Antiulcer Activity and Isolation of Chemical Constituents from Ethanolic Stem Bark Extract of Ficus Bengalensis

N. Anupama a, Jennifer Fernandes a* and K. Adarsha Govinda a

a Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, NITTE (Deemed to be University), Mangaluru-575018, Karnataka, India.

ABSTRACT

Aims: The screen the antiulcer activity and to isolate the phytoconstituents of stem bark extract of Ficus bengalgensis.

Methodology: The cleaned, dried and powdered stem barks of Ficus bengalgensis were subjected to extraction. The extracts were then packed into column chromatography for the isolation of phytoconstituents and they were characterized by IR, 1HNMR, 13CNMR and mass spectroscopy. Invivo antiulcer activity were performed by 4-H pylorus ligation and ethanol induced ulcer methods.

Results: Preliminary phytochemical studies showed the presence of steroids, terpenoids, flavonoids, carbohydrates. Isolation of extracts led to give compounds like triterpenoid, a steroid and a flavonoid. The extract was found to be safe up to 2000mg/kg body weight. Antiulcer activity was found significant at level P=05 when compared with control models in experimental animals.

Conclusion: From petroleum ether extract isolated alpha-amyrin acetate, stigmasterol and quercetin from chloroform extract. The presence of triterpenoids, steroids and flavonoid might be responsible for the antiulcer activity of the stem bark extract of Ficus bengalgensis.

Keywords: Ficus bengalgensis; antiulcer activity; stigmasterol; quercetin; Moraceae; 4-Hpylorus ligation model.

*Corresponding author: E-mail: fernandesj@nitte.edu.in;
1. INTRODUCTION

Plants serve as vital source of drugs in different ancient forms of medications around the world. Sushrutha Samhita, Charaka Samhita, Rig Veda, etc. have detailed emphasis about medicinal values of herbs. India being one of major biodiversity centre and also home of the oldest system of medicine Ayurveda helps us to understand medicinal values of various herbs and their preparations. Mainly, pharmacological and phytochemical features helps in evaluation of herbs for its medicinal values. This includes different instrumental techniques like microscopy, chromatography, etc. With growing awareness in using traditional medications because of their safety, appropriate scientific evaluation of traditional medication is very important [1]. Banyan tree which is scientifically known as *Ficus bengalensis* (FB) (Moraceae) is known for its medicinal and traditional values in India, in Ayurveda it is called as Vata or Vada tree [2]. This plant is widespread in Sri Lanka, India and Bangladesh. Since, Hindu merchants that is Banias assembled under this tree for trading, Britishers gave the name ‘Banyan’. Triad which includes Banyan tree, Ganges and Himalayas symbolizes India. So, Banyan tree is considered to be Indian National tree. In scientific name Ficus means fig and bengalensis indicates Bengal [3]. It is one of the four holy trees Nalpamara (Ksirivrksas) supposed to be planted around temples and home [4].

Since it leans on another tree and ends up suffocating it, it is also called as “strangler fig”. Banyan tree is found vastly in monsoon and rainforests, it is evergreen and fast growing with its height going up to 20m. They are resistant to frost and drought. Tree gives rise to propagating roots that grows downward as aerial root. After they reach ground, they become supportive woody trunks. Birds like Indian Myna eat figs of these plant and seeds are likely to get germinated and sprout after excretion [5]. The banyan tree has been used for many medicinal purposes from ancient times. Its bark and leaves both possess analgesic and anti-inflammatory properties. The bark of the Banyan tree is considered useful in burning sensation, ulcers, and painful skin diseases. It can also be used in inflammation and tooth ache. Therefore, the present study is undertaken to screen the antiulcer activity and isolate the chemical constituents of *Ficus bengalensis* stem bark extracts.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of the plant

The stem bark of *F. bengalensis* was collected from Mangaluru, Karnataka, during May 2010. It was authenticated by Dr. Noeline J. Pinto, Professor and Head, Dept. of Botany, St. Agnes College Mangaluru.

2.2 Preparation of Ethanolic Extract

Barks were dried under shade, powdered (5 kg) and macerated for three days along with ethanol and Ethanol was decanted thereafter. It was repeated thrice. Initially solvent in the obtained extract was distilled off and is evaporated to dryness (500g) [6,7]. Preliminary phytochemical tests were performed for the presence of secondary metabolites [8,9].

2.3 Fractionation of Ethanolic Extract

Ethanolic extract (400g) was made into two equal parts. These were placed in distilled water (500 ml) and extracted using petroleum ether (60–80 °C, 8x500 ml), diethyl ether (8x500 ml) and chloroform (8x500 ml) in succession. Distilled water (30 ml) was used to wash the fractions, then these were dried over anhydrous sodium sulfate followed by distillation to make it free from solvent. Thus ethanolic extract was fractionated to diethyl ether soluble fraction (34 g), chloroform soluble fraction (35 g) and petroleum ether (60–80 °C) soluble fraction (40 g).

2.4 Isolation of Compounds from Petroleum Ether Extract

CHCl₃ (20 ml) was employed to dissolve pet. Ether extract (20 g) and was adsorbed onto silica gel (20 g). It was loaded into column of silica gel (150 g) prepared in petroleum ether (60–80 °C) after solvent evaporation. First pet. ether 100 % (60–80 °C) was eluted through the column which was followed by graded mixtures of petroleum ether (60–80°C): CHCl₃ (95:5, 90:10, 80:20), CHCl₃ 100 %, graded mixture of CHCl₃: MeOH (95:5, 90:10, 80:20) and with methanol. TLC was employed to monitor the elution (Silica gel G; vanillin-sulphuric acid reagent was used for visualization of spots by heating at 110 °C). Every time 10ml were collected and same amount of eluates were combined and was
concentrated to 5ml and was placed inside dessicator.

Elution which was performed using petroleum ether (60-80°C): CHCl₃ graded mixture (95:5) gave one spot I TLC (pet. ether: CHCl₃; 95:5), it was named as compound I (50 mg). Elution which was performed using petroleum ether (60-80°C): CHCl₃ graded mixture (90:10) also gave one spot on TLC, it was named as compound II (55 mg). Other eluates resulted in brown colored resin mass and were unprocessed.

2.4.1 Hydrolysis of the compound III

Add 5 ml of 2N HCl to solution of compound III (10 mg) in same volume of methanol. Mixture obtained was reflushed for 2h at 100°C and under reduced pressure it was evaporated till dryness. Distilled H₂O (5 ml) was added, extracted with ether and concentrated. Then it was dried using an Na₂SO₄ which resulted in deposition of yellow colored solid. Yellow needles were obtained on crystallization (MeOH) of aglycone which had m.p. 314-317°C. With Mg/HCl it gave orange color.

Acetylation of compound III: The compound III (5 mg) was mixed with freshly fused NaOAc (0.5 g) and freshly distilled acetic anhydride (2 ml). Resultant mixture was reflushed for 2h at 140°C. Contents were then poured to cold water. The crude solid thus obtained was filtered, cleaned using cold water and dried. Colorless prisms are obtained from recrystallization of crude acetate from methanol, m.p. 199-200°C.

2.4.2 Isolation of compounds from Chloroform extract

CH₃OH (30 ml) along with extract (20 g) was made into solution and was loaded onto column of silica gel (150 g) prepared in ethyl acetate. Ethyl acetate, 1 %, 2 % and 5 % methanol in ethyl acetate were used in elution. TLC was employed to monitor the elution (EtOAc: MeOH: CHCl₃, 100:16.5:13.5) and UV/NH₃ was used for visualization. In TLC ethyl acetate and 1 % MeOH in Ethyl acetate eluates showed existence of similar compounds, which was concentrated to yield crystalline yellow solid. Obtained solid material was filtered, cleaned and recrystallized (MeOH) which resulted in formation of yellow compound (32 mg). Only resinous matter was obtained on concentration of eluates of the 2 % and 5 % methanol in ethyl acetate and these were unprocessed.

2.5 Acute Toxicity Studies

As per “Up and Down” [10] method and OECD 425 guidelines [11] adult female albino rats of 150-200 g were selected for acute toxicity studies. Test extract (suspended in 0.6% Na CMC) was given orally to overnight fasted animals 1000 mg/kg body weight. Once in 30 min. for 4h these animals were observed for their neurological, general behavioral and autonomic profiles and lastly for death after 24h.

2.5.1 Selection of animals

Albino Wistar rats (150-250 g) of either sex were procured from the central animal house of NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore. In a temperature controlled room rats were caged with standard conditions, food, water access and 12:12 hours light/dark cycles. Each group of rats are used only once and were chosen randomly. Under IAEc of KSHEMA, Deralakatte, Mangalore (KSHEMA/AEC/26/2010) all the studies were carried out.

2.5.2 Selection of doses [12]

Three dose levels were chosen to assess antiulcer activity in a way that, mid dose was approx. 1/10th of the max. dose given during acute toxicity study (i.e., 200 mg/kg body weight - 1/10th of 2000 mg/kg body weight), a high dose that was two times of 1/10th doses (i.e., 400 mg/kg body weight) and lower dose that was half of 1/10th dose (i.e., 100 mg/kg body weight)

2.6. In vivo Antiulcer Activity Methods:

2.6.1 4H-Pylorus ligated ulcer model

Albino Wistar rats in weight range of 180-250 g were made into 5 groups with each group having 6 rats. 1st group was considered as control, whereas 2nd group was considered for std. drug and finally 3-5th group were considered as test drug treatment at graded doses of 100,200 and 400mg/kg bodyweight. For 7 days this treatment was continued. 24h before pyloric ligation rats will be fasted i.e, on 7th day but water was allowed adlibitum. In order to avoid corography care was taken. Rats were anaesthesitized with the help of anaesthetic ether on 8th day and by midline incision abdomen was opened. Stomach was lifted out and pyloric end part was ligated. In order to prevent occlusion of blood vessels care was taken. Instrumental gasping on stomach was
avoided. Replacement of stomach was done carefully and by interrupted structures abdomen wall was closed. After 4h by overdosing with anaesthetic ether rats were sacrificed and cardiac end of stomach was ligated. Stomach was pulled out and cut along greater curvature.

In centrifuge tubes, gastric juice was collected and subjected for centrifugation to estimate total and free acidity and remaining biological parameters such as total carbohydrates and proteins. Gastric juice volume and pH were measured.

Running tap water was used to wash stomachs to check stomachs glandular portions for presence of ulcers. Macroscopically, no of ulcers were counted and their severity was scored with hand lens (10x) [13] Ulcer index was then calculated and obtained values were analysed statistically.

Mean ulcerative index was calculated as follows:

I. Incidence of single sub mucosal punctiform hemorrhages, edema and hyperemia.

II. Incidence of sub mucosal hemorrhagic lesions with small erosions.

III. Incidence of deep ulcers with invasive lesions erosions

![Ulcer index formula here]

The percentage inhibition was determined as follows:

\[
\text{Percentage Inhibition} = \frac{\text{Control mean lesion index} - \text{Test mean lesion index}}{\text{Control mean lesion index}} \times 100
\]

2.6.2 Method for estimation of free acidity and total acidity [14]

Collection of gastric juice: From pylorus ligated rats gastric juice was collected. Collected gastric juice was centrifuged, pH and gastric juice volume was noted. Then biochemical estimation of gastric juice was carried out as follows.

Determination of free acidity and total acidity in gastric juice: 1ml of gastric juice was pipetted into a conical flask (100ml), 2 drops of Topfer’s reagent was added and then it was subjected for titration with 0.01 N sodium hydroxide (standardized with 0.01 N oxalic acid) until disappearance of red color and the color of the solution was yellowish orange. Added alkali volume was noted which matches to free acidity. Phenolphthalein solution (2-3 drops) was added and titration was continued until appearance red tinge appears. Total added alkali volume was noted which matches to free acidity. Acidity was calculated by using the formula [15,16].

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1}
\]

Acidity was expressed in meq/1/100 g; the data was subjected to statistical analysis. For 7 days above mentioned treatment was given. 14-16 hours prior to experiment food was withdrawn. One hour after the final dose of the drug, irrespective of bodyweight 90% ethanol was administered to the overnight fasted rats of all groups on 7th day, at a dose of 1ml per animal, via oral route. After 2h of ulcerogenic dosing by cervical dislocation animals were sacrificed. The stomach was removed, incised along the greater curvature and washed with running tap water.

2.6.3 Ethanol induced ulcer model

Either sex of Albino Wistar rats weighing in range of 180-250 g were made into five groups with six animals in every group. 1st group was considered as control, whereas 2nd group was considered for std. drug and finally 3-5th group were considered as test drug treatment at graded doses of 100,200 and 400mg/kg bodyweight. For 7 days above treatment was given. 14-16 hours prior to the experiment food was withdrawn. One hour after the final dose of the drug, irrespective of bodyweight 90% ethanol was administered to the overnight fasted rats of all groups on 7th day at a dose of 1ml per animal, via oral route. After 2h of ulcerogenic dosing by cervical dislocation animals were sacrificed. The stomach was removed, incised along the greater curvature and washed under running tap water. Mucosal erosions and ulcers scores were recorded macroscopically by using Hand lens (10X) [17,18].

2.7 Statistical Analysis

The results were expressed as mean ± SEM. The total variation present in the data was analysed by one way analysis of variance (ANOVA) followed by Post hoc test (Dunnett’s test).
3. RESULTS AND DISCUSSION

3.1 Analysis of Compound I

Physical state: Pearl white crystals. Rf value: 0.48 [Solvent system; petroleum ether: chloroform (95:5)]. Melting point: 227° C

Spectral Data (cm⁻¹): C-H stretching in CH₃ & CH₂ 1712 (C=O stretching), 1456.2 (C-H deformation of CH₂) 1365.5 (str. of gem dimethyl) 1241.0 (C-O stretching), 1172.6 (C-O deformation), 5 0.85 (s, 3H, 28) δ .86 to δ 0.89 (m, 12H-H-23, 24, 29, 30) δ 1.08 (s, 3H, H-27), δ 2.068 (s, 3H, OAc), δ 4.52 (t, 1H, H-3), δ 5.15 (s, 1H, H-12). The signals due to methylene and methine protons overlapped with each other and appeared as multiplet in the region of δ 1.0-5.12 integrating for 23 protons.

Molecular formula: C₅₀H₅₂O₃. Molecular weight: 468; EIMS (m/z): 468 (M+, C₅₀H₅₂O₃, 60%), 453 (M⁺ - CH₃, 30%), 408 (M⁺ - CH₃COOH, 14%), 368 (18%), 358(22%), 325(10%), 297 (32%), 270(26%), 249(16%), 218(100%).

3.1.1 Hydrolysis of compound I

Compound I (5 mg) was saponified with 20% ethanolic KOH (100 ml) for 2 h. This was then evaporated to remove all traces of EtOH, the lost volume being replaced by water from time to time. The unsaponifiable portion was then extracted with ether (4x50 ml). Each extraction was then washed with distilled water (20 ml) and dried over anhydrous sodium sulphate. All the ethereal fractions were combined and evaporated leading to the isolation of triterpenic alcohol m.p. of which (184 °C) was in good agreement with that for α-amyrin. Further it was confirmed from the melting point, IR, ¹HNMR and mass spectral data.

α - Amyrin acetate: m.p. 227 °C. It gave a characteristic colour reaction for triterpenes. It gave a green colour with LB test. An ester linkage was discernible from the characteristic IR adsorption at 1735.8 cm⁻¹. The acetate nature of the ester was indicated by the characteristic acetyl protons singlet at δ 2.069 further more mass fragment m/z 408 is suggestive of the loss of acetic acid (60) from the molecular ion (M+) 468. The peaks at 250 and 218 (Base peak) m/z were due to Retro-Diels-Alder fragmentation with the usual hydrogen transfer characteristic of the left and right half arising from triterpene having δ 12 – oleanane / ursane structure. The peak at 190 (250 – 60) m/z was due to loss of acetate moiety from the left half providing conclusive proof for the attachment of the acetate grouping at C-3 [19].

3.2 Analysis of Compound II

Physical state: Pearl white crystals, Rf value:0.5 (solvent system; petroleum ether: chloroform 90:10), Melting point:168 °C.

IR Spectral Data (cm⁻¹): 3433.0 (br, OH), 2929.0 (C-H str. in CH₃), 2854.6 (C-H str. in CH₂), 1634.5 (C=O str.) 1462.6 (C-H deformation in gem dimethyl) 1042.4 (C-O str of secondary alcohol). 607.8 (rocking vibration of CH₂).¹HNMR Spectral data: δ 0.65 to δ 1.1 (m, 18H, 6xCH₃) δ 1.1 to δ 1.26 (m, 18H, 9xCH₂) δ 1.26 to δ 1.86 (8H, 8xCH), δ 3.59 (br, 1H CHO), δ 5.4 (t, 1H, vinylic protons) δ 5.2 (2H, br, allylic protons). GC-MS (m/z): 412 (M+, C₂₉H₄₁O), the other peaks appeared at 397, 369, 351, 327, 300, 271.

Stigmasterol: m.p. 167-170 °C it showed positive response to Libermann-Burchard’s test and Salkowski test. The IR spectrum exhibited strong absorption at 3433 cm⁻¹ IR peak at 2929.0 cm⁻¹ and 2854.6 cm⁻¹ indicating the presence of C-H stretching in CH₃ and CH₂ respectively. IR peak at 1634.5 cm⁻¹ indicates C=O stretching. Peak at 1462.6 cm⁻¹ indicates C-H deformation in gem dimethyl. Peak at 1042.4 cm⁻¹ indicates C-O stretching of secondary alcohol. The ¹HNMR spectra of this compound II exhibited the presence of six methyl group at position 18, 19, 21, 26, 27 and 29 in between δ 0.65 to δ 1.1. The ¹HNMR spectra also showed vinylic proton at δ 5.4 and allylic proton at δ 5.2 The GC-MS spectra showed the molecule ion peak at 412[M+] corresponding to the molecular formula C₂₉H₄₁O. Its identity was also confirmed by co-chromatography with an authentic sample [20].

3.3 Analysis of Compound III

Physical state: Yellow Crystals, Rf Value: 0.027 (Solvent system: EtOAc: MeOH 99:1) Melting Point: 345°C.

Spectral Characterization of compound III: IR Spectral Data (cm⁻¹): 3407.4 (O-H str.), 2945 (C-H str.), 1660.2 (C=O str.), 1552.2, 1460.62 (C=C str), 1200.31 (C-O str). ¹HNMR Spectral data: δ 12.5 (s, 1H, 5- OH), δ 10.9 (s, 1H, 3- OH), δ 9.6 (s, 1H, 7- OH), δ 9.3 (1H, 4'-OH), δ 9.4 (1H, 5'-OH), δ 6.8 (1H d, 1H, H-8), δ 6.39 (d, 1H, H-7), δ 6.16, 6.17 (1H d, H 5'-d) δ 7.17 (d, 1H, H-2).¹³CNMR
spectral data: $\delta$ 145.05 (C-2), 135.72 (C-3), 175.83 (C-4), 160.70 (C-5) 98.34 (C-6), 163.8 (C-7), 93.34 (C-8), 156.13 (C-9), 121.96 (C-10), 147.69 (C-11), 115.6 (C-12), 119.9 (C-13), 115.0 (C-14), 145.05 (C-15), 147.69 (C-16), 98.34 (C-17), 93.34 (C-18), 156.13 (C-19), 103.01 (C-20), 121.96 (C-21), 115.0 (C-22), 145.05 (C-23), 147.69 (C-24), 98.34 (C-25), 93.34 (C-26), 128 (8%), 91(6%) 9 (6%), 69 (20%).

Quercetin: m.p.314-317 °C, gave orange color with shinoda’s test & yellow color with NH3. The IR spectra showed absorption band for hydroxyl at 3467cm$^{-1}$. The singlet of aromatic 5-OH of compounds $\delta$12.5 was observed. $^{13}$C NMR spectra of compound showed carbonyl signal (C=O) at $\delta$ 175.83. $^{13}$CNMR spectral data matched with exactly with that of quercetin. Its identity as quercetin was further supported by the mass spectral data and confirmed by Co. Chromatography with an authentic sample of quercetin [21].

### 3.4 Acute Toxicity Studies

The groups were treated with an oral dose upto 2000 mg/kg body weight of ethanolic extract of *Ficus bengalensis* observed with no signs of toxicity or mortality and at this dose the extract were found to be safe.

#### 3.5 Antiulcer activity of Ethanolic Extract of Stem Bark of *Ficus bengalensis*

##### 3.5.1 4H-Pylorus ligated ulcer model

The volume of gastric HCl in sucralfate treated group has decreased significantly up to 2.9±0.23 compared to control group in which the values was 6.00±0.17 (p=0.05).In groups treated with ethanolic extract the volume of gastric HCl was reduced to 5.36±0.28, 4.33±0.31, 4.03±0.18 (p=0.05) at a dose of 100, 200 and 400 mg/kg respectively when compared to control group.

The pH of gastric HCl in sucralfate treated group have increased significantly up to 4.8±0.08 compared to control group in which the value was 1.51±0.08 (p=0.05).In groups treated with ethanolic extract the pH of gastric HCl was increased to 3.24±0.01, 3.85±0.04, 4.0±0.08 (p=0.05) at a dose of 100, 200 and 400 mg/kg respectively when compared to control group.

### Table 1. Effect of Ethanolic extract of stem bark of *Ficus bengalensis* on volume of gastric juice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment(mg/kg)</th>
<th>Vol.HCl</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(0.6% CMC)</td>
<td>5ml/Kg</td>
<td>6.00±0.17b</td>
<td>-</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>400</td>
<td>2.9±0.23ac</td>
<td>51.67</td>
</tr>
<tr>
<td>Ethanolic Extract</td>
<td>100</td>
<td>5.36±0.28ac</td>
<td>10.55</td>
</tr>
<tr>
<td><em>Ficus bengalensis</em></td>
<td>200</td>
<td>4.33±0.31ab</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>4.03±0.18a</td>
<td>32.80</td>
</tr>
</tbody>
</table>

* a - P = 0.05 when compared with control, b - P = 0.05 when compared with standard

### Table 2. Effect of Ethanolic extract of stem bark of *Ficus bengalensis* on pH of gastric juice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment(mg/kg)</th>
<th>pH of gastric HCl</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(0.6% CMC)</td>
<td>5ml/Kg</td>
<td>1.51±0.08b</td>
<td>-</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>400</td>
<td>4.8±0.08a</td>
<td>217.89%</td>
</tr>
<tr>
<td>Ethanolic Extract</td>
<td>100</td>
<td>3.24±0.01ab</td>
<td>115.03%</td>
</tr>
<tr>
<td><em>Ficus bengalensis</em></td>
<td>200</td>
<td>3.85±0.04a</td>
<td>155.43%</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>4.00±0.08a</td>
<td>164.9%</td>
</tr>
</tbody>
</table>

* a - P= 0.05 when compared with control, b - P = 0.05 when compared with standard

### Table 3. Effect of Ethanolic extract of stem bark of *Ficus bengalensis* on Free acidity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment(mg/kg)</th>
<th>Free Acidity</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5ml/Kg</td>
<td>89.90±0.08a</td>
<td>-</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>400</td>
<td>20.27±0.18a</td>
<td>77.45%</td>
</tr>
<tr>
<td>Ethanolic Extract</td>
<td>100</td>
<td>78.63±0.13a</td>
<td>12.54%</td>
</tr>
<tr>
<td><em>Ficus bengalensis</em></td>
<td>200</td>
<td>42.97±0.76ad</td>
<td>52.20%</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>27.47±0.40a</td>
<td>69.44%</td>
</tr>
</tbody>
</table>

* a - P= 0.05 when compared with control, b - P = 0.05 when compared with standard
Table 4. Effect of Ethanolic extract of stem bark of Ficus bengalensis on total acidity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment(mg/kg)</th>
<th>Total Acidity</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5ml/Kg</td>
<td>113.2±1.43a</td>
<td>-</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>400</td>
<td>33.80±0.26a</td>
<td>70.13%</td>
</tr>
<tr>
<td>Ethanolic Extract Ficus</td>
<td>100</td>
<td>82.13±0.65b</td>
<td>27.43%</td>
</tr>
<tr>
<td>bengalensis</td>
<td>200</td>
<td>58.03±0.27ab</td>
<td>48.72%</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>42.2±0.49a</td>
<td>62.71%</td>
</tr>
</tbody>
</table>

a - P= 0.05 when compared with control, b - P = 0.05 when compared with standard

Table 5. Effect of Ethanolic extract of stem bark of Ficus bengalensis on ulcer index

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment(mg/kg)</th>
<th>Ulcer Index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5ml/Kg</td>
<td>23.67±1.03b</td>
<td>-</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>400</td>
<td>6.66±0.51a</td>
<td>71.82%</td>
</tr>
<tr>
<td>Ethanolic Extract Ficus</td>
<td>100</td>
<td>19.33±0.51b</td>
<td>18.34%</td>
</tr>
<tr>
<td>Ficus bengalensis</td>
<td>200</td>
<td>11.67±0.51a</td>
<td>50.70%</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>9.33±0.51a</td>
<td>60.06%</td>
</tr>
</tbody>
</table>

a - P= 0.05 when compared with control, b - P = 0.05 when compared with standard

Table 6. Effect of Ethanolic extract of stem bark of Ficus bengalensis on Ulcer index

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment(mg/kg)</th>
<th>Ulcer Index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5ml/Kg</td>
<td>18.33±1.03b</td>
<td>-</td>
</tr>
<tr>
<td>Succralfate</td>
<td>400</td>
<td>3.66±1.03a</td>
<td>80.0%</td>
</tr>
<tr>
<td>Ethanolic Extract Ficus</td>
<td>100</td>
<td>12.33±0.51b</td>
<td>32.73%</td>
</tr>
<tr>
<td>Ficus bengalensis</td>
<td>200</td>
<td>8.66±0.51b</td>
<td>52.3%</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>6.66±0.51a</td>
<td>63.61%</td>
</tr>
</tbody>
</table>

a - P= 0.05 when compared with control, b - P = 0.05 when compared with standard

Free acidity in sucralfate treated group have decreased significantly up to 20.27±0.18 compared to control group in which the values was 89.90±0.08 (p=0.05). In groups treated with ethanolic extract the free acidity was reduced to 78.63±0.13, 42.97±0.76, 27.47±0.40 (p=0.05) at a dose of 100, 200 and 400 mg/kg respectively when compared to control group.

Total acidity in sucralfate treated group have decreased significantly up to 33.80±0.26 compared to control group in which the value was 113.2±1.43 (p=0.05). In groups treated with ethanolic extract the total acidity was reduced to 82.13±0.65, 58.03±0.27, 42.2±0.49 (p=0.05) at a dose of 100, 200 and 400 mg/kg respectively when compared to control group.

3.5.2 Ethanol induced ulcer model

In 4H-pylorus ligation model, the ulcer index in sucralfate treated group have decreased significantly up to 6.66±0.51 compared to control group in which the value was 23.67±1.03 (p=0.05). In groups treated with ethanolic extract the ulcer index was reduced to, 19.33±0.51, 11.67±0.51 , 9.33±0.51 (p=0.05) at a dose of 100, 200 and 400 mg/kg respectively when compared to control group.

4. CONCLUSION

Chemical investigation of the stem bark of Ficus bengalensis Linn. led to isolation of compounds namely α-amyrin acetate, stigmasterol from petroleum ether extract and quercetin from chloroform extract. The constituents isolated from the stem bark of Ficus bengalensis Linn can be categorized under Triterpenoids, Steroids and Flavonoids. All these isolated components are reported for the first time from the stem bark of Ficus bengalensis. The ethanolic extract of stem bark was subjected to anti-ulcer activity. The results indicate that ethanolic extract was found to have good anti-ulcer activity justifying their use in traditional system of medicine.

CONSENT

It is not applicable.
ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" (KSHEMA/AEC/26/2010)

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Competing interests

Authors have declared that no competing interests exist.

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