Biomedical & Pharmacology Journal, March 2025. Vol. **18**(1), p. 957-963

Antioxidant and Anti-inflammatory Effects of *Cayratia auriculata* (Roxb): A Traditional South Indian Medicinal Plant

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https://dx.doi.org/10.13005/bpj/3144

(Received: 04 October 2024; accepted: 11 February 2025)

The phytochemical components of most medicinal plants contain diverse pharmacological properties like antioxidant, anti-inflammatory to protect it from environmental stress and insects. In the recent years many studies are conducted to identify the therapeutic effect of such plant extracts as they can be used for treating many degenerative diseases. Cayratia auriculata(Roxb) is a local south Indian species with many medicinal properties. Hence it becomes essential to identify its antioxidant, anti-inflammatory properties. Aim: To assess the anti-inflammatory and anti-oxidant effects of C.auriculata. C.auriculata was collected in November 2018 from the Araku valley, Visakhapatnam, Andhra Pradesh, India. Preliminary phytochemical qualitative and quantitative analysis was carried out. Then in vitro DPPH radical scavenging activity, BSA Denaturation procedures were conducted to assess its anti-oxidant and anti-inflammatory actions. Antioxidant Property was assessed based on the DPPH absorbance reduction indicating the free radicals scavenging action of the extract. IC50 value was obtained and calculated as 1386.207μg/mL. Anti-inflammatory property was assessed based on 1% Bovine serum albumin assay results which showed different concentrations exhibit different level of inhibition. IC50 Value was calculated and observed to be 12.564μg/mL. The antioxidant and anti-inflammatory property of ethanolic leaf extract of C. auriculata can serve as a reliable pharmacological therapy for many degenerative disorders caused due to chronic cellular stress and inflammations. Alternative herbal medicines from traditional sources have the potential to be an effective, affordable, and accessible remedy.

Keywords: Anti-inflammation; Anti-oxidants; Cayratia auriculata (Roxb); Herbal medicine; In vitro; Phytochemical analysis.

The disruption in the free radical formation and the detoxification process of our biological system is mainly due to oxidative stress.[1,2] The underlying cause for many medical ailments is the enzymatic and non-enzymatic reactions happening inside the cells due to the production of free radicals.[3] However these cells survive these injuries with the help of antioxidants.[4]

With this base, we have evaluated the anti-oxidant and anti-inflammatory property of ethanol extract of *C.auriculata*. 1,1-Diphenyl–2picryl hydrazyl, and hydroxyl radicals were used to investigate the free radical scavenging property in dose dependent manner of the ethanolic extract.[5] Some of the antioxidants that aid in free radical scavenging are phenolic acids, polyphenols and flavonoids.[6] The most potential host defence mechanism is inflammation which helps to combat the tissue damage, infection and irritation which are due to the release of enzymes during tissue damage and repair.[7] Few plants and herbs are found to retain such anti-inflammatory properties along with their antioxidant actions.[8]

C.auriculata is a local south Indian species. It is a climber commonly seen in dry evergreen to dry deciduous woods of Andhra Pradesh[9, 10], Tamilnadu[11], also seen in areas of Maharashtra[12], Madhya Pradesh[13], Orissa[14], Gujarat, Goa, Karnataka, Kerala, Bihar, West Bengal, Bangladesh, Myanmar and Rajasthan[15]. Ethno medicinal study on *C.auriculata* shows that this plant is perhaps the most ordinarily utilized in old stories medication, for cardiovascular problems, as blood purifier, in intestinal worm diseases[16], wound boil, ear infection as tonic[17], dog bites, as astringent, hydrocele, tumors, cold and hack. In veterinary medication it is utilized to treat animals' diarrhoea and blood dysentery[18]. Our study is the first of its kind to assess the antioxidant and anti-inflammatory property of *C.auriculata*

Objective

To assess the anti-inflammatory and anti-oxidant effects of *Cayratia auriculata* (Roxb)

MATERIALS AND METHODS

Collection of plant and Authentication *Cayratia auriculata*

C.auriculata was collected in November 2018 from the Araku valley in Eastern Ghats of Visakhapatnam, Andhra Pradesh, India. The taxonomic identification of *C.auriculata* was confirmed by Dr.S.B.Padal, Botany Professor from the University of Andhra Pradesh. Plant herbarium was arranged and kept at Botany Department Herbarium, Andhra University, India (Herbarium no.AUB.D.H–22228). Undesirable residue molecule from new plant material was taken out by washing under running faucet water and with refined water at that point conceal dried for 14 days. The shade dried plant material was then mixed to a fine powder, put away in impermeable compartments at 4 æ%C until additional utilized.

Preparation of extracts from *Cayratia auriculata*

The fresh leaves of *C.auriculata* were washed, dried and powdered. Then 75 g of the powder (75g/250ml) was used to prepare the extract using the device soxhlet extractor for 10-12 hours. The extract obtained was then concentrated and dried. Dried extracts are stored in 20°C and used whenever needed.[19]

Phytochemical analysis

The qualitative preliminary phytochemical analysis of the ethanolic extract of *C.auriculata* was done to assess the presence of phytochemicals like phenols, flavonoids, steroids, terpenoids, glycosides following the methods used in the study conducted by Sofowora and team.[20] Later quantitative analysis to assess the presence of phenol and flavonoids were performed using the Folin -Ciocalteau Assay and Aluminium chloride colorimetric assay.

Total Phenolic Determination

Folin-Ciocalteu Assay was used to assess the presence of total phenolic content of ethanolic extract of *C.auriculata*. 20 µl of the extract was added to the reagent. Later the solution was mixed well and incubated for 40mins in dark and spectrophotometrically read at 725nm. Gallic acid calibration curve was plotted and the total phenolic content was expressed in mg of gallic acid equivalent (mg GAE/g extract).[12]

Total Flavonoid Estimation

Total flavonoid content determination was done as per the method (Aluminium chloride colorimetric assay) followed by researchers like Senguttuvan.[21] 1 mL plant extract was diluted in 200µL distilled water and 150µL of sodium nitrite (5%) was added. The mixture was incubated for 5mins and 150µL of aluminium chloride (10%) was added to it. After 6mins 2ml sodium hydroxide (4%) was added. Then distilled water was added to make the composition 5mL. Rutin was the standard. After shaking the mixture, it was kept for 15mins at room temperature. Absorbance measured was found to be 510nm. The pink colour obtained confirmed the presence of flavonoids. Later using the standard curve, the total flavonoid content was

expressed in mg of rutin equivalent (mg RE/g extract) on a dry weight basis.

Antioxidant property determination

The antioxidant activity of ethanolic extract of *C.auriculata* was evaluated by the standard DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.[22] 0.1mM of DPPH was prepared in methanol. 200il of sample solution in various concentrations (100, 200, 300, 400 and 500ig/mL) was added to 800il of the DPPH solution. 100ìg/mL of butylated hydroxytoluene was selected as standard. After vigorously shaking the mixture wait for 30mins at room temperature. Using a spectrophotometer the absorbance was noted at 517nm.

The DPPH scavenging effect was assessed using the formula:

$$\text{Inhibition \%} = ((A0 - A1)/A0) \times 100$$

Where A0=Absorbance of control; A1=Absorbance in presence of test or standard.[23]

Anti-inflammatory activity estimation

The anti-inflammatory activity of ethanolic extract of *C.auriculata* was assessed using the albumin denaturation inhibition method.[24] Various concentrations of leaf extracts (10, 25, 50, 75 & 100 ìg/mL) were taken in separate test tubes. 1% bovine serum albumin was added to the test tubes. 1N Hcl was added to make the pH 6.3. After incubating the mixture for 10-15mins, it was heated for 20mins. Then the solution was cooled at room temperature and the absorbance was noted at 700nm. Acetyl salicylic acid was taken as a positive control.

The anti-inflammatory action was assessed using the formula:

$$\text{Inhibition \%} = ((A1 - A2)/A2) * 100),$$

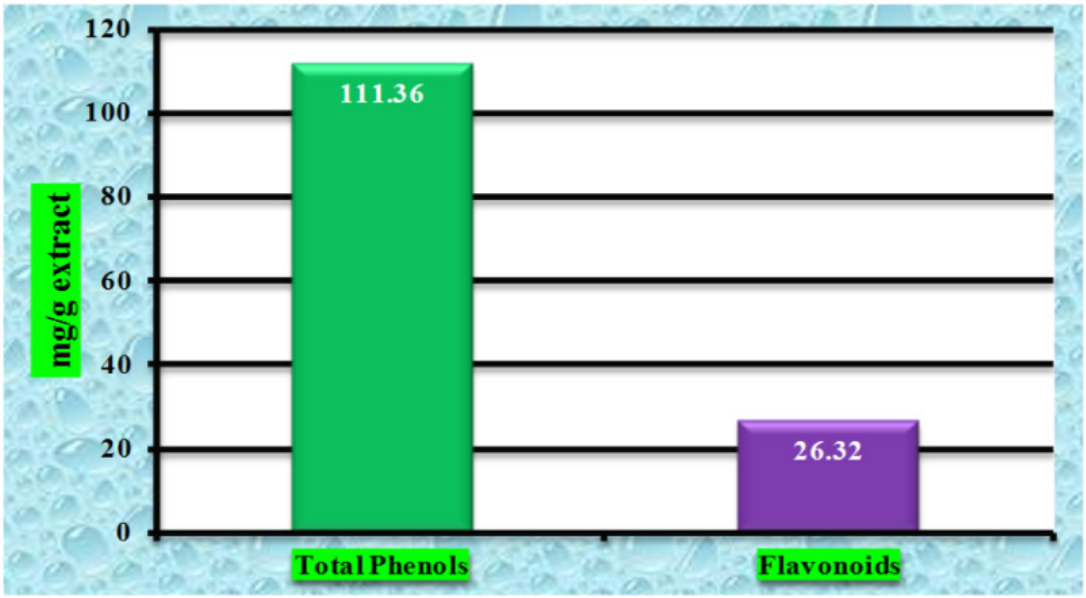
Where A1=Absorbance of sample; A2=Absorbance of control

Statistical Analysis

Experiment was done in triplicates manner. Results were expressed as Mean±Standard Deviation.

RESULTS

Preliminary Qualitative phytochemical analysis identified the presence (+) of phytoconstituents such as alkaloids, flavonoids, phenols, coumarins, saponins, tannins, terpenoids, steroids and cardiac glycosides. Absence (-) of anthroquinones was reported as shown in Table 1. Quantitative analysis for the total phenol and total flavonoid content revealed the concentrations of



Graph 1. Determination of total phenolic and flavonoid content

phenols and flavonoids in *C.auriculata* extract as shown in Graph 1.

Following this antioxidant, anti-inflammatory effects were tested for ethanolic extract of *C.auriculata* leaves. Results revealed that extract exert a significant effect in a dose dependent pattern. Table 2 Depicts the DPPH (1-Diphenyl-2-picrylhydraxyl) assay of ethanolic extract of *C.auriculata*. It is used to assess the capability of the extract to scavenge/neutralize free radicals. Ethanolic extracts of *C.auriculata* with various concentrations (100 to 500µg/mL) was observed to render different level of radical scavenging ability. The percentage of inhibition is directly proportional to the concentration of *C. auriculata* (Roxb). From this, IC50 value was obtained and calculated as 1386.207µg/mL. Table: 3 Shows the assessment of in-vitro anti-inflammatory activity of ethanolic extracts of *C.auriculata* with various concentrations (10, 25, 50, 75, and 100 µg/mL). 1% bovine serum albumin was added and the percentage inhibition for albumin denaturation was used to assess the anti-inflammatory property of extracts. Results obtained show that different concentration exhibit different level of inhibition. Acetyl salicylic acid was used as the standard. From this, the IC50 Value was calculated and observed to be 12.564 µg/mL.[25]

Table 1. Preliminary qualitative phytochemical analysis of ethanolic extract of *C. auriculata*

Sl. No	Parameter	Result
1	Alkaloids	+
2	Anthraquinones	-
3	Flavonoids	+
4	Phenols	+
5	Coumarins	+
6	Saponins	+
7	Tannins	+
8	Terpenoids	+
9	Steroids	+
10	Cardiac Glycosides	+

DISCUSSION

Preliminary phytochemical investigations of ethanolic extract of *C.auriculata* was carried out to analyse the components of the extract. Alkaloids, flavonoids, phenols, terpenoids, steroids, and cardiac glycosides were identified as phytoconstituents; anthraquinones were not detected. Similar to our study, the findings of a study conducted in 2021 by Balakrishnan and colleagues demonstrate that these phytochemicals prevent oxidative damage by reducing oxidative stress, increasing glutathione, preventing lipid peroxidation, modifying the release of inflammatory cytokines, controlling the

Table 2. DPPH Free Radical Scavenging (Antioxidant) Activity of ethanolic leaf extract of *C.auriculata*. Values expressed in Mean±SD

Concentration (µg/mL)	Mean±SD, Absorbance 510nm	% Inhibition
100	0.621±0.003	1.74
200	0.601±0.003	4.91
300	0.547±0.001	13.45
400	0.518±0.002	18.04
500	0.425±0.004	32.75
Standard (BHT)	0.832±0.005	92.36
Control	0.632±0.003	-

Table 3. Anti-Inflammatory Activity of Ethanolic Extract of *C.auriculata*: BSA Denaturation Study. Values expressed in Mean±SD

Concentration (µg/mL)	Mean±SD, Absorbance 700nm	% Inhibition
10	0.440±0.003	33.333
25	0.210±0.002	68.182
50	0.140±0.003	78.788
75	0.070±0.001	89.394
100	0.070±0.002	89.394
Standard (Acetyl salicylic acid)	0.179±0.003	72.879
Blank/ control		0.000

expression of inflammatory mediators, and their related signalling pathways.[26]

Apart from qualitative analysis of phytochemicals, quantitative assay of the most vital phytochemicals such as phenols and flavonoids were executed in the present study. Results suggest the significant presence of both phenols and flavonoids in sufficient amounts in the ethanolic extract of *C.auriculata.*[27, 28] These polyphenolic compounds are divided into several further classes which include flavonoids, phenol acids, phenolic alcohols, tannins and lignans. Polyphenols have extremely powerful antioxidant properties that are mainly attributed to their free radical scavenging and iron chelating activity. The therapeutic effects of polyphenols include antibacterial, antiviral, antioxidant, anti-inflammatory, neuroprotective and anticancer activities. Flavonoids exhibit various medicinal properties which include antimicrobial, antiinflammatory, antithrombotic, anticancer, and immunomodulatory activities.[29] Flavonoids were found to show neuroprotective effect revealed from the inhibition of various inflammatory factors like nitric oxide, prostaglandin E2, tumor necrosis factor á. Flavonoids also observed to reduce the loss of dopaminergic neurons.[30]

In addition, these plant based drugs are multi-targeted and hence can manage the main pathological condition and also aid in the maintenance of system homeostasis. While the synthetic drugs are single targeted, hence using synthetic drugs may not be effective in the regulation of system homeostasis. Further, it is reported that the neurotransmission network is a highly complex process and using single target drugs are found to be not effective which is suggesting the need for multitarget therapy to regulate the molecular processes in CNS related disorders. Similarly Kennedy and Wightman, 2011 stated that herbal compounds help us to avoid the various limitations of synthetic drugs by targeting multiple features which includes neuronal communication modulation or alteration of neurotransmitter synthesis.[31]

Using antioxidant supplements in pathophysiological conditions is primarily intended to preserve cell homeostasis and the related cell signalling, which will ultimately lead to the restoration of normal physiological processes by controlling cell proliferation, cell death, necrosis, autophagy, metabolic response, gene expression, etc.[32-34] Therefore, the DPPH radical scavenging assay was used to assess the antioxidant efficacy of *C. auriculata.* It was found to be differentially effective in inhibiting DPPH radical upon incubation with varying concentrations, indicating that it can be used as an antioxidant supplement to maintain oxidative stress. BSA Denaturation Study was done to assess the anti-inflammatory activity of ethanolic extracts of *C.auriculata*. Research studies stated that pro-inflammatory cytokines and chemokines are involved in the amplification of immune response and thus may be directly involved in many neuro-degeneration diseases.[35]

CONCLUSION

Phytochemical analysis using *in vitro* DPPH radical scavenging activity, and BSA denaturation studies of the ethanolic leaf extract of *C.auriculata* revealed the presence of antioxidant and anti-inflammatory properties. Our study demonstrates that studied extract exhibits these beneficial properties and hence has the potential to serve as an effective pharmacological treatment for various degenerative disorders caused by chronic cellular stress and inflammation. As a result, alternative herbal medicine from traditional sources is increasingly recognized as essential, as these remedies have the potential to be an effective, affordable, and accessible

ACKNOWLEDGEMENT

I thank Sri Ramachandra Institute of Higher Education and Research especially the management, department of Physiology, Pharmacology and the technical assistance for providing the environment for succeeding in my project.

Funding Source

'GATE funding"- Grant approval (Ref 29/ DOF/2020) by Sri Ramachandra Institute of Higher Education and Research

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Authors contribution

Lalitha Surulichamy – Literature survey, Data Collection, Data entry, Data Analysis, Preparing final report; Deepika Chandrasekaran – Literature Survey, Intellectual Assistance, Draft writing; Anusha Dakshinamoorti – Supervision, Intellectual Assistance, Reviewing the manuscript.

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