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Investigating *Cocculus hirsutus* and *Calycopteris floribunda* for Antioxidant and Antiulcer Therapy: A Comparative Study

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ABSTRACT
This study aimed to investigate the phytochemical constituents, antioxidant activity, and antiulcer potential of ethanol extracts from *Cocculus hirsutus* and *Calycopteris floribunda* leaves. The phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, tannins, and steroids in both extracts. The total phenolic and flavonoid content of *C. hirsutus* was higher than those of *C. floribunda*. Additionally, the ethanol extract of *C. hirsutus* exhibited a higher antioxidant activity than *C. floribunda*, as evidenced by the DPPH radical scavenging assay. The antiulcer activity of the ethanol extracts was evaluated in a drug-induced ulcer model in rats. The results showed that both extracts had significant antiulcer activity, with the highest activity observed in the ethanol extract of *C. floribunda*. The extract of *C. hirsutus* also showed significant antiulcer activity, albeit to a lesser extent than *C. floribunda*. The findings of this study suggest that the ethanol extracts of *C. floribunda* and *C. hirsutus* could be potential candidates for the development of natural antiulcer agents. In conclusion, the present study highlights the potential of *C. hirsutus* and *C. floribunda* as sources of natural antioxidants and antiulcer agents. The higher phenolic and flavonoid content and antioxidant activity of *C. floribunda* suggest it may be a more potent source of natural antioxidants than *C. hirsutus*. The observed antiulcer activity of both extracts, particularly *C. floribunda*, indicates their potential as natural antiulcer agents. However, further research is required to validate the findings in human subjects and to elucidate the underlying mechanisms of the observed antiulcer activity.
Keywords: *Cocculus hirsutus, Calycopteris floribunda,* antioxidant, and antiulcer activity

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INTRODUCTION

Herbal medicines have been used for healthcare since ancient times, with approximately 80% of the world's population depending on them, owing to their safety and efficacy. Traditional herbal drugs have been reported to be effective in treating various diseases, such as skin diseases, tuberculosis, diabetes, jaundice, hypertension, mental disorders, cancer, and AIDS [1]. In India, China, South America, and Egypt, herbal drug usage is widespread, with approximately 25,000 herbal-based drugs being used traditionally and 2000 tons of herbs being consumed in the production of herbal medicines [2].

According to the World Health Organization, peptic ulcer is a common medical condition affecting 10% of the world's population. It is characterized by an open sore in the stomach, duodenum, or esophagus lining that causes skin rupture or mucous layer. The condition is mostly caused by the bacterium Helicobacter pylori or prolonged use of nonsteroidal anti-inflammatory drugs [3]. The symptoms of peptic ulcer include nausea, vomiting, weight loss, and abdominal pain. The condition is more common in elderly people, and if left untreated, it can lead to serious complications like perforation, bleeding, and obstruction. Proper diagnosis and treatment of peptic ulcer is essential to prevent its complications and improve the quality of life of affected individuals [4].

C. hirsutus, also known as broom creeper, is a perennial climber shrub from the Meinspermaceae family. It is native to Asia and Africa, with the plant mostly found in Kerala and Maharashtra, India [5-6]. *C. hirsutus* leaves are used to treat stomach aches, and decoction is used to treat female sterility. The leaves are simple and alternate, with a petiole of 1 cm. The flowers are small and green, and the seeds are curved [7].

C. floribunda, also known as "Ukshi," is a climbing shrub that belongs to the Combretaceae family. It is mostly grown in India and Bangladesh. Different parts of *C. floribunda* treat various diseases, such as ulcers, jaundice, colic, leprosy, neurotoxicity, dysentery, skin diseases, and inflammation [8]. The fruits

treat jaundice and ringworms, while the leaves show laxative, antiulcer, astringent, and anti-malaria properties. The stems are used to treat snake bites. Therefore, due to their traditional use and potential medicinal properties, *C. hirsutus* and *C. floribunda* were selected for the study to investigate their phytochemical composition and antiulcer properties [9-10].

MATERIAL AND METHODS

Plant material

Cocculus hirsutus(authentication) and *Calycopteris floribunda* were collected in February 2019 in the Osmania University Campus.

Extraction

The whole herb of *Cocculus hirsutus* (3kg) and leaves of *Calycopteris floribunda*(3kg) was dried under shade for 7 days and powdered with subsequent extraction. Extraction was performed using Soxhlet extraction with ethanol and prior defatting with n-Hexane. The extract solution was evaporated under a vacuum to get the solvent-free crude solid extract. The percentage yield was calculated[11].

Phytochemical screening

Preliminary phytochemical screening for the extracts was performed according to standard established procedures [12].

Invitro screening:

Total Phenolic content estimation:

Preparation of Standard Gallic Acid for Calibration Curve:

Standard Gallic acid solutions with varying concentrations from 25 to 100 g/mL were prepared by dissolving pure Gallic acid in methanol, and 10 mL of 10% Folin-Ciocalteu reagent and 8 ml of (7.5% w/v) Sodium carbonate was also added to make a final volume of 20 ml, which was then incubated at room temperature for 2 hours. The resulting-colored solution was measured at 765 nm with a UV-visible spectrophotometer, and Calibration curve was plotted.

Estimation of total Phenolic content:

With some modifications, the Singleton et al. (1965) method was used to determine the overall phenolic content. Gallic acid was first prepared as a stock solution and then diluted with ethanol to different concentrations, ranging from 25-100 µg/mL. Next, a mixture of Folin-Ciocalteu reagent (10%) and sodium carbonate (7.5% w/v) was added, and the resulting solution was allowed to incubate for two hours at room temperature. The absorbance was measured in triplicate using a UV-visible spectrophotometer at 765 nm. A calibration curve was then created, and the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) [13].

Total Flavonoid content estimation:

Dowd method is used to estimate the flavonoid content, and the procedure is to measure the total flavonoid by taking 1ml of extract and mixed with 0.2ml of 10%AlCl3 solution in methanol and adding 5.6ml of water were mixed. After 30 minutes of incubation at room temperature, absorbance at 415 nm was measured against a blank. Using a calibration curve, the total flavonoid content was demonstrated as g of quercetin equivalents per mg dry matter (g QE/ mg dry weight). All experiments were carried out in triplicate. The mean and standard deviations were computed [14].

Invitro antioxidant activity:

DPPH radical scavenging assay

To assess the capability of ethanolic extracts to scavenge free radicals, the DPPH radical scavenging assay was utilized. In this assay, 0.2mL of the extract solution was mixed with a 0.5mM DPPH solution (2mL) and left to incubate at room temperature for 20 minutes. Ascorbic acid was used as a reference standard, and the absorbance of each extract was measured in triplicate at 515 nm. The formula provided was used to calculate the antioxidant activity [15].

% Free radical scavenging activity = $[(A_0\text{-}A_s)/A_0] \times 100$

Where,

A_0 is the absorbance of blank (DPPH solution alone)

A_0 is the absorbance of the test (DPPH + sample)

Nitric oxide radical scavenging assay:

C. hirsutus and *C. floribunda* were screened for nitric oxide radical scavenging activity by mixing 0.5 mL of the test solution with 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH-7.4) was be mixed with 0.5 mL of the test solution and incubated for 150 min at 25 °C. Ascorbic acid solution and DMSO served as standard and control, respectively. Equal volumes (0.5mL each) of Griess reagent and test samples were incubated for 30 min at 25 °C [16]. The absorbance was recorded at 540 nm, and the percentage of nitric oxide inhibition was calculated as:

Percentage of nitric oxide radical scavenging assay = $[(A_0\text{-}A_s)/A_0] \times 100$

Where,

A_0 was the absorbance of control

A_s was the absorbance of the treated sample

Experimental animals

Adult Wistar rats around 8weeks old of either sex were selected for the study. Animals were randomly grouped before the study; the rats were accommodated in polypropylene cages for 10 days under controlled temperature (26 ± 2ºC), relative humidity (45-55%), and a dark/light cycle of 12 hrs. A rodent pellet diet was supplied, and water *ad libitum*. The institutional animal ethics committee (IAEC) has approved (CPCSEA/IAES/JLS/006/01/17/005) the study protocol before the commencement of the experiment.

Acute oral toxicity study

The extract underwent oral acute toxicity studies on albino rats, following the OECD guidelines 423 with minor modifications. A maximum test dose of 2000mg/kg was administered to the rats, and it was determined that a dose of 1000mg/kg body weight was deemed safe [17].

Antiulcer activity screening

The antiulcer activity was evaluated using the indomethacin induced gastric ulcer model in female Wistar rats. The Indomethacin-induced antiulcer activity model is a commonly used experimental model to evaluate the potential of drugs or natural compounds in preventing or treating ulcers induced by nonsteroidal anti-inflammatory drugs (NSAIDs). Indomethacin is a commonly used NSAID known to cause gastric mucosal damage and ulceration in animals and humans, making it a reliable model for studying the antiulcer potential of test substances. The animals were subjected to gastric ulceration with a single oral dose of indomethacin (30 mg/kg body weight) and 24 hours of food deprivation but free access to water before the ulcer induction. After four hours of indomethacin administration, the animals were divided into group 2 (disease group; treated with saline), group 3 (positive control) received Esomeprazole (20mg/kg b.w.), groups4 and 5 received low and high doses (200 and 400 mg/kg, respectively) of *C. hirsutus* extract, and group 6 and 7 received low and high doses (150 and 300 mg/kg, respectively) of *C. floribunda* extract. A control group was also used for the comparison. The animals were sacrificed after 6 hours, and the stomachs were examined for ulcer index, and ulcer inhibition rate [18].

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening

The methanolic extract of *C. hirsutus* and *C. floribunda* was subjected to a preliminary phytochemical study, revealing diverse secondary metabolites. Specifically, the extracts contained alkaloids, saponins, flavonoids, tannins, amino acids, and carbohydrates.

Total phenolic content

Phenols play a crucial role in protecting the body from cellular stress [19]. The Folin-Ciocalteu method was used to determine the total phenolic content of the methanolic extract of *C. hirsutus* and *C. floribunda*, with gallic acid as the reference standard. A calibration curve was generated from the absorbance values of different gallic acid concentrations, and the extracts' total phenolic content was calculated using the regression equation ($y = 6.7073x + 0.218$, $R^2 = 0.9923$). The results were expressed in mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic content of the methanolic extract of *C. hirsutus* was high as 93±0.78 mgGE/g, while that of *C. floribunda* was 121±1.21 mgGE/g.

Total flavonoid content

Polyphenolic compounds, particularly flavonoids, are essential adaptogens that aid the body in adapting to harsh environments while enhancing human health by addressing chronic ailments [20]. In this study, the flavonoid content was measured using the Dowd technique's colorimetric method and was found to be 28±0.21mg of gram equivalence of Quercetin in *C. hirsutus* and 64±1.23of gram equivalence of Quercetin in *C. floribunda* at 415 nm. The calibration curve was established through linear regression, and the results were triplicate. By applying the regression equation of the calibration curve ($y = 7.4699x + 0.0876$, $R^2 = 0.9971$), the total phenolic content of the extract was computed and reported as mg Quercetin equivalents per gram of dry weight (mg QE/g). The study found that *C. floribunda* contains a significantly higher total phenolic content than *C. hirsutus*.

Antioxidant activity:

2,2-diphenyl-1-picrylthydrazyl(DPPH) assay

The table 3 shows the results of the DPPH assay for *Cocculus hirsutus*, and *Calycopteris floribunda*, compared with ascorbic acid at various concentrations ranging from 10 µg/ml to 75 µg/ml.The data indicate that as the concentration of each compound increases, its ability to reduce the DPPH radicals increases, as evidenced by the decreasing IC_{50} values.Ascorbic acid shows the highest antioxidant activity

with the lowest IC_{50}value of 42.33 µg/ml, followed by *C. floribunda* with an IC_{50}value of 48.27 µg/ml and *C.hirsutus* with an IC_{50}value of 57.67 µg/ml. At the highest concentration tested (75 µg/ml), ascorbic acid was the most effective antioxidant, reducing DPPH radicals by 75.31%, while *C.hirsutus* and *C. floribunda* reduced DPPH radicals by 57.13% and 67.80%, respectively. At lower concentrations, the differences in antioxidant activity between the compounds become more pronounced. This suggests that ascorbic acid is the most potent antioxidant of the three compounds tested, followed by *C.hirsutus* and *C. floribunda*.

Table 3: DPPH scavenging activity

Concentration µg/ml	Ascorbic acid	*Cocculus hirsutus*	*Calycopteris floribunda*
75	75.31 ± 0.12	57.13±0.65	67.80 ±0.82
50	64.96± 0.95	48.65±0.79	57.26±1.06
25	40.35±1.05	34.80±0.54	37.12±1.35
15	22.14±0.63	17.33±0.39	19.16±0.97
10	18.22±0.18	14.20±0.25	15.25±0.85
IC_{50}	**42.33µg/ml**	**57.67µg/ml**	**48.27µg/ml**

NO Free radical Scavenging activity

The NO free radical scavenging assay is an important technique used to evaluate the ability of different substances to scavenge nitric oxide free radicals. The data presented in this study showed the percentage of scavenging activity at different concentrations for *Cocculus hirsutus*, and *Calycopteris floribunda*, compared with ascorbic acid. The results demonstrated that ascorbic acid had the highest scavenging activity, with a percentage of 86.32% at a concentration of 75 µg/ml, followed by*C. floribunda* and *C.hirsutus* with percentages of 54.10% and 57.83%, respectively. The IC_{50} values for each substance were also determined, with ascorbic acid having the lowest IC_{50}value of 40.63 µg/ml, followed by *C. floribunda*(50.99µg/ml)and *C.hirsutus* (68.33µg/ml)indicating a better ability to scavenge free radicals. The findings suggest that *C. floribunda* has the most potent nitric oxide scavenging activity, next to ascorbic acid, among the three substances tested.

Table:4 NO Scavenging activity

Concentration µg/ml	Ascorbic acid	*Cocculus hirsutus*	*Calycopteris floribunda*
75	86.32±1.06	54.10±0.95	57.83±1.55
50	60.51±0.96	45.72±0.58	59.48±1.64
25	34.69±1.35	30.75±0.93	36.49±1.07
15	24.40±0.68	15.31±1.03	16.35±0.67
10	18.15±1.32	12.41±0.57	14.32±0.65
IC_{50}	**40.63µg/ml**	**68.33µg/ml**	**50.99µg/ml**

Indomethacin Induced antiulcer activity

The antiulcer activity was estimated by calculating the average ulcer index (UI) and percentage of ulcer inhibition. The results indicate that the ethanol extract of *C. floribunda* at a dose of 150 mg/kg b.wt and 300 mg/kg b.wt produced a significant reduction in ulcer formation with an average UI of 4.37±0.33 and 4.85±0.37, respectively, and a high percentage of ulcer inhibition of 66.47 and 62.85, respectively. In comparison, the ethanol extract of *C. hirsutus* showed antiulcer activity, but to a lesser extent, with an average UI of 9.29±0.54 and 8.39±0.49 and a percentage of ulcer inhibition of 28.74 and 35.69, respectively, at doses of 200 mg/kg b.wt and 400 mg/kg b.wt.

The standard drug Esomeprazole at a dose of 20mg/kg b.wt produced the highest antiulcer activity with an average UI of 2.60±0.19 and a percentage of ulcer inhibition of 80.07. The results suggest that the ethanol extracts of *C. floribunda* and *C. hirsutus* possess antiulcer activity, with *C. floribunda* showing the most potent activity.

Table 3: Results of Antiulcer activity of ethanol extract in Drug-induced Ulcer model

Groups	Description	Ulcer index (UI)	% Ulcer Inhibition
Group 1	Normal Control	0	--
Group 2	Disease control (Indomethacin 30mg/kgb.w.)	13.04±067	--
Group 3	Positive control (96% Ethanol) + Esomeprazole 20mg/kgb.w. (Standard)	2.60±0.19	80.07
Group 4	CH low dose (96% Ethanol) + (*C. hirsutus* 200 mg/kg b.wt)	9.29±0.54	28.74
Group 5	CH high dose (96% Ethanol) + (*C. hirsutus* 400 mg/kg b.wt)	8.39±0.49	35.69
Group 6	CF low dose (96% Ethanol) + (*C. floribunda* 150 mg/kg b. wt)	4.37±0.33	66.47
Group 7	CF high dose (96% Ethanol) + (*C. floribunda* 300 mg/kg b. wt)	4.85±0.37	62.85

CONCLUSION

C. hirsutus and *C.flouribunda* are traditional medicinal plants that treat various diseases. This study aimed to evaluate the phytochemical constituents of the ethanolic leaf extracts of these plants. The study showed that both plants contain alkaloids, glycosides, flavonoids, tannins, and steroids. However, the total phenolic and flavonoid content in *C. flouribunda* was higher than in *C.hirsutus*.The study also investigated the antiulcer activity of the ethanol extracts of *C. hirsutus* and *C.flouribunda* in a drug-induced ulcer model. The experiment results revealed that both plants have antiulcer activity, with *C.flouribunda* showing the highest ulcer protective activity. The ethanol extract of *C. hirsutus* also exhibited antiulcer activity, albeit to a lesser extent. These findings suggest that *C. hirsutus*and *C.flouribunda* could be potential candidates for further research and development of natural antiulcer agents. However, more research is needed to validate the findings in human subjects and to understand the underlying mechanisms of the observed antiulcer activity. Overall, these results highlight the potential of traditional medicinal plants as sources of natural antiulcer agents.

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