EVALUATION OF ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF *CAYRATIA TRIFOLIA*

Running Title: Evaluation of properties of *C.trifolia*

ABSTRACT:

Introduction: Cayratia trifolia is a plant belonging to the family Vitaceae and genus Cayratia. Anti-inflammatory activity is the property of a substance or treatment that reduces inflammation or swelling. Antioxidants are defined as substances that delay or inhibit oxidative damage to a target molecule and its principal function is its ability to trap free radicals. The alcoholic leaf extract of *Cayratia trifolia* has anti-inflammatory as well as antioxidant properties against inflammations and swelling and DPPH free radicals respectively and can be evaluated using both in vitro and in vivo assays. The principal aim of the study is to employ in-vitro assays to evaluate the anti-inflammatory and antioxidant properties of *Cayratia trifolia*.

Materials and Methods: Plant extract of *Cayratia trifolia* was purchased and tested for anti-inflammatory and antioxidant properties. In case of evaluating the antioxidant activity, the methanolic extract of the plant was utilised. The examination of the anti-inflammatory properties of *Cayratia trifolia* involves acetyl salicylic acid as a positive control and aspirin and aspirin was employed as a standard anti-inflammatory drug.

Results: The anti-inflammatory activity of Cayratia trifolia extract was examined and it was observed that the plant extract showed an increased percentage of inhibition of trypsin with increasing concentration. Similarly, the antioxidant activity was compared to Vitamin C and the percentage of inhibition of DPPH free radicals increased with the increase in concentration of the extract.

Conclusion: Within the limits of the study, the plant extract of Cayratia trifolia was observed to possess both antioxidant and anti-inflammatory properties.

Keywords: *Cayratia trifolia*; Antioxidant; Anti-inflammatory; Ecofriendly; Innovative technique , Novel method

Comment [A1]: Is it ethanolic extract or methanolic extract? state the appropriate one used

Comment [A2]: Using which methods?

Comment [A3]: Delete repeated words.

Comment [A4]: What are the limits you are referring to because you did not explain the method of how you checked these properties, equipment used, quantity utilized and how many methods did you employ before concluding that the plant extract contains such properties.

INTRODUCTION:

Cayratia trifolia is a plant belonging to the family Vitaceae and genus Cayratia. It is a perennial climber having trifoliate leaves with 2-3 cm long petioles and ovate to oblong-ovate leaflets. It is commonly known as fox-grape or bush- grape (1). It consists of black-coloured berries and its leaves contain several flavonoids, such as cyanidin and delphinidin. Hydrocyanic acid is present in the stem, leaves and roots of the plant (2). Leaves contain stilbenes, piceid, resveratrol, viniferin, ampelopsin (3). This plant has medicinal uses, where the root, ground with black pepper, is applied to boils and is also used as an astringent medicine. The bark extract shows antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activity. Upon preliminary phytochemical screening, the whole plant of Cayratia trifolia was observed to contain steroids, terpenoids, flavonoids & tannins, and yellow waxy oil (4).

Inflammation is the body's first response to infection or injury and is critical for both innate and adaptive immunity. Anti-inflammatory activity is the property of a substance or treatment that reduces inflammation or swelling (5). The main action of anti-inflammatory activity is the inhibition of protein synthesis and pro-cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins. There are two principal types of anti-inflammatory drugs, namely, steroidal anti-inflammatory drugs, which reduce inflammation by binding to cortisol receptors and nonsteroidal anti-inflammatory drugs, which decrease damage by inhibition of cyclooxygenase enzymes (6). Non-steroidal anti-inflammatory drugs generally include aspirin, ibuprofen and naproxen. The different plants or herbs that help reduce inflammation include cucurmin (7), black tea, capsaicin, rosemary, Uncaria tomentosa (8), etc. Antioxidants are defined as substances that delay or inhibit oxidative damage to a target molecule (9). The principal function of antioxidants is its ability to trap free radicals. Free radicals are oxygen-containing molecules that can cause large chain chemical reactions in the body since their reaction with other molecules. These reactions are called oxidation (10). The presence of free radicals can result in central nervous system related diseases like Alzheimer's, and can also cause rheumatoid arthritis, cancer, diabetes, cataract, and genetic degenerative diseases like Huntington's disease. Few powerful antioxidants include alpha-tocopherol which is an active form of vitamin E in humans, and vitamin C (11).

Comment [A5]: Pls check this sentence ,it is not complete

The alcoholic leaf extract of *Cayratia trifolia* has anti-inflammatory as well as antioxidant properties against inflammations and swelling and DPPH free radicals respectively and can be evaluated using both in vitro and in vivo assays. Our team has extensive knowledge and research experience that has translate into high quality publications(12),(13),(14),(15),(16),(17), (18), (19), (20),(21),(22),(23),(24),(25),(26), (27),(28),(29),(30),(31) The principal aim of the study is to employ in-vitro assays to evaluate the anti-inflammatory and antioxidant properties of *Cayratia trifolia*.

Comment [A6]: Ethanolic or methanolic

Comment [A7]: For purposes like this I would have suggested notable and most recent publications be cited.to support what you are saying and not just highlighting all of these references

MATERIALS & METHODS:

Plant extract of *Cayratia trifolia* was purchased and tested for anti-inflammatory and antioxidant properties.

Comment [A8]: You bought the extract? or did you prepare the extract, how did you prepare it.

***In Vitro* Anti-inflammatory activity:**

Protease inhibition assay

Inhibition of trypsin was evaluated by the method of Oyedepo and Femurewas (1965) and Sakat et al. (2010). 100 µL of bovine serum albumin was added to 100 µl of plant extracts (0.1 to 0.5mg/ml) with an increase in concentrations (100-500µg/ml). This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 250 µl of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Acetyl salicylic acid was used as a positive control. The experiment was carried out in triplicates and percent inhibition of protease inhibition was calculated. In this study, Aspirin was used as a standard anti-inflammatory drug.

Calculation: $\%\ \text{Inhibition} = 100 - ((A_1 - A_2)/A_0) * 100)$

***In Vitro* Antioxidant activity:**

DPPH radical assay

The DPPH free radical scavenging assay was performed by Liyana Pathirana and Shahidi method [Kikuzaki and Nakatan, 1993]. 200 µL of 0.1 mM DPPH prepared in methanol was

added to 100 µL of the plant extract with an increase in concentration (100-500µg/ml). The resulting mixture was incubated at room temperature in the dark for 15 minutes. Absorbance was observed at 517 nm. BHT was taken as a positive control. The experiment was carried out in triplicates and percentage inhibition of the DPPH radical scavenging activity was calculated.

Calculation: % Inhibition=((A_0-A_1)/A0)*100,

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Statistical analysis:

The data were analyzed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p<0.05$.

RESULTS:

The results obtained from the study were plotted in the form of graphs. Figure 1 refers to the graph plotted to compare the anti-inflammatory activity between aspirin and *C.trifolia* using the protein denaturation inhibition assay. The antioxidant activity between *C.trifolia* and vitamin C is represented by figure 2.

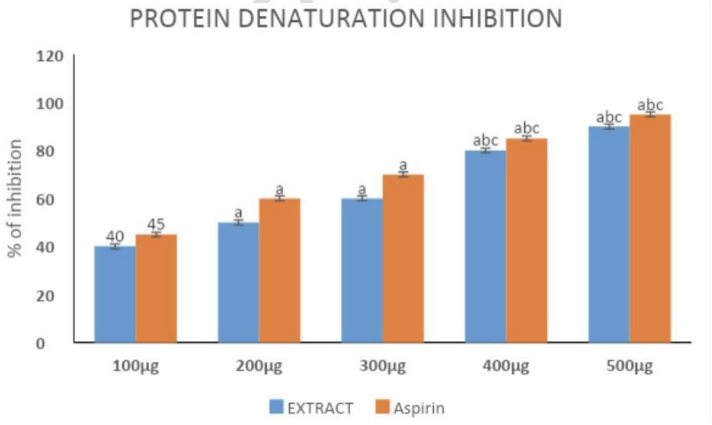

Figure 1: The graph depicts the anti-inflammatory activity of *Cayratia trifolia*. Each bar represents the mean ± SD of 6 observations. Significance at the levels of $p<0.05$. a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.

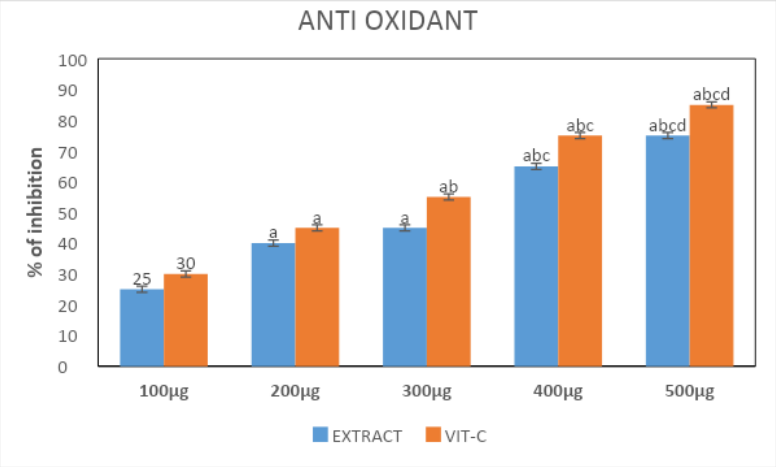

Figure 2: The bar chart represents the antioxidant activity of Cayratia trifolia extract. Each bar represents the mean ± SD of 6 observations. Significance at the levels of $p<0.05$. a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.

DISCUSSION:

The anti-inflammatory activity of Cayratia trifolia extract was examined and it was observed that the plant extract showed an increased percentage of inhibition of trypsin with increasing concentration. Similarly, the antioxidant activity was compared to Vitamin C and the percentage of inhibition of DPPH free radicals increased with the increase in concentration of the extract.

The anti-inflammatory property of aspirin was comparatively higher than that of C. trifolia due to the increased inhibition of proteins. According to previous studies, it was proved that aspirin

was generally used to reduce inflammation, and also possessed analgesic properties (32). Aspirin blocks the production of prostaglandins, which is considered as an important mediator of inflammation (33).

The plant extract of Cayratia trifolia exhibited anti-inflammatory properties, but showed slightly less activity than aspirin, which is also known as acetyl salicylic acid. Previous research has indicated that the methanolic leaf extract of Cayratia trifolia was observed to have anti-ulcer properties (34). Hence, C.trifolia exhibited anti-inflammatory properties by inhibiting the synthesis of trypsin.

Vitamin C is known to possess a potent antioxidant property by trapping free radicals and by destroying them. Vitamin C, otherwise known as ascorbic acid, acts as an antioxidant and a pro-oxidant in-vitro and also provides protection against oxidative stress-induced cellular damage, by scavenging free radical species. The plant extract of Cayratia trifolia has antioxidant activity against DPPH free radicals and scavenges them through the DPPH free radical scavenging assay. Preceding works of various authors proved that the ethanolic extract of Cayratia trifolia contains natural sources of antioxidants and possessed good free radical scavenging activity .

The limitations of this study was that it was carried out only as an in-vitro study. In-vivo studies can also be carried out for obtaining better results in the future.

The future scope of this study based on Cayratia trifolia is that it can be used as a natural remedy for treatment of inflammations and ulcers or swellings. The possession of antioxidant property by the plant extract can also be used for treating multiple diseases like diabetes.

Comment [A9]: You can not make such a statement here because you did not work on diabetic cases in this work to making this strong assertion. So pls remove

CONCLUSION:

Within the limits of the study, the plant extract of Cayratia trifolia was observed to possess both antioxidant and anti-inflammatory properties. Though its activity was not as effective as the standard drug used for comparison, it can be used as a biological alternative for treating inflammations, cancer or heart diseases.

Comment [A10]: How? Especially cancer and heart disease you mention.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES:

1. Kumar D, Kumar S, Gupta J, Arya R, Gupta A. A review on chemical and biological properties of Cayratia trifolia Linn. (Vitaceae). Pharmacogn Rev. 2011 Jul;5(10):184–8.

2. Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta A. Pharmacognostic evaluation of Cayratia trifolia (Linn.) leaf. Asian Pac J Trop Biomed. 2012 Jan;2(1):6–10.

3. Perumal PC, Sophia D, Raj CA, Ragavendran P, Starlin T, Gopalakrishnan VK. In vitro antioxidant activities and HPTLC analysis of ethanolic extract of Cayratia trifolia (L.) [Internet]. Vol. 2, Asian Pacific Journal of Tropical Disease. 2012. p. S952–6. Available from: http://dx.doi.org/10.1016/s2222-1808(12)60299-0

4. Meganathan B, Palanisamy CP, Panagal M. Antioxidant, antimicrobial and cytotoxicity potential of n-hexane extract of Cayratia trifolia L. Bioinformation. 2021 Mar 31;17(3):452–9.

5. Azab A, Nassar A, Azab AN. Anti-Inflammatory Activity of Natural Products. Molecules [Internet]. 2016 Oct 1;21(10). Available from: http://dx.doi.org/10.3390/molecules21101321

6. Vane J, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs [Internet]. Vol. 1, The FASEB Journal. 1987. p. 89–96. Available from: http://dx.doi.org/10.1096/fasebj.1.2.3111928

7. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa). J Altern Complement Med. 2003 Feb;9(1):161–8.

8. Aguilar JL, Rojas P, Marcelo A, Plaza A, Bauer R, Reininger E, et al. Anti-inflammatory activity of two different extracts of Uncaria tomentosa (Rubiaceae). J Ethnopharmacol. 2002 Jul;81(2):271–6.

9. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed. 2012 Dec;2(12):960–5.

10. Khan MA, Rahman AA, Islam S, Khandokhar P, Parvin S, Islam MB, et al. A comparative study on the antioxidant activity of methanolic extracts from different parts of Morus alba

L. (Moraceae) [Internet]. Vol. 6, BMC Research Notes. 2013. p. 24. Available from: http://dx.doi.org/10.1186/1756-0500-6-24

11. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J-H, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr. 2003 Feb;22(1):18–35.

12. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3297–305.

13. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). J Photochem Photobiol B. 2019 Aug;197:111518.

14. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, Journal of Photochemistry and Photobiology B: Biology. 2020. p. 111773. Available from: http://dx.doi.org/10.1016/j.jphotobiol.2019.111773

15. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020 Nov;131:110702.

16. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from Albizia lebbeck on HCT-116 colon cancer cell lines. Artif Cells Nanomed Biotechnol. 2020 Dec;48(1):1206–13.

17. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from Trichosanthes kirilowii in colon cancer cells through the induction of apoptotic pathway. Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3577–84.

18. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. Biomed Pharmacother. 2018 Nov;107:1514–22.

19. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea [Internet]. Food Reviews International. 2021. p. 1–20. Available from: http://dx.doi.org/10.1080/87559129.2021.1889581

20. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. Environmental Nanotechnology, Monitoring & Management. 2020 Dec 1;14:100333.

21. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitroandin vivostudies. Arabian Journal of Chemistry. 2020 May 1;13(5):5501–16.

22. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. Crit Rev Food Sci Nutr. 2020 Oct 27;1–8.

23. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.

24. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.

25. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021 May;31(3):436–41.

26. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from Scutellaria baicalensis on human osteoblast-like MG-63 cells [Internet]. Vol. 119, Materials Science and Engineering: C. 2021. p. 111656. Available from: http://dx.doi.org/10.1016/j.msec.2020.111656

27. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. Environ Toxicol. 2021 Jun;36(6):1011–20.

28. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of Scutellaria barbata and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). Saudi J Biol Sci. 2021 Jul;28(7):3633–40.

29. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021 Jul;28(7):3641–9.

30. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15, Journal of Dental Sciences. 2020. p. 562–3. Available from: http://dx.doi.org/10.1016/j.jds.2020.02.009

31. Theertha M, Sanju S, Priya VV, Jain P, Varma PK, Mony U. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis. Cell Mol Immunol. 2020 Oct;17(10):1114–6.

32. Morris T, Stables M, Hobbs A, de Souza P, Colville-Nash P, Warner T, et al. Effects of

low-dose aspirin on acute inflammatory responses in humans. J Immunol. 2009 Aug 1;183(3):2089–96.

33. Al-Swayeh OA, Clifford RH, Del Soldato P, Moore PK. A comparison of the anti-inflammatory and anti-nociceptive activity of nitroaspirin and aspirin [Internet]. Vol. 129, British Journal of Pharmacology. 2000. p. 343–50. Available from: http://dx.doi.org/10.1038/sj.bjp.0703064

34. Gupta J, Kumar D, Gupta A. Evaluation of gastric anti–ulcer activity of methanolic extract of Cayratia trifolia in experimental animals [Internet]. Vol. 2, Asian Pacific Journal of Tropical Disease. 2012. p. 99–102. Available from: http://dx.doi.org/10.1016/s2222-1808(12)60024-3