EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN 2663-0990 (Print) & 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-1 | Issue-3 | May-Jun-2019 |

Research Article

Phytochemical Screening and Evaluation of Antiulcer and Antioxidant Activity of Hydroalcoholic Extract of *Costus Speciosus* Rhizome

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Abstract: Peptic ulcer is a digestive disorder most commonly found in clinical practice. Given the many side effects of modern medicine, the initial acquisition of fewer side effects and medication of indigenous drugs, it should be considered as a better alternative for the treatment of peptic ulcer. *Costus speciosus (C. speciosus*, Zingiberaceae) is an important medicinal plant widely used for the treatment of various ailments. The plant has multiple active ingredients and has been found to possess many pharmacological activities such as antioxidant, anticancer, anti-inflammatory, antidiabetic, hypolipidemic, hepatoprotective, steroidogenic, adaptogenic and antimicrobial effects. Therefore present study was designed to evaluate antiulcer and antioxidant activity of hydroalcoholic extract of *C. speciosus* rhizome in rats. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity and *in vivo* the anti-ulcer activity of hydroalcoholic extract of rhizomes of *C. speciosus* was assessed against DPPH model and pylorus-ligated-induced peptic ulcers model in rats. Depending on the model, outcome measures were volume and pH of gastric fluid, free acidity, total acidity and ulcer index as well as percent inhibition of ulcer index. Preliminary phytochemical screening revealed the presence of flavonoids, carbohydrates, saponins, polyphenols and amino acids. The total phenolics content of *C. speciosus* extract was (0.789 mg/100mg), followed by flavonoids (0.647mg/100mg) respectively. The IC_{50} value for DPPH radical scavenging of the ascorbic acid and extract was found 14.11 and 67.37µg/ml respectively. Further hydroalcoholic extract of 200 and 400mg/ kg / p.o significantly ($p<0.01$) reduced the gastric volume, pH, ulcer number, ulcer index, free acidity and total acidity in pylorus ligation induced ulcer models in rats. The findings of this study confirmed that *C. speciosus* extract has anti-ulcer pharmacologic activity due to one or more of the secondary metabolites present in it. Therefore, this study validates its anti-ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

Keywords: *C. speciosus*, Phytochemical constituents, Antiulcer, Antioxidant activity, Pylorus ligation.

INTRODUCTION

Peptic ulcer is one of the most prevalent GIT disorders with increased morbidity which affects approximately 5-10% of people during their life (Falk, G.W. 2001). Peptic ulcer disease is a disease of multiple etiologies, till date there is continuous research to elucidate the exact pathogenesis of peptic ulcer, although scientist and researcher proposed a common ground to understand the possible pathogenesis of peptic ulcer. Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors in the stomach (Tripathi, K.D. 1999). Such factors could range from natural causes, infections and lifestyle (Ojewole, E.B. 2004; Rastogi, S., & Rawat, A.K.S. 2008). Various treatment options (proton pump inhibitors, histamine receptor antagonists prostaglandins analogs, and cyto protective agents) are available for the management of peptic ulcer. But majority of these drugs generate several undesirable adverse reactions (headache, abdominal pain, bowl upset, dizziness, constipation and diarrhoea) and also may alter normal biochemical homeostasis of the body on chronic use (elevated serum aluminium levels due to antacids and sucralfate, reduced calcium

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Journal homepage:
http://www.easpublisher.com/easjpp/

Article History
Received: 15.05.2019
Accepted: 25.05.2019
Published: 29.06.2019

absorption by proton pump inhibitors) (Ariyphisi, I. *et al.,* 1986). In recent years, a lot of work has been carried out on natural drugs to elucidate their potential effectiveness in gastric ulcer prevention. Herbal medication is promising as an alternative treatment to available synthetic drugs for the treatment of ulcer probably due to availability, affordability, lesser adverse effects and proved effectiveness (Bi, W.P. *et al.,* 2014; Ayala, G. *et al.,* 2014). Many natural herbs have been pharmacologically reported possessing potent anti-ulcer activity (Vela, S.M. *et al.,* 1997; Aguwa, C.N. 2004). Antioxidants apparently protect the living system from oxidative insults, which is a hallmark feature of cancer, cardiovascular disease, and diabetes (Siriwatanametanon, N. *et al.,* 2010). This oxidative damage is caused by reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl, and nitric oxide (NO) radical (Cerutti, P.A. 1991). These ROS accumulations lead to damage to crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, carbohydrates, and DNA in living system also directly stimulate histamine release from mast cells (Farombi, E.O., & Fakoya, A. 2005). Most of the antioxidantpresents in vascular plants such as Vitamin C and E, carotenoids, flavonoids, and tannins (Park, H.S. *et al.,* 2010). The naturally polyphenolic compounds, especially flavonoids have been largely studied for their strong antioxidants capacity (Rice-Evans, C. 2001). *Costus speciosus* (Koen.) SM. is a plant belonging to the Zingiberaceae family, which is found in various parts of India, Taiwan and Malaysia (Sivarajan, V.V., & Balachandran, I. 1994). Commonly known as Pushkara, Kashmeera, Keu and Kust, the plant is described as a succulent plant with long simple spirally arranged leaves and spirally twisted stems which can grow up to a height of 1-3 metres and have horizontal rhizomes which are used in traditional medicine. The rhizomes are used in constipation, skin diseases, fever, asthma, bronchitis, inflammation and anemia and are medicinally tried to utilize its antihelminthic, astringent, purgative and aphrodisiac properties (Warrier, P.K. *et al.,* 1994). The alkaloidal fraction from *C. speciosus* was evaluated for anticholinesterase activity (Bhattacharya, S. K. *et al.,* 1972) and was later demonstrated to possess papaverine like smooth muscle relaxant action, diuretic, cardiotonic and central nervous system depressant activities (Bhattacharya, S. K. *et al.,* 1973). It was recognized as a source of diosgenin (Dasgupta, B., & Pandey, V.B. 1970) and the saponin content was found to have antifungal activity (Singh, U.P., & Srivastava, B.P. 1992). Therefore, current research is focused on the discovery of natural antiulcer and antioxidant compounds from the plants for new and safer treatment options with fewer side effects. Thus, the objective of the present investigation was to evaluate *invivo* antiulcer and *invitro* antioxidant activity of *C. speciosus* rhizomes extract.

MATERIALS AND METHODS

Plant material

The rhizomes of *C. speciosus* were collected from local area of Bhopal (M.P.) in the month of February, 2018.

Chemicals and reagents

All the drugs, solvents and chemicals used in the study were of analytical grade. Omeprazole was obtained as a gift sample from Scan Research Lab, Bhopal, Madhya Pradesh, India. All other chemicals e. g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India.

Extraction by maceration process

Rhizomes of *C. speciosus* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether. The extraction was continued till the defatting of the material has been taken place. 50 gm of dried powdered rhizomes of *C. speciosus* has been extracted with hydroalcoholic solvent (30:70) using maceration process for 48 hrs. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening

Hydroalcoholic extract of *C. speciosus* rhizomes was subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures (Khandelwal, K.R. 2005; Evans, W.C. 2005).

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso, O.O., & Afolayan, A.J. (2011). A volume of 2 ml of rhizomes of *C. speciosus* extracts or standard was mixed with 5 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso, O.O., & Afolayan, A.J. (2011). 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of

the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

DPPH Free Radical Scavenging Assay

DPPH scavenging activity was measured by modified method (Olufunmiso, O.O., & Afolayan, A.J. 2011). DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

Animal

Healthy adult Swiss albino wistar rats of either sex were selected randomly for the study. The rats weighing between 180-220 gm, were used for the experiment. Rats we're group housed (n= 6) under a standard 12h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00hr. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute Toxicity Test

Acute toxicity study was carried out using the limit test dose of 2000 mg/kg as described by OECD 420 guideline. Three female albino rats were fasted for 24 hours but allowed free access to water. 2000 mg/kg of hydroalcoholic extract of *C. speciosus* rhizome was administered and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing continuously for 2 hours, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days (Guideline Document on Acute oral Toxicity Testing. 1996).

Pylorus Ligation Model

The albino rats were randomly divided into five groups of six animals each (Samyuktha, K. *et al.*, 2017; Abebaw, M. *et al.*, 2017).

Experimental Design

- **Group I:** Normal control animals
- **Group II:** Disease control (pylorus ligated)
- **Group III:** Hydroalcoholic extract of *C. speciosus* (200 mg/kg p.o) suspended in 1% *w/v* CMC
- **Group IV:** Hydroalcoholic extract of *C. speciosus* (400mg/kg p.o) suspended in 1% *w/v* CMC
- **Group V:** Standard treated Omeprazole (20mg/kg) suspended in 1% *w/v* CMC

Procedure

On the day of experiment, animals of Group III, IV and V were treated with low, high doses of *C. speciosus* extract and omeprazole respectively with the help of an oral feeding tube. The control group was treated with normal saline only. After one hr of drug treatment, Group II, III, IV and V animals were anaesthetized with the help of anaesthetic ether the abdomen was opened by a small midline incision. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by sutures. Rats were sacrificed by an over dose of anaesthetic ether after six hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acid. Each stomach was examined for lesions in the fore stomach portion and indexed according to the severity.

Biochemical Estimations

Determination of gastric volume

After sacrificing the rat, the stomach portion was removed. The gastric contents were transferred into the centrifuge tube, centrifuged and filtered. The supernatant liquid was then transferred to a measuring cylinder and the volume was measured.

Determination of pH of gastric content

One ml of the gastric juice was collected and the pH was directly measured by using Digital pH meter.

Determination of ulcer index

The stomachs were opened along the greater curvature; the number of ulcers was counted.

Ulcer scoring was done by the following scoring system:

- 0=no ulcer,
- 1=superficial ulcer,
- 2=deep ulcer,
- 3=perforation.

Ulcer index was calculated by using following formula

UI=UN+US+UP×10-1

Where,

UI=ulcer index,
UN=mean of ulcer number,
US=mean of ulcer score,
UP=ulcer probability for each group.

Determination of free acidity and total acidity

The total volume of gastric content was measured. The gastric contents were centrifuged and filtered. One ml of the gastric juice was pipette out and the solution was titrated against 0.1N sodium hydroxide using 2 to 3 drops of topfer's reagent as indicator, to the end point when the solution turned to yellowish orange colour was observed. This indicated the volume of NaOH required neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red colour appears. The difference between the two readings indicated the volume of NaOH required neutralizing the combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH}}{\text{Volume of gastric juice used}}$$

RESULTS AND DISCUSSIONS

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *C. speciosus* extracts was 2.3 %w/w. The results of preliminary phytochemical screening of hydroalcoholic extract of *C. speciosus* rhizome are shown in Table 1. The extract showed the presence of polyphenolic compounds, saponins, flavonoids, carbohydrate, proteins and amino acids. The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.042X+0.002, R^2= 0.999**, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: **Y=0.040X + 0.009, R^2=0.999**, where X is the absorbance and Y is the quercetin equivalent (QE). Results was shown in table 2 and fig. 1& 2

Table 1 Result of phytochemical screening of extracts of *C. speciosus*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	-
2.	Glycosides	-
3.	Flavonoids	+
4.	Saponins	+
5.	Phenolics	+
6.	Amino Acids	+
7.	Carbohydrate	+
8.	Proteins	+
9.	Diterpenes	-

Table 2 Total phenolic and total flavonoid content of rhizomes extract of *C. speciosus*

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extract
	Rhizomes of *C. speciosus*	
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.789
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.647

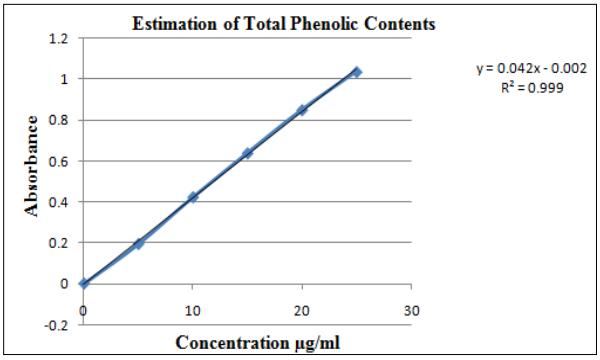

Figure 1: Graph of estimation of total phenolic content

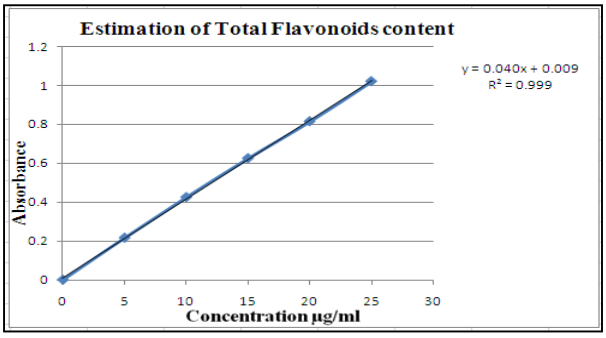

Figure 2: Graph of estimation of total flavonoid content

The presence of various compounds may be the result for the potential activity of antioxidant property of *C. speciosus.* The DPPH radical scavenging effect was studied and the results are presented in the table 3 and fig.3 The IC_{50} value for DPPH radical scavenging of the ascorbic acid and extract was found 14.11 and 67.37µg/ml respectively.

Table 3 % Inhibition of ascorbic acid and *C. speciosus* extract using DPPH method

S. No.	Concentration (µg/ml)	Ascorbic acid % Inhibition	*C. speciosus* extract % Inhibition
1.	10	40.92	12.58
2.	20	48.70	21.36
3.	40	73.48	34.47
4.	60	89.04	48.74
5.	80	90.20	57.98
6.	100	91.35	67.65
	IC 50	14.11	67.37

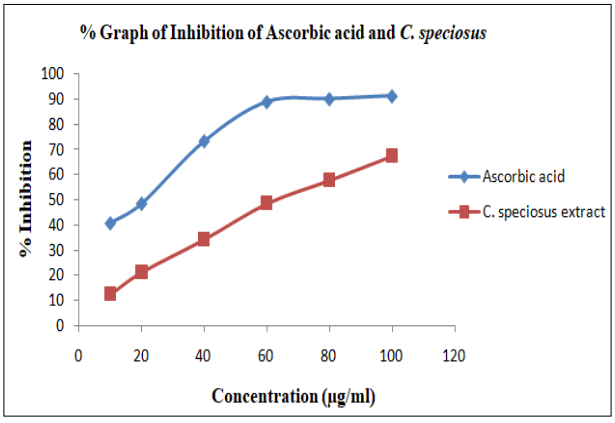

Figure 3 Graph of % inhibition of ascorbic acid and *c. speciosus* using DPPH method

The acute oral toxicity study was done according to the OECD 425 guidelines. No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *C. speciosus* rhizomes. This indicates that 2000 mg/kg is maximum safe dose. So $1/10^{th}$ and $1/5^{th}$ *i.e.* 200 and 400 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-ulcer effects. Pylorus ligation induced ulcer was used to study the effect of hydroalcoholic extract of *C. speciosus* rhizome on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The fasting of rats for 24 h followed by ligation of pyloric end of the stomach, the ulcer index is determined 4 h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. The hydroalcoholic extract of *C. speciosus* and omeprazole significantly decreased the total acidity and free acidity; and significantly enhance the pH; this suggests that it having an anti-secretory effect. Pylorus ligation induced ulcer control rats shown perforated ulcer, deep ulceration of granular epithelium and almost reducing the sub-mucosa. The hydroalcoholic extract of *C. speciosus* at 200 mg/kg dose has shown mucosal erosion, the partial healing of ulcer with few inflammatory cells and the dose 400 mg/kg has shown the healed ulcer, normal mucosa and no inflammatory cells (Table 4-6).

Table 4 Effect on gastric volume and pH in pylorus ligated gastric ulcer

Group	Gastric Volume	Gastric pH
Group I: Control (CMC)	2.40 ± 0.46	2.89±0.26
Group II: Pylorus ligated	7.65± 0.45**	2.20 ±0.24**
Group III: Treated with *C.speciosus* (200mg/kg)	6.40 ± 0.28**	2.59 ± 0.18*
Group IV: Treated with *C.speciosus* (400mg/kg)	4.89 ± 0.26**	3.28 ± 0.41**
Group V: Treated with *Omeprazole* (20 mg/kg)	3.74 ± 0.46***	3.72 ± 0.16***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

Table 5 Effect on number of ulcer and ulcer index in pylorus ligated gastric ulcer

Group	Number of Ulcer	Ulcer Index
Group I: Control (CMC)	0.22±0.18	2.80±0.21
Group II: Pylorus ligated	4.68±0.27**	26.26±0.26**
Group III : Treated with *C.speciosus* (200mg/kg)	3.62±0.14*	18.20±0.14*
Group IV : Treated with *C.speciosus* (400mg/kg)	2.30±0.48**	15.90±0.32**
Group V : Treated with *Omeprazole* (20 mg/kg)	1.62±0.47***	12.60±0.30***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

Table 6 Effect on free acidity and total acidity in pylorus ligated gastric ulcer

Group	Free acidity content	Total acidity content
Group I: Control (CMC)	4.60± 0.24	5.60 ± 0.21
Group II: Pylorus ligated	7.86 ±0.22**	8.46 ± 0.16**
Group III : Treated with *C. speciosus* (200mg/kg)	6.69±0.21**	7.62 ±0.20*
Group IV : Treated with *C. speciosus* (400mg/kg)	5.01± 0.18**	5.21 ± 0.18**
Group V : Treated with *Omeprazole* (20 mg/kg)	3.62±0.20***	3.98± 0.10***

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

CONCLUSION

The preliminary phytochemical investigation of hydroalcoholic extract of *C. speciosus* rhizomes showed the presence of flavonoids, saponins, polyphenols ect. Hydroalcoholic extract was screened for acute oral toxicity and was found to be non toxic. Hydroalcoholic extract of *C. speciosus* rhizomes possesses significant anti-ulcer activity in addition to potent oxidant and antioxidant activity. In conclusion, our results showed that the anti-ulcer activity of the extract was a result of the probable gastric ulcer healing mechanism (anti-secretory, cytoprotective and the antioxidant properties) of its active phytoconstituents. These findings suggest the potential for use of *C. speciosus* as an adjuvant in the treatment of gastric ulcer. Further, studies are needed for the isolation of active constituents responsible for the anti-ulcer activity and to elucidate the exact mechanism of action in gastric ulcer healing.

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