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Evaluation of antiulcer activity of the terpenoid fraction from the leaves of *Thespesia populnea* (L) (Malvaceae) in albino rats

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ABSTRACT

The plant *Thespesia populnea* (L.) used for the treatment of ulcer, in the folk medicine of different cultures. The present study was undertaken to determine the anti-ulcer potential of the terpenoid fraction from the leaves of *Thespesia populnea*. The terpenoid fraction (TF) from the leaves of *Thespesia populnea* were tested orally at the doses of 50, 100 and 200 mg/kg, on gastric ulcerations experimentally induced by pylorus ligation, aspirin induced ulcer, aspirin induced ulcerogenesis in pylorus ligated rats and analysed for ulcer index, gastric volume, pH, free and total acidity, sodium and potassium ion output. Bio-chemical estimations like total proteins, total hexoses, hexosamine, fucose, sialic acid were also made. Terpenoid fraction decrease ulcer index in dose dependent manner. The aggressive factors like gastric volume, free and total acidity decreases showing the antisecretory mechanism. Increase in pH and K⁺ ion output. The terpenoid fraction significantly decreased the protein level and increased the total carbohydrate (TC). Mucin activity (TC: P) significantly increased at the tested dose level 200mg/kg. Terpenoid fraction from *T. populnea* leaves showed significant antiulcer activity in experimentally induced ulcer in rat model by decreasing the gastric secretions and by enhancing glycoprotein levels. Thus the results of the present study substantiate the folk claims that the use of *Thespesia populnea* is beneficial in gastric ulcer.

Keywords: Antiulcer; *Thespesia populnea*; Terpenoid fraction; Pylorus ligation; Aspirin; Aspirin plus pylorus ligation.

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INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to H₂ antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ECL), hyperplasia and haemopoietic changes [1]. Considering the several side effects of modern medicine, should be looked for as a better alternative for the treatment of peptic ulcer. Thus, there is a need for more effective and safe anti-ulcer agents. However, plants are the most important source for the new drug development due to the resurgence of the interest in the use of herbal preparations. In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers [2,3].

T. populnea is common plant found in India. Several studies reported isolation and characterization of various constituents from *T. populnea*. Few studies on pharmacological activity of this plant such as wound healing, anti-inflammatory and antinociceptive activity, antioxidant activity, antifertility activity, alzheimer's disease have been reported scientifically. The plant used for the treatment of ulcer, in the folk medicine of different cultures. The presence of active constituent's viz. terpenoids, flavonoids, glycosides and sterol have been reported from the plant. Terpenoid have been investigated for numerous biological activities. There are number of studies available regarding the antiulcer activity of terpenoid [4-12].

However, so far there is no work earlier reported on the antiulcer activity of leaves of *T. populnea*. The present investigation was undertaken to demonstrate the pharmacological potential of Terpenoid fraction by using animal model.

MATERIAL AND METHOD

Preparation of the plant extract

The plant materials were collected from tropical dry deciduous forests of Satpuda hills, India (20°38' and 22°3' N Latitude and 72°11' E Longitude) in August, 2008. The plant specimen was authenticated by Dr. D. A. Patil Botanist, S. S. V. P. S. College of Science, Dhule, India.

The plant material powdered in a pulverizer (Drone, 9500) equipped with a 60 no. sieve. 250 gm of powder were extracted with ethyl acetate during 15 min at 40^o c (250 mL, three times). After filtration, the acidic compounds were extracted with aqueous KOH 5% (100mL, three times) followed by the extraction of the basic compounds with aqueous HCL 5% (100 mL, three times).The organic fraction, which contained the neutral compounds, was washed with

100 mL of water and concentrated in a rotatory evaporator to 50 mL, being then centrifuged during 10 min at 6000rpm, to remove the suspended particles. The solvent was evaporated to dryness [13]. (Figure1).

Test animals

Albino Wistar rats (120–160 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25⁰C with free access to feed and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Registration No. 651/02/C/CPCSEA).

Phytochemical analysis

The terpenoid fraction was analyzed for the presence of phytochemicals by qualitative chemical analysis. Examined for terpenoids by developing HPTLC pattern on solvent system pet ether: ethyl acetate (8:2) and sprayed with anisaldehyde H₂SO₄ reagent. The GC-MS analysis was performed using Autosystem XL GC+ Perkin Elmer instrument and having NIST Mass spectra library.

Acute oral toxicity studies

Acute oral toxicity studies were performed according to OECD-425 guidelines (Up and Down method). Starting dose was selected to be 2g/kg body wt. as specified in guideline. Animals were observed initially after dosing for first 30 minutes and at least once for next 14 days. At 2g/kg body wt. no mortality was observed and considered the cutoff point.

Antiulcer studies

Modified pyloric ligated (Shay) rats [14]

Rats were divided into groups of six animals each and were placed in cages with grating floor to avoid coprophagy and fasted for 48 h allowing free access to water. One group received 0.5% CMC (5 ml/kg) and was served as control. Omeprazole (10 mg/kg) was selected as reference drug. In the test group, the animals were grouped into three, receiving terpenoid fraction at a dose level of 50, 100 and 200 mg/kg b.w. The test drugs and reference drug were administered, orally, for 7 days prior to and 1 h before pyloric ligation. The animals were deprived of both food and water during the postoperative period. The animals were anaesthetised with ether. Four hours after ligation, animals were sacrificed.

Aspirin induced ulcer [14]

In aspirin-induced ulcer, ulceration induced by aspirin at a dose of 500 mg/kg orally in a suspension prepared in 0.5% CMC with water (time interval between reference drug and aspirin should be 1 h) and the process described above was followed.

Aspirin-induced ulcerogenesis in pylorus ligated rats [15]

The animals were divided into six groups, each containing six animals. Group I served as PL control, which received vehicle only. Group II served as Aspirin (200 mg/kg, p.o.) + PL control. Group III received Ranitidine (50 mg/kg, p.o.) as standard drug + Aspirin + PL. Groups IV, V and VI received terpenoid fraction at the dose of 50, 100 and 200 mg/kg, p.o respectively + Aspirin + PL. Groups III–VII received the assigned drug treatment for the respective 7 days daily. From days 5 to 7, animals of Groups II, III, IV, V, and VI received aspirin orally as an aqueous suspension at the dose of 200 mg/kg, 2 h after the administration of the drugs. Animals in all groups were fasted for 48 h, anaesthetised and the pyloric was ligated. Four hours after ligation, animals were sacrificed by excess anaesthesia.

Biochemical parameters.

The stomach was excised carefully keeping the oesophagus closed, opened along the greater curvature and the luminal contents were removed. The gastric contents were collected in a beaker and centrifuged at 1000 rpm for 10 min. The samples were analysed for gastric volume, pH, free and total acidity and sodium and potassium output. Biochemical estimations like total proteins, total hexoses, hexosamine, fucose, sialic acid and pepsin were also made. The mucosa was flushed with saline and stomach pinned on a frog board and scored.

Collection of gastric juice

Gastric juice was collected from the pylorus-ligated rats. The gastric juice thus collected was centrifuged and the volume of gastric juice as well as pH of gastric juice was measured. Then the gastric juice was subjected to bio-chemical estimation as follows.

Determination of ulcer index [16]

A score for the ulcer was made as follows:

- 0: normal colored stomach.
- 0.5: red coloration.
- 1: spot ulcers.
- 1.5: haemorrhagic streak.
- 2: ulcers.
- 3: perforation.

Mean ulcer score for each animal was expressed as ulcer index.

Determination of free and total acidity in gastric juice [17]

One milliliter of gastric juice was pipetted into a 100 ml conical flask, added 2-3 drops of Topfer's reagent and titrated with 0.01 N NaOH until all traces of the red colour disappears and the colour of solution was yellowish orange. The volume of alkali added was noted. The volume corresponds to free acidity. Then 2-3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity. Acidity was calculated by using the formula:

Sodium and potassium ion concentration in gastric juice [18].

This was carried out in Systronics mediflame 127-flame photometer. Stock solution was prepared. Sodium stock solution was prepared by dissolving 2.542 g NaCl in 1 l of distilled water. It contains 1 mg Na per ml (i.e. 1000 ppm). Stock solution was diluted to give four solutions containing 10, 5, 2.5 and 1 ppm of sodium ions. Then potassium stock solution was prepared by dissolving 1.909 g KCl in 1 l of distilled water. It contains 1 mg potassium per ml (i.e. 1000 ppm). Stock solution was diluted to give four solutions containing 20, 10, 5 and 2 ppm of potassium ions.

Total proteins [19]

The dissolved protein in gastric juice was estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice in 9:1 ratio. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken in another test tube, to this 4 ml of alkaline mixture was added and kept for 10 min. Then 0.4 ml of phenol reagent was added and again 10 min was allowed for colour development. Reading was taken against blank prepared with distilled water at 610 nm in micro plate reader (Bio-Tek, U.S.A.). The protein content was calculated from standard curve prepared with bovine albumin and was expressed in terms of mg/ml of gastric juice.

Total carbohydrates [20].

The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice in 9:1 ratio. Briefly the method consists of taking two aliquots of gastric juice and treated as described. To 1 ml of gastric juice, 9 ml of 90% alcohol was added. The mixture was kept for 10 min before it was centrifuged. The supernatant was discarded. The precipitate was dissolved in 0.5 ml of 0.1 N NaOH. To this 1.8 ml of 6 N HCl was added. The mixture was hydrolysed in water bath at 100°C for 2 h. The hydrolysate was neutralised by 5 N NaOH using phenolphthalein as indicator and the volume was made upto 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose as described. To the other aliquot of 0.5 ml of gastric juice, 4.5 ml of alcohol was added. The mixture was shaken for 10 min and centrifuged to obtain precipitate. The precipitate was dissolved in 0.5 ml of 0.1 N H₂SO₄. This reconstituted solution was

transferred to glass-stoppered tubes and then hydrolysed in a water bath at 100°C for 1 h. After hydrolysis, the volume restored to 0.5 ml; 0.2 ml of this hydrolysate was used for the estimation of sialic acid. After obtaining the concentration (mg/ml) of individual carbohydrates namely hexoses, hexosamine, fucose and sialic acid, the total carbohydrate content was calculated by adding the concentration of individual carbohydrates. Mucosubstances activity has been expressed as ratio of total carbohydrates to total proteins.

Hexoses [21]

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling waterbath at 60°C for 15 min. This was then cooled under running tap water and intensity of the colour was read in micro plate reader (Bio-Tek, U.S.A.) at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D (+)-galactose-mannose and has been expressed in mg/ ml of gastric juice.

Hexosamine [22]

About 0.5 ml of the hydrolysate fraction was taken. To this 0.5 ml of acetyl-acetone reagent was added. The mixture was heated in boiling waterbath at 60°C for 20 min, then cooled under running tap water. About 1.5 ml of 90% alcohol was added and allowed for 30 min. The colour intensity was measured in micro plate reader (Bio-Tek, U.S.A.) at 530 nm against blank prepared by using distilled water instead of hydrolysate. Hexosamine content was determined from the standard curve prepared by using D (+)-glucosamine hydrochloride and concentration has been expressed in mg/ml of gastric juice.

Fucose [22]

In this method, three test tubes were taken. In one tube 0.4 ml of distilled water was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysates were taken. To all three tubes 1.8 ml of H₂SO₄: water (6:1) was added by keeping the test tubes in ice-cold waterbath to prevent breakage due to strong exothermic reaction. The mixture was then heated in boiling waterbath for exactly 3 min. The tubes were taken out and cooled. To the blank and to one of the hydrolysate containing tube (unknown), 0.1 ml of cysteine reagent was added while cysteine reagent was not added to the last test tube containing the hydrolysate (unknown blank). It is then allowed for 90 min to complete the reaction. The reading was taken in micro plate reader (Bio-Tek, U.S.A.) at 396 and 430 nm setting zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D (+)-fucose. The fucose content was expressed in terms of mg/ml of gastric juice.

Sialic acid [23]

To 0.5 ml of the hydrolysate in 0.1 N H₂SO₄, 0.2 ml of sodium periodate was added and mixed thoroughly by shaking. A time of 20 min was allowed to elapse before addition of 1 ml of sodium arsenite solution to this mixture. The brown colour produced disappeared after shaking. Then 3 ml of thiobarbituric acid was added and the mixture was heated in boiling waterbath for 15 min. After cooling the tubes, 4.5 ml of cyclohexanone was added and thorough shaking was done for 15 s till all the colour was taken up by the cyclohexanone supernatant. The mixture was centrifuged to get a clear pink layer of cyclohexanone. This supernatant was pipetted out and intensity of colour was measured in micro plate reader (Bio-Tek, U.S.A.) at 550 nm. The sialic acid content of the sample was determined from the standard curve of sialic acid and has been expressed in terms of mg/ml of gastric Juice.

Histopathological examination [15]

For histopathological examination, the stomach was washed thoroughly with saline, dehydrated in gradual ethanol (50–100%), cleared in xylene and embedded in paraffin. Sections (4–5 mm) were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation (magnification 100 X).

Statistical analysis

For Modified pyloric ligated (Shay) rats and Aspirin induced ulcer

The data represent Mean \pm SEM. Results were analyzed statistically using one-way ANOVA followed by Dunnett's multiple comparisons. The minimum level of significance was set at $p < 0.05$.

For Aspirin-induced ulcerogenesis in pylorus ligated rats

The data represent Mean \pm SEM. Results were analyzed statistically using one-way ANOVA followed by Tukey's multiple comparisons. The minimum level of significance was set at $p < 0.05$.

RESULTS

Phytochemical analysis

TLC. TLC pattern of TF in the mobile phase containing Pet. ether: Ethyl acetate (8:2) v/v indicated the presence of 6 compounds with R_f at 0.11, 0.27, 0.48, 0.51, 0.66 and 0.73 respectively. The R_f of Lupeol matched with that of TF at 0.66 which was further confirmed by HPTLC.(Fig.2).

HPTLC. HPTLC pattern of TF showed the presence of 6 compounds with R_f at 0.20, 0.36, 0.43, 0.48, 0.56 and 0.70 respectively. In which compound with R_f 0.56 matched with that of standard lupeol, in solvent system Pet. ether: Ethyl acetate (8:2) v/v.

GC-MS. GLC analysis of TF of *T. Populnea* showed the presence of 8 major peaks, the peak 41.44 was further forwarded to Mass spectroscopy.(Fig. 3).

Modified pyloric ligated (Shay) rats

It was observed that in the vehicle treated control group the ulcer index was 1 ± 0.06 and the maximum number of ulcers was of the score 1.5 and 2. In this group we observed some of rat shows number of perforated ulcers (score 3). TF was found to produce a decrease in ulcer index in all the three doses in dose dependant manner. Aggressive factors like free acidity, total acidity and gastric volume decreased in all three dose levels. There is an increase in the pH and K^+ contents. The protein content was decreases significantly also supported the result.(Table 1).

Aspirin induced ulcer

Animals in the Aspirin induced group showed a significant ($P < 0.01$) increase in the ulcer index and acid secretory parameters like gastric volume, pH, total acidity when compared with those of vehicle treated group. Administration of TF of *T. Populnea* produced significant ($P < 0.01$) decrease in ulcer index in a dose dependent manner. The TF of *T. Populnea* also significantly reduced the gastric volume, total acidity, and increased the pH of the gastric fluid, proving its antisecretory activity.(Table 2).

Aspirin-induced ulcerogenesis in pylorus ligated rats

Table 3 shows the effect of drugs on secretory parameters like total and free acidity, pH, gastric volume and ulcer index. Animals in the Aspirin + PL group showed an increase in the ulcer index and acid secretory parameters like gastric volume, free and total acidity when compared with those of vehicle treated group. In the rats of this group, a number of perforated ulcers (score 3) were also observed. Administration of TF produced significant decrease in ulcer index in a dose dependent manner. The TF also significantly reduced the gastric volume, total and free acidity, and increased the pH of the gastric fluid, proving its antisecretory activity. The drug treatments significantly increase in the K^+ contents at the dose of 200mg/kg. Table 4 indicates the effect of TF of *T. Populnea* on Mucin activity of Aspirin + PL rats. In control group (Aspirin + PL) rats, there was increase in protein concentration, but decrease in individual as well as total carbohydrate levels, when compared with control group (PL). The TF of *T. Populnea* treatment significantly decreased the protein level and increased the total carbohydrate (TC) level when compare with control group. The drug TF at the tested dose level 200mg/kg significantly ($p < 0.001$) increased the TC: P ratio. The Group VI (TF, 200 mg/kg p.o.) showed greater activity that is comparable to the other two doses of drug.

In histological study, pretreatment with TF of *T. Populnea* was found to preserve the functional cytoarchitecture of the entire gastric mucosa. TF of *T. Populnea* treatment showed not only the maintenance but also the regeneration of gastric mucosa in the damaged regions. These findings confirm the cytoprotective nature of TF.(Fig.4).

DISCUSSION

Present study showed the protective effects on gastric ulcer models, ulcer healing and antisecretory property of TF (Terpenoid fraction) of *T. populnea*, which is important plant in herbal medicinal practice. Toxicity studies of TF carried out in rats indicate no lethal effect at least up to an oral dose of 2.0 g/kg for 14 days indicating that LD₅₀ of TF will be higher than that dose. Phytochemical analysis showed the presence of terpenoids. The Terpenoid fraction, confirmed by TLC and HPTLC analysis was found to match with Lupeol. In GC-MS analysis the mass fragmentation patterns of the peak resemble that with the triterpene moiety Hop-22(29)-en-3beta.-ol, which is closed to Lupeol (Lup-20(29)-en-3beta.-ol). The position of double bond is the only differentiating point between the Lupeol [20(29)-en] and Hop-22(29)-en-3beta.-ol [22(29)-en].

Gastric ulcers have multiple etiopathogenesis. Although in most of the cases the etiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism [24]. Ulcer caused by pylorus ligation is due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa and break down of the gastric mucosal barrier [25]. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion [26]. NSAID like aspirin, induce gastric ulceration. The reason being attributed to inhibition of biosynthesis of 'cytoprotective prostaglandins', e.g. PGE'S and PGI2 resulting in overproduction of leukotrience and other products of 5-lipoxygenase pathway Increasing acid secretion and back diffusion of H⁺ ions [27]. Aspirin was administered to PL rats; thus, aspirin further aggravated the acidity and the resistance of the gastric mucosa was decreased thereby causing extensive damage to the glandular regions of the stomach [15].

Antiulcer effect is supported by the decrease in the aggressive factors like gastric volume, decrease in free and total acidity and an increase in the resistance factors like pH showing the antisecretory mechanism. It is significant to note when the pH reached 3, the ulcer score appeared less [16]. The antiulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandins. Prostaglandins have a vital protective role. They stimulate the secretion of bicarbonate and mucus, maintain mucosal blood flow and regulate mucosal cell turnover and repair [28]. The increase in the K⁺ ion inturn reflects in the increase in H⁺ ion concentration and bicarbonate ion concentration. The increase in bicarbonate ion concentration plays an important role in protecting the gastric and duodenal mucosa against hydrochloric acid. The mucosal defense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to H⁺ ions thereby forming a physical barrier [18].

Mucus serves as first line of defense against ulcerogens. Mucus is secreted by the mucus neck cells and covers the gastric mucosa. Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucus barrier [29]. Increased mucus secretion by the gastric mucosal cells can

prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid in gastric juice and by acting as an effective barrier to back diffusion of H⁺ ions [15].

TF in the dose 200mg/kg significantly increased mucus secretion as observed from the increase in TC: P ratio, which is taken as reliable marker for mucin secretion. The increase in total carbohydrate: protein (TC: P) ratio is the direct reflection of mucin activity, which is indicated by the enhanced level of individual mucopolysaccharides like hexose, hexosamine, fucose and sialic acid [20]. Decrease in protein content in the gastric juice also signifies decrease in leakage from the mucosal cells indicating mucosal resistance. The wide distribution of adherent mucus content in the gastrointestinal tract plays a pivotal role in cytoprotection and repair of the gastric mucosa. The results showed increased levels of adherent mucus content of gastric tissue pretreated with TF indicating its cytoprotective action on experimentally induced gastric ulcer [29]. In histological study, pretreatment with TF of *T. Populnea* was found to preserve the functional cytoarchitecture of the entire gastric mucosa. TF of *T. Populnea* treatment showed not only the maintenance but also the regeneration of gastric mucosa in the damaged regions. These findings confirm the cytoprotective nature of TF.

Terpenoids have been shown to inhibit gastric acid secretion and enhancement in gastric mucus content against several experimental ulcer models. The plant constituents present in TF of *T. populnea* leaves especially lupeol might have the ability to protect against ulceration induced by aspirin, pylorus ligation and aspirin induced ulcerogenesis in pylorus ligated rats.

CONCLUSION

Significant activity of TF in gastric ulcer models suggests its usefulness in control of ulcer. Thus the results of the present study substantiate the traditional claims that the use of *Thespesia populnea* is beneficial in gastric ulcer.

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Fig. 1. Scheme for Extraction and Fractionation

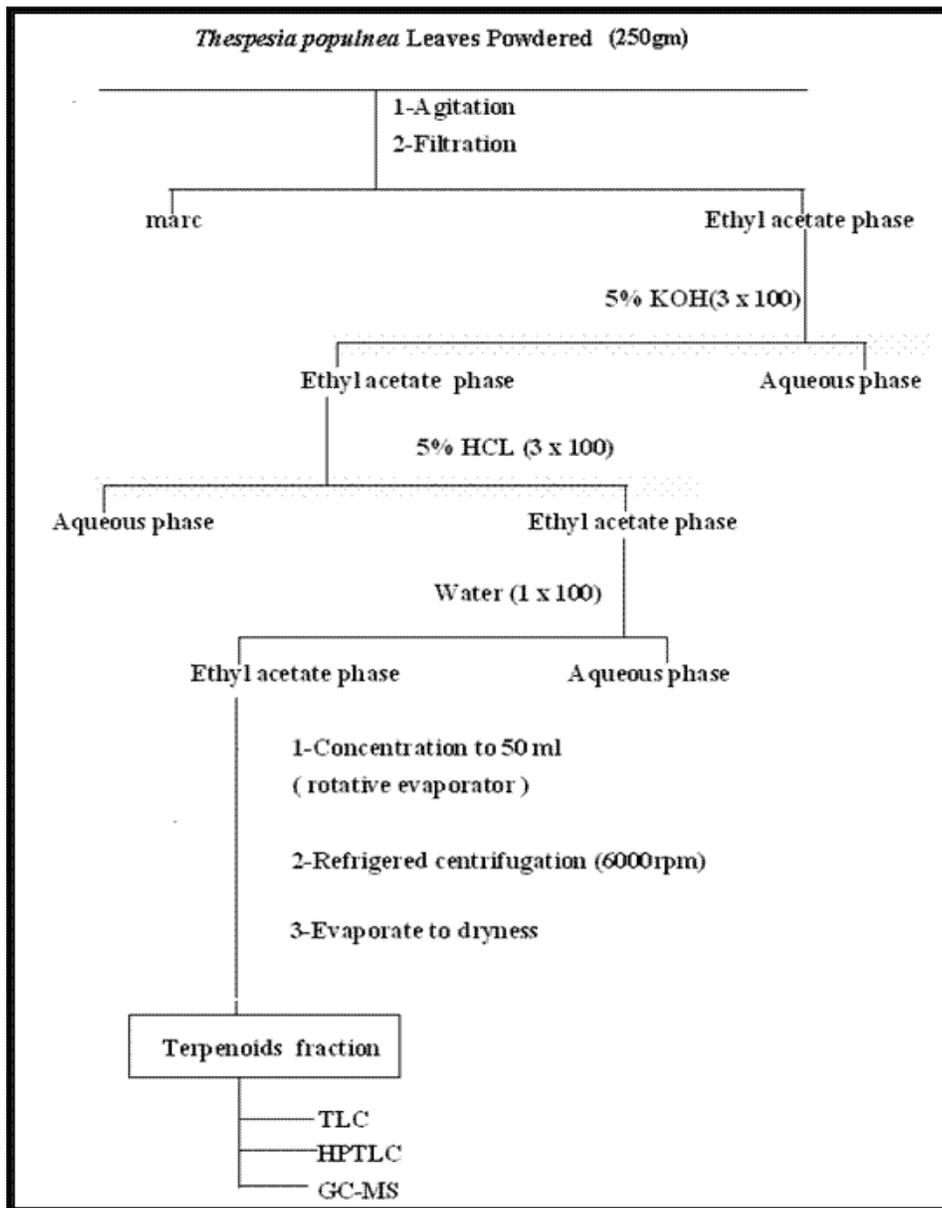
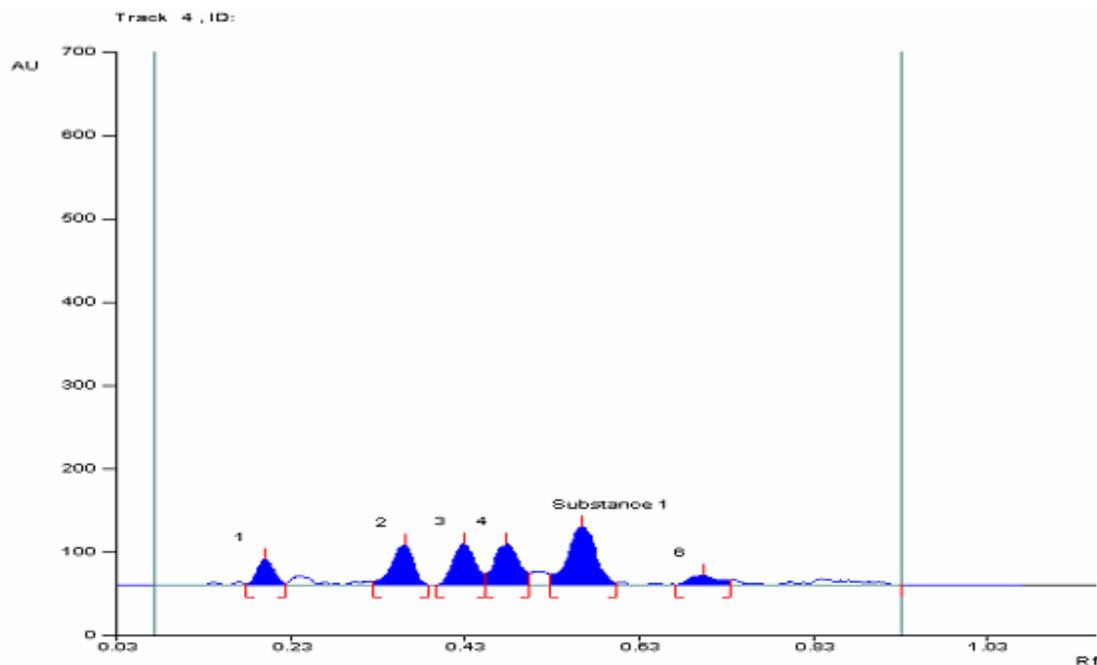


Fig. 2. HPTLC Fingerprinting of Terpenoid Fraction of *Thespesia populnea*



Wavelength: 366nm

[Rf]

Peak #	Start		Track 4, Analysis terpenoid fraction			End		Area		
	Rf	H	Rf	H	[%]	Rf	H	F	[%]	
1	0.18	1.1	0.20	30.5	11.78	0.22	1.9	478.5	8.38	
2	0.32	4.0	0.36	47.9	18.54	0.39	0.1	974.4	17.06	
3	0.40	0.1	0.43	49.1	19.00	0.45	13.4	1001.8	17.54	
4	0.45	13.8	0.48	49.2	19.02	0.50	14.3	1128.6	19.76	
5	0.53	12.4	0.56	69.9	27.04	0.60	3.2	1813.5	31.75	
6	0.67	0.6	0.70	11.9	4.62	0.73	5.2	314.1	5.50	
			Total height=2585			Total area=			5710.9	

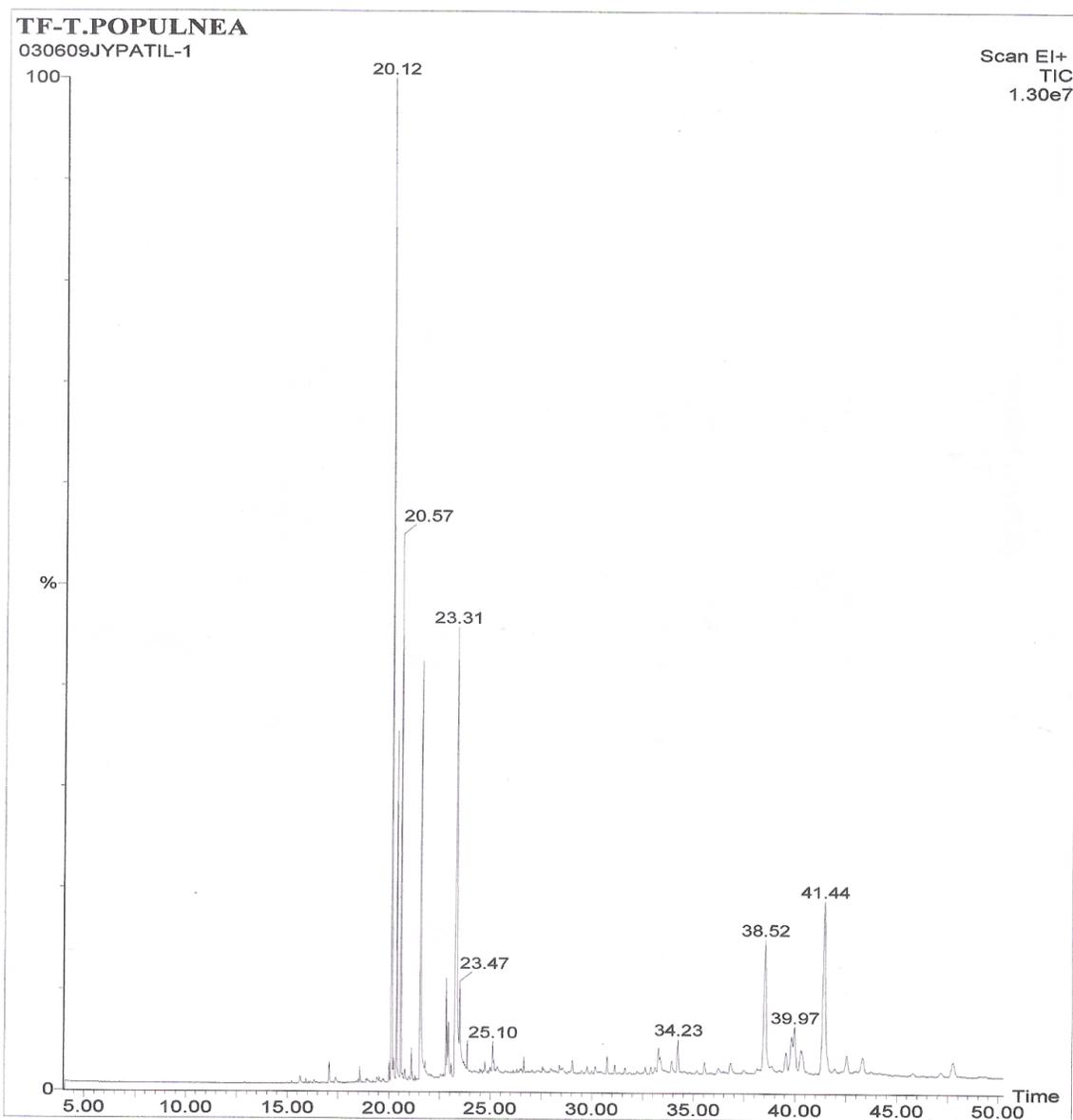
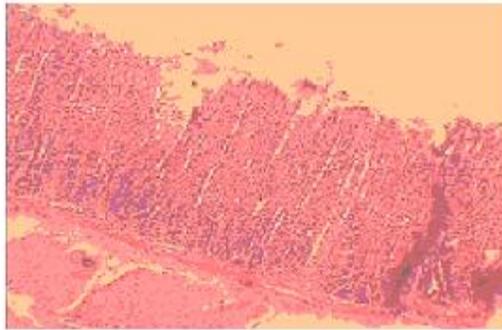
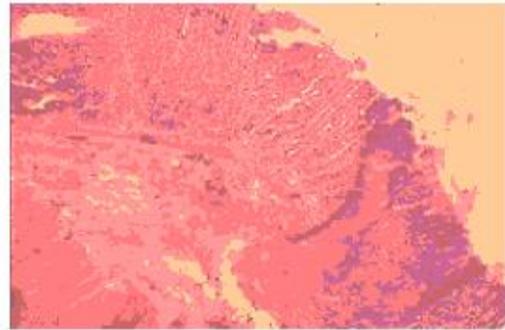


Fig. 3. GLC analysis of Terpenoid Fraction of *Thespesia Populnea*

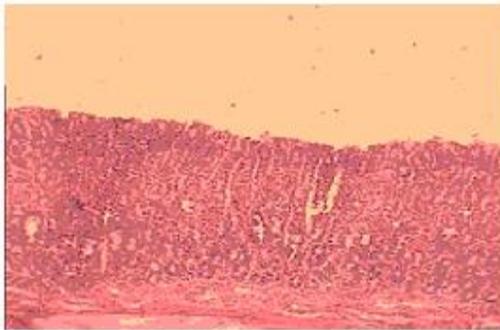
Histopathological sections of stomach mucosa



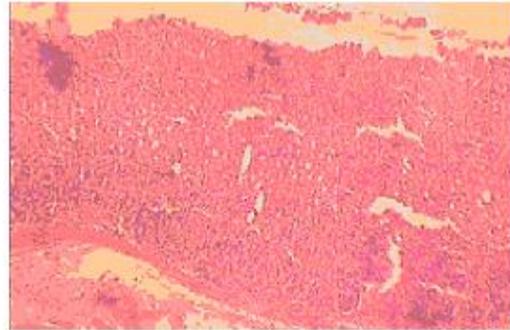
(a) Group I



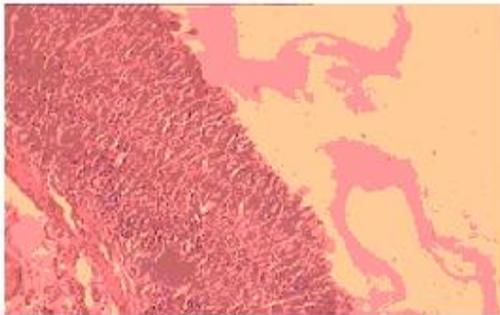
(b) Group II



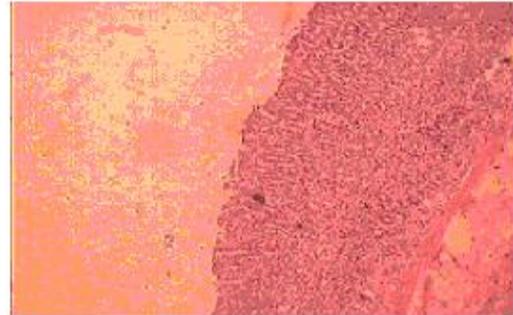
(c) Group III



(d) Group IV



(e) Group V



(f) Group VI

Fig. 4. Histopathological sections of stomach mucosa. (a) Group I-shows discontinuity in the lining epithelium with exudates in the lumen sub-mucosal edema. (b) Group II- shows hemorrhage and discontinuity in the lining epithelium hyperplastic mucosal glands. (c) Group III- normal mucosa with no ulcer. (d) Group IV-slight discontinuity in the lining epithelium with edema of sub mucosa. (e) Group V-no ulcer, hyperplastic mucosa, mild edema of the sub-mucosa. (f) Group VI-normal mucosa and muscularis mucosa with mild edema in the sub-mucosa.

Table1. Effect of TF on Biochemical parameter in Pylorus ligation in rats

Treated groups	Ulcer index	Gastric volume (ml)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)	Na ⁺⁺ (mEq/L)	K ⁺ (mEq/L)	Total Protein (ug/ml)
0.5% CMC (5 mg/kg)	1±0.06	4.0±0.21	2.29±0.16	55.67±3.73	81.83±4.67	43.91±3.47 ₄	11.05±0.9863	1212±79.76
Omeprazole (10 mg/kg)	0.56±0.02**	2.06±0.15**	3.58±0.18**	30.50±1.94**	39.17±3.61**	34.31±2.94 ₉	18.62±1.557* _*	655.2±59.5**
TF (50 mg/kg)	0.83±0.60*	3.63±0.18	2.93±0.16*	46.17±2.37	66.0±5.55*	37.03±2.77 ₄	13.54±1.241	1058±62.31
TF (100 mg/kg)	0.77±0.05**	3.03±0.18**	3.27±0.16**	40.83±3.29**	55.5±4.08* _*	40.03±3.32 ₇	15.89±1.773	855.3±62.3**
TF (200 mg/kg)	0.63±0.04**	2.83±0.14**	3.47±0.18**	36.0±2.0**	45.17±2.97**	42.09±3.32 ₁	17.29±1.71* _*	713.5±72.46* _*

Results were analyzed statistically using one way ANOVA followed by Dunnett's multiple comparisons. The minimum level of significance was set at p < 0.05. Each value is the mean ± S.E.M of six determinations. * p < 0.05, **p < 0.01, ***p < 0.001. Dunnett test as compared to control.

Table2. Effect of TF on Biochemical parameter in Aspirin induced ulcer in rats

Treated groups	Ulcer index	Gastric volume (mL)	pH	Total acidity (mEq/L)
0.5% CMC (5mg/kg)	0.99±0.06	3.7±0.22	2.5±0.16	77±3.6
Omeprazole (10mg/kg)	0.58±0.03**	1.9±0.20**	3.7±0.19**	33±2.9**
TF (50mg/kg)	0.76±0.06*	2.7±0.25*	3.1±0.15	53±4.5**
TF (100mg/kg)	0.66±0.04**	2.3±0.20**	3.3±0.17*	40±3.4**
TF (200mg/kg)	0.58±0.03**	2.0±0.17**	3.6±0.17**	35±2.9**

Results were analyzed statistically using **one way ANOVA** followed by **Dunnets multiple comparisons**. The minimum level of significance was set at $p < 0.05$.

Each value is the mean \pm S.E.M of six determinations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Dunnet test as compared to control.

Table3. Effect of TF on Biochemical parameters in Aspirin plus pylorus ligated induced ulcerogenesis in rats

Groups	Ulcer index	Gastric volume (ml)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)	Na+ output (mEq/L)	K+ output (mEq/L)
Group I	1.1±0.078	3.6±0.22	2.4±0.15	48±4.8	79±4.9	40±2.6	13±1
Group II	1.3±0.078	4.3±0.23	2.1±0.19	65±6.3	96±6.4	58±3.7 a [#]	7.9±0.61
Group III	0.66±0.075 a [#] b*	2.6±0.18 a [®] b*	3.8±0.17 a* b*	28±3 a [®] b*	58±3.1 a [®] b*	43±4.3 b [®]	16±1.6 b [#]
Group IV	0.92±0.043 b [#]	3.3±0.17 b [®]	3.1±0.12 b [#]	44±4.1 b [®]	76±4	50±3.6	10±1.4
Group V	0.8±0.042 b*	3±0.2 b [#]	3.5±0.14 a [#] b*	36±2.6 b*	68±3.4 b*	54±3	12±1.2
Group VI	0.73±0.066 a [#] b*	2.7±0.19 a [®] b*	3.7±0.19 a* b*	31±3.2 b*	62±3.2 b*	56±2.6 a [®]	14±1.7 b [®]

Results were analyzed statistically using **one-way ANOVA** followed by **Tukey's multiple comparison**.

Values are Mean ± SEM, n = 6 animals in each group. Group I – vehicle control (PL), Group II – aspirin (200 mg/kg body wt./p.o.) + PL control, Group III – ranitidine (50 mg/kg body wt./p.o.) + aspirin + PL, Groups IV- TF treated (50 mg/kg body wt./p.o.) + aspirin + PL, Groups V- TF treated (100 mg/kg body wt./p.o.) + aspirin + PL and VI – TF treated (200 mg/kg body wt./p.o.) + aspirin + PL.

Comparisons were made between: **(a)** Group I vs. II, III, IV, V and VI; **(b)** Group II vs. III, IV, V and VI; **(c)** Group III vs. IV, V and VI.

Symbols represent statistical significance: *p<0.001, [#]p<0.01, [®]p<0.05

Table4. Effect of TF on Biochemical parameters in Aspirin plus pylorus ligated induced ulcerogenesis in rats

Groups	Total Protein (Ug /ml)	Hexose (A) (Ug /ml)	Hexosamine (B) (Ug /ml)	Fucose (C) (Ug /ml)	Sialic acid (D) (Ug /ml)	Total carbohydrates(A+B+C+D)	TC:TP
Group I	1090±68	358±36	129±15	88±9.8	34±3.3	607±26	0.57±0.017
Group II	1264±73	352±48	141±17	92±9.6	37±5	621±58	0.54±0.032
Group III	584±37 a* b*	732±84 a [#] b [#]	233±26 a [®]	100±12	52±7.2	1117±98 a [#] b [#]	2±0.23 a* b*
Group IV	889±65 b [#]	567±66	186±20	97±11	59±6.9	907±81	1.1±0.14 c [®]
Group V	736±69 a [#] b*	656±66 a [®] b [®]	208±23	101±12	65±6.3 a [®] b [®]	1030±81 a [#] b [#]	1.5±0.16 a [®] b [®]
Group VI	632±62 a* b*	671±71 a [®] b [®]	227±28 a [®]	102±14	70±8.2 a [#] b [#]	1062±108 a [#] b [#]	1.8±0.3 a* b*

Results were analyzed statistically using **one-way ANOVA** followed by **Tukey's multiple comparison**.

Values are Mean ± SEM, n = 6 animals in each group. Group I – vehicle control (PL), Group II – aspirin (200 mg/kg body wt./p.o.) + PL control, Group III – ranitidine (50 mg/kg body wt./p.o.) + aspirin + PL, Groups IV- TF treated (50 mg/kg body wt./p.o.) + aspirin + PL, Groups V- TF treated (100 mg/kg body wt./p.o.) + aspirin + PL and VI – TF treated (200 mg/kg body wt./p.o.) + aspirin + PL.

Comparisons were made between: **(a)** Group I vs. II, III, IV, V and VI; **(b)** Group II vs. III, IV, V and VI; **(c)** Group III vs. IV, V and VI.

Symbols represent statistical significance: *p<0.001, [#]p<0.01, [®]p<0.05

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