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Determination Of Anti-Inflammatory Potential Of Endangered Plant Species Of Western Ghats

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Abstract
NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Therefore, there is resurgence to search for orally active molecules that are more effective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms. Hence, current study aimed to assess the anti-inflammatory activities of endangered plant species of western ghats against murine monocytic macrophage RAW 264.7 cell line. Results depicted that all the three endangered plant species of Western ghats viz. C. zedoaria, S. travancorium, and P. vettiveroides, exhibited anti-inflammatory properties. However, stem extract of endangered plant species of western ghat viz. S. travancorium has shown highest anti-inflammatory activity with mean inhibition of 39.75 compared with other two plant species viz. C. zedoaria and P. vettiveroides. In conclusion, stem extract of endangered plant species of western ghat S. travancorium possesses potent anti-inflammatory properties, and hence it could be considered for the development of natural anti-inflammatory drug agents.

Keywords: *Anti-inflammatory, Murine macrophage RAW 264.7 cell line, Stem extract, S. travancorium, Natural drug agents*

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Proinflammatory cytokines (e.g., tumor necrosis factor - a [TNF-a], interleukin [IL-6], and IL-1b) are produced in large quantities by activated macrophages/monocytes that stimulate cellular responses via increasing prostaglandins (PGs) and reactive oxygen species. In addition, lipid peroxidation (malondialdehyde) is produced by free radicals attacking the cell membranes. Thus, inflammatory effect results in the accumulation of malondialdehyde.[1]

Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. It is not surprising that from conception to market most

compounds face an uphill battle to become an approved drug. For approximately every 5,000 to 10,000 compounds that enter preclinical testing, only one is approved for marketing. Drug research and development is comprehensive, expensive, time-consuming and full of risk. On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs.[2]

Moreover, non-steroidal anti-inflammatory drugs (NSAIDs) that are mainly used in the treatment of pain and inflammation related to a large variety of pathologies have been prepared and marketed. These have been of immense help in the management of various inflammatory conditions like rheumatism, arthritis and breast pain.[3] However, their long-term uses are associated with several serious adverse effects. Hence, the discovery of new and safe analgesic and anti-inflammatory drug is needed.[4] Western Ghats is one of the major repositories of medicinal plants.[5]

Most of the economically important plants are usually assumed to be semi-public products in India because of which the harvesting is un-regulated from the forest. Among the species of medicinal plant species globally, 58 species of the Western Ghats region alone are extremely endangered because of excess of harvesting, according to the analysis of the rare endangered threatened status of medicinal plants.[6] The pharmacological efficiency of the medicinal plant extracts can be studied by separating out the active components.[7] Plants are found to be the richest source of medicinal drugs which play an essential role in the scope of traditional medicines, nutraceuticals, modern medicines and synthetic drugs.[8]

Furthermore, plant-derived products are verified to be a major resource of bioactive compounds, several of which are on the way to becoming new lead chemicals to be developed as pharmaceuticals. Another merit is the possibility to discover new drugs with reduced adverse effect as there are many cancers or pathogens which are resistant toward existing drugs or develops resistance during prolonged chemotherapy.[9] Unfortunately, only a few species of plants have been scientifically studied among an abundance of medicinal plants worldwide. With this context, present study was conducted with the main purpose to determine anti-inflammatory potential of endangered plant species of western ghats.

MATERIALS AND METHODS

Collection of Plant Species

The endangered plant species of western ghats *viz. Curcuma zedoaria*, *Syzygium travancorium*, and *Plectranthus vettiveroides* (Figure 1) have been selected in the present study were collected from the Foundation for Revitalisation of Local Health Traditions (FRLHT), Yelahanka, Bengaluru. The plants were classified and authenticated for taxonomic identity. The plants and their parts were collected in plastic bags and transported to the laboratory. Roots, stem and leaves are shade dried, powdered and stored in refrigerator until the time of analysis.

Figure 1. Showing endangered plant species of western ghats collected from FRLHT, Yelahanka, Bengaluru

Extraction

The collected samples were separated into leaves, stem and roots. All the parts were cleaned and shade dried and powdered in a blender. 15g of powdered sample without moisture content was elicited in the Soxhlet apparatus with methyl alcohol. The extraction was run for a minimum of 20 cycles or till the solvent in the sample container turned colorless (Figure 2).

Figure 2. Showing Soxhlet extraction of phytoactives from endangered plant species of western ghats

Determination of Anti-inflammatory Activities of Plant Extracts

The anti-inflammatory activities of endangered plant species of western ghats *viz. C. zedoaria, S. travancorium,* and *P. vettiveroides* was carried out by the modified method of Dirsch et al. Briefly, the murine monocytic macrophage RAW 264.7 cell line was cultured in Dulbecco's Modified Eagle Media (DMEM). The cells were cultured in an atmosphere of 37°C temperature and 5% CO_2 and sub-cultured twice a week. They were seeded

in 96-well tissue culture plates (1×10^6 cells/ml) and incubated for 24 hours at 37°C temperature and 5% CO_2. 100µl of the extract in DMSO was serially diluted to obtain two-fold concentration variations and then added onto cells. These cells were stimulated with 10µg/ml *Escherichia coli* lipopolysaccharide (LPS) and incubation was carried out for an additional 17 hours at 37°C. From each well of cell culture plates, 100µl of supernatant was relocated to the 96-well microtitre plates. The same volume of Griess reagent was added and incubation was carried out for 24 hours. A microtitre plate reader was used to determine the absorbance at 550 nm of the resulting solutions in the wells of the microtiter plate after 10 min. Regression analysis was used to calculate nitrite concentrations and a serial dilution of sodium nitrite was utilized as a standard. Percentage of inhibition was determined facilitated by the capability of extracts to inhibit nitric oxide production by cells in comparison with the control (cells in the media which does not contain extracts with DMSO and triggering agents), which was considered as 0% inhibition.

RESULTS

Effect of stem extracts of endangered plant species of western ghat *viz. C. zedoaria,* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line was represented in Table 1 and illustrated in Figure 3. Results revealed that stem extract of *C. zedoaria* at the concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml, and 320 µg/ml showed mean inhibition percentage of 8.92, 13.41, 20.62, 23.81, 29.64, and 34.60 respectively. The IC_{50} value for stem extract *C. zedoaria,* against murine monocytic macrophage RAW 264.7 cell line was found to be 15.04 µg/ml.

Table 1. Effect of stem extract of *C. zedoaria* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line

Samples	**Conc. (µg/mL)**	**Inhibition %**	**IC_{50} (µg/mL)**
Control	0.00	0.00	
Stem extract of *C. zedoaria*	10	8.92 ± 0.635	15.04
	20	13.41 ± 0.760	
	40	20.62 ± 0.514	
	80	23.81 ± 0.609	
	160	29.64 ± 0.370	
	320	34.60 ± 0.580	

Values were expressed as mean ± standard error of mean (SEM)

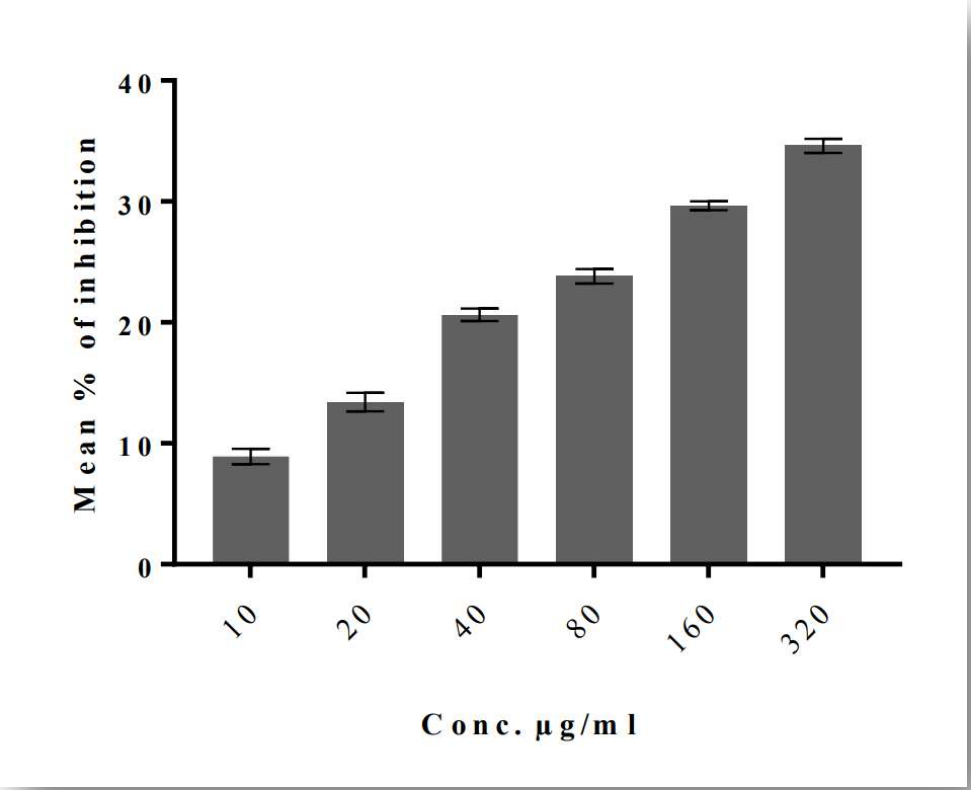

Figure 3. Effect of stem extract of *C. zedoaria* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line

Effect of stem extracts of endangered plant species of western ghat *viz. S. travancorium,* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line was represented in Table 2 and plotted in Figure 4. Results revealed that stem extract of *S. travancorium* at the concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml, and 320 µg/ml showed mean inhibition percentage of 8.10, 15.10, 22.82, 29.21, 37.23, and 39.75 respectively. The IC_{50} value for stem extract of *S. travancorium,* against murine monocytic macrophage RAW 264.7 cell line was found to be 15.04 µg/ml.

Table 2. Effect of stem extract of *S. travancorium* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line

Samples	**Conc. (µg/mL)**	**Inhibition %**	**IC_{50} (µg/mL)**
Control	0.00	0.00	

Stem extract of *S. travancorium*	10	8.10 ± 0.593	15.04
	20	15.10 ± 0.395	
	40	22.82 ± 0.783	
	80	29.21 ± 0.750	
	160	37.23 ± 0.748	
	320	39.75 ± 4.101	

Values were expressed as mean ± standard error of mean (SEM)

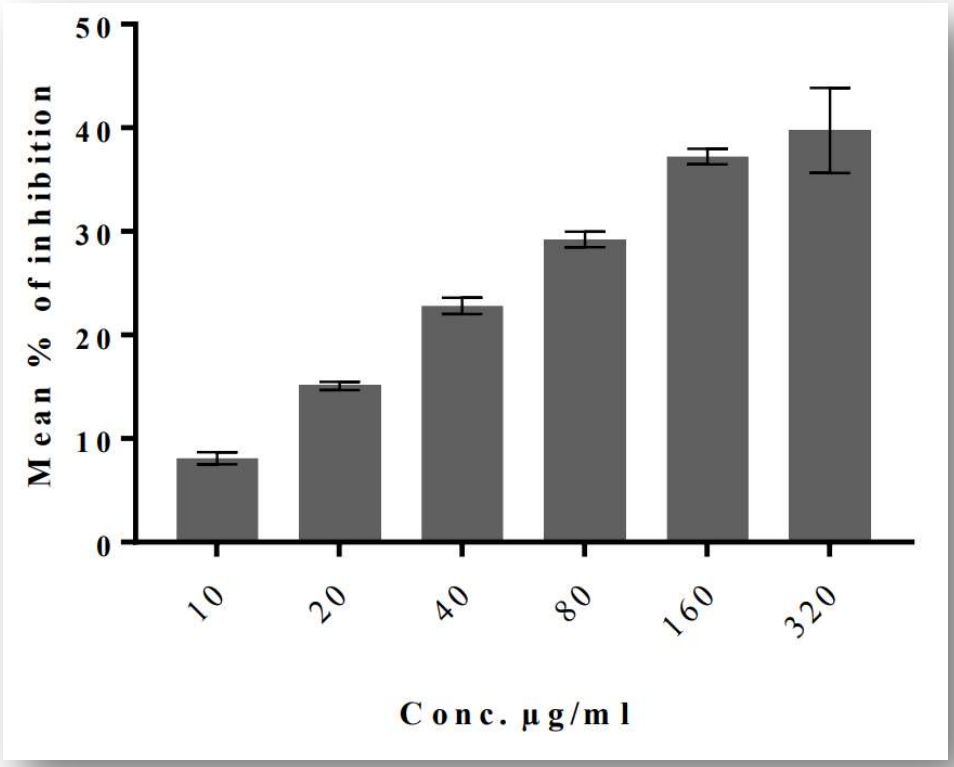

Figure 4. Effect of stem extract of *S. travancorium* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line

Effect of leaf extracts of endangered plant species of western ghat *viz. P. vettiveroides,* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line was represented in Table 3 and illustrated in Figure 5. Results implied that leaf extract of *P. vettiveroides* at the concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml, and 320 µg/ml showed mean inhibition percentage of 7.15, 10.56,

16.65, 25.36, 32.53, and 38.22 respectively. The IC_{50} value for leaf extract of *P. vettiveroides,* against murine monocytic macrophage RAW 264.7 cell line was found to be 15.04 µg/ml.

Table 3. Effect of leaf extract of *P. vettiveroides* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line

Samples	**Conc. (µg/mL)**	**Inhibition %**	**IC_{50} (µg/mL)**
Control	0.00	0.00	
Leaf extract of *P. vettiveroides*	10	7.15 ± 0.621	15.04
	20	10.56 ± 0.707	
	40	16.65 ± 0.583	
	80	25.36 ± 0.630	
	160	32.53 ± 0.718	
	320	38.22 ± 0.825	

Values were expressed as mean ± standard error of mean (SEM)

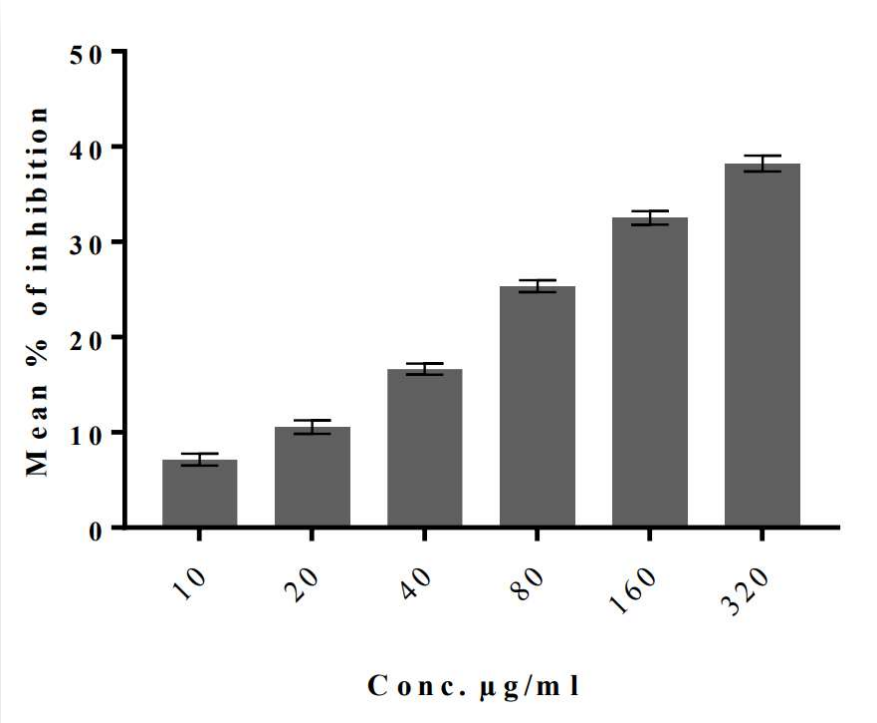

Figure 5. Effect of leaf extract on *P. vettiveroides* on anti-inflammatory activity against murine monocytic macrophage RAW 264.7 cell line

Summarily, among the three plant samples evaluated for anti-inflammatory activities, stem extract endangered plant species of western ghat *S. travancorium* has shown highest anti-inflammatory activity with mean inhibition of 39.75 than others.

DISCUSSION

A number of factors, such as bacterial infection, chemical injury, and environmental pollution, can cause inflammation, which is a complicated process that can cause cell damage or death.[10] The most frequent inflammatory related complaints are pain and fever. The most widely used drugs in the world today are NSAIDs.[11] NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Market demand exists for orally active molecules that are more effective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms.[12] Medicinal plant based traditional system of medicines are playing an important role in providing health care to large section of population, especially in developing countries.[13] Moreover, active research has been driven in recent years on plant-based products due to their biologically beneficial effects since plant products over synthetic compound in the treatment of diseases are needed because of no deleterious effects on man.[14] Therefore, present study was designed with the main objective to assess the anti-inflammatory activities of endangered plant species of western ghats against murine monocytic macrophage RAW 264.7 cell line.

Results of our study revealed that among the three endangered plant species of western ghat *viz. C. zedoaria*, *S. travancorium*, and *P. vettiveroides*, stem extract of endangered plant species of western ghat *S. travancorium* has shown highest anti-inflammatory activity with mean inhibition of 39.75 compared with other two plant species *viz. C. zedoaria* and *P. vettiveroides.* These findings were comparable with literature studies reported by various other research investigators.

The anti-inflammatory activity of the ethanolic leaf extract of *Cyclanthera pedata* was evaluated by Rajmohan et al., using cotton pellet granuloma and granuloma pouch models in rats. An attempt to evaluate the possible mechanisms of action was also done on various *in- vitro* models. It was shown that treatment with the *C. pedata* extract significantly reduced the formation of granuloma and reduced the severity of inflammatory process in both the animal models.[15] Sharma, et al., conducted tests on the extract of *S. jambos*. The compounds myricitrin and ursolic acid were isolated from the extract which suppressed 74 to 99% of the release of TNF-α and IL-8, two cytokines that cause inflammation.[16] Furthermore, the anti-inflammatory effect of phytochemicals has already been documented to be the result of regulating the various enzymes and compounds (COX-2, iNOS), secondary messengers (cAMP, cGMP, calcium, protein kinases), cytokines (TNF-α, IL-1β), transcription factors (NF-kB, AP-1, protooncogenes), proteases and neuropeptides which are directly involved in inflammatory reactions.[17]

Literature reports depicted that the phytoactives flavonoids and saponins in present in the plants could inhibit the phospholipase A2. Phospholipase A2 is an enzyme that acts to break down the Sn-2 fatty acid of membrane phospholipid that encloses arachidonic acid which plays an important role in the inflammatory process. The arachidonic acid is subsequently converted to prostaglandin by COX-1 and COX-2 enzymes. COX-2 is an enzyme responsible for the inflammatory process and its activity is induced by inflammatory, hormonal, and growth factors. Flavonoids and saponins are also known to have an activity that can inhibit COX-2 which is a non-steroidal anti-inflammatory drug target.[13] Moreover, Akhila and Keshamma reported that decrement of paw volume following treatment with methanolic fruit extract of Ashwagandha might be due to the presence of phenolic and flavonoid compounds present in the fruit extract of Ashwagandha.[2]

CONCLUSION

In conclusion, results of this study portrayed that all the three endangered plant species of Western ghats *viz. C. zedoaria, S. travancorium,* and *P. vettiveroides,* exhibited anti-inflammatory properties. However, stem extract of endangered plant species of western ghat *S. travancorium* possesses potent anti-inflammatory properties, and hence it could be considered for the development of natural anti-inflammatory formulations.

REFERENCES

1. Keshamma E. A Study On Assessment Of In-Vitro Anti-Inflammatory Activity Of Garlic (Allium Sativum) Extract Using Albumin Denaturation Method. Nat Volatiles & Essent Oils. 2018;05(2):167-172.
2. Akhila A, Keshamma E. Assessment Of In-Vivo Anti-Inflammatory Potential Of Fruit Extract Of Ashwagandha (Withania Somnifera) Using Carrageenan Induced Paw Edema Rat Model Study. Journal Of Advanced Zoology. 2023;44(S2):3570-3579.

3. Akhila A, Keshamma E. Evaluation Of Anti-Inflammatory Activity Solanum Lycopersicum (Tomato). Int J Food Nut Sci. 2022;11(11):2560-2571.
4. Sharada Devi Jn, Keshamma E. A Study On Assessment Of Anti-Inflammatory Activity Of Ethanolic Extracts Of Tamarind (Tamarindus Indica) Seeds. Int J Food Nut Sci. 2022;11(9):3285-3297.
5. Krishnan Pn, Decruse Sw, Radha Rk. Conservation Of Medicinal Plants Of Western Ghats, India And Its Sustainable Utilization Through In Vitro Technology. In Vitro Cellular & Developmental Biology-Plant. 2011; 47:110-22.
6. Ravikanth G, Jagadish Mr, Vasudeva R, Shaanker Ru, Aravind Na. Recovery Of Critically Endangered Plant Species In India: Need For A Comprehensive Approach. Cur Sci. 2018:504-11.
7. Gunalan G, Krishnamurthy V, Saraswathy A. Gc-Ms And Hptlc Fingerprinting Of Bauhinia Variegata Leaves For Anticancer Activity. World J Pharm Res. 2014;3(9):1313-39.
8. Jayappa Md, Ramaiah Ck, Kumar Ma, Suresh D, Prabhu A, Devasya Rp, Sheikh S. Green Synthesis Of Zinc Oxide Nanoparticles From The Leaf, Stem And In-Vitro Grown Callus Of Mussaenda Frondosa L.: Characterization And Their Applications. Applied Nanoscience. 2020; 10:3057-74.
9. El Sayed K, Khanfar M, Muralidharan A, Awate B, Youssef D, Shah G. Targeting Cytoskeleton Signaling In Cancer: Rationale Design Of Semisynthetic Latrunculin Analogs As Inhibitors For Metastatic Prostate Cancer. Cancer Research. 2008 May 1;68(9_Supplement):610-.
10. Keshamma E. Evaluation Of In-Vitro Anti-Inflammatory Activity Of Seed Extract Of Zea Mays Using Albumin Denaturation Method. Int J Curr Microbiol App Sci. 2019;8(07): 3015-3024.
11. Mamatha N, Keshamma E. A Study On Assessing The Anti-Inflammatory Efficiency Of Abelmoschus Moschatus (Bhendi). Neuroquantology. 2010;8(2):262-268.
12. Keshamma E. Quantitative Estimation Of Phytochemicals And Assay Of In-Vitro Anti-Inflammatory Activity Of White Ekka (Calotropis Gigantea) Using Albumin Denaturation Method. Int J Curr Microbiol App Sci. 2014; 3(11): 1047-1054.
13. Keshamma E. Phytochemical Screening And Evaluation Of In-Vitro Anti-Inflammatory Activity Of Leaf Parts Of Elephantopus Scaber. Res Militaris. 2023;13(4):218-25.
14. Keshamma E And Shobha Rani K. A Study On Assessment Of Anti-Inflammatory Potential Of Moringa Oleifera. Int J Food Nut Sci. 2021;10(2):729-736.
15. Rajmohanan Tp, Nair S. Evaluation Of Anti-Inflammatory Activity Of Cayratia Pedata (Lam.) Juss. Leaf Extract In Some In Vivo And In-Vitro Experimental Models, International Journal For Pharmaceutical Research Scholars (Ijprs). 2015:4(3):163-175.
16. Sharma R, Kishore N, Hussein A, Lall N. Antibacterial And Anti-Inflammatory Effects Of Syzygium Jambos L. (Alston) And Isolated Compounds On Acne Vulgaris. Bmc Complementary And Alternative Medicine. 2013; 13:1-0.
17. Kundu Jk, Surh Yj. Inflammation: Gearing The Journey To Cancer. Mutation Research/Reviews In Mutation Research. 2008;659(1-2):15-30.