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ANTIULCER ACTIVITIES OF THE METHANOL LEAF EXTRACT AND FRACTIONS OF *COMMELINA ASCENDENS* (*COMMELINACEAE*) USING INVITRO AND INVIVO MODELS

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Abstract

Recently, research has focused on utilizing medicinal plants in the discovery of new antiulcerogenic drugs. *Commelina ascendens* has been used to treat many diseases in folk medicine especially gastric disorders and as an antimicrobial agent for topical wounds, but its efficacy has not been validated. This study aimed to explore the potential gastro-protective action of the whole leaf extract and solvent fractions of *Commelina* ascendens.

The effect of the Methanol leaf extract (MCA), n-hexane fraction (HCA), Ethylacetate fraction (ECA) and butanol fraction (BCA) of *Commelina ascendens* on peptic ulcer disease induced by various ulcerogens; ethanol, indomethacin and hypothermic stress in rats and its effect on gastrointestinal motility, isolated gut tissue preparations of guinea pig and rabbit, as well as antimicrobial activities were explored. MCA and fractions at all doses (100, 200 and 400 mg/kg) exhibited significant ($p<0.05$) and dose-related protection of rats against all ulcerogen induced ulcers. Gastrointestinal propulsion in mice was also significantly ($p<0.05$) reduced in a concentration dependent manner by the extract. MCA, HCA, ECA and BCA inhibited acetylcholine-evoked contractile response in rabbit jejenum with IC_{50} of 17.15, 27.23,5.66, 42.51 µg/ml respectively. The microorganisms used in the study were inhibited by the extracts and fractions with HCA appearing to exhibit better activity against the fungi and bacteria. Preliminary phytochemical studies indicated the presence of saponins, tannins, flavonoids, terpenoids, steroids, acidic acid, glycosides, proteins and resins. These findings indicate that the aerial parts of *C. Ascendens,* possess some antiulcer and antimicrobial properties.

Keywords: *Anti-ulcerogenic, Commelina ascendens, gastro-protection, peptic ulcer disease, antimicrobial*

Introduction

One of the most common forms of gastrointestinal ulcers widely distributed worldwide is the Peptic Ulcer disease (PUD). About 14.5 million people are estimated to be affected by peptic ulcer with 4.08 million deaths yearly [1,2].

Peptic ulcers are wound on the epithelial layer of the gastric surface accompanied by mucosal irritation with diameter of 5mm or more in depth reaching down to the submucosa. Although the mechanism for gastric ulcers is complex, the disease etiology usually involves an imbalance between mucosal defensive mechanisms (mucin, prostaglandin, bicarbonate, nitric oxide, growth factors) and various endogenous offensive factors such as acid, pepsin and *Helicobacter pylori* [3,4,5,6]. *Helicobacter pylori* (*H. pylori*), a spiral-shaped, highly motile bacteria is a major etiologic factor that has significant influence on susceptibility to peptic ulcer disorder [7]. The efficacy of currently available agents is limited by their numerous adverse effects including gastrointestinal dysfunction, mental state changes and an increased risk of respiratory and enteric infections, hence leading researchers to investigate medicinal plants and herbs which could provide an excellent source for safer, economical and efficacious newer and natural drugs or molecules [8,9]. Medicinal plants are known as an important source of compounds for the treatment of gastric ulcers and new drugs discovery [10]. One of such a plant is *Commelina ascendens* J.K. Morton, belonging to the family Commelinaceae. It is commonly found in the primary and secondary lowland rain-forest, often by rivers or streams [11]. As a scandent herb, it grows up to a length of 8ft. It has stems rooting at lower nodes, lanceolate leaves up to 11 cm long and 3 cm broad, and pale blue flowers opening early in the day and fading within 3 h of dawn (about 7-9 am) [11,12]. Traditionally, it is used in the eastern part of Nigeria for the treatment of boils, skin ulcers, cuts, wounds and other ailments.

However, there are no documented reports on the anti-ulcer effect of the leave extract of *Commelina ascendens.* In the limelight of its traditional use, the present study aimed to investigate and assess the gastroprotective potentials of the methanol leave extract of *Commelina ascendens* and its fractions using *in vivo* and *in vitro* antiulcerogenic models in order to substantiate its ethnopharmacological claim of providing relief in peptic ulcer disease and support the usage of herbs for nutraceutical development. The antimicrobial effect of the extracts and fractions was also investigated.

Methods

Collection and authentication of Plant materials

Fresh leaves of *Commelina ascendens* were collected from bushes in Orba, Nsukka, Enugu State, Nigeria. The plant material was authenticated by Mr. A. Ozioko, a plant Taxonomist, of the Bioresources Development and Conservation Programme (BDCP) center, Nsukka, Enugu State, Nigeria. A dried voucher specimen was preserved thereafter for future reference with specimen voucher no. Inter-CEDD/16095.

Preparation of plant material

The plant leaves were cleaned, sliced into smaller pieces and air-dried on an aluminum plate maintained at room temperature and then pulverized using a manual blender. The fine powder of the leave (3.2 kg) was extracted by cold maceration for 48 h with methanol with intermittent shaking. The solvent extract was filtered and the filtrate was concentrated *in vaccuo* using rotary evaporator at 40 ^{o}C.

The percentage yield of the powdered leave sample was determined accordingly as 92g (2.875%, w/w). The dried extract was stored in aliquots and preserved in a refrigerator at -20 ^{o}C for future use in experiments and labelled as methanol leaf extract of *Commelina ascendens* (MCA).

Solvent-guided fractionation of extract

A portion of the methanol extract (MCA) (60g) was partitioned using solvents of increasing polarity in the following order: n- hexane, ethyl acetate and butanol. The fractionation was achieved by mixing MCA (60 g) with each solvent separately in a separating funnel and the mixture was shaken vigorously and allowed to stand for 30 min. The fractions were collected and the solvents evaporated to dryness as described earlier. The fractions obtained were HCA, ECA and BCA for n-hexane, ethylacetate and butanol respectively.

Phytochemical Analysis

Preliminary phytochemical tests were carried out using standard procedures [13,14].

Animals

Adult healthy animals of either sex comprising of Wister rats (100 - 180 g), Swiss albino mice (20 - 30 g), guinea pigs (350 - 400 g) and New Zealand rabbits (1.5 - 3.0 kg) were used in the study. The animals were procured from the laboratory animal facility of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were housed in the institutional facility under standard condition (25 ± 2°C and 12 h light/dark cycle) and were maintained on standard livestock pellet (Vital feeds, Jos, Nigeria). The rabbits and guinea pigs were maintained on guinea grass (*Panicum maximum*). All the animals had access to clean water *ad libitum*. The study was done in accordance to local Institutional Ethical guideline and in line with the European Union Directives for the Protection of Animals used for Experimental and other Scientific Purposes [15].

Acute toxicity (LD_{50}) test

The acute toxicity (LD_{50}) of MCA was estimated in mice by the oral route using the method of [16]. The tests involved two phases: in the first phase, the toxic dose range was determined. The mice were placed in groups (n=3) and MCA (10, 100, or 1000 mg/kg) was administered using oral gavage (per os). The treated mice were then monitored for 24 h for signs of toxicity and mortality. In the second phase, four different doses of MCA (1600, 2900, 3600 and 5000 mg /kg body weight) were administered per os based on the earlier outcomes. The mice were then observed for lethality and signs of acute intoxication for 24 h. The LD50 was then calculated as the geometric mean of the highest non-lethal dose and the least toxic dose.

Evaluation of anti-ulcer activity induced by different ulcerogenic models

The methanol extract, n-hexane, ethyl acetate and butanol fractions (MCA, HCA, ECA and BCA) were tested for antiulcer activity, using six gastric ulcer experimental models.

Gastric lesions induced by Ethanol

The anti-ulcerogenic assay was adapted from the method described by Amani and Esraa [17] with slight modification. Briefly, rats were randomized into groups of 5 animals each (n=5) and deprived of food 18 h prior to the experiment. Group I served as the negative control and received 5 ml/kg of 3% Tween 80. Rats in group II were treated with omeprazole (20 mg/kg) and served as the positive control. Rats in groups III, IV V were administered MCA (100, 200 and 400 mg/kg; *per os*). One (1) hr after drug treatment, ethanol (1 mL of 96 %v/v) was administered to the animals orally to induce gastric ulceration. One hour later, all the animals were euthanized under chloroform anaesthesia and sacrificed [18], the stomachs were excised and opened along with the greater curvature to expose the gastric mucosal layer. In the incised stomach, the mucosa was rinsed slowly with water and the stomach pinned flat on a corkboard and observed using a hand lens. Erosions formed on the glandular portions of the stomach were counted and each given a severity rating on a 0-3 scale based on the diameter of the ulcer (0- no ulceration; 1-ulcers ≤ 1mm; 2- ulcers ≥ 1 mm ≤ 2 mm; 3- ulcers > 3 mm). The total ulcer score for each stomach divided by a factor of 10 was calculated for each animal and expressed as ulcer index (U.I). The degree of ulcer protection was calculated as a percentage with respect to the mean ulcer index of the negative control group. The procedure was repeated for the n-hexane (HCA), ethyl acetate (ECA) and butanol (BCA) fractions at 100, 200 and 400 mg/kg doses.

Indomethacin-induced gastric ulcer

The effect of MCA and its fractions on gastric ulcer in rats was evaluated by the ulcerogen, indomethacin. The rats were randomly assigned into groups of five animals each (n=5) and pretreated as in the ethanol-induced ulcer. Rats were fasted for 18 h before treatment. Indomethacin (100 mg/kg) was administered orally 1 h after drug treatment. After 8 h, the animals were euthanized and sacrificed under chloroform anaesthesia, their stomachs dissected, opened along the greater curvature, rinsed and stretched on cork boards. The degree of ulcer protection [19] was also calculated as in the ethanol-induced model. The procedure was repeated for the n-hexane (HCA), ethyl acetate (ECA) and butanol (BCA) fractions at 100, 200 and 400 mg/kg doses.

Hypothermic resistant stress induced gastric ulcer

Adult swiss albino rats (of either sex) were divided into groups of five animals each (n=5) and pretreated as in ethanol-induced ulcer. The rats were then deprived of food for 18 h, but allowed free access to water. One (1) h after oral drug administration, the rats were immobilized individually in retraining cages at a temperature of 4

± 1°C in a refrigerator for 2 h. The stomachs were excised, opened along the greater curvature, washed, stretched on cork plates and the inner surface examined for the presence of lesions with a hand lens. The ulcer index per animal was also calculated [20,21] as in the ethanol-induced ulcer model. The procedure was repeated for the n-hexane (HCA), ethyl acetate (ECA) and butanol (BCA) fractions at 100, 200 and 400 mg/kg doses.

Gastrointestinal Transit time Tests

Swiss albino mice were deprived of food for 24 h prior to the experiment but were allowed unrestricted access to water prior to the experiment. They were randomized into groups of five animals each (n=5). Group I served as the negative control and received 3% Tween 80 (20 mL/kg). Group II served as the positive control and received atropine (10 mg/kg). Groups III, IV and V were treated with various doses of MCA (100, 200 and 400 mg/kg, respectively) per oral. The procedure was repeated for n-hexane, ethyl acetate and butanol fractions at 100, 200 and 400 mg/kg doses. One hour after drug treatment, each mouse received 0.5 mL of charcoal meal (5% deactivated charcoal in 10% aqueous solution tragacanth powder) orally. Thirty (30) min later, each animal was euthanized with chloroform and the intestine carefully removed and displayed. The intestinal distance moved by the charcoal meal from the pylorus was measured and expressed as a percentage of the distance from the pylorus to the ileocaecal junction for each animal [22]

Studies on isolated gut preparations

The effects of MCA on isolated guinea pig ileum and isolated rabbit jejunum preparations were studied. Segments of the tissues, 2-3 cm long were suspended in 50 ml organ bath filled with Tyrode solution of composition (mM/L): NaCl-8.0, KCl-0.2, $CaCl_2$-0.2, $NaHCO_3$-0.1, $NaHPO_4$-0.05, $MgCl_2$ - 0.1 and glucose - 1.0, maintained at 37 ± 1°C and aerated with air. The preparations were set up under resting tension of 1 g and allowed to equilibrate for 60 min during which the bathing fluid was changed every 15 min. At the end of the equilibration period, the effects of graded concentrations of extracts and fractions (MCA, HCA, ECA and BCA) on the guinea pig ileum preparation and on the rhythmic movement of the rabbit jejunum preparation were determined. The various concentrations of the extracts and fractions were tested. The effect of the extract on histamine and acetylcholine-evoked contractile responses of guinea pig ileum and rabbit jejenum was also studied. The contact time of activity for each treatment concentration was 15 sec with a tissue recovery period of 1 min between drug tests. Responses were determined in triplicate and recorded on a kymograph 10550 through a frontal writing lever.

Antimicrobial screening

Helicobacter pyroli, the enteric organism implicated in peptic ulcer disease was not used in this screening test because of the difficulty in culturing the organism. However, other entheropathogenic and related microorganisms were employed. The organisms used were: *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi, Candida albican and Aspergillus niger*. They were clinical strains isolated from the Bishop Shannahan Hospital, Nsukka, cultured and subcultured in the Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka. The organisms were maintained by weekly sub-culturing and incubating at 37 °C (for bacteria) and 25 °C (for fungi and yeast). The 24 h old cultures of the microorganisms were used in the screening. The agar disc diffusion method was employed [23]. Wells of 6 mm diameter were bored on seeded agar gel dish containing 1.0 x 10^6 cfu/ml of the respective organism and varying concentrations of the extract and fractions applied to the appropriately labelled wells. The plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi and yeast. The effects of the extract on the growth of the microorganisms were studied by observing the zones of inhibitions. The experiments were carried out in triplicates and the mean inhibition zone diameter obtained in each case.

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). Data were analyzed by using Statistical Programme for Social Sciences (SPSS) (Version 11.0). The mean values of test groups were compared with those of the control groups using ANOVA and Dunnett's post *hoc* least significant different (LSD) test. Differences between mean observations were considered significant at *p* values<0.05.

Results

Acute Toxicity and Lethality (LD50)

Oral administration of MCA caused no death or no signs of acute intoxication at doses up to 5000 mg kg^{-1}. The oral LD_{50} of MCA in mice was thus established to be greater than 5,000 mg kg^{-1} for the single-dose administration of the test extract.

Phytochemical Studies of Extract and Fractions

The methanol leaf extract of *Commelina ascendens* yielded 92g (2.875%, w/w). Preliminary phytochemical analysis showed that MCA and BCA showed the presence of alkaloids, saponins, tannins, carbohydrates, reducing sugar, flavonoids, terpenoids, steroids, oil, glycosides, proteins and resins. The ECA fraction did not give positive reactions for reducing sugar while HCA in addition to reducing sugars did not give positive reaction for carbohydrate and alkaloids.

Effect of Oral Extract and Fractions on Ethanol-induced Gastric Ulcers

In this experiment, the gastroprotective effect of MCA and its fractions is presented in Table 1. It was observed that the rats pretreated with different doses of MCA, HCA, BCA, ECA and Omeprazole significantly ($p<0.05$) reduced the gastric lesions as compared to the normal negative control in a dose dependent manner. MCA at all doses (100, 200 & 400 mg/kg) produced gastroprotective effects by as much as 46.52, 67.40 and 66.90% respectively comparable to the standard drug, Omeprazole (83.91%). ECA (400 mg/kg) produced the most potent inhibition of gastric lesions at 90.00% when compared to Omeprazole (20 mg/kg).

Effect of MCA and Fractions on Indomethacin-induced Gastric Lesion

The methanol leave extract of *Commelina ascendens* MCA (100, 200 and 400mg/kg) and its fractions at all doses showed significant ($p<0.05$) and dose related protection of rats against Indomethacin-induced ulcers. Pretreatment of rats with MCA at 100, 200 and 400 mg/kg elicited a dose dependent protection up to 50.34, 67.80 and 84.32% from indomethacin induced ulceration comparable to the effect of Omeprazole (20 mg/kg). Fractions ECA and BCA (400 mg/kg) also produced potent inhibitions. Omeprazole (20 mg/kg) exhibited more protection (92.80%) to the rats from indomethacin induced gastric ulcers (Table 2).

Effect of Extract and Fractions on Hypothermic Restraint Stress-Induced Gastric Ulcer

In the hypothermic restraint stress ulcer model, the pretreatment with MCA and fractions of *Commelina ascendens* markedly ameliorated the ulcer index in the cold stress induced ulcers in rats. The oral administration of MCA (100, 200 and 400 mg/kg) significantly ($p<0.05$) reduced the gastric ulcer indices to 6.7 ± 1.102, 7.30 ± 1.586 and 3.1 ± 0.557 respectively compared to the negative control group (21.14 ± 2.496). These findings were comparable to the reduction produced by the standard drug, Omeprazole (20 mg/kg) (4.40 ± 0.797) (Table 3).

Similarly, factions HCA, ECA and BCA at 400 mg/kg dose produced significant ($p<0.05$) decreases in the gastric ulcer index by as much as 8.40 ± 0.87, 4.20 ± 1.24 and 5.60 ± 1.08 respectively.

Effect of Extract and Fractions on Gastrointestinal Propulsions

The extract and fractions elicited significant ($p<0.05$) dose-related reduction in charcoal meal transit time except HCA (100 mg/kg). MCA (200 mg/kg and 400 mg/kg) reduced the distance covered by the charcoal meal in the gastrointestinal tract of mice by 60.54 and 58.54% respectively. These values were comparable to that produced by atropine (10 mg/kg) 61.00% (Table 4).

Effect of Extract and Fractions on the Isolated Guinea Pig Ileum

The extract and fractions neither contracted nor relaxed the isolated guinea pig ileum. However, they inhibited contractions induced by acetylcholine in the isolated rabbit jejenum and guninea pig ileum to varying extents (Figure 1, 2 and 3). The IC_{50} derived from the effects of crude extract and fractions on rabbit jejunum are 17.15, 27.23, 5.66, 42.51 mg/ml for the methanol extract of *Commelina ascendens* (MCA), n-hexane fraction (HCA), ethylacetate fraction (ECA) and butanol fraction (BCA), respectively (Figure 1-4).

Antimicrobial Studies on Extracts and Fractions

The sensitivity of the microorganisms to the crude extract and fractions is shown in Table 5. The extract MCA and fractions HCA, ECA and BCA inhibited the growths of all organism, bacteria and fungi. *Psuedomonia aeruginosa* and *Salmonella paratyphi* showed least sensitivity to the extracts and fractions tested. *Klebisiella preumonia* showed

the highest sensitivity with the n-hexane fraction. The n-hexane fraction appears to show better activity against the microorganisms when compared to MCA, ECA and BCA (Table 5).

Discussion

Investigation into the pathogenesis of peptic ulcers, which are benign lesions of the gastric or duodenal mucosa, reveals that it results from a shift in the balance between aggressive action of peptic acid secretion (in addition to other factors) and the maintenance of mucosal integrity through endogenous defence mechanisms, although the aetiology remains complex. Therefore, therapeutic strategies are aimed at balancing aggressive factors against defensive factors. No doubt, currently available medicines have brought about remarkable changes in peptic ulcer therapy, reports on their clinical evaluation shows incidences of adverse effects and danger of drug interactions in ulcer therapy [24]. Hence the search for new and novel antiulcer drug continues and has been extended to medicinal plants and herbs [25] which may afford better protection and reduced incidences of relapse. The present study evaluated the gastroprotective effect of the methanol extracts and fractions of the aerial parts of *C. ascendens* in different models of experimentally induced gastric ulcers as well as their antimicrobial activity.

The oral acute toxicity (LD_{50}) of MCA was found to be >5000 mg/kg; this suggests that MCA might be considered to have a high degree of relative safety, devoid of toxicity and possibly safe for a single dose [16]. However, toxic effects due to repeated and chronic administration have not been determined. Preliminary phytochemical showed that the extract of *C. ascendens* contains typical phytoconstituents; alkaloids, saponins, tannins, carbohydrates, reducing sugar, flavonoids, terpenoids, steroids, oil, acidic acid, glycosides, proteins and resins. The presence of flavonoid and tannins groups indicates its possible potential antioxidant activity which could play an important role in the treatment of many diseases especially gastrointestinal disorder [17,26.27].

The effect of the extracts and fractions of *C. ascendens* was assessed in ulcer model induced by ethanol. It is well established that ethanol is a 'gastrotoxic' agent and its ingestion in large amount promotes gastric lesions in humans and experimental animals as well as decreases the number of gastric defense mechanisms such as disruption of blood flow, degranulation of mast cells, reduction of prostaglandin and mucus/bicarbonate release [28,29,30]. It is also responsible to promote the generation of free radicals and oxidative stress that significantly injures cells and membranes [31,32]. Oral administration of ethanol in the control group of experimental animals promoted necrotic lesions and bleeding characteristics however MCA and its fractions reduced lesion areas. Agents that are effective against the detrimental effect of ethanol could possess gastric mucosal membrane protective action. The mechanism of ethanol antiulcerogenic effect may result from an increase in prostaglandin synthesis or mucus adherence to the gastric mucosa [28,33].

The methanol extract and fractions of *C.ascendens* were observed to significantly reduce mucosal damage in the indomethacin-induced ulcer model suggesting the possible mobilization and involvement of prostaglandin [34]. Indomethacin and related Non-steriodal anti-inflammatory drugs (NSAIDs) are known to induce gastric ulceration by inhibiting the biosynthesis of endogenous prostaglandins [28,35,36] which has been demonstrated to serve useful gastro-protective functions by maintaining gastric microcirculation [37], stimulating mucous and bicarbonate secretions and inhibition of gastric acid secretion [38]. Additionally, inhibition of prostaglandin leads to the over production of leukotrienes which could induce mucosal vasoconstriction, thereby reducing blood flow [39]. Consequently, the potential of the extract and fractions to significantly confer protection to the animals against gastric lesions induced by indomethacin suggest possible cytoprotective actions probably mediated by enhancing the synthesis or inhibition of the depletion of prostaglandin [40].

Hypothermic restraint stress model is associated with an increase in gastric acid secretion and a decrease in pH [41]. Cold restraint is known to cause psychological and physical stress to rats thereby releasing histamine in the stomach leading to increased gastric acid secretion and decreased

mucus production ultimately leading to ulcers [8,36]. Previous studies have also suggested a possible role for leukotriene-C4 (LTC4), a lipoxygenase derived metabolite of arachidonic acid in stress induced ulcers. Hence histamine is believed to have an essential role in the pathogenesis of stress ulcers since it is a potent stimulator of gastric acid secretion [42]. Histaminne released from mast cells and non-mast cell sources produces gastric mucosal microcirculatory changes which are responsible for gastric ulceration [43]. The extract MCA and its fractions caused a dose dependent significant reduction in the ulcer index in this model, suggesting the role of histamine in its mechanism [8]. Although more studies are required to further elucidate the mechanism.

The extract MCA and its fractions significantly and dose dependently inhibited the charcoal meal peristaltic propulsive movement time, indicating a dose dependent inhibition of gastrointestinal motility. Prolongation of charcoal meal transit time is usually a result of antimotility action leading to delay in gastric emptying and reduction in gastrointestinal propulsion [44] to enable the antiulcer drug or extract have extended time contact with the gastrointestinal mucosa to effect gastric protection or healing. Previous studies have shown that the reduction in intestinal motility ameliorates ulcer pains and hastens the healing of ulcers. The reduction in gastrointestinal motility/peristalsis exhibited by the methanol leave extract of *C.ascendens* and its fractions corroborated the inhibitions of the spontaneous pendular movement of the jejenum and may be related to the inhibition of contractile responses evoked by Ach on the isolated guinea pig ileum and rabbit jejenum in the *invitro* studies and found useful in the management of peptic ulcer [45]. The extract and its fractions also inhibited the rhythmic contraction of the isolated rabbit jejenum. The neurotransmitters Ach and histamine have been implicated in the pathogenesis of ulcer and is known to stimulate and regulate gastric acid secretion. Therefore, inhibition of any of their activities is important in providing beneficial effect in antiulcer therapy by reducing gastric acid secretion. Although the extract and fractions inhibited the contractile response to Ach on isolated tissue, conclusions cannot be drawn to associate the antiulcer properties of *C. ascendens* to the antagonism of the mediator Ach which is important in the pathology of peptic ulcer.

The colonization of the gastrointestinal system by microbes has been associated with variety of peptic ulcer diseases [38,46] Although *H. pylori* could not be cultured for use in the antimicrobial studies, the effect of the extract and fractions against other Gram negative enteric organisms which belong to the same class as *H. pylori* were ascertained and they inhibited microbial growth which could be relevant to the antiulcer activity of the plant extract and its fractions.

Conclusion

The methanol leaf extract and fractions of *C. ascendens* protected rats against ulcers induced by different ulcerogens. The traditional use of *C. ascendens* decoction in the treatment of peptic ulcer disease and other gastrointestinal disturbances could be justified by the result of the study.

In conclusion the methanol leaf extract and fractions of *C. ascendens* protected rats against ulcers induced by different ulcerogens. The traditional use of *C. ascendens* decoction in the treatment of peptic ulcer disease and other gastrointestinal disturbances could be justified by the result of the study.

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Table 1: Effect of Methanol leaf extract (MCA), N-hexane fraction (HCA), ethyl acetate fraction (ECA) and butanol fraction (BCA) on ulcer index in Ethanol - induced gastric ulcer in rats.

Treatment	Dose (mg/kg)	Ulcer index	Protection (%)
Tween 80	5ml/kg	23.00±1.31	0.00
Omeprazole	20	3.70±1.09*	83.91
MCA	100	12.30±1.12*	46.52
HCA	100	15.20±2.21*	33.91
ECA	100	6.40±0.88*	72.17
BCA	100	11.40±1.24*	50.44
MCA	200	7.50±1.95*	67.40
HCA	200	13.00±1.68*	43.48
ECA	200	6.60±1.44*	71.30
BCA	200	8.30±0.80*	63.91
MCA	400	7.80±1.09*	66.09
HCA	400	8.80±0.90*	61.74
ECA	400	2.30±0.66*	90.00
BCA	400	3.30±0.30*	85.65

Values are expressed as mean±SEM (n= 5). * *P*<0.05 versus untreated control group

Table 2: Effect of Methanol leaf extract (MCA), N-hexane fraction (HCA), ethyl acetate fraction (ECA) and butanol fraction (BCA) on ulcer index in indomethacin - induced gastric ulcer in rats.

Treatment	Dose (mg/kg)	Ulcer index	Protection (%)
Tween 80	5ml/kg	23.60±1.81	0.00
Omeprazole	20	1.70±0.77*	92.80
MCA	100	11.72±1.92*	50.34
HCA	100	13.80±1.89*	41.53
ECA	100	8.80±1.19*	62.71
BCA	100	5.40±1.54*	77.12
MCA	200	7.60±1.239*	67.80
HCA	200	13.90±2.05*	41.10
ECA	200	6.20±1.46*	73.73
BCA	200	5.20±1.01*	78.00
MCA	400	3.70±0.77*	84.32
HCA	400	9.30±0.99*	60.60
ECA	400	2.30±1.11*	90.25
BCA	400	3.00±1.34*	87.29

Values are expressed as mean±SEM (n= 5). * *P*<0.05 versus untreated control group.

Table 3: Effect of Methanol leaf extract (MCA), N-hexane fraction (HCA), ethyl acetate fraction (ECA) and butanol fraction (BCA) on ulcer index in Hypothermic resistant stress - induced gastric ulcer in rats.

Treatment	Dose (mg/kg)	Ulcer index	Protection (%)
Tween 80	5ml/kg	21.14±2.49	
Omeprazole	20	4.40±0.79*	79.19
MCA	100	6.70±1.10*	68.31
HCA	100	16.40±1.50	22.42
ECA	100	11.80±2.22*	44.18
BCA	100	10.80±1.96*	48.91
MCA	200	7.30±1.59*	65.47
HCA	200	7.60±0.87*	64.05
ECA	200	7.40±1.26*	65.00
BCA	200	5.60±1.08*	73.51
MCA	400	3.10±0.55*	85.34
HCA	400	8.40±0.87*	60.25
ECA	400	4.20±1.24*	80.13
BCA	400	2.60±0.87*	87.70

Values are expressed as mean±SEM (n= 5). * *P*<0.05 versus untreated control group.

Table 4: Effect of Methanol leaf extract (MCA), N-hexane fraction (HCA), ethyl acetate fraction (ECA) and butanol fraction (BCA) on gastrointestinal motility in rats.

Treatment	Dose (mg/kg)	Percentage distance traveled (%)	Percentage inhibition (%)
Tween 80	5ml/kg	69.94±5.92	
Atropine	10	27.27±1.97*	61.00
MCA	100	35.34±5.12*	49.47
HCA	100	49.08±4.76*	28.97
ECA	100	26.72±4.24*	61.79
BCA	100	29.34±1.71*	58.05
MCA	200	27.60±4.77*	60.54
HCA	200	39.56±4.35*	43.44
ECA	200	24.64±4.39*	64.77
BCA	200	29.58±6.59*	57.71
MCA	400	29.00±4.27*	58.54
HCA	400	43.62±6.37*	37.63
ECA	400	29.66±1.20*	57.59
BCA	400	27.68±1.78*	60.42

Values are expressed as mean±SEM (n= 5). * *P*<0.05 versus untreated control group.

Table 5: Effect of Methanol extract (MCA), N-hexane fraction (HCA), ethyl acetate fraction (ECA) and butanol fraction (BCA) on Sensitivity of microorganism.

Microorganisms	Sensitivity (mean IZD mm)			
	MCA	HCA	ECA	BCA
Aspergillus niger	7	12	12	8
Candida albiian	7	12	10	8
Eschesichia coli	7	8	10	9
Staphylococcus aurous	12	13	11	11
Pseudomonas aeruginosa	7	7	7	7
Salmonella paratyphi	7	7	8	7
Klebsiella preumona	7	16	10	9

Figure 1: Effects of acetylcholine and n-hexane on Rabbit jejunum

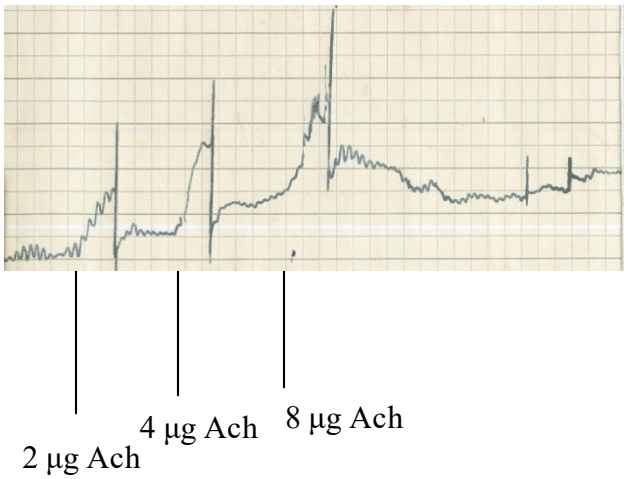

Figure 2: Effects of acetylcholine and n-hexane on Rabbit jejunum

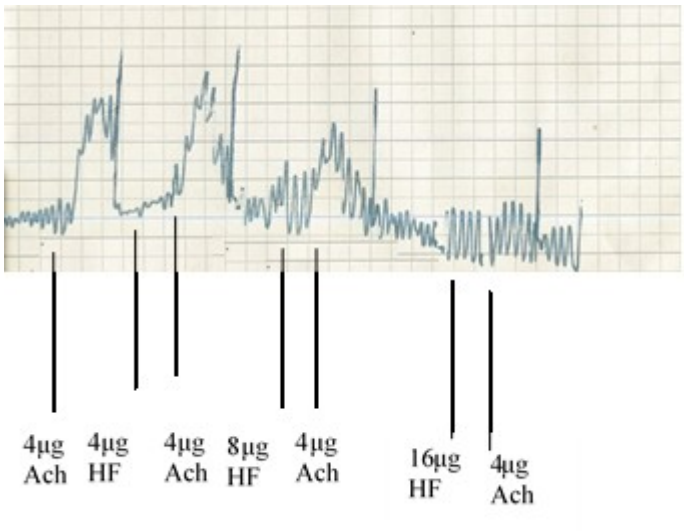

Figure 3: Effects of acetylcholine and n-hexane on Rabbit jejunum

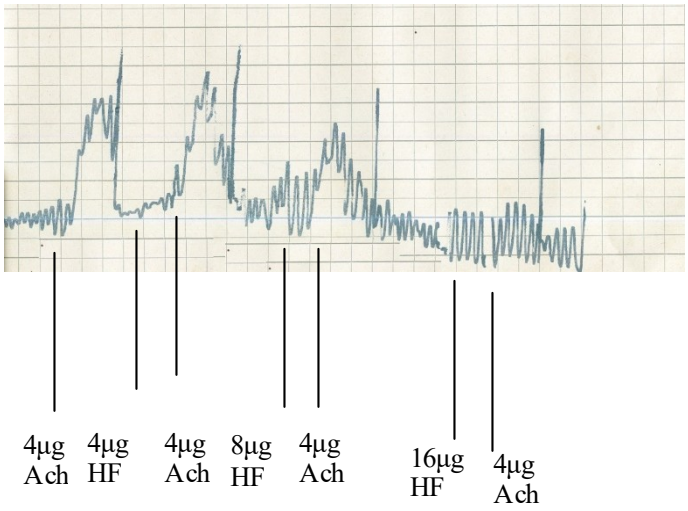

Figure 4: Effects of Acetylcholine and ethanol extract on Guinea pig ileum