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THE ANTI-ULCEROGENIC ACTIVITY OF THE CRUDE METHANOLIC EXTRACT OF *BERGIA SUFFRUTICOSA*

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ABSTRACT

This study was carried out to evaluate the anti-ulcerogenic activity of the methanolic extract of ***Bergia suffruticosa*** to validate its traditional uses in treatment of stomach disturbances. The methanolic extract of Bergia suffruticosa was screened for its anti-ulcerogenic activity against induced-ulcer in four different models. The phytochemical studies of the plant showed that flavonoids,sterols, triterpenes, tannins, saponins and cuomarins are the main conistituents of the plant. The results showed that the methanolic extract of Bergia suffruticosa at a dose of 300 mg/kg was markedly decreased the incidence of ulcer that induced by aspirin in rat stomach and reduced ulcer index from 19.1±1.6 that induced by 150mg/kg aspirin to 10.8±07. Also inhibited H^+ion concentration that had been stimulated by administration of histamine (2µg/kg) in rat stomach when the plant was given simultaneously at a dose 400ug/kg,with histamine and elevate the pH to 4.34±1.03 compared with the pH 3.14±1.20 that induced by administration of 2ug/ml histamine alone. Also B.s extract was antagonized histamine effect in contracting rat uterus and blocked the stimulant effect of histamine on guinea pig atrium.Also the extract antagonized ulcer induced by HCl/ethanol in mice by40.09%ulcer inhibition, compared with 50.54% that of sucralfate. The extract reduced water immersion stress induced ulcer in rat by 47.59%ulcer inhibition whereas that of omeprazole was 100%.The results obtained from this

study confirmed the anti-ulcerogenic activity of the methanolic extract of Bergia suffruticosa.

KEYWORDS: Anti-ulcer; A Bergia suffruticosa; spirin-induced ulcer; H^+ ion concentration.

INTODUCTION

Peptic ulcer is one of common diseases spreading throughout the world. Man kind lived with it since ancient times. Peptic ulcers are seemed as holes extend from mucosal surface to submucosa. It include gastric ulcers (Gus) and duodenal ulcers (DUs) which are resemble in common features in term of pathogensis, diagnosis and treatment. It is chronic inflammatory conditions in which injury to stomach and duodenal is caused by offending factors that disturb the gastric mucosal parrier and thus promote ulcer development. These conditions include ulcer secondary to the use of conventional nonsteroidal antiinflamatory drugs (NSAIDs), gastric infection with Helicobacter pylori bacterium, ulcer due to Zollinger-ellison syndrome (ZES) due to gastrin producing tumer, gastro-esophageal reflux disease (GERD), benign & malignant peptic ulcer, stress related mucosal injuries &injuries due to other factors e,g alcohol consumption, sigarette smoking,spicey diet etc. Treatment of symptomatologies related to gastric ulcer or gastritis with medicinal plants are quite common in traditional medicine worldwide e.g. extract of licorice (Fabaceae) has been used for treatment of peptic ulcer since ancient Egyptian, Greek, Roman and in traditional Chinese medicine, The Glinus lotoidus plant (Aizoaceae) distributed in warm temperate areas worldwide. It grows in tropical and subtropical Eurasia and Africa. It used to treat many diseases in such areas. used as antidiabetic and skin ailments (El-Hamidi, 1967).Treat diarrhea, abdominal diseases and weekness in children (Kirtikar and Basu, 1995). Antihelmintic. The present study is conducted to evaluate the traditional use of Bergia suffruticosa to treat gastric disorders.

MATERIALS AND METHODS

Plant

The plant was collected from Nile bank, identified and authenticated by the herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI).

Extraction

The plant was freed from foreign parts and coarsely powdered, then 10g were extracted with 100 ml of 80% methanol in a shaker for 1hr., filtered and concentrated under reduced

pressure, kept under fan to get the solid mass (13.01%) that kept frozen for pharmacological investigation.

Animals

Albino Wister rats, healthy males of(200-250g)

Guinea pigs

Swiss albino mices of both sex (30g) wt.

Which were fed on wheat, grain , grass,oil,and meat,water ad libidum, in the animal house of (MAPRI)

METHODS

General phytochemical screening of the methanolic extract was carried out using the method described by **Martinez and Valencia, 2003) Sofowora (1993), Harborne (1984)** and **Wall et al (1952).**The results obtained revealed the presence of triterpenes,sterols,tannins, Flavonoids, Cuomarins and saponins.

Evaluation of antiulcerogenic activity against Hcl-ethanol induced ulcer in mice

The experiment was performed as described by Yesilada et al. (1997).Swiss albino mice of either sex were divided into three groups, each group consists of six animals. Group 1 received 1.0ml/kg per.os. 1%Sodium carboxymethylcelulose (SCMC) as vehicle control, group2 received 100mg/kg, p.o. sucralfate as standard control. Group 3 received 300mg/kg, p.o. methanolic extract of test plant. After 1hr all the animals were treated with 0.2ml of Hcl ethanol mixture p.o. (0.3M hydrochloric acid and ethanol 60%) to induce gastric ulcer. Animals were killed by cervical dislocation with anesthesia, 1hr. after administration of Hcl-ethanol mixture and the stomach was excised. The ulcer lesions were counted by counting the red spots on the stomach surface of each rat in the group (n=6).. Mean lesion index for each group was calculated. Percentage of ulcer inhibition was calculated for each group on comparison with vehicle control group.(Mean lesion index of treated group/(Mean lesion index of untreated group x 100).

Evaluation of Untiulcerogenic activity using experimentally water immersion stress induced ulcer in rats

Stress ulcers were induced by forcing the Wistar albino rats of either sex to swim in the glass cylinder (Bhattacharya and Bhattacharya, 1982 and Alder, 1984) containing water to the height of 35 cm maintained at 25 °C for 3 h. animals were fasted for 24 h prior to the

experiment and divided into three groups each group consist of six animals. Group 1 received 1.0 ml/kg p.o. 1% (SCMC) as vehicle control, Group 2 received 20 mg/kg, p.o. Omeprazole as standard control, Group 3 received 300 mg/kg, p.o. ethanolic extract of test plant. After the drug treatment animals were allowed to swim in water for 3 h. thereafter, the animals were killed by anesthetic ether. The stomach of each animal was cut longitudinally along the greater curvature. The ulcer lesions were counted. Mean lesion index for each group was calculated. Percentage of ulcer inhibition was calculated for each group in comparison with vehicle control group.

Evaluation of untiulcerogenic activity against the effect of gastric acid secretion (PH) in rats.

The experiment was performed as described by Esplugues et al. (1990) and Lippe et al.,(1989). In this experiment male wister rat(250-300) was anaesthetized with urethane.A soft polyethene catheter (inner diameter 0.8mm) was inserted into the stomach through the esophagus and was connected to a peristaltic pump for infusion of warm saline at 37c.Another polyethylene canula (internal diameter 3mm) was inserted via duodenum into the stomach and tied in place for collection of gastric outflow. The temperature was maintained at 37c. The stomach was flushed with worm (37c) saline to remove all solid contents. Then the stomach was been perfused at a rate of 0.9ml/min. One hour later, 9ml fractions (at 10min intervals) of gastric perfusate were collected and their ph were determined by digital Ph-meter After stabilization of gastric acid output, the gastric acid secretion was stimulated calculated as (H).by passing histamine 2µg/ml through the stomach. Then the influence of certain doses of the test extract on the secretogogues induced gastric secretion examined by passing different concentrations before the stimulants. The readings of ph-meter were scored and calculated as Hydrogen ion concentration $\{H^+\}$

Evaluation of antiulcerogenic activity against Aspirin-induced ulcers in rat stomach and duodenum

This experiment was prepared according to Selye and Szabo (1973), Robert et al., (1974) and Scarpignato and Zappia, (1987), peptic ulcer can be induced in fasting male rats by specific H_2 receptors stimulation through intravenous injection of a single large dose of an ulcerogenic agent that stimulate the secretion of HCl from the parietal cells.Also ulcer can be induced by oral administration of ulcerogenic drug to fasting rat that decrease the strength of

gastric mucosa. In this experiment ulcers were induced in the stomach of fasting rats by oral administration of aqueous suspension of acetyl salicylic acid (150 mg/kg).

Twenty four male rats were housed in a suitable environment of lighting, temperature, food and water supply for a week to be acclimatized. Then the rats were been housed individually in cages. On the day before the experiment, the rats were divided in four groups each of six. and were marked. The rats were fastened from food for 24 hrs but free access to water was allowed. On the day of experiment, the rats were weighed individually and calculated doses of the test extract 300mg/kg, 150mg/kg, 75mg/kg, in a volume of 2 ml/kg was administered orally by oral gavage to three groups while the fourth group was given normal saline. Fourty five minutes later aqueous oral suspension of aspirin 150mg/kg was administered to all rats and rats were returned to their cages. Twenty–four hours later, rats were killed by dislocating the necks and the abdomens were opened to expose the stomach and duodenum. Both organs (stomach and duodenum) were removed and opened carefully (the stomach opened along the greater curvature and washed with saline interiorly clean. Using a were identified high power magnifier, red spots (representing ulcer lesions) were identified and counted.

Histopathological studies

The stomach tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethano(l 50-100%) cleared in xylene and embedded in paraffin sections (4-5µm) were prepared and then stained with hematoxylin and eosin (H.E) dye for photomicroscopic observations.

Statistical analysis of results

Results were expressed as mean ± S.E.M and were analysed statistically by one –way Anova followed by Tukeys multipie comparison test using SPSS software students version.

RESULTS

Table1: Effect of Omeprazole and ME of *B.suffruticosa* against water immersion stress induced- ulcer in rats.

Treatment	Dose(mg/kg)	Mean ulcer score	%ulcer inhibition
Control	1%SCMC	10.17±2.982	
Omeprazole	20mg/kg	00±00***	100%
B.suffruticosa	300mg/kg	5.17±1.222	47.59%

N=6 ***p<0.007

Table 2: Effect of ME 0f B.suffruticosa against HCL/ethanol induced- gastric lesions in mice.

Treatment	Dose(mg/kg)	Mean lesion index	%Ulcer inhibition
Control	1%SCMC	30.33±10.938	
Sucralfate	100mg/kg	15.0±5.825*	50.54%
B.suffruticosa	300mg/kg	18.17±7.068	40.09%

N=6 *=p<0.05

Table 3: Effect of ME of *Bergia suffruticosa* on Aspirin-induced ulcer in rats

Treatment	Number of ulcer Lesion (mean ±S.E.M)
Aspirin150mg/kg(control)	19.1±1.6
B. suffruticosa.75mg/kg+Asp.150mg/kg	18.9±0.5
B. suffruticosa.150mg/kg+Asp.150mg/kg	14.8±0.1*
B. suffruticosa.300mg/kg+Asp.150mg/kg	10.8±0.7**

N=6 *=P<0.02 * *=P<0.001

Table 4: Effect of methanolic extract of B. suffruticosa on gastric acid secretion (pH).

Treatment	pH(M±S.E.M)	$[H^+]$
Normal saline	3.57 ± 0.13	0.000269
2 µg/ml Hist.	3.14 ±1.20	0.000724
100 µg/ml.B.suffruticosa +2 µg/ml.His	3.40±0.82	0.000398
200µg/ml. B.suffruticosa +2 µg/ml Hist.	3.50± 0.47*	0.000316
400 µg/ml. B.suffruticosa + 2 µg/ml .Hist	4.34 1.03*	0 .0000457

N=10 *=P<0. 5

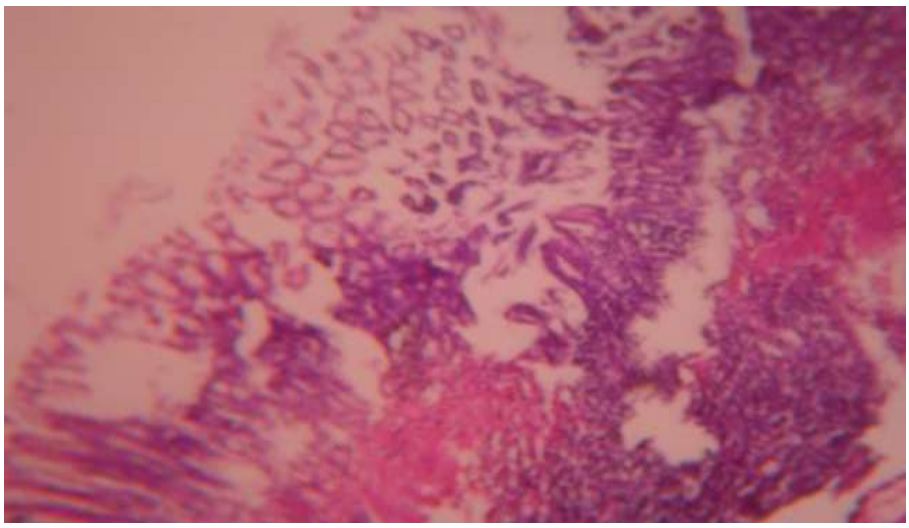

Fig.1: Photomicrograph of gastric mucosa showed sever mucous degeneration, necrosis, sloughing and sever accumulation of the inflammatory cells in submucosal, of fasting rat treated with aqueous oral suspension of aspirin at dose 150mg/kg (H&E stain), X100.

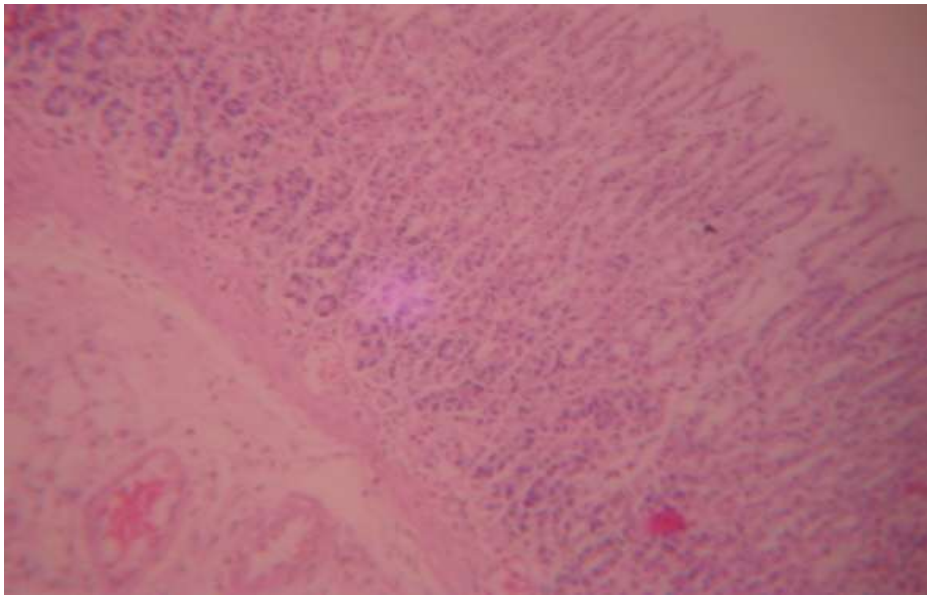

Fig.2: Photomicrograph showed mild mucous degeneration of the gastric mucosa and vascular changes in submucosa, of fasting rat treated with methanolic extract of Bergia suffruticosa at dose 300mg/kg after it had been treated with aspirin at dose 150mg/kg (H&E stain),X100.

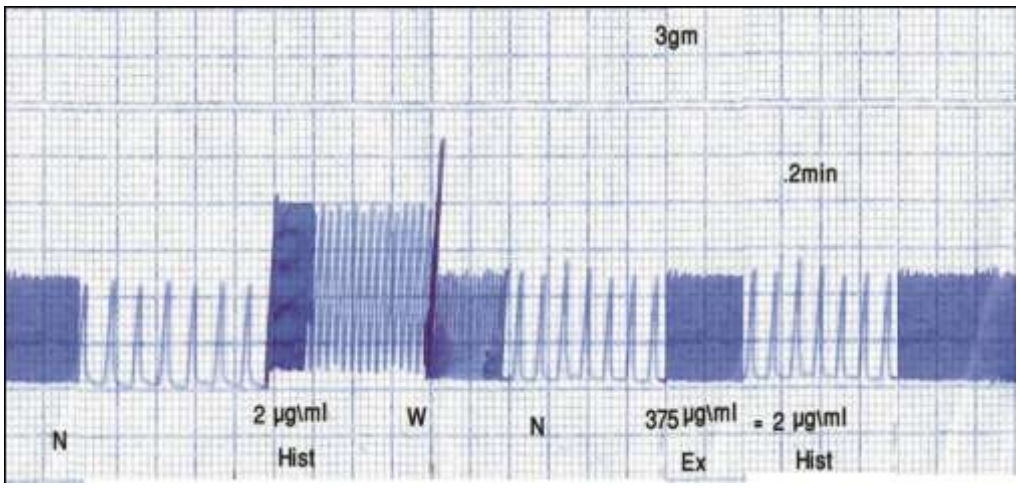

Fig 3: Effect of methanolic extract (Ex) of *Bergia suffruticosa* on guinea pig atrium. Histamine in a concentration of 2µg ml^{-1}increased remarkably the rate and the force of the contraction of the isolated guinea pig atrium, 375µg $ml^{-1}$of methanolic extract of *Bergia suffruticosa* blocked the stimulatory effect of 2µg ml^{-1} histamine.

N=Normal ,W=Wash ,Ex= methanolic extract of *B.sufruticosa* , hist.=histamine.

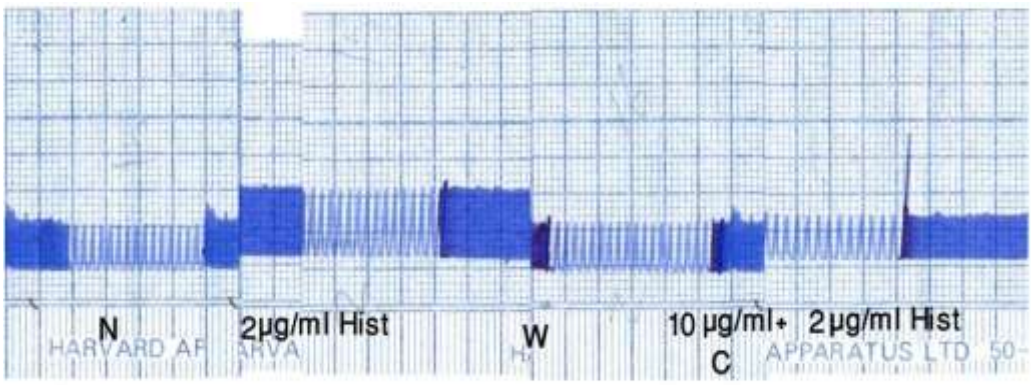

Fig 4: Effect of cimetidine on guinea pig atrium.Histamine in a concentration of 2µg ml^{-1}increased the rate and the force of contraction of the isolated guinea pig atrium.10µg ml^{-1} of cimetidine blocked the stimulatory effect of 2µg ml^{-1}histamine.

N=Normal W=Wash hist=histamine C=Cimetidine

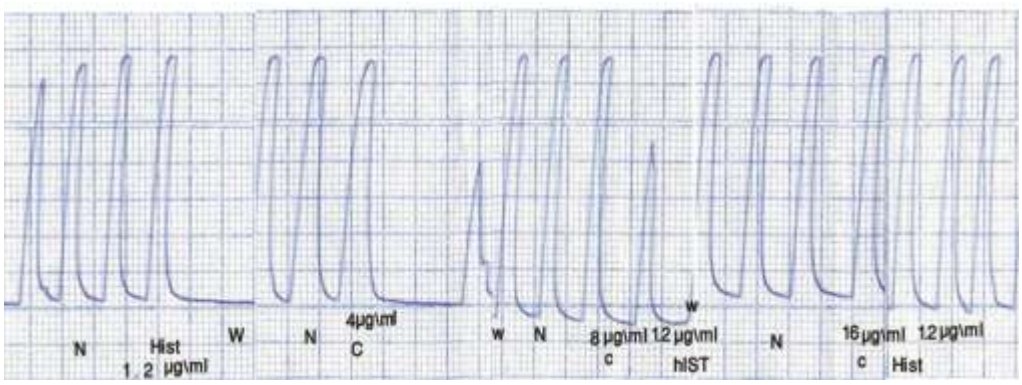

Fig 5: The effect of cimetidine on contracting rat uterus.16µg ml^{-1}Produced complete blockade of the relaxing response of histamine (1.2 µg ml^{-1})

Hist=histamine N=normal W=wash C=cimetidine

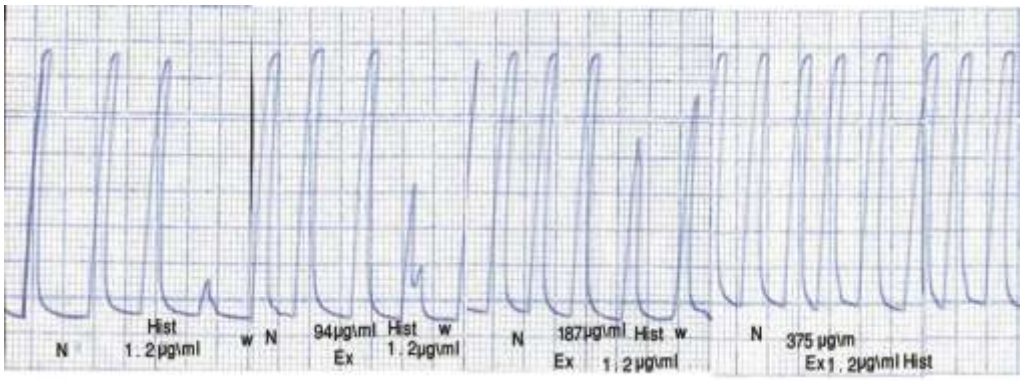

Fig 6: The effect of methanolic extract of *Bergia suffruticosa* on contracting rat uterus .375µg ml^{-1}produced complete blockade of the relaxing response of histamine(1.2µg ml^{-1}) Hist=histamine N=Normal W=Wash Ex=methanolic extract og *Bergia suffruticosa.*

DISCUSSION

The methanolic extract (ME) of, ***Bergia suffruticosa*** which was examined against induced ulcer in four different models, inhibited ulcer induced by water immersion stress in rats by (47.59%) ulcer inhibition as shown in (Table 1).Also ME of ***B. suffruticosa*** inhibited ulcer formation in mice stomach that induced by ethanolic HCl.by (40.09%) ulcer inhibition . (Table2) Aspirin is one of noxious agents that aggravate acid secretion which in turn leads to ulcer formation and gastric mucosal injury. The gastric damage induced by aspirin may possibly be due to leukotrienes production and involvement of libooxygenase. The gastroprotective effect against gastric damage in this model may be due to protection against cycloxygenase and leukotrienes pathway possibly by stimulation of prostaglandin synthesis and/or stimulation of mucin and bicarbonate secretion which in turn protect and strengthening the gastric mucosal parrier.ME of ***B. suffruticosa*** showed a remarkable reduction in incidence(10.8±0.7) of gastric ulcer that induced by aspirin in aspirin-induced ulcer in rats compared to that of the control(19.1±1.6)(Table 3) The antiulcer activity is recognized by reduction of acid secretory parameters (i.e total acid or free acid) suggesting that acid inhibition accelerates ulcer healing as stated by (Nunes et al., 2009) gastroprotective and antiulcerogenic effects are related to the inhibition of gastric acid secretion and elevation of gastric pH which is the main factor for ulcer healing . The low pH is important factor of acid secretion which is a main factor of ulcer formation. The ME of ***B. suffruticosa*** at a dose (400µg/ml) elevated stomach pH to (4.34±1.03)in presence of (2µg/ml) histamine that lowered the stomach pH to (3.14±1.20) (Table4) . Gastric mucosa of fasting rat treated with aqueous oral suspension of aspirin at dose 150mg/kg,showed sever mucous degeneration, necrosis, sloughing and sever accumulation of the inflammatory cells in sub mucosa, (Figure1) Methanolic extract of ***B. suffruticosa*** at a dose 300mg/kg minimized the degenerative effect of aspirin (Figure2). The methanolic extract of ***B. suffruticosa*** blocked the histamine stimulatory effect in guinea pig atrium,(Figure 3) ,wheras cimetidine blocked the stimulatory effect of 2µg ml^{-1}histamine.(Figure4), . As same as cimetidine blocked the histamine effect in isolated rat uterus(Figure 5) methanolic extract of ***B. suffruticosa*** did (Figure6).These results concluded thatThe methanolic extract of B. ***suffruticosa*** possesses antisecretory activity through the blockage of H_2receptors which may account for antiulcer activity. The anti-ulcerogenic activity may contributed to the presence of triterpenes, flavonoids, taninns , saponin, sterols and cuomarins which were all documented for their anti-ulcerogenic activity). The aqueous extracts of *Phoradendron crassifolium* and *Franseria artemisioides* being the most active, exerting a cytoprotective activity comparable to atropine.

The analysis of the chemical constituents of the extracts studied showed the presence of tanins, saponins, flavonoids and coumarins. (Gonzales et al., 2001). The intraduodenal administration of *Combretum leprosum* in four-hour pylorus-ligated rats increased the volume and pH of gastric juice while decreasing the acid output and produced a significant increase in gastric wall mucus content. The major compounds detected in a preliminary phytochemical screening were triterpenes, flavonoids, taninns and saponins. This study provides evidence that the ethanolic extract of *Combretum leprosum* possesses gastroprotective and anti-ulcerogenic effects, which are related to the inhibition of the gastric acid secretion and an increase of mucosal defensive factors such as mucus and prostaglandin. (Nunes et al., 2009).

CONCLUSION

The results obtained concluded that the methanolic extract (ME) of, ***Bergia suffruticosa*** possesses dose related anti-ulcerogenic effect which may be due to antisecretory and cytoprotective mechanism.

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