native of drug is Australia, China, India, and Sri Lanka[6]. It is expanded in WA,NT,CYP,NEQ,CEQ and southwards to south- eastern queensland. It naturally grows in grassy fields, waste places and thickets, on ridges, and along roadsides, in Java. *Tephrosia purpurea* is an erect, annual or lived perennial legume shrub growing to a height of 40-80 (-150) cm. It has a long stout taproot, many slender branches, erect or decumbent at the base[7]. The stems are cylindrical, woody at the base, with stiff coarse hairs, frequently reddish in colour[8]. Stems show good therapeutic activity like antioxidant, anti-inflammatory and anticancer. But there are less activities proved like antiulcer, anticancer, anthelmintic , wound healing for stem part[9]. This study was aimed at investigating the occurrence of constituents in *Tephrosia purpurea* (L.) plant extracts prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by considerable decrease in superoxide dismutase, H+K+ATPase and increase in catalase activity. The H+K+ATPase are the dimeric enzyme responsible for H+ secretion by the gastric parietal cells. H+K+ATPase are selectively blocked by the action of stem part[10].

MATERIALS AND METHODS:

Processing of Plant Material: The collected plant material has identified by Dr.S. Prasad rao, botanist, Sir C R Reddy College of Autonomous sciences, Eluru.A voucher specimen (Voucher No.ATC28/09/2021) has been deposited at the herbarium unit of the Department of Botany, Sir C R Reddy Degree College, Eluru, west godavari(District), Andhra Pradesh, India. The plant was washed with tap water 3 times and sterilized by sprinkling with 70% alcohol. The plant is then dried in shade at room temperature and checked regularly for fungal contamination (if any). Once the plant is dried completely, it is then grinded into fine powder with pestle and motor. The fine powder is collected and used for extraction of the crude drug in aqueous solvents by Soxhlet extraction method.

Chemicals used: Aluminium hydroxide, sodium hydroxide, hydrochloric acid, sodium cmc, Tween80, Sodium benzoate, Orange oil and magnesium hydroxide other chemicals used were analytical grade.

Extraction by Soxhlet Apparatus : Extraction by Soxhlet apparatus: this extraction procedure has been practiced for a long time for crude drugs. The mode of extraction process depends on the plant materials water content to be extracted. Usually, the crude extract has taken from the Soxhlet apparatus with the aqueous solvent. This apparatus consists of three parts; a round bottom flask to take the solvent, the main jar is loaded with material from which the compounds have to be extracted. And a condenser in which condensation of solvents vapours takes place. 100 g of the powder of plant material is taken into Soxhlet main jar. The solvent is poured into the round bottom flask and extract condensation under reduced pressure, and in a temperature of 60-80 °C set to boil through the regulated heating mantle. The vapour of the solvent pass-through drive tubes enters the condenser through the main jar and gets condensed where there is a continuous flow of water in the condenser[11]. The condensed solvent falls back on the packed material in the main jar before collecting in a jar itself. The collection and extraction of material take place simultaneously in the main jar, as seen by the colouring of the solvent as a compound of material gets dissolved in the solvent. Thus, the crude plant material extract has been obtained, and it usually takes 7-8 h to complete an extraction. The solvent has evaporated, and finally, it yields brown extract; this has been stored in the refrigerator for further studies.

Chemical constituents of *Tephrosia Purpurea* (l.) stem aq. extract: By doing Qualitative Phytochemical Analysis the extract contains alkaloids, flavanoids, glycosides, saponins and tannins results are shown in Tables: 1. The stem of *Tephrosiapurpurea* (L.) contains Purpurin, Quercitin, (−)-Purpurindehydroisoderricin, (−)-Maackiainpseudosemiglabrin, (−)-semiglabrin, Terpurinflavone, (-)-Isolonchocarpin, Isoglabratephrin, Tephropurpulin A, Rutin, Serratin 7-O-β-D-glucopyranosyl-(1→4)-O-βD-galoctopyranoside.

Test for Alkaloids:

Dragendorff's test: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Test for Saponins:

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. No layer of foam indicates the absence of saponins.

Test for Glycosides:

Legal's test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. No formation of pink to red colur shows absence of glycosides.

Test for Carbohydrates:

Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Test for Tannins:

Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

Test for Flavonoids:

The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

Test for phenol:

Bromine Water: Take 5ml of bromine add 100ml of distilled water and shake well. Decant off the clear liquid.

Test for Proteins:

Biuret test: Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins[12].

Preparation of herbal suspension dosage form

The composition of formulation for preparing 100 ml of suspension of *Tephrosia purpurea* powder was as shown in Table 1. The 60-mesh size fine particles of the drugs are properly mixed by triturating[13]. the drug mixed in solvents which are different additive such as Tween-80, sodium carboxymethyl cellulose (CMC),as sweetening agent, flavoring agent like orange oil, and stabilizing agent sodium benzoate during shelf life of formulation. the study of antiulcer suspension formulation in vitro antiulcer study is to be conducted after In-vitro Evaluation of Antiulcer Activity of aq. extract of stem of *Tephrosia purpurea* (l.)

STABILITY PARAMETERS FOR SUSPENSION

Physical test of herbal suspension

The physical test of suspension aq. extract of stem of *Tephrosia Purpurea* (l.) formulation was conducted at room temperature (±28°C) and 45°C. The results were tabulated in Table 2.

Sedimentation volume

The sedimentation volume is the ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension as the suspension settles in a cylinder under standard condition. It was determined by keeping a measured volume of suspension in a graduated cylinder in an undisturbed state for a certain period of time and note that the volume of the sediment which is expressed as ultimate height.

Accelerated stability studies

The accelerated stability studies were conducted for herbal suspension. The different parameters such as sedimentation volume, flow rate, redispersibility, viscosity, pH and crystal growth were studied for the formulation and observation[14].

Redispersibility

The suspension was allowed to settle in a measuring cylinder. The mouth of the bottle was closed and was shaked up and down for 30 times and the number of inversion necessary to restore a homogeneous suspension was determined.

Rheology

The time required for suspension sample to flow through a pipette was determined the apparent viscosity was using the equation. Flow rate = Volume of pipette (ml)/Flow time.

Viscosity

The viscosity of the sample was determined at room temperature using Brookfield viscometer at 50 rpm by using spindle no. 3.

pH

The pH of aq. extract of stem of *Tephrosia Purpurea* (l.) suspension was determined using pH meter.

Crystal growth

Stability of suspension will also decrease because of crystal growth, which usually occurs from temperature fluctuation during storage and form broad particle size distribution. Crystal formulation was determined at 4°C, Room temperature (RT) and 47°C. The resulting parameters of suspension for all formulation are shown in Tables 3 and 4.

Determination of Microbial limit test:

A microbial limit test[15] was performed as per I.P 2014.

In-vitro Evaluation of Antiulcer Activity:

The aqueous extract of acid- neutralizing capacity value are 100mg, 200mg, 300mg, 400mg, 500mg, 750mg, 1000mg and 1500mg was done. The aluminium hydroxide and magnesium hydroxide (500mg) have compared for the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl was immediately titrated until the pink colour is attained .The titre values are noted in the table no. 6

The moles of acid neutralized is calculated by,

Moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH)

Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided / Grams of Antacid or Extract.

Acid Neutralizing Capacity: The neutralizing effect of the aqueous extract is studied for concentration (100mg, 200mg, 300mg, 400mg, 500mg, 750mg, 1000mg, and 1500mg) and standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2](500mg) was used in our study. The results obtained are tabulated in table no. 6 and graph No-1 is drawn between concentration verses ANC.

RESULTS AND DISCUSSION:

The medicinal crude drug plant, stems of Tephrosiapurpurea (L.) belonging to the family of Fabaceae has been investigated in a systematic way covering preliminary Phytochemical, antiulcer suspension formulation and pharmacological aspects in an attempt to rationalize its use as drug of therapeutic importance. The stems of Tephrosiapurpurea (L.) was powdered and successively extracted with distilled water, the yield was 20.6%.The stems of Tephrosiapurpurea (L.) aqueous extracts were subjected to Phytochemical test to find out the

active constituents which shows the presence of alkaloids, flavanoids, glycosides, saponins and tannins results are shown in Tables:1.

It was observed that all these three formulation **aq. extract of stem of *Tephrosia Purpurea* (l.) herbal suspensions F1, F2 and F3** have similar organoleptic characteristics such as liquid in nature, orange brown shade in colour, sourness taste.

In **F1** it was observed that, sedimentation volume 2.26, PH slightly alkaline pH 6.66±0.57, viscosity 54.6cP rapid flow rate (6.33SEC) per 5 ml of formulation (**Table No. 4**). In **F2** it was observed that, sedimentation volume 2.29, PH slightly alkaline pH 7.26±0.57 , viscosity 51cP rapid flow rate (4.78SEC) per 5 ml of formulation (**Table No. 4**). In **F3** it was observed that, sedimentation volume 3.16, PH slightly alkaline pH 7.89±0.57, viscosity 50cP rapid flow rate (3.49SEC) per 5 ml of formulation (**Table No. 4**).

The **F1** suspension had a pleasant appearance and texture at different temperature and did not exhibit any change. There were no noticeable changes in sedimentation volume as time increases because it is near to 2 which is the acceptable limit (Table no. 4).

To assess the standard and shelf life of the F1, F2 and F3 formulations total aerobic bacterial count was performed. Unintentional contamination, like fungal contamination throughout the production stage, may cause deterioration in safety and quality as the risk of hemorrhagic colitis, profuse bleeding. headache, dehydration, and changes in blood pressure and heart rate Feeling stressed,Having unmanaged diabetes and Having a weak immune system. For the evaluation of microbial contamination, total aerobic count, Total Fungal count, Escherichia coli, Candida albicans and Salmonella spp. the count was determined as per Indian Pharmacopoeia. It was observed that F1 Formulation there is presence of TAC (4.3 $\times 10^2$) cfu, TFC (18) cfu, and absence of Escherichia coli, Salmonella and Candida albicans which is within limits of standardization parameters (Table no-6)

Acid Neutralizing Capacity: The neutralizing effect of the aqueous extract was studied for four concentration (100mg, 500mg, 1000mg, 1500mg) and standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2](500mg). The results obtained envisage that the extract at concentration 100mg , 200mg , 300mg , 400mg , 500mg, 750mg, 1000mg and 1500mg showed a significant reduction in acid neutralizing capacity (ANC), i.e., 39.08,16.52, 4.506, 0.94, 2.226, 4.266, 1.596, 3.504, respectively, as compared to standard Al(OH)3+Mg(OH)2 (500 mg) which is 39.78. The extracts of stems of *Tephrosiapurpurea* (L.) at a concentration of 100 mg has been found to neutralize acid more significantly as compared to standard. The results have tabulated in Tableno. 7 & Graph no. 1.The acid-neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize, and it has been measured by a process known as back titration. In ANC, the aqueous extract at 100mg concentration showed a significant reduction in ANC 39.08 of stems of *Tephrosia purpurea* (L.) and aq. extract of stem of *Tephrosia Purpurea* (l.) herbal suspension studies are going in future.

TABLE NO.1

Qualitative phytochemical analysis of aqueous extract of the powdered medicinal crude drugs

Phytoconstituents	T.P Aq. EXTRACT
Alkaloids	Present
Saponins	Present
Glycosides	Present
Carbohydrates	present
Tannins	Present
Flavonoids	Present
Steroids	Present
phenols	present
Proteins	present
Terpenoids	Absent
Fats	Absent
Gums and Mucilages	Absent

T.P =Tephrosiapurpurea *(L)*

Table No. 2: Composition of aq. extract of stem of *Tephrosia Purpurea* (l.) herbal suspension

S.No	Ingredients list	Quantities in suspension		
		F1	F2	F3
1.	T.P aq.extract	1gm	1.5gm	2gm
2.	Sodium CMC	0.6%	0.6%	0.6%
3.	Tween80	0.1w/v	0.1w/v	0.1w/v
4.	Sodium benzoate	1.5gm	1.5gm	1.5gm
5.	Orange oil	1ml	1ml	1ml
6.	Purified water q.s	100ml	100ml	100ml

Table No. 3: Physical test for aq. extract of stem of *Tephrosia Purpurea* (l.) herbal suspension

S.No.	Parameter	F1	F2	F3
1.	Nature	Liquid	Liquid	Liquid
2.	Colour	Orange brown	Orange brown	Orange brown
3.	Texture	suspension	suspension	suspension
4.	Odour	citrus	citrus	citrus
5.	Taste	sourness	sourness	sourness

Table No. 4: Accelerated stability studies of aq. extract of stem of *Tephrosia Purpurea* (l.)

S.No.	Parameter	F1	F2	F3
1.	Redispersibility	Good	Medium	Medium
2.	pH	6.66±0.57	7.26±0.57	7.89±0.57
3.	Flow rate	5 ml/6.33 seconds	5 ml/4.78 seconds	5 ml/3.49 seconds
4.	Viscosity	54.6 cP	51 cP	50 cP
5.	Sedimentation	2.26	2.29	3.16

Table No. 5: Crystal formation of suspension formulation of aq. extract of stem of *Tephrosia Purpurea* (l.)

Parameter	Time duration (hrs)								
	24hrs			48hrs			72HRS		
Temperature (°C) & Crystal formulation	F1								
	4°C	28°C	45°C	4°C	28°C	45°C	4°C	28°C	45°C
	NO	NO	NO	NO	NO	NO	NO	LIGHT	NO
	F2								
	4°C	28°C	45°C	4°C	28°C	45°C	4°C	28°C	45°C
	NO	NO	NO	NO	LIGHT	NO	NO	NO	NO
	F3								
	4°C	28°C	45°C	4°C	28°C	45°C	4°C	28°C	45°C
	NO	NO	NO	NO	NO	NO	NO	NO	NO

Table No. 6 : Microbial Limit test for suspension formulation of aq. extract of stem of *Tephrosia Purpurea* (l.)

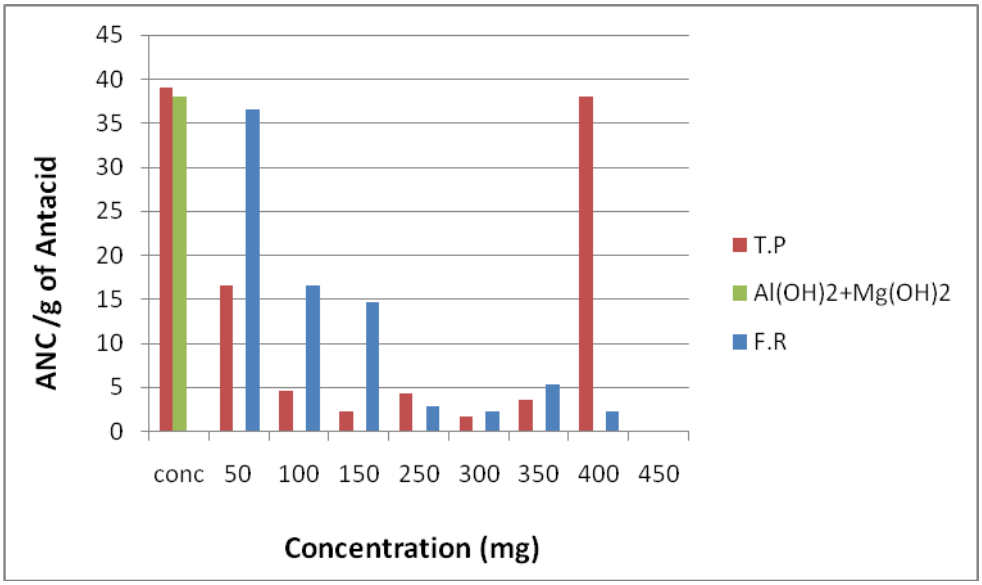
S.NO	Sample	Total Aerobic Count (cfu per ml)	Total Fungal count (cfu per ml)	Escherichia coli (per ml)	Salmonella sp. (per ml)	Candida albicans (per ml)
1.	**F1**	4.3×10^2	18	absent	absent	absent
2.	**F2**	6.6×10^2	25	absent	absent	present
3.	**F3**	8.9×10^2	30	present	present	present

Table No. 7 : Effect of aqueous extract of stem of *Tephrosia Purpurea* (l.) on acid neutralizing capacity

S.no.	Concentration of extract (mg)	Volumeof NaOH consumed(ml)	mEq of Acid Consumed	ANC per gram of Antacid
1.	100mgT.P	53.5	3.908	39.08
2.	200mgT.P	52	3.304	16.52
3.	300mgT.P	56	1.352	4.506
4.	400mgT.P	58	0.376	0.94

5.	500mgT.P	55	1.113	2.226
6.	750mgT.P	51	3.20	4.266
7.	1000mgT.P	55.6	1.596	1.596
8.	1500mgT.P	48	5.256	3.504
9.	Al(OH)3+ Mg(OH)2 500mg	49	3.978	39.78

GRAPH NO 1: EFFECT OF AQUEOUS EXTRACT OF ON ACID NEUTRALIZING CAPACITY



Conclusion: -

The present investigation revealed the presents of bioactive compounds such as phenolic, flavonoids, terpenoids, steroids, alkaloids and glycoside in the **aqueous extract of stem of *Tephrosia Purpurea* (l.).**

There are so many changes were observed in sedimentation, viscosity and other physicochemical parameters after performing stability studies at variable temperature with different concentration of aq.extract of stems of *Tephrosia purpurea* (L.) in suspension . As per the result of accelerated stability studies of F1, F2 and F3 suspension formulations it is confirmed that due to changes in mian herbal ingredient quantity affects an increase in viscosity of the formulation, decreases the flow rate of formulation simultaneously. It also affects sedimentation volume and PH of the suspension. There were no noticeable changes in the organoleptic and physicochemical properties of the of F1, F2 and F3 suspension formulations. In of F1, F2 and F3 suspension formulations, Formulation- F1 had all the stability parameters are stable acceptable, and optimum at variable temperature.

On the basis of the results, we may conclude that the aqueous extracts of stems of *Tephrosia purpurea* (L.) of the species may be considered as a sole source of novel antiulcer drugs. However, a detailed study on the isolation of active constituents from this species and its

underlying mechanism of action responsible for its antiulcer effect is to be studied in the future.This may be due to the fact that the extracts may contain more concentration of the active ingredients responsible for antiulcer activity. Thus, efforts to formulate an herbal antacid suspension were found to be satisfactory.

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