ANTIULCER ACTIVITY AND ISOLATION OF CHEMICAL CONSTITUENTS FROM ETHANOLIC STEM BARK EXTRACT OF FICUS BENGALENSIS

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, ~~ethanol,~~ 4-Hpylorus ligati model.

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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 bio diversity 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 microscop chromatography, etc. With growing awareness in using traditional medications because of their safety, appropria 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 4 holy trees Nalpamara (Ksirivrksa supposed to be planted around temples and home [4].

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Since it leans on another tree and ends up suffocating it, it is also called as "strangler fig". Banyan tree is fou vastly in monsoon and rainforests, it is evergreen and fast growing with its height going up to 20m. They a 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. MATERIAL 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 3 days along with ethanol. Ethanol was decanted after 3 days. It was repeated thrice. Initially solvent in the obtained extract was distilled off and is evaporated to dryness (500g) [6,7]. Phytochemical tests were performed using ~~std. methods~~ [8, 9].

2.3. Fractionation of Ethanolic extract

Ethanolic extract (400g) was made into 2 (or two) equal parts. These were placed in distilled water (500 ml) and extracted using pet. 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 an. 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 per. 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 pet. 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 $CHCl_3$: 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 pet. ether (60-80°C): $CHCl_3$ 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 pet. ether (60-80°C): $CHCl_3$ 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 refluxed 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. Na_2SO_4 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.

2.4.1.1. 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 refluxed 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

CH_3OH (30 ml) along with extract (20 g) was made into solution and was loaded on to 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: H_2O, 100:16.5:13.5) and UV/NH_3 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

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Albino Wistar rats (150-250 g) of either sex were procured from the central animal house of NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangaluru. In a climate-controlled room 4 rats were caged with std 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 anti-ulcer 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., 100mg/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 anaesthetized 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 = (no of lesions. I) + (no of lesion. II) x 2 + (no of lesion III) x 3

The percentage inhibition was determined as follows

$$\frac{\text{(control mean lesion index - test mean lesion index) X 100}}{\text{Control mean lesion index}}$$

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

2.6.1.1. 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.

2.6.1.2. 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

IR Spectral Data(cm-1):2921.7, 2850.5(C-H stretching in CH3 &CH2) 1712 (C=O stretching),1456.2 (C-H deformation of CH3) 1365.5 (str. of gem dimethyl) 1241.0 (C-O stretching),1172.6 (C-O deformation),δ 0.85 (s, 3H,28) δ .0.86 to δ 0.89 (m, 12,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 multipart in the region of. δ 1.0- δ1.92 integrating for 23 protons.

Molecular formula: $C_{32}H_{52}O_2$, Molecular weight: 468; EIMS (m/z):468 (M+, $C_{32}H_{52}O_2$,60%), 453 (M^+ - CH_3,30%),408 (M+ -CH_3COOH, 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 tritpenic alcohol m.p. of which (184 °C) was in good agreement with that for α-amyrin. From the melting point, IR, ^{1}HNMR and mass spectra, compound I was ascertained as α-amyrin acetate.

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 1735.8cm^{-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 ºC.
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=C str.)1462.6 (C-H deformation in gem dimethyl) 1042. 4 (C-O str of secondary alcohol),607.8 (rocking vibration of CH2).^{1}HNMR Spectral data:δ 0.65 to δ 1.1 (m, 18H, 6xCH_3) δ 1.1 to δ 1.26 (m, 18H, 9xCH2) δ 1.26 to δ 1.86 (8H, 8xCH),δ 3.59 (br, 1H CHOH),δ 5.4 (t,1H, vinylic protons) δ 5.2 (2H, br,alllylic protons).GC-MS(m/z):412 (M+, $C_{29}H_{48}O$),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 CH_3 and CH_2 respectively.IR peak at 1634.5 cm-1 indicates C=C 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 δ0.65 to δ1.1.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 $C_{29}H_{48}O$. Its identity was also confirmed by co-chromatography with an authentic sample [20].

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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),1552.36,1460.62(C=Cstr),1200.31 (C-O str). ^{1}HNMR 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'-6') δ 7.17 (d 1H, H-2'),^{13}CNMR spectral data:δ 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),103.01 (C-10), 121.96 (C-1'),115.0 (C-2'),145.05 (C-3'), 147.69 (C-4'),115.6 (C-5'),119.9(C-6').Molecular formula: $C_{15}H_{10}O_7$, Molecular weight: 302 EIMS (m/z):302(M+, $C_{15}H_{10}O_7$, 100%) 274(15%), 246(11%),137(26%), 128 (8%) 91(6%), 69 (20%).
Quercetin : m.p.314-317 oC , 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 δ12.5 was observed.^{13}C NMR spectra of compound showed carbonyl signal (C=O) at δ 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].

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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

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

Groups	Treatment(mg/kg)	Vol.HCl	% Protection
Control(0.6% CMC)	5ml/Kg	6.00±0.17^{b}	-
Sucralfate	400	2.9±0.23^{a}	51.67
Ethanolic Extract Ficus bengalensis	100	5.36±0.28^{b}	10.55
	200	4.33±0.31^{ab}	27.8
	400	4.03±0.18^{a}	32.80

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

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.

Table 2: Effect of Ethanolic extract of stem bark of Ficus bengalensis on pH of gastric juice

Groups	Treatment(mg/kg)	pH of gastric HCl	% Protection
Control(0.6% CMC)	5ml/Kg	1.51±0.08^{b}	-
Sucralfate	400	4.8±0.08^{a}	217.89%
Ethanolic Extract Ficus	100	3.24±0.01^{ab}	115.03%

bengalensis	200	3.85±0.04[a]	155.43%
	400	4.00±0.08[a]	164.9%

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

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, 4.00±0.08 (p=0.05) at a dose of 100, 200 and 400 mg/kg respectively when compared to control group.

Table 3: Effect of Ethanolic extract of stem bark of Ficus bengalensis on Free acidity

Groups	Treatment(mg/kg)	Free Acidity	% Protection
Control	5ml/Kg	89.90±0.08[b]	-
Sucralfate	400	20.27±0.18[a]	77.45%
Ethanolic Extract Ficus bengalensis	100	78.63±0.13[b]	12.54%
	200	42.97±0.76[ab]	52.20%
	400	27.47±0.40[a]	69.44%

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.

Table 4: Effect of Ethanolic extract of stem bark of Ficus bengalensis on total acidity

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

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

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 400mg/kg respectively when compared to control group.

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

Groups	Treatment(mg/kg)	Ulcer Index	% Protection
Control	5ml/Kg	23.67±1.03[b]	
Sucralfate	400	6.66±0.51[a]	71.82%
Ethanolic Extract Ficus bengalensis	100	19.33±0.51[b]	18.34%
	200	11.67±0.51[a]	50.70%
	400	9.33±0.51[a]	60.06%

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

3.5.2. Ethanol induced ulcer model

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

Groups	Treatment(mg/kg)	Ulcer Index	% Protection
Control	5ml/Kg	18.33±1.03[b]	
Sucralfate	400	3.66±1.03[a]	80.0%
Ethanolic Extract Ficus bengalensis	100	12.33±0.51[b]	32.73%
	200	8.66±0.51[b]	52.3%
	400	6.66±0.51[a]	63.61%

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

In 4H-pylorous 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. In alcohol induced ulcer model , the ulcer index in sucralfate treated group have decreased significantly up to 3.66±1.03 compared to control group in which the value was 18.33±1.03 (p=0.05).In groups treated with ethanolic extract the ulcer index was reduced to 12.33±0.51, 8.66±0.51, 6.66±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.

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)

NOTE:

The study highlights the efficacy of "ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

REFERENCES

1. Kirtikar KR and Basu BD. Indian medicinal plants. India Press Publisher, Allahabad. 1989; Vol. 2, 2389.
2. Manoj A, Urmila A, Bhagyashri W, Meenakshi V, Akshaya W, Kishore NG. Anthelmintic activity of Ficus bengalensis. Int. J. Green Pharm. 2008; 2(3), 170-172.

3. Patil VV, Pimprikar RB, Patil VR. Pharmacognostical studies and evaluation of anti-inflammatory activity of Ficus bengalensis. J. Young Pharm. 2009; 1: 49-53.
4. The Wealth of India, Volume- (F-G). In: A Dictionary of Indian Raw Materials and industrial products. New Delhi: Council of Scientific and Industrial Research. 1999; Vol. 4, 24- 26.
5. Midya, S.; Brahmachary, R. L. (1991) "The Effect of Birds upon Germination of Banyan (Ficus bengalensis) Seeds". Journal of Tropical Ecology. 7(4):537-538.
6. Rajisha K,Fernanandes J.Pharmacognostic and Preliminary Phytochemical Investigation of Exacum bicolor Roxb.Research J.Pharm.and Tech.2020;13(4):1752-56 doi:10.5958/0974-360X.2020.00316.9
7. Lobo AP, Arpitha KV, Shetty DK, Rai ST, Nayak P, Kumar A, Kumar P and Pai A. Phytochemical and Pharmacological Evaluation of Yellow and Green Variety of Cocus nucifera Water, Journal of Pharmaceutical Research International. 2021; 33(51A):180-186. doi:10.9734/jpri/2021/v33i51A33483.
8. Nayak P, Sandeep DS, Hameed A, Priya S, Kumar P, Kumar A. A Study of Antioxidant and Anti-bacterial activities of Borassus flabellifer. Journal of Pharmaceutical Research International.2021 33(25B):53-60. doi: 10.9734/jpri/2021/v33i25B31461.
9. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. The Scientific World Journal.2017;3:1-7.https://doi.org/10.1155/2017/5873648.
10. Ghosh MN. Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984. pp. 153-157.
11. OECD/OCED 425 OECD guidelines for testing of chemicals acute oral toxicity up and down procedure. 2001; 26: 1-26.
12. Vogel GH, Vogel WH. Drug discovery and evaluation.2nd ed. Springer Verlag Berlin Heidelberg.NewYork; 2002:347.
13. Brodie DA, Hanson HM. A study of the factors involved in the production of gastric ulcers of the resistant technique. Gastroenterol.1960; 30:353-60.
14. Kaminski JJ,Doweyko AM. Antiulcer agents .6. Analysis of the in vitro biochemical and in vivo gastric antisecretory activity of substituted imidazo[1,2-a] pyridines and related analogues using comparative molecular field analysis and hypothetical active site lattice methodologies.Journal of Medicinal Chemistry.1997;40(4):427-36.DOI10.1021/jm950700s
15. Hawk PB, Oser B L,Summerson HW. Practical Physiological Chemistry.12th edn,Churchill, London,1947:347.
16. Kulkarni S K. Hand Book of Experimental Pharmacology.Vallabh Prakashan, 1999:148-49.
17. Rao CV,Ojha SK, Radhakrishnan K, Govindarajan R ,Rastogi S, Mehrotra S, Pushpangadan P. Antiulcer activity of Utleria salicifolia rhizome extract.Journal of Ethnopharmacology2004;91(2-3):243-49.DOI10.1016/j.jep.2003.12.020
18. Gulcin I,Kufrevioglu OI, Oktay M,Buyukokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). Journal of Ethnopharmacology.2004;90(2-3):205-15.DOI10.1016/j.jep.2003.09.028
19. Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FB. Beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of Alstonia boonei display profound anti-inflammatory activity.Pharm Biol. 2014 ; 52(11):1478-86. doi: 10.3109/13880209.2014.898078. PMID: 25026352
20. Heymann H, Tezuka Y, Supriyatna S.Constituents of Sindora sumatrana MIQ.I. Isolation and NMR Spectral Analysis of Sesquiterpenes from the Dried Pods. Chemical and Pharmaceutical Bulletin1994; 42(1):138-46 DOI:10.1248/CPB.42.138.
21. Zahoor, M., Shafiq, S., Ullah, H. et al. Isolation of quercetin and mandelic acid from Aesculus indica fruit and their biological activities. BMC Biochem 19, 5 (2018). https://doi.org/10.1186/s12858-018-0095-7