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PHYTOCHEMICAL INVESTIGATION AND SCREENING FOR INFLAMMATORY BOWEL DISEASE ACTIVITY OF ETHANOLIC EXTRACT OF *TECTONA GRANDIS* LINN. BARK

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Keywords:

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ABSTRACT: Inflammatory bowel disease (IBD) is a multifactorial process that causes ulcerative colitis and Crohn's disease due to unknown mechanisms associated with release of proinflammatory mediators. Aim of the present study is to evaluate inhibitory activity by bark extract *Tectona grandis* Linn. (TGE) against experimental induced IBD. Two different models were used to induce IBD *viz*., Indomethacin-induced enterocolitis and acetic acid induced ulcerative colitis in rats. Male wistar rats were pretreated with TGE the dose of 10 and 25 mg/kg p.o. daily for a period of 7 days. On 8th and 9th day indomethacin was administered (7.5 mg/kg, s.c.) for to induce enterocolitis. In case of acetic acid induced ulcerative colitis model, two ml (3%, v/v) acetic acid in saline was instilled into the rectum of a rat. Quantification of inflammation was done using myeloperoxidase assay (MPO), lactate dehydrogenase (LDH), lipid peroxidase (LPO). All parameters were altered in ulcerated rats, and improved in animals receiving TGE an effect that was comparable to that of the standard sulfasalazine, especially at the highest dose level. Evaluation based on macroscopic features showed significantly lower score values for drug treated and standard drug treated groups compared to the disease control groups. Histological examination of disease control group showed massive necrosis of the mucosa and submucosa. Drug treated group showed mild lesions, regeneration and inflammatory reaction. The sulfasalazine treated group showed suppressed inflammatory reaction. The results observed from MPO, LDH and LPO assays showed significant improvement of disease with extract treated groups compared to disease control group. Mechanism of action was determined by studying COX and prostaglandin inhibition studies. Results indicated that TGE shows inhibition of COX-1 is 42.35% and that of COX-2 is 45.8% and also produces action by inhibiting the synthesis of prostaglandin. The results obtained established the efficacy of the TGE against inflammatory bowel diseases possibly by its anti-inflammatory properties.

INTRODUCTION: Inflammatory bowel disease (IBD) is a spectrum of chronic idiopathic inflammatory intestinal conditions. IBD causes significant gastrointestinal symptoms that include diarrhoea, abdominal pain, bleeding, anemia and weight loss.

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IBD conventionally is divided into two major subtypes: Ulcerative colitis and Crohn's disease. Ulcerative colitis is characterized by confluent mucosal inflammation of the colon starting at the anal verge and extending proximally for a variable extent (*e.g.*, proctitis, left - sided colitis, or pancolitis).

Crohn's disease, by contrast, is characterized by transmural inflammation of any part of the gastrointestinal tract but most commonly the area adjacent to the ileocecal valve [1]. Both diseases increase the risk of adenocarcinoma of the colon in the affected area [2]. Ulcerative colitis is most

prevalent in North America, North Europe and Australia. The prevalence is roughly 10 times lower in southern and Eastern Europe, Africa, Asia and South America. Some reports have indicated that disease is now seen with increased frequency in parts of Asia such as India, Bangladesh and Japan [3].

Specific goals of pharmacotherapy in IBD include controlling acute exacerbations of the disease, maintaining remission, and treating specific complications such as fistulas [1]. The main drugs used in the treatment of ulcerative colitis and Crohn's disease are the aminosalicylates and corticosteroids [4]. Although many types of treatment have been proposed and clinically proven, additional therapeutic approaches are needed because many patients do not satisfactorily respond to the currently available options or show significant side effects due to their prolonged use.

Therefore there is need to develop safe and effective alternative therapeutic agents for treatment of IBD. Considering oxidative stress as one of the factor in IBD, antioxidants could be expected to provide relief [5].

Keeping this in view, an attempt was made to search for the plants containing antioxidants for the evaluation of effectiveness in improving IBD. In our literature search, *Tectona grandis* Linn. Bark, which contains phytoconstituents such as flavanoids, alkaloids, tannins, anthraquionones and saponins [6], has been already proved for its anti-oxidant activity.

Therefore it is been hypothesized that the bark may be useful in treating IBD. However there are no scientific data regarding the utility of *Tectona grandis* Linn. Bark extract in the treatment of IBD. Thus has been selected for the present study and probable mechanism of action is also determined by studing COX and prostaglandins inhibition.

MATERIALS AND METHODS:

Collection and Authentication of the Plant Material: *Tectona grandis* Linn. barks were collected from surrounding areas of Kudal, Sindhudurg. The plant was washed with distilled water, dried at room temperature under shade. The sample was identified and authenticated by Nikhil Analytical and Research Laboratory, Sangli, Maharashtra.

Preparation of the Extract: The stem bark of *Tectona grandis* Linn. was collected from Kudal, Sindhudurg in the month of June-August. The plant material was washed and dried in shade. The dried plant material (1.5 kg) was then crushed and continuous hot extraction method using Soxhlet apparatus with 90% ethanol. The extract was filtered in hot condition and evaporated in a rotary flash evaporator to remove the alcohol and residue was dried in a vacuum desiccator over calcium chloride, to yield ethanolic extract [TGE].

Phytochemical Screening: Preliminary qualitative Phytochemical analysis of the extract was carried out using various procedures [7 - 9] to detect the presence of several phytochemical like tannins, terpenoids, alkaloids, flavonoids, steroids and free amino acid *etc*.

HPTLC Analysis: HPTLC analyses were carried out using CAMAG linomat HPTLC instrument. Sample preparation was done by taking weighed quantity of extract residue was dissolved in 1ml of chromatographic grade methanol which was used for sample application on pre-coated silica gel 60 F 254 aluminum sheets.

Developing Solvent System: A number of solvent systems were tried, for each extract for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the solvent of tolnaftate: ethyl alcohol: formic acid :: 10:3:1.

Sample Application: Application of bands of each extract was carried out (15 mm in length and 3 µl in concentration) using spray technique. Samples were applied in duplicate on pre-coated silica gel 60 F254 aluminium sheets (5 × 10 cm) with the help of linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of Chromatogram: After the application of sample, the chromatogram was developed in twin trough glass chamber 10 × 10 cm saturated with and solvent system in and tolnaftate: ethyl alcohol: formic acid:: 10:3:1 for aqueous extract.

Detection of Spots: 3 mg/ml concentration of extracts were prepared in methanol of chromatographic grade and then filtered by

Whatman filter paper No. 1. prepared samples of different extracts were applied on TLC aluminum sheets silica gel 60 F 254 (Merck) 07 µl each with band length of 8 mm, sample applicator set at a speed of 150 nl/sec.

GC-MS Analysis: GC-MS analysis was carried out on a TURBOMASS 2017. The column used was capillary column measuring 30 m × 0.25 mm with a film thickness of 0.25 mm composed of 95% dimethyl polysiloxane. Sample injection volume of 1 micro litter was utilized and inlet temperature was maintained as 250 °C. The oven temperature was programmed initially at 110 °C for 4 min, then an increase to 240 °C. And then programmed to increase to 280 °C at a rate of 20 °C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200 °C. The source temperature was maintained at 180 °C. GC-MS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library.

Animals Care and Handling: The experiment was approved by the institutional animal ethics committee of S. E. T. College of Pharmacy, S. R. Nagar Dharwad. The experiment was carried out on Albino rats of Wistar strain and Swiss albino mice of both sexes weigh around 200 grams and 25 grams respectively. Animals were procured from Venkateshwara Enterprises, Bangalore and were housed in the animal house.

The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25 ± 2 °C relative humidity 44 - 56% and light and dark cycles of 12:12 h, fed with standard pallet diet and water *ad libitum* during experiment. The animals were kept in polypropylene cages in groups. The research was conducted in accordance with the ethical rules on animal experimentation.

Screening for Inflammatory Bowel Disease:

Indomethacin-induced Enterocolitis in Rats: The male Wistar albino rats (200 - 250 gm) were selected and randomized into five groups of six animals in each group. Group 1 served as Normal or untreated animals, group 2 was positive control (induced) animals receive only indomethacin (7.5 mg/kg) s.c. group 3 was treated group with lower dose received indomethacin (7.5 mg/kg) s.c along with TGE, group 4 was treated group with higher dose received indomethacin (7.5 mg/kg) s.c along with TGE and group 5 standard group received indomethacin (7.5 mg/kg) s.c along with prednisolone (2 mg/kg p.o). Animals pretreated with *Tectona grandis* Linn. bark extract for 7 days will be administered Indomethacin (7.5 mg/kg, s.c.) on 8th and 9th day of treatment. Extract will be administered till 11th day. On the 11th day the animals will be sacrificed by cervical dislocation and dissected. Ileum and colon will be taken out to assess inflammation, based on physical parameters, macroscopy and microscopic features. Quantification of inflammation would be done using biochemical assay (MPO, lipid peroxides, GSH) [10, 11].

Acetic Acid-induced Colitis in Rats: The male Wistar albino rats (200 - 250 gm) were selected and randomized into five groups of six animals in each group. Group 1 served as normal or untreated animals, group 2 was positive control (induced) animals receive 2 ml of 4% (v/v) acetic acid. group 3 was treated group with lower dose received 2 ml of 4% (v/v) acetic acid along with TGE, group 4 was treated group with higher dose received 2ml of 4% (v/v) acetic acid along with TGE and group5 standard group received 2 ml of 4% (v/v) acetic acid along with Prednisolone (2 mg/kg p.o). Animals will be treated with *Tectona grandis* Linn. bark extract for 7 days. On the 8th day, overnight fasted animals will be anaesthetized using pentobarbitone sodium and 2 ml of 4% acetic acid solution will be instilled into rectum. After 48 h animals will be sacrificed by cervical dislocation and dissected to remove colon. Waste material will be removed from colon and it will be flushed with saline gently. Inflammation will be assessed based on physical parameters, macroscopy and microscopic features. Quantification of inflammation would be done using biochemical assay (MPO and lipid peroxides) [10, 11].

Anti-oxidant Activity of Bark Extract of *Tectona grandis* Linn: The bark extract of *Tectona grandis* will be taken and subjected to antioxidant screening by chemical methods at different concentration.

The free radical scavenging property of extracts will be analysed by 1, 2-diphenyl 1-phenyl hydrazil. Antioxidants status of all scavenge those free radicals at different concentration will be analysed [12].

Evaluation of the Disease: The disease induced in experimental animals was evaluated based on its macroscopic and microscopic characteristics. Evaluation pattern for macroscopic characteristics, given by [12] was used after some modifications. The inflammation was quantitated using myelo-peroxidase assay.

Evaluation Based on Macroscopic Characters:
Scoring for Rat Colon: [13] For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, and slightly cleaned in physiological saline to remove faecal residues. Pieces of rat ileum and colon (10 cm long each) were scored for macroscopic features using following scoring pattern.

TABLE 1: SCORING FOR RAT COLON

Score	Macroscopic changes
0	No visible change
1	Hyperemia at sites
2	Lesions having diameter 1mm or less
3	Lesions having diameter 2mm or less (number < 5)
4	Lesions having diameter 2mm or less (number 5 – 10)
5	Lesions having diameter 2mm or less (number > 10)
6	Lesions having diameter more than2mm (number < 5)
7	Lesions having diameter more than2mm (number 5 – 10)
8	Lesions having diameter more than2mm (number > 10)

Percentage Area Affected of Rat Colon: [13] Rat colon (5 cm long) was scored for macroscopic features using following scoring pattern.

TABLE 2: PERCENTAGE AREA AFFECTED OF RAT COLON

Score	Percentage area affected
0	0
1	1-15
2	5 -10
3	10 -25
4	25-50
5	50-75
6	75-1000

Score for an individual rat was calculated as the combined score of ileum, colon, and caecum.

Evaluation Based on Microscopic (Histologic) Characters: The colon from each animal was removed after sacrificing the animal and was collected and preserved in 10% formalin solution. The samples were submitted to Jeevan Regional diagnostic health care and research centre Pvt. Ltd., (Belgaum, India) for histological examination.

Myeloperoxidase Assay for Quantification of Inflammation: Pieces of inflamed tissues (colon-4 cm) were taken. The tissue was then rinsed with ice-cold saline, blotted dry, weighed and excised. Minced tissue was homogenized in 10 volumes of ice-cold potassium phosphate buffer (pH 7.4), using Remi tissue homogenizer (RQ-127A). The homogenate was centrifuged at 3500 rpm for 30 min at 4 ºC (Remi centrifuge C23). The supernatant was discarded. 10 ml of ice- cold 50 mM potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB) and l0 mM EDTA was then added to the pellet. It was then subjected to one cycle of freezing and thawing and brief period (l5 s) of sonication.

After sonication solution was centrifuged at 15,000 rpm for 20 min. (Remi centrifuge, R24). Myelo-peroxidase (MPO) activity was measured spectro-photometrically as follows. 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer containing 0.167 mg/ml *O*-dianisidine hydrochloride and 0.0005% H_2O_2. The change in absorbance was measured spectrophotometrically (Shimadzu UV 1 60A UV-VIS spectrophotometer), at 460 nm. One unit of MPO activity is defined as the change in absorbance per minute by 1.0 at room temperature, in the final reaction.

Calculation of MPO Activity:

MPO activity (U/g) = X / (Wt of piece of tissue taken)

Where X = (10x Changes in absorbance per minute) / (Volume of supernatant taken in the final reaction)

Measurement of Colonic Lipid Peroxides Concentration: Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species was measured as thiobarbituric acid reactive substance. The amount of colonic lipid peroxides

was measured by the thiobarbituric acid assay (TBA). Briefly, 0.5 ml of colonic tissue homogenates prepared were reacted with 2 ml of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl. Samples were boiled for 15 min, cooled and centrifuged. Absorbance of the supernatants was spectrophotometrically measured at 532 nm. TBARS concentrations were calculated by the use of 1, 3, 3, 3 tetra-ethoxypropane as a standard. The results were expressed as µmol/g wet tissue weight.

COX Inhibition Assay: The assay was performed by using Colorimetric COX (human ovine) inhibitor Screening assay kit. A reaction mixture contains, 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2), and 10 µl of TGE (1 mg/ml). The percent COX inhibition was calculated by colorimetrically by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm. Aspirin was used as a standard drug. The percent COX inhibition was calculated using following equation [14]:

$$\% \text{ Inhibition of COX} = 1 - (T/C) \times 100$$

Where T = Absorbance of the inhibitor well at 590 nm. C = Absorbance of the 100% initial activity without inhibitor well at 590 nm.

Screening of Prostaglandins Inhibition: Screening of Prostaglandins inhibition was determined by inhibition of Castor oil induced diarrhea in rats. In the present study animals were divided into three groups of six rats each. Group 1 was administered vehicle orally and served as control. Group 2 served as standard and received aspirin (150 mg/kg), orally.

Group 3 was administered with TGE 300 mg/kg by orally. Before administration of test and standard drugs animals were fasted overnight but allowed free access to water. After 30 min of administration of above dose all the rats were given with 1 ml of castor oil orally. The numbers of wet fecal dropping were measured for four hours [15].

Statistical Analysis: All data was expressed as mean ± standard error of the mean (S.E.M.) of 6 rats per experimental group. Statistical analysis was performed using Graph pad prism 5.0 statistical software. Parametric one way analysis of variance (ANOVA) followed by Tukey's post test. The minimal level of significance was identified at $p < 0$.

RESULTS AND DISCUSSION: Extraction of *Tectona grandis* Linn. was carried out by continuous hot extraction method by Soxhlet apparatus using ethyl alcohol and temperature of apparatus was kept around 60 - 70 ºC. Thus obtained ethanolic extract was further concentrated by rotary evaporator to get grayish brown colored slurry with specific teak wood smell and around 16.01% yield was obtained. Extracts was subjected for preliminary qualitative analysis to detect the presence of several phytochemicals

The results indicated that the extract showing positive tests for Carbohydrates, Glycosides, Flavonoids, Tannins and phenolic compounds and Alkaloids Steroids. In order to find out number constituents present the TLC was carried out and it was tried find out major constituents present and result indicated the presence of the tannins and confirmation of the band was done by the standard tannin samples.

TABLE 1: RESULTS OF % YIELD OF ETHANOLIC EXTRACT OF *TECTONA GRANDIS* LINN. BARK

Batch	Initial weight of powder drug in grams	Weight of extract	% yield
I	20.1	3.4	16.92
II	20.5	2.3	11.22
III	19.6	3.9	19.90
		Average	16.01

HPTLC profiling of ethanolic extract showed the presence of 11 constituents at different R_f values. The developed TLC plates and HPTLC chromatograms are given in **Fig. 1**. Peak 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 got R_f value of 0.01, 0.03, 0.08, 0.11, 0.14, 0.15, 0.17, 0.23, 0.27, 0.33 and 0.41 respectively. TGE showed the presence of andrographolide and which was confirmed with retardation factor R_f 0.6. The KEE showed the presence of tannins in the respective R_f region, which matched very well with that of standard tannins **Fig. 1** and **Table 2**.

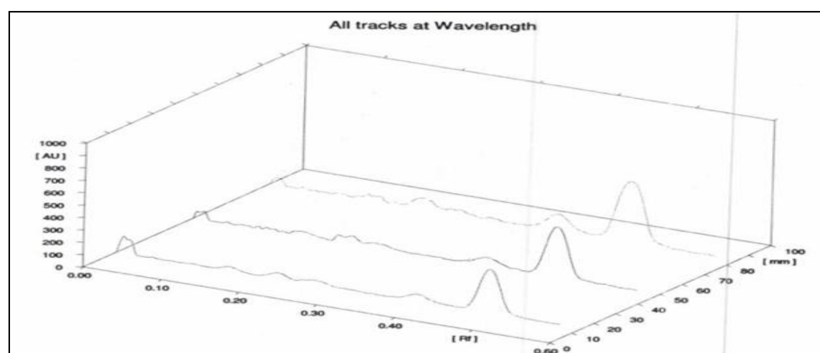

FIG. 1: HPTLC CHROMATOGRAMS OF ETHANOLIC EXTRACT OF *TECTONA GRANDIS* LINN. BARK

TABLE 2: RESULTS OF R_f VALUES OBTAINED FROM HPTLC CHROMATOGRAMS OF ETHANOLIC EXTRACT OF *TECTONA GRANDIS* LINN. BARK

Peak	Start R_f	Start height	Max. R_f	Max. height	Max. %	End R_f	End height	Area	Area %
1	0.01	53.9	0.01	57.5	5.93	0.02	0.8	428.1	1.93
2	0.03	1.1	0.04	14.0	1.44	0.06	6.3	122.8	0.55
3	0.08	1.7	0.10	16.2	1.67	0.10	1.5	147.1	0.66
4	0.11	0.8	0.12	46.4	4.79	0.13	37.9	542.1	2.44
5	0.14	37.0	0.14	45.4	4.69	0.15	4.0	336.0	1.52
6	0.15	0.0	0.16	16.4	1.69	0.17	6.6	95.0	0.43
7	0.17	7.1	0.20	85.8	8.86	0.22	26.4	1940.9	8.75
8	0.23	26.9	0.25	40.8	4.21	0.27	0.0	769.6	3.47
9	0.27	1.5	0.28	20.5	2.11	0.30	0.9	237.4	1.07
10	0.33	4.2	0.37	135.6	13.99	0.41	14.8	3555.1	16.03
11	0.41	15.2	0.46	490.5	50.62	0.52	5.6	14000.1	63.14

GC-MS Analysis: GC-MS analysis *Tectona grandis* Linn. bark is presented in **Fig. 2** and found a major fractions at RT 22.275 with % area of 56.66. Interpretation these fractions were done by confirming data bank of national institute of standards and technique by comparing the molecular weight and molecular formula. The spectrum showed the presence of 1, 2, 3, 5-cyclohexanetetrol and various analogs of quinic acid in ethanolic extract of *Tectona grandis* Linn. bark.

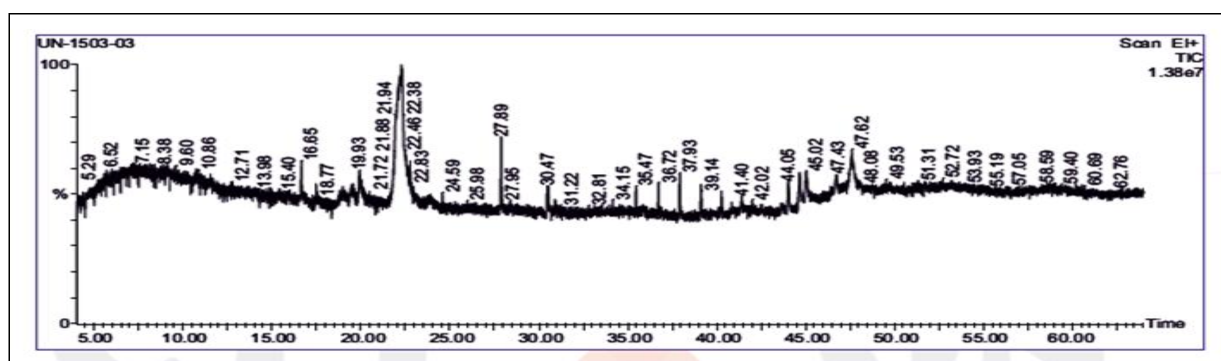

FIG. 2: GC-MS ANALYSIS ETHANOLIC EXTRACT OF *TECTONA GRANDIS* LINN. BARK

Indomethacin-Induced Enterocolitis in Rats: Two days treatment with indomethacin (7.5 mg/kg, s.c.), produced severe inflammation in rat intestine. The middle portion of the small intestine i.e. jejunum and proximal ileum showed more inflammation compared to proximal portion of the small intestine. Caecum was the most severely affected part, showing hemorrhagic spots. The ileum showed many lesions, which were transmural. In between there were skip areas of normal tissue. In some animals the large intestine was found to be affected with hemorrhagic lesions. Evaluation based on macroscopic features showed significantly ($P<0.001$) lower score values for drug treated and standard treated group compared to the positive control group. Score values of the drug treated group were comparable with the scores obtained in standard treated group **Table 3**.

TABLE 3: EFFECT OF *TECTONA GRANDIS* EXTRACT ON MACROSCOPIC FEATURES IN INDOMETHACIN-INDUCED ENTEROCOLITIS IN RATS

S. no.	Treatment groups	Mean of macroscopic scores ± S.E.M	
1	Normal or untreated animals.	0	
2	Control animals receive only indomethacin (7.5mg/kg) s.c.	8.33	± 0.56
3	Animals treated with indomethacin (7.5 mg/kg) s.c + lower dose. (TGE)	5.33	± 0.88*
4	Animals treated with indomethacin (7.5 mg/kg) subcutaneous + higher dose. (TGE)	3.33	± 0.56[x]
5	Animals, which will receive Prednisolone (2 mg/kg p.o) and indomethacin (7.5 mg/kg)	1.5	± 0.22[x]

Table represents significant reduction in macroscopic score value compare to Indomethacin alone. Each value represents mean of macroscopic scores ± S.E.M. (n = 6). Significant in macroscopic score values according to one-way ANOVA followed by Tukey's post-test, P values of *< 0.05 were considered statistically significant. [X] P < 0.001 compare to indomethacin alone.

The myeloperoxidase assay showed significant increase in MPO activity of positive control group compared to normal untreated group. The drug treated and standard treated group showed significant (P < 0.01, P < 0.001) reduction in MPO activity compared to the positive control group. MPO activity of the drug treated group was comparable with the standard treated group **Table 5** and **Fig. 3**. The lactate dehydrogenase (LDH) assay showed significant (P < 0.001) increase in LDH activity of positive control group compared to normal untreated group. The drug treated and standard treated group showed significant (P < 0.01, P < 0.001) reduction in LDH activity compared to the positive control group. LDH activity of the drug treated group was comparable with the standard treated group **Table 4** and **Fig. 4**.

The Lipid peroxidase (LPO) assay showed significant (P < 0.001) increase in LPO activity of positive control group compared to normal untreated group. The drug treated and standard treated group showed significant (P < 0.01, P < 0.001) reduction in LPO activity compared to the positive control group. LPO activity of the drug treated group was comparable with the standard treated group **Table 5** and **Fig. 5**. Histological examination of positive control group showed advanced lesions as necrosis of even payers patches and fragmentation of nuclei. The drug treated group showed reduced intensity of lesions without any evidence of necrosis, regeneration or inflammatory reaction. Standard treatment showed suppressed inflammatory reaction.

TABLE 4: EFFECT OF *TECTONA GRANDIS* EXTRACT ON MPO, LDH AND LPO ACTIVITY IN INDOMETHACIN-INDUCED ENTEROCOLITIS

Groups	Treatment Groups	MPO activity (U/g) ± S.E.M		LDH activity (U/L) ± S.E.M		LPO Activity (µmol/g) S.E.M	
I	Normal or untreated animals.	2.17	±0.31	513.67	± 6.38	0.16	±0.04
II	Control animals receive only Indomethacin (7.5mg/kg) s.c.	12.67	±0.71	1158.67	±60.68	0.70	±0.06
III	Animals treated with Indomethacin (7.5mg/kg) s.c. + lower dose (TGE)	8.33	±0.84[B]	909.17	±75[b]	0.47	±0.09[c]
IV	Animals treated with Indomethacin (7.5mg/kg) s.c.+ higher dose(TGE)	4.00	±1.13[C]	839.0	±61[a]	0.3	±0.08[a]
V	Animal treated group, which will receive Prednisolone (2mg/kg p.o) and indomethacin 7.5mg/kg) s.c.	1.67	±0.21[C]	555.67	±100[b]	0.15	±0.02[b]

Each value represents mean of MPO activity (U/g), LDH (U/L), LPO (µmol/g) ± S.E.M. (n = 6). (One-way ANOVA followed by Tukey's post-test)

c. Significant increase in MPO, LDH, LPO activity P<0.001 w.r.t normal group
a. Significant decrease in MPO, LDH, LPO activity P<0.01 w.r.t positive control group
b. Significant decrease in MPO, LDH, LPO activity P<0.001 w.r.t positive control group

Acetic Acid-Induced Colitis in Rats: Intra-rectal instillation of acetic acid caused inflammatory reaction in the colon. The inflammation covered rectum and distal colon portion. The visible changes included severe epithelial necrosis and ulcerated mucosa. Drug treated and standard treated group showed significantly (P < 0.001) lower score values of macroscopic evaluation as compared to positive control group and values obtained for the drug treated group were comparable with the standard treated group **Table 5**.

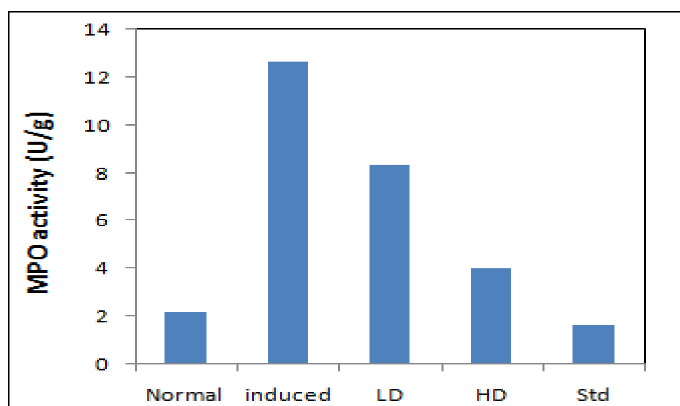

FIG. 3: EFFECT OF *TECTONA GRANDIS* EXTRACT ON MPO ACTIVITY IN INDOMETHACIN-INDUCED ENTEROCOLITIS IN RATS

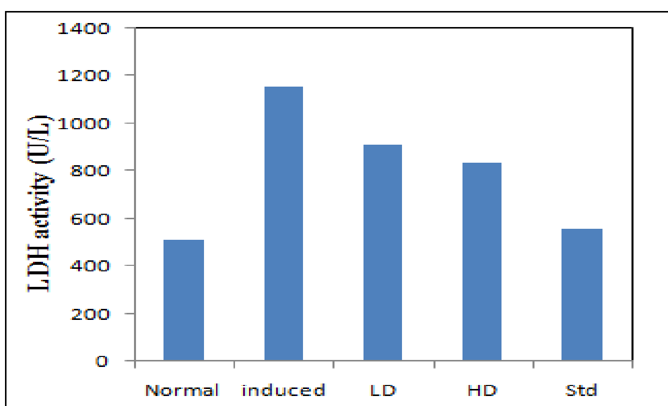

FIG. 4: EFFECT OF *TECTONA GRANDIS* EXTRACT ON LDH ACTIVITY IN INDOMETHACIN-INDUCED ENTEROCOLITIS IN RATS

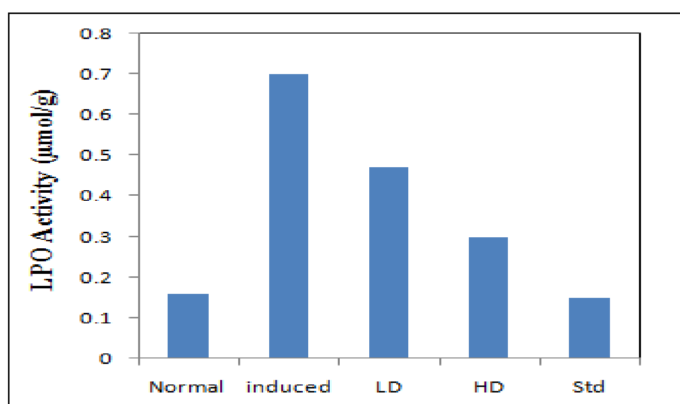

FIG. 5: EFFECT OF *TECTONA GRANDIS* EXTRACT ON LPO ACTIVITY IN INDOMETHACIN-INDUCED ENTEROCOLITIS IN RATS

TABLE 5: EFFECT OF *TECTONA GRANDIS* ETHANOLIC EXTRACT MACROSCOPIC FEATURES IN ACETIC ACID INDUCED COLITIS IN RATS

S. no.	Treatment groups	Mean of macroscopic scores ± S.E.M	
1	Normal	0	
2	Positive control- Acetic acid in saline alone 2 ml (4%, v/v) (once, intra-rectally)	10.17	± 0.40
3	Ethanolic extract of *Tectona grandis* + Acetic acid in saline 2 ml (4%, v/v) (once, intra-rectally) [TGE]	6.67	± 0.80*
4	Ethanolic extract of *Tectona grandis* + Acetic acid in saline 2 ml (4%, v/v) (once, intra-rectally) [TGE]	2.83	± 0.48x
5	Standard (500 mg/kg, p.o.) + Acetic acid in saline 2 ml (4%, v/v) (once, intra-rectally)	2.17	± 0.17x

Table represents significant reduction in macroscopic score value compare to acetic acid alone. Each value represents mean of macroscopic scores ± S.E.M. (n = 6). Significant in macroscopic score values according to one-way ANOVA followed by Tukey's post-test, P values of < 0.05 were considered statistically significant. cP < 0.001 compare to acetic acid alone

The myeloperoxidase assay showed significant (P < 0.001) increase in MPO activity of positive control group compared to normal group. The drug treated and standard treated groups showed significant (P < 0.01, P < 0.001) decrease in MPO activity compared to positive control group **Table 6** and **Fig. 6**. The lactate dehydrogenase (LDH) assay showed significant (P < 0.001) increase in LDH activity of positive control group compared to normal group. The drug treated and standard treated groups showed significant (P < 0.01, P < 0.001) decrease in LDH activity compared to positive control group **Table 6** and **Fig. 7**.

The lipid peroxidase (LPO) assay showed significant (P < 0.001) increase in LPO activity of control group compared to normal group. The drug treated and standard treated groups showed significant (P < 0.01, P < 0.001) decrease in LPO activity compared to positive control group **Table 6** and **Fig. 8**.

Histological examination of control group showed massive necrosis of the mucosa and submucosa. Payers patches appeared distorted with karyohexis and karyolysis. Drug treated group showed mild lesions, regeneration and inflammatory reaction. The standard treated group showed suppressed inflammatory reaction.

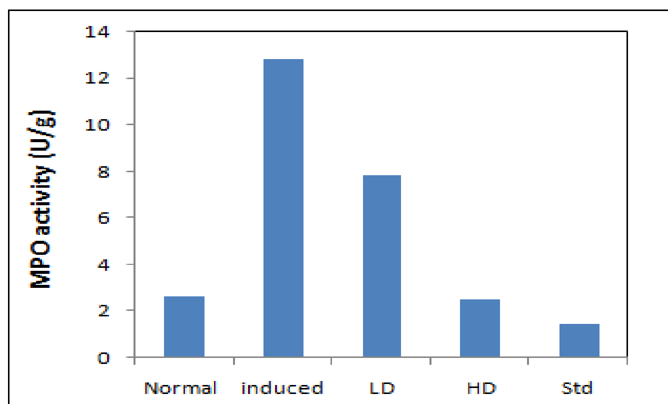

FIG. 6: EFFECT OF *TECTONA GRANDIS* EXTRACT ON MPO ACTIVITY IN ACETIC ACID-INDUCED ENTEROCOLITIS IN RATS

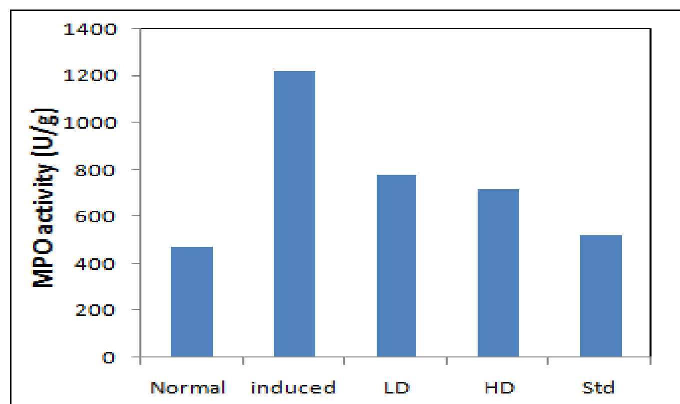

FIG. 7: EFFECT OF *TECTONA GRANDIS* EXTRACT ON LDH ACTIVITY IN ACETIC ACID-INDUCED ENTEROCOLITIS IN RATS

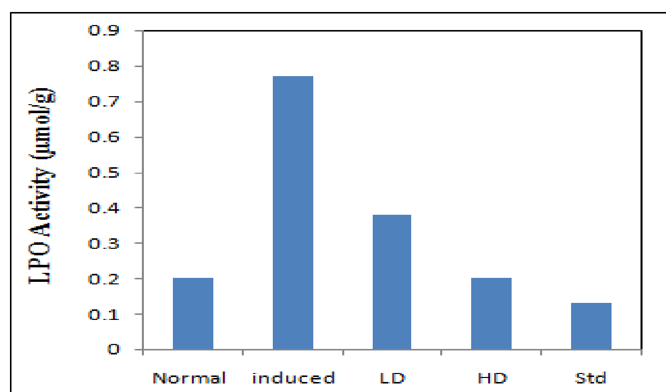

FIG. 8: EFFECT OF *TECTONA GRANDIS* EXTRACT ON LPO ACTIVITY IN ACETIC-INDUCED ENTEROCOLITIS IN RATS

TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF *TECTONA GRANDIS* ON MPO, LDH AND LPO ACTIVITY IN ACETIC ACID-INDUCED COLITIS IN RATS

Groups	Treatment groups	MPO activity (U/g) ± S.E.M		LDH activity (U/L) ± S.E.M		LPO Activity (µmol/g) S.E.M	
I	Normal	2.67	0.21	472.33	46.22	0.20	0.04
II	Positive control- Acetic acid in saline alone 2 ml (4%, v/v) (once, intra-rectally)	12.83	0.87	1221.83	55.88	0.77	0.05
III	Ethanolic extract of *Tectona grandis* + Acetic acid in saline 2 ml (4%, v/v) (once, intra-rectally [TGE]	7.83	0.87	778.50	96.20	0.38	0.09
IV	Ethanolic extract of *Tectona grandis* + Acetic acid in saline 2 ml (4%, v/v) (once, intra-rectally) [TGE]	2.50	0.56	721.50	95.91	0.20	0.03
V	Standard (500 mg/kg, p.o.) + Acetic acid in saline 2ml (4%, v/v) (once, intra- rectally)	1.50	0.22	523.33	83.62	0.13	0.02

Each value represents mean of MPO activity (U/g), LDH (U/L), LPO (µmol/g) ± S.E.M. (n = 6). (One-way ANOVA followed by Tukey's post-test)

c. Significant increase in MPO, LDH, LPO activity P<0.001 w.r.t normal group
a. Significant decrease in MPO, LDH, LPO activity P<0.01 w.r.t positive control group
b. Significant decrease in MPO, LDH, LPO activity P<0.001 w.r.t positive control group

TGE was evaluated for its *in vitro* COX-1 and COX-2 inhibitory activities by using colorimetric COX (human ovine) inhibitor screening assay kit. The results showed that TGE inhibits COX-1 about 42.35% and that of COX-2 is 45.8% see **Table** 7. Prostaglandins inhibition was studies by inhibition

of Castor oil induced diarrhea in rats. Administration of Castrol induced diarrhea around 9 fecal drops with total weight of 5.5 grams in 4 h. This was reduced in both test and standard groups. Indicating TGE produces action by inhibiting the synthesis of prostaglandins see **Table 8**.

TABLE 7: EFFECT OF KEE ON COX INHIBITORY ACTIVITY

Extract	Inhibition of COX-1 (%)	Inhibition of COX-2 (%)
TGE	43.17	48.67

TABLE 8: DETERMINATION OF PROSTAGLANDINS INHIBITION

Groups	Treatment and dose	Mean frequency of diarrhea ± SEM	Mean no. of fecal drops ± SEM	Mean wt. of feaces ± after 4 hrs (gm)
1	Control (0.5 ml of DI water)	7.17 ±1.47	9.67 ±1.51	5.17 ±1.17
2	Aspirin150 mg/kg	1.67 ±0.82	3.67 ±1.21	3.33 ±1.86
3	TGE	1.33 ±0.52	4.67 ±3.08	3.17 ±1.33

DISCUSSION: Bark extract of *Tectona grandis* Linn. is screened for inflammatory bowel disease. Various parts of the plant have showed activities like astringent, depurative, diaphoretic, diuretic expectorant, febrifuge, odontalgic and ophthalmic. The aerial part of this plant has been reported to contain pyrrolizidine alkaloids and flavonoids [2 - 3]. The aqueous extract of the aerial parts have shown antimicrobial activity [4]. The plant is astringent, sweet, thermogeic, ntipyretic and antiasthmatic. Aqueous and methanolic extract of ES leaves have been shown to progressively reduced rat paw edema induced by sub plantar injection of albumin, which suggest that this extract might have anti-inflammatory principals. The bark extract of *Tectona grandis* Linn. has antioxidant and antiproliferative effects on human cancer cells [4]. In a study *Tectona grandis* Linn. stem bark extracts showed significant *in vivo* analgesic and anti-inflammatory activities [5].

The models used for study of IBD are acetic acid induced ulcerative colitis and indomethacin induced enterocolitis. Intrarectal instillation of acetic acid in rats affected only the distal colon portion. The inflammation was not transmural. Massive necrosis of mucosal and submucosal layers was observed. This model shares many of the histologic features of ulcerative colitis in human beings including mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration [68]. The mechanism by which acetic acid produces inflammation appears to involve the entry of the protonated form of the acid into epithelium, where it dissociates to liberate protons causing intracellular acidification that most likely accounts for the epithelial injury observed. The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipooxygenase pathways and generation of inflammatory mediators like prostaglandins and leukotrienes. Excess production of reactive oxygen metabolites *e.g.* superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid and oxidant derivatives such as N-chloramines are detected in inflamed mucosa and may be pathogenic in IBD. Also, there is an increase in proinflammatory cytokine TNF -α production in colonic mucosa after acetic acid instillation. Indomethacin, a non-selective COX inhibitor produces enterocolitis in rats on sub cuteneous administration which is characterized by linear ulceration, thickening and transmural inflammation.

The mechanism of indomethacin induced enterocolitis have not been fully illustrated, but previous reports suggests that, inhibition of protective prostaglandins PGE1, PGE2 and prostacyclin (PG12) may be one of the mechanism by which indomethacin induces injury. In addition, bacteria and bacterial products, biliary secretion and food intake have been demonstrated to be important for the development of the intestinal lesions.

The treatment with bark extract *Tectona grandis* Linn. has shown a decrease in the macroscopic scores for the inflammation. Since the intestine is in a constant state of controlled inflammation, thus amplification of the inflammatory response activates infiltration of inflammatory cells that triggers pathological responses and symptoms of

IBD [16]. Our study showed that acetic acid raised the levels of colonic MPO, indicating infiltration of neutrophils and perturbation of the inflammatory system [70]. This fact is documented in both animal models, [17] and patients with IBD [18]. *Tectona grandis* Linn. bark extract ameliorated neutrophil infiltration as evidenced by suppression of colon MPO and improvement of histological features.

Histopathology examination of drug treated group revealed less damage compared to control group. A significant decrease in MPO activity was also observed. All these observations support the findings that the barks extract of *Tectona grandis* Linn. was able to offer significant protection in both the models studied. Acetic acid-induced colitis and indomethacin-induced enterocolitis simulate two different disease conditions, which are ulcerative colitis and Crohn's disease respectively. On this basis we can say that the constituents extract under study may be useful in treating UC as well as CD in humans. The prednisolone treatment has shown significant protection in both the animal models under our study. The bark extract *Tectona grandis* Linn. was found comparable with standard drug. The role of oxygen-derived free radicals, such as hydroxyl radical and superoxide radical, in the inflammatory process is well known. It is also generally assumed that most of the antioxidants possess anti-inflammatory effect. Bark extract of *Tectona grandis* Linn. may account for the observed anti-inflammatory properties.

CONCLUSION: Bark extract of *Tectona grandis* Linn. has potent protection against inflammatory bowel activity in both indomethacin induced enterocolitis and acetic acid induced ulcerative colitis. Collectively, these findings indicate that the anti-oxidant effect of fraction may be an important contributor to its anti-inflammatory activity in IBD. This present investigation has also opened avenues for treatment of IBD from the title plant.

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CONFLICTS OF INTEREST: Nil

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