Research Article

In vitro Antioxidant and Gastroprotective Effect of *Cayratia pedata* var. *Glabra* on Ethanol Induced Ulcer Model

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ABSTRACT

To assess the free radical scavenging activities and gastroprotective effect of aerial plant extracts of *Cayratia pedata* var. *glabra*. The different extracts of study plant was screened for *in vitro* antioxidant activity by oxygen and nitrogen radical scavenging such as hydroxyl radical, nitric oxide radical, superoxide radical, β-carotene / linoleic acid and phosphomolybdenum antioxidant assay at different concentrations. The gastroprotective effect of ethanolic extract of *Cayratia pedata* var. *glabra* were studied on ethanol induced ulcer model. Different extracts of *Cayratia pedata* var.*glabra* possess different range of free radical scavenging activities, which is in dose dependent manner. The ehanolic extract of study plant produced gastro protective effect in ethanol induced rats showed the decreased level of ulcer index, gastric volume, free acidity, total acidity, total protein and increased level of percentage of protection and pH were calculated from the effective concentrations of plant sample (400 mg/kg) compared with Omeprazole (10 mg/kg). It is concluded that, the *Cayratia pedata* var. *glabra* contains natural source of antioxidants and different extracts of this plant possesses good free radical scavenging activity. The antioxidant property may be related to the phenols, tannins and flavonoids present in the extract. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of various free radicals and inhibition of lipid peroxidation. This present study clearly indicates that, *Cayratia pedata* var. *glabra* is effective against free radical mediated disease and the ethanolic extract of this study plant have potential anti-ulcer property in ethanol induced ulcer model. In future, it can be used as a bioactive source of natural antioxidants and are potential natural resources for pharmacology of functional foods.

Keywords: Gastroprotective, *Cayratia pedata* var. *glabra,* radical scavenging, Omeprazole.

INTRODUCTION

Medicinal plants and plant extracts represent the oldest and most widespread form of medication. At least 25% of the active compounds in currently prescribed synthetic drugs were first identified in plant sources.[1] According to WHO more than 80% of the world's population relies on traditional herbal medicine for their primary health care.[2] In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000plants have been studied during the last 5 year period. However, due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day.[3]

Cayratia pedata (Lam.) Gagnep.var. *glabra* Gamble is a weak climber commonly known as kattuppirandai, ainthilaikodi (5-pedata) in tamil, goalilata in hindi, godhapadi in sanskrit and veluttasorivalli in malayalam. It belongs to family Vitaceae. This species can be found in Thiashola and Korakundah range and scrambling over the hedges and trees. The photochemical analysis of plant has proved to contain calcium, magnesium, potassium, sodium, manganese, zinc, iron and copper.[4] The study plant contains vitamin A, vitamin E, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_{12}, Niacinamide and vitamin B_9. Concentration of Lead was less than 1 ppm and chromium occurred in traces. The other heavy metals like mercury, cadmium and arsenic were totally absent. The preliminary phytochemical screening revealed the presence of carbohydrates, proteins, aminoacids, alkaloids, anthroquinones, flavonoids, glycosides, phenols and tannins, steroids and sterols, triterpenoids and volatile oil.[5]

The leaf decoction of the study genus is used for treating uterine and other fluxes; lukewarm leaf juice is used as ear drops for fungal infections; leaves are astringent, refrigerant and also used to cure ulcers; stem paste is applied for healing bone fracture; whole plant is useful in acrid, refrigerant and beneficial in hysteria, burning of the skin and diarrhea.[4] Antioxidant are the substances which scavenge free radicals and they play an important role in the prevention of free radical-induced diseases by donating hydrogen radicals to the primary radicals which gets reduced to non-radical chemical compounds and then get converted to oxidize antioxidant radicals.[6,7]The findings of the present study can be useful to progress and surge further scientific investigation on the aerial parts of this species and also developing a standardized profile of aerial parts of *Cayratia pedata* var. *glabra* which would be of immense use to assess anti-ulcer activity of this plant species.

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MATERIALS AND METHODS

Plant collection and extraction

Aerial plant of *Cayratia pedata* var. *Glabra* were collected from Thiashola, Manjoor, Nilgiris South Division, Western Ghats and the voucher herbarium specimen was processed followed by standard methods Jain and Rao.[8] The collected plants were identified with the help of the existing Floras[9-11]and the identity is authenticated with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore (No. BSI/SRC/5/23/2010-11/Tech. 1300), Tamil Nadu. Some quantities of leaves and total aerial parts were air dried, powdered and stored in air–tight containers for extraction purpose.

Successive solvent extraction

The air dried, powdered plant material was extracted in Soxhlet apparatus successively with different solvents in the increasing order of polarity [Acetone (56.5°C), Ethanol (78.5°C) and Water (99.98°C)]. Each time, before extracting with the next solvent, the powdered material was dried in a hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 hrs and the water extract was filtered. The different solvent extracts were concentrated, vacuum dried and weighed. The extracts were dried over anhydrous sodium sulfate, stored in sealed vials in refrigerator (5-8°C) until analysis.[12]

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of plant extracts was determined using the method reported by Klein et al.,1991.[13]Various quantities of extracts (250–1250 µg) were added with 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018%) and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min. in a water bath. After incubation the reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and raised to 1 litre with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula:

HRSA % = 1 - (difference in absorbance of sample / difference of blank) x 100

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity of the plant extracts was measured by the standard method of Sreejayan and Rao.[14]Three ml of 10 mM sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (50-250 µg/ml) of solvent extracts and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was read at 546 nm. Tannic acid, BHT and the same mixture of the reaction without sample extracts were employed as positive and negative control. Percentage radical scavenging activity of the sample was calculated as follows: NO radical scavenging activity % = (control OD - sample OD / control OD) x 100

Superoxide radical scavenging activity

The superoxide radical scavenging activity of the plant extracts was measured by the standard method Beauchamp and Fridovich,[15] Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations (50–250 µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept in dark served as blank. The percentage inhibition of superoxide anion generation was calculated as:% Inhibition = (control OD - sample OD / control OD) x 100

β-carotene / linoleic acid antioxidant activity

β-carotene / linoleic acid antioxidant activitywas determined using the method reported by Taga et al.,[16] One milliliter of a β-carotene solution in chloroform (1 mg/10 ml) was pipetted in to a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45ºC for 4 min and, 50 ml of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 200 µl of the antioxidant (extracts, BHT or Tannic acid) solution at 1 mg/ml concentration and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50ºC and the absorbance was monitored at 15 min intervals until 180 min. All determinations were carried out in triplicates. The antioxidant activity of the sample extracts and standard was evaluated in terms of bleaching of β-carotene using the following formula,

$$AA = \left[\frac{1 - (A_0 - A_t)}{A_0^1 - A_t^1} \right] \times 100$$

Where, A_0 and A_0^1 are the absorbances measured at zero time of incubation for the test sample and control, respectively, and A_t and A^1 t are the absorbances measured in the test sample and control, respectively, after incubation for 180 min.

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Phosphomolybdenum assay

The phosphomolybdenum antioxidant assay was carried out according to the procedure reported by Prieto et al.[17] An aliquot of 100 µl of sample solution (in 1 mM dimethyl sulfoxide, DMSO) was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95º C for 9 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents / 100 g extract.

Antiulcer property

Animals

After approval of the Institutional Ethics committee, pathogen-free Wistar strain Albino rats mass ranging from 150-200 g of male breed were used for ethanol induced ulcer model. The rats were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. All *in vivo* experiments were carried out as suggested by the Institutional Ethical Committee - CPCSEA (Reg. No. 722 / 02 / a / CPCSEA). The animals were housed in polypropylene cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard environmental conditions (14 h dark / 10 h light cycles; temp 25 ± 2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad-libitum*. The rats were acclimatized to the environment for two weeks prior to experiment use. Animals were fasted over night before the experimental schedule, but have free access for water *ad-libitum*.

Sample preparation

Coarse powder from the shade dried aerial plant parts of *C. pedata* var. *glabra* (500 g) was exhaustively extracted using Soxhlet apparatus with absolute ethanol (78.5°C). The extract was dried (free of solvent) using a vacuum evaporator for condensation. The extract thus obtained was stored in refrigerator and used for antiulcer activity. The residual extract was dissolved in distilled water at the time of study period.

Ethanol-induced gastric ulcer

Six groups of male Wistar rats (n = 6) were fasted over night prior to the start of the experiment but have free access to water *ad-libitum*. The first and the second groups received distilled water (10 ml/kg /day p.o.), while the third group was treated with omeprazole (10 mg/kg /day p.o.) whereas fourth, fifth and sixth groups were administered with the ethanol extract of *C. pedata* var. *glabra* (100, 200 and 400 mg/kg /day p.o.). On day third ethanol (1ml/kg) was given as a single oral dose to the groups 2 - 6 to induce gastric ulcers, after 60 min of omeprazole and *C. pedata* var. *glabra* extracts treatments. After 1 h the animals were sacrificed with the over dose of diethyl ether and each stomach was examined for ulcer index.[18]

Experimental design

Group I: Untreated control rats

Group II: Ethanol induced ulcer (1 ml/kg p.o.) treated rats.

Group III: Standard drug Omeprazole was administered to the rats for 3 days + Ethanol (1 ml/kg p.o.) treated rats.

Group III: *C. pedata* var. *glabra* extract (100 mg/kg p.o./day) + Ethanol (1 ml/kg p.o.) treated rats.

Group IV: *C. pedata* var. *glabra* extract (200 mg/kg p.o./day) + Ethanol (1 ml/kg p.o.) treated rats.

Group V : *C. pedata* var. *glabra*extract (400 mg/kg p.o./day) + Ethanol (1 ml/kg p.o.) treated rats.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated as described by Djahanguiri,[19] using the following formula:

$$\% I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

Biochemical Estimations

Determination of gastric volume

After sacrificing the rat, the stomach portion was removed. The gastric contents were transferred in to the centrifuge tube, and centrifuged at 1000 rpm for 10 minutes. The supernatant liquid was then transferred to a measuring cylinder, and the volume was measured.

Determination of pH of gastric content

1 ml of the gastric juice was collected, and pH was directly measured by using pH strip.[20]

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 pipetted out and the solution was titrated against 0.1N sodium hydroxide using 2 to 3 drops of Topfer's reagent as indicator, to the endpoint 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.

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Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The difference between the two readings indicated the volume of NaOH required neutralizing the Acidity was calculated by using formula;

$$\text{Acidity} = \frac{\text{Vol. of NaOH X Normality of NaOH}}{\text{Vol. of Gastric juice used}} \text{ m. Eq. /dl.}$$

combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.[21]

Estimation of total proteins

Reagents

Alkaline copper reagent

Solution A: 2% sodium carbonate in 0.1N sodium hydroxide

Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate. 50 ml of solution A was mixed with 1 ml of solution B just before use. Folins phenol reagent. One volume of folins reagent was diluted with two volumes of distilled water just before use. 20 mg of standard bovine serum albumin was dissolved in 100 ml of distilled water. Few drops of NaOH was added to it aid complete dissolution of bovine serum albumin and to avoid frothing, it was allowed to stand overnight in a refrigerator.

Procedure

The dissolved proteins in gastric juice were estimated in the alcoholic precipitate obtained by adding 90 % of alcohol with gastric juice in 9: 1 ratio respectively. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken in another test tube. To this 4 ml of alkaline copper reagent was added and kept for 10 min. Then 0.5 ml of phenol reagent was added and again 10 min. was allowed for color development. Reading was taken against blank prepared with distilled water at 640 nm. The protein content was calculated from standard curve prepared with bovine serum albumin and has been expressed in terms of µg/ ml of gastric juice.[22]

RESULTS

***In vitro* antioxidant activity**

Hydroxyl radical scavenging activity

In the present investigation, IC_{50} values (Figure 1) of the plant extracts (acetone, ethanol, water) and standards (BHA, Tannic acid) in this assay were 396.8 ± 2.17 µg/ml, 549.45 ± 1.29 µg/ml, 108.23 ± 2.25 µg/ml, 9.72 ± 1.29 µg/ml and 5.48 ± 0.1 µg/ml respectively. Among the extracts, the water extract appears to have the highest potential for hydroxyl radical scavenging activity indicated by the lowest IC_{50} value (108.23 ± 2.25 µg/ml). This value is comparable with the IC_{50} value of the standard Tannic acid(5.48 ± 0.1 µg/ml). All the solvent extracts exhibited hydroxyl radical scavenging activity (2.53% - 19.60%) at 250 µg concentration in the reaction mixture.

Nitric oxide radical scavenging activity

Nitric oxide radicals are generated from sodium nitroprusside were found to be inhibited by different solvent extracts of *C. pedata* var. *glabra*. In the present investigation, the IC_{50} values (Figure 2) of the plant extracts (acetone, ethanol, water) and standards (BHA, Tannic acid) in this assay were 170.88 ± 0.1 µg/ml, 85.29 ± 1.7 µg/ml, 144.01 ± 0.9 µg/ml, 2.94 ± 0.1 µg/ml and 1.45 ± 0.3 µg/ml respectively. The ethanol extract appears to be more potent nitric oxide scavenger, exhibiting the lowest IC_{50} value of 85.29 ± 1.7 µg/ml. This value is comparable with the IC_{50} value of the standard Tannic acid (1.45 ± 0.3 µg/ml).

Superoxide radical scavenging activity

The results on the effect of different solvent extracts of *C. pedata* var. *glabra* were found to scavenge the superoxide generated by riboflavin photoreduction method are shown in Table 1. The superoxide radical scavenging effect was found to increase with increasing concentration of plant extracts regardless of the nature of preparation.The percentage inhibition of the plant extracts and standards ranged between 4.72 ± 1.8% and 85.44 ± 1.4%.At concentrations 50–250 µg/ml, plant extracts showed a scavenging rate ranging from 9.55% to 63.23% for acetone extract, 8.20% to 23.47% for ethanol extract and 5.31% to 36.76% for water extract.Although both water and methanol extract exhibited a lower superoxide scavenging activity than acetone extract, which is comparable with the percentage value of the standard BHA (34.05% to 85.44%) and tannic acid (4.72% to 31.63%) at concentrations 10–50 µg/ml.

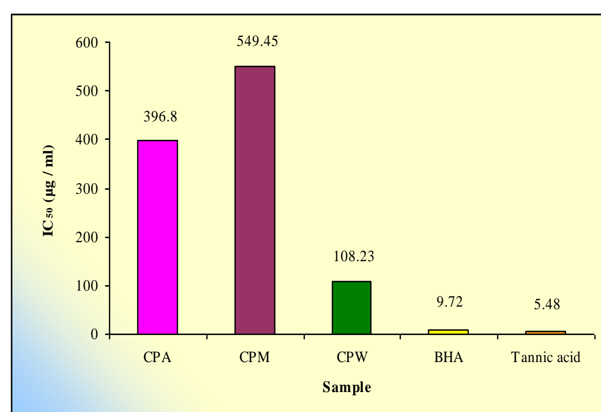

Figure 1: Hydroxyl radical scavenging activity of different solvent extracts of C. pedatavar. Glabra

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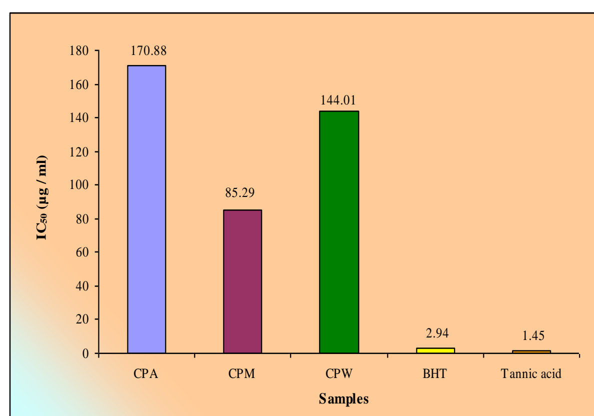

Figure 2: Nitric oxide radical scavenging activity of different solvent extracts of C. pedatavar. Glabra

Values are means of triplicate determinations ± SD
CPA - Acetone extract of*C. pedata* var. *glabra;* **CPE** - Ethanolic extract of *C. pedata* var. *glabra*
CPW - Water extract of *C. pedata* var. *glabra;* **BHA** - Butylatedhydroxynisole

β-carotene / linoleic acid peroxidation inhibition property

In the β-carotene-linoleic acid coupled oxidation model system, the linoleic acid free radical (LOO•) formed attacks the highly unsaturated β-carotene molecules and in the absence of an antioxidant rapidly bleaches the typically orange colour of β-carotene which is monitored spectrophotometrically at 420 nm.

Table 1: Superoxide radical scavenging activity of different solvent extracts of *C. pedata*var.*glabra*

S. No.	Extraction medium	Concentration (µg/ml)	% activity #
1.	CPA	50	09.55 ± 1.1
		100	14.16 ± 3.2
		150	39. 60 ± 2.7
		200	44.34 ± 0.4
		250	63.23 ± 3.2
2.	CPE	50	08.20 ± 0.9
		100	13.33 ± 0.5
		150	14.60 ± 1.0
		200	19.51 ± 0.1
		250	23.47 ± 0.3
3.	CPW	50	05.31 ± 1.1
		100	11.80 ± 0.4
		150	15.25 ± 0.7
		200	17.54 ± 0.9
		250	36.76 ± 0.1
4.	BHT	10	34.05 ± 0.6
		20	56.86 ± 0.1
		30	81.22 ± 2.1
		40	84.34 ± 0.3
		50	85.44 ± 1.4
5.	Tannic acid	10	04.72 ± 1.8
		20	14.56 ± 0.6
		30	20.20 ± 1.6
		40	26.81 ± 2.1
		50	31.63 ± 0.6

Values are means of triplicate determinations ± Standard Deviation; **CPA** - Acetone extractof*C. pedata* var. *glabra;* **CPE** - Ethanolic extractof*C. pedata* var. *glabra;* **CPW** - Water extract of*C. pedata* var. *glabra;* **BHT** -Butylatedhydroxytolune

The antioxidant activities of the extracts of acetone, ethanol, water and standards of BHT, tannic acid after 180 minutes reaction time were 84.42 ± 0.66%, 66.19 ± 1.90%, 84.88 ± 1.77%, 91.37 ± 2.20% and 94.11 ± 1.50% respectively (Table 2). In this study, the order of antioxidant activity towards β-carotene oxidation was Tannic acid > BHT > water > acetone > ethanol. The water extract displayed the highest inhibition effect (84.88 ± 1.77%). Tannic acid (94.11 ± 1.50%) and BHT (91.37 ± 2.20%) registered higher peroxidation inhibition than the sample extracts. All the extracts studied, displayed moderate potential of quenching linoleate free radicals (generated from linoleic acid peroxidation) and shielding of the carotenoid from bleaching.

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Table 2: β-carotene / linoleic acid peroxidation inhibition property of different solvent extracts of *C. pedata* var.*glabra*

S. No.	Sample	Percentage inhibition #
1	CPA	84.42 ± 0.66
2	CPE	66.19 ± 1.90
3	CPW	84.88 ± 1.77
4	BHT	91.37 ± 2.20
5	Tannic acid	94.11± 1.50

Values are means of triplicate determinations ± Standard Deviation

CPA - Acetone extract of *C. pedata* var. *glabra*

CPE -Ethanolic extractof*C. pedata* var. *glabra*

CPW - Water extract of*C. pedata* var. *glabra*

BHT -Butylatedhydroxytolune

Phosphomolybdenum assay

Total antioxidant capacity of plant extracts, expressed as equivalents of ascorbic acid (µg/ml of extract) is depicted in Table 3. The antioxidant capacity of *C. pedata* var. *glabra* plant extracts was found to decrease in the order acetone extract < ethanol extract <water extract. Among the different extracts, acetone extract exhibited the maximum antioxidant activity followed by ethanol extract. The lower antioxidant activity was seen in water extract (86.9 ± 1.50 mg/g).

Table 3: Phosphomolybdenum assay of different solvent extracts of *C. pedata* var.*glabra*

S. No.	Extraction medium	AEAC (mg AAE / g extract)* #
1	Acetone	405.3 ± 2.60
2	Ethanol	230.2 ± 3.00
3	Water	086.9 ± 1.50

Values are means of triplicate determinations ± Standard Deviation

* Ascorbic acid equivalent antioxidant capacity (mg equivalent of ascorbic acid / g extract) through the formation of phosphomolybdenum complex

***In vivo* acute toxicity studies**

Assessment of the behaviour of animals (Wistar albino rats) was carried out by general observations of each animal immediately after the administration of the drug at hourly basis (0 hr, 1 hr, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 24 hrs and 72 hrs). Any change or abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behaviour before and after the administration of an oral dose of ethanolic extract of *C. pedata* var. *glabra* (Table 4).

Table 4: Toxicological evaluation of ethanolic extract of *C. pedata* var.*glabra* on acute toxicity test in Wistar albino rats

S. No.	Response	Animals	
		Before treatment	After treatment
1	Alertness	Normal	Normal
2	Grooming	Absent	Absent
3	Restlessness	Absent	Absent
4	Touch response	Absent	Absent
5	Torch response	Normal	Normal
6	Pain response	Normal	Normal
7	Tremors	Absent	Absent
8	Convulsion	Absent	Absent
9	Righting reflex	Normal	Normal
10	Gripping strength	Normal	Normal
11	Pinna reflux	Present	Present
12	Corneal reflux	Present	Present
13	Writhing	Absent	Absent
14	Pupils	Normal	Normal
15	Urination	Normal	Normal
16	Salivation	Normal	Normal
17	Skin colour	Normal	Normal
18	Lacrimation	Normal	Normal

Ethanol induced ulcer model

The anti ulcerogenic effect of the extracts on ethanol induced gastric ulcer is presented in Table 5. In the ethanol induced ulcer model, administration of ethanol produced haemorrhagic gastric lesions in the gastric mucosa of the control group. It was observed that the treatment with ethanolic extract of *C. pedata* var. *glabra* at 400 mg/kg significantly ($p<0.05$) reduced at higher doses (100, 200 and 400 mg/kg bw.). The test extract showed gastroprotection in a dose-dependent manner and 73.26% protection at 400 mg/kg dose level (Plate 1).

The level of gastric volume, free acidity, total acidity and protein content was increased in the Group-II (ethanol treated) when compared to standard drug omeprazole and plant extracts at different concentrations (100 mg/kg, 200 mg/kg and 400 mg/kg). *C. pedata* var. *glabra* ethanolic extraction in400 mg/kg treated group shown a decrease in the level of free acidity which is more or less equal to that of standard omeprazole. Decreased level of gastric volume was observed in 400 mg/kg plant extract treated group. The level of pH was decreased in the Group-II (ethanol treated) when compared to the standard drug omeprazole and plant extracts at different concentrations (100 mg/kg, 200 mg/kg and 400 mg/kg) (Table 6).

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Table 5: Effect of ethanolic extracts of *C. pedata*var.*glabra* against ethanol induced gastric ulcer in Wistar albino rats

S.No.	Treatment	Dose (mg/kg b.w.)	Ulcer Index	Protection (%) #
1	Control	-	-	-
2	Ethanol	1m1/kg	30.9 ± 2.83[a]	-
3	Omeprazole + ET	10 mg/kg + 1m1/kg	6.44 ± 0.95[d]	79.15
4	CPE + ET	100 mg/kg + 1m1/kg	21.26 ± 2.24[b]	31.19
5	CPE + ET	200 mg/kg + 1m1/kg	14.95 ± 1.80[c]	51.61
6	CPE + ET	400 mg/kg + 1m1/kg	8.26 ± 0.89[d]	73.26

Values are means of independent analysis ± Standard Deviation (n=5)

Mean values within a column with no common superscript differ significantly (p<0.05)

ET – Ethanol; **CPE** - Ethanolic extract of *C. pedata* var. *glabra*

Table 6: Effect of ethanolic extract of *C. pedata* var.*glabra* against indomethacin induced gastric ulcer in Wistar albino rats – Biochemical parameters

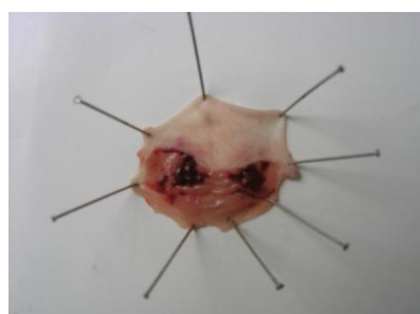
S.No.	Treatment	Dose (mg/kg b.w.)	Gastric Volume (ml/100g)	pH	Free acidity (mEq/dl)	Total acidity (mEq/dl)	Total protein (µg/ml)
1	Control	-	4.266 ± 0.25	1.25± 0.16	3.458± 0.39	4.506 ± 0.02	310.70 ± 7.01
2	Ethanol	1m1/kg	8.032± 1.38	3.17 ±0.12	7.621± 0.24	8.359 ± 0.23	475.11 ± 5.49
3	Omeprazole + ET	10 mg/kg + 1m1/kg	3.640 ± 0.17	4.14 ± 0.17	4.069±0.14	5.121 ± 0.13	255.47 ± 5.75
4	CPE + ET	100 mg/kg + 1m1/kg	7.335± 0.21	3.36 ± 0.10	6.598±0.11	7.028 ±0.35	388.64 ±5.81
5	CPE + ET	200 mg/kg + 1m1/kg	6.822 ± 0.16	3.18± 0.17	5.594±0.18	6.671±0.37	334.45±4.89
6	CPE + ET	400 mg/kg + 1m1/kg	4.649 ± 0.22	3.55±0.48	4.122± 0.31	5.46 ± 0.20	320.21 ± 7.46

Values are means of independent analysis ± Standard Deviation (n=5) **CPE** - Ethanolic extractof*C. pedata* var. *glabra;* **ET** – Ethanol

Plate 1: Effect of ethanolic extract of *C. pedata*var.*glabra* against ethanol induced gastric ulcer in Wistar albino rats

Stomach of control group

Stomach of rats treated with ethanol (1 ml/kg p.o.)



Stomach of rats treated with ethanol and standard drug omeprazole (10 mg/kg p.o.)

Stomach of rats treated with ethanol and test drug at the rate of 100 mg/kg p.o.

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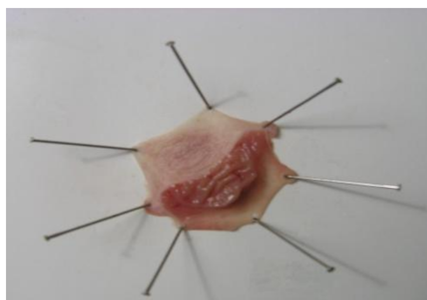
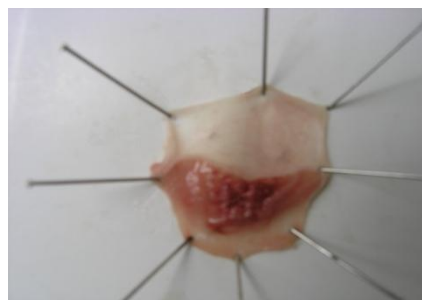
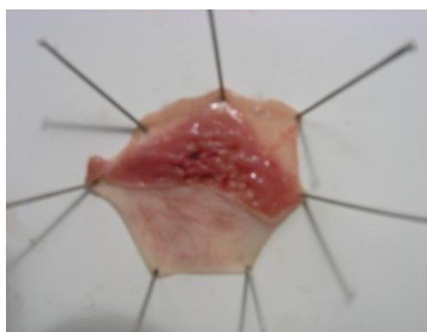
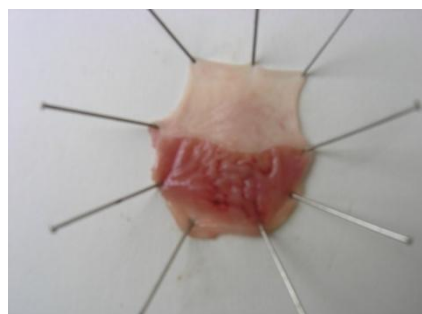


Stomach of rats treated with ethanol and test drug at the rate of 200 mg/kg p.o.



Stomach of rats treated with ethanol and test drug at the rate of 400 mg/ kg p.o.





DISCUSSION

In hydroxyl radical scavenging activity, the water extract appears to have the highest potential for scavenging hydroxyl radical indicated by the lowest IC_{50} value (108.23 ± 2.25 µg/ml). This value is comparable with the IC_{50} value of the standard Tannic acid (5.48 ± 0.1 µg/ml). Hydroxyl radical scavenging activity was quantified by measuring inhibition of the degradation of the deoxyribose by free radicals.[23] Hydroxyl radical scavenging activity was assessed by generating the hydroxyl radicals using ascorbic acid - iron EDTA. The hydroxyl radicals formed by the oxidation, reacts with dimethyl sulfoxide to yield formaldehyde, which provide a convenient method to detect hydroxyl radicals by treatment with Nash reagent.[24]

The ethanol extract appears to be more potent nitric oxide scavenger, exhibiting the lowest IC_{50} value of 85.29 ± 1.7 µg/ml. This value is comparable with the IC_{50} value of the standard Tannic acid (1.45 ± 0.3 µg/ml). The large quantity of nitric oxide produced in response to mutagens plays an important role in end toxemia and inflammatory conditions.[25]Excessive amount of nitric oxide has been implicated in the pathogenesis of many diseases like septic shock, chronic degenerative diseases and rheumatoid arthritis. [26]

At concentrations 50–250 µg/ml, plant extracts showed a superoxide radical scavenging rate ranging from 9.55% to 63.23% for acetone extract, 8.20% to 23.47% for ethanol extract and 5.31% to 36.76% for water extract. The superoxide is biologically important radical since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals. It is very harmful to the cellular components in a biological system.[27, 28]All the extracts studied in β-carotene assay, displayed moderate potential of quenching linoleate free radicals (generated from linoleic acid peroxidation) and shielding of the carotenoid from bleaching. The bleaching of β-carotene could be inhibited by antioxidants, which are capable of reducing the rate of chain reaction initiated during lipid peroxidation and transforming the reactive end product to a more stable form.[29] The extracts reduced the extent of β-carotene bleaching by neutralising the linoleate - free radical and other free radicals formed in the system.[30]Among the different extracts studied in phosphomolyptenum assay, acetone extract exhibited the maximum antioxidant activity followed by ethanol extract. This assay is based on the reduction of Mo (VI) to Mo (V) in presence of the antioxidant compounds and the subsequent formation of a green phosphate / Mo (V) complex at acidic pH, which is measured at 695 nm.

Ulcers are an open sore of the skin or mucus fluid layer portrayed by sloughing of aggravated dead tissue. There are numerous sorts of ulcer, for example, mouth ulcer, throat ulcer, peptic ulcer and genital ulcer. Of these peptic ulcer is seen among numerous individuals. Gastric ulcers are situated in the stomach, portrayed by agony; ulcers are basic in more seasoned age bunch.[31] It was observed that the treatment with ethanolic extract of *C. pedata* var. *glabra* at 400 mg/kg significantly ($p<0.05$) reduced ulceration at higher doses (100, 200 and 400 mg/kg bw.). The test extract showed gastroprotection in a dose-dependent manner and 73.26% protection at 400 mg/kg dose level.

The level of gastric volume, free acidity, total acidity and protein content was increased in the Group-II (ethanol treated) when compared to standard drug omeprazole

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and plant extracts at different concentrations (100 mg/kg, 200 mg/kg and 400 mg/kg). *C. pedata* var. *glabra* ethanolic extraction in400 mg/kg treated group shown a decrease in the level of free acidity which is more or less equal to that of standard omeprazole. In similar lines, phytosterols which have been shown various properties that is necessary for protection against ulcer induction. Since flavonoids and sterols shown to be present in the, chloroform extract of *Cayratia pedata* Lam. these constituents may be responsible for the anti-ulcer activity.[32]

CONCLUSION

On the basis of the results of this study, it clearly indicates that *C. pedata* var. *glabra*aerial part extracts had powerful *in vitro* antioxidant capacity against various antioxidant systems as hydroxyl, nitric oxide, superoxide, β-carotene/linoleic acid peroxidation inhibition and phosphomolybdenum scavenger. From our results, the antioxidant activity of *C. pedata* var. *glabra* exhibit concentration dependent activity. The extracts could exhibit antioxidant properties approximately comparable to commercial synthetic antioxidants as BHT and Tannic acid. The result of the study points indicates that the ethanol extract of *C. pedata* var. *glabra* contains maximum phytochemical agents with antioxidant and free radical scavenging properties. The results of estimation of total phenolic and total flavonoid content points to the fact that the antioxidant and scavenging activities may be attributed to the presence of these secondary metabolites. On the basis of the results of this study, it clearly indicates that *C. pedata* var. *glabra* had powerful *in vitro* antioxidant capacity. From our results obtained, it can be concluded that this plant extract have a potential to be used as an anti-ulcer drug in combination with other drugs or alone. Though the mechanism of the anti-ulcer action of the plant extract remains to be studied in detail. In order to use the aerial parts of *C. pedata* var. *glabra* for its potential anti-oxidant and anti-ulcer activity, further studies like isolation, identification, chemical characterization and *in vivo* studies of these compounds may be carried in future.

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