International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Evaluation of Anti-inflammatory Activity of *Millingtonia hortensis* Leaf Extract

Krishnaveni Manubolu[1], Raveesha Peeriga[2]*, Mohan Gandhi Bonthu[2], Bhaskara Raju Vatchavai[3], Nirmala Korukola[4], Edward Raju Gope[5], Devi Kadirvel[6]

[1]*Narayana Pharmacy College, Chintharedypalem, Nellore-524002, Andhra Pradesh India*

[2]*V. V. Institute of Pharmaceutical Sciences, Seshadri Rao Knowledge Village, Gudlavalleru-521301, Andhra Pradesh, India*

[3]*Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Tadepalligudem-534101, West Godavari District, Andra Pradesh, India*

[4]*Department of Pharmacognosy, KGRL College of Pharmacy, Bhimavaram, Andhra Pradesh, India.*

[5]*Dr Samuel George Institute of Pharmaceutical Sciences, Markapur ANUCPS, Prakasam District, Andhra Pradesh, India.*

[6]*Department of Pharmacology, Annai Veilankanni's Pharmacy College, Saidapet, Chennai- 600015, Tamil Nadu, India.*

Abstract

In the present study "*Millingtonia hortensis*", a herbal aqueous extract of leaves are investigated to find anti-inflammatory activity by invitro evaluation. *Millingtonia hortensis* (*BIGNONIACEAE*) subjected for Invitro anti-inflammatory activity was evaluated by albumin protein denaturation method. *Millingtonia hortensis* leaf aqueous extract was prepared by cold maceration method. *Millingtonia hortensis* aqueous extract was screened and gave postitive results for alkaloids, flavonoids, carbohydrates, phenols and other constituents. The extract gave more significant anti-inflammatory activity relative to reference standard drug sample ibuprofen. *Millingtonia hortensis* leaf aqueous extract showed noticeable anti-inflammatory activity and it indicates that it has potential application in the conditions of inflammatory conditions like arthritis, diabetes mellitus and cancer where natural anti-inflammatory agents are having beneficial action.

Keywords: *Millingtonia hortensis*, herbal extract, anti-inflammatory, albumin denaturation

Full-length article **Corresponding Author*, e-mail: drprsha@gmail.com

1. Introduction

This quickly growing tree sheds its flowers early in the morning and flowers at night. Hortensis means "grown in gardens," and the name *Millingtonia* is derived from the English botanist Thomas Millington. The tree is a gardener's favorite. Because its corky bark is used to process a subpar cork, it is also known as the "cork tree." The biological name of the tall, drought-resistant tree is *Millingtonia hortensis*, and it belongs to the bignoniaceae family.

The flowers have a rich, pleasant scent. Propagation using suckers and seeds. This crude drug is also known by the names Akas Nim, Nim chameli, betali nim, mini chameli, and karkku. It has an eternal lifespan. It's a tall tree, deciduous. It increases meter by meter. The leaves have multiple pinnate layers. Dried flower is a good lung tonic, used in cough diseases; bark is used to make yellow dye. Grows in tropical forests in central India [1]. Trees that are 8–25 meters tall. 40–100 cm in length, with elliptic, ovate, or ovate ot oblong leaflets that are 1.5–4 cm, glabrous, base rounded, oblique, margin entire, and apex acuminate; four or five lateral veins on the side of the midrid [2]. Cymose – paniculate inflorescence. Tiny, cupular, 2-4 mm, sino-late lobed calyx with slightly reflexed lobes. White corolla, tube 3-7 cm, diameter 2-3 mm. Glabrous ovary with many, four-rowed ovules.

Habitat low altitude slopes, usually located between 500 and 1200 meters at an elevation of 0 to 922 meters (0 to 3,025 feet). The medicinal uses include apoptosis and antiproliferation against RKO colon cancer [3]. It exhibits antimicrobial activity. Essential oil of flowers [4]. In Hispidulin and ethanoic extracts from Millingtonia hortensis and Oroxylum indicum (L) Kurz were chemically characterized and shown to have ant arboviral activity [5-8].

2. Materials & Methods

2.1. Plant Material Collection

The Indian cork tree, or *Millingtonia hortensis Linn.* (Bignoniaceae), is a perennial flowering tree that grows widely throughout India's plains and is planted in gardens. We gathered newly matured leaves from the campus of our institute Narayana pharmacy college, Nellore in the month of August 2023, authenticated by botanist in D.R. Womens college, Gudur, Nellore (Specimen no.DRWG/BOT/128). Freshly collected mature leaves were washed with distilled water so that foreign debris removed. These were dried at room temperature to remove excess moisture present in the living tissues. Initial weight of powdered drug was 399 gm and blended until coarse particles were produced. This powder was extracted thoroughly with distilled water using cold maceration method. The solvents were evaporated under steam distillation (solvent recovery) and the extract obtain and preserved in airtight container for further use.

2.2. Phytochemical screening

The prepared *Millingtonia hortensis* aqueous leaf extract was tested for different phytochemical constituents' presence by standard methods [9-10].

2.3. Invitro Anti-inflammatory activity

2.3.1. Preparation of reference drug and test solutions

2.3.1.1. Preparation of stock solution

The reference medication NSAID (ibuprofen). Prednisolone was finely ground into a powder. 1mg/ml of ibuprofen solution was prepared using distilled water. Using a vortex, the solution was thoroughly combined. For herbal extract also, a similar process was followed.

2.3.1.2. Preparation of working solutions

Ibuprofen was the reference drug, and *Millingtonia hortensis* extract was serially diluted from 100 µg/ml - 0.01 µg/ml. Each substance to be tested had a total volume of 5.0 milliliters. 0.2 ml of egg albumin and a volume of phosphate-buffered saline (pH 6.4) sufficient to produce 3ml and were used to create reaction mixtures. Then the reaction mixtures were gently mixed with 2 ml of herbal extract from each different concentration. Prednisolone and ibuprofen, the reference medications utilized as positive controls in this investigation, underwent a similar procedure. Distilled water was also employed as a negative control.

2.3.1.3. Invitro albumin denaturation test

According to this procedure sample solutions of reaction mixtures were incubated for 15–20 minutes at 37°C ± 2°C in a water bath, reaction mixtures were heated to 70°C for a period of 5 minutes. And after that, the reaction mixture was given fifteen minutes to bring at room temperature. Using a colorimeter, the absorbance of samples was allowed to read at 680 nm for each concentration (100 to 0.01 µg/ml) before and after denaturation for absorbance. The mean absorbance was measured after each test was conducted three times. Percentage protein inhibition of samples was measured, by the below mentioned formula [9, 13].

$$\text{Inhibition percentage}\,(\%) = \frac{(absorbance\ of\ control - absorbance\ of\ test)}{(absorbance\ of\ control)} * 100$$

2.3.1.4. Statistical analysis

% protein denaturation inhibition values of herbal extract and reference drug were tested using unpaired t test at $p<0.05$ value to test the variance among the results using graph pad prism 5.

3. Results and Discussion

The prepared herbal extract exhibited black, brown in colour, odourless and exhibited good solubility in water.

3.1. Invitro albumin denaturation test

Millingtonia hortensis leaf aqueous extract's anti-inflammatory properties were assessed using the egg albumin denaturation method. 100 µg/ml concentration is the one with the highest inhibition rate was noted cold maceration water extracts. Findings are expressed in mean±standard deviation The main goal of the egg albumin denaturation assay is to ascertain whether certain substances or agents can prevent or impede the denatured state of egg albumin in specific situations. The process by which a protein loses its biological activity and undergoes structural changes is known as denaturation. In the experiment, egg albumin is used as a model protein, and it is desaturated by subjecting it to high or low temperatures, changes in pH, or other denaturing agents. During denaturation, the original conformation of egg albumin is broken, altering its physical properties and rendering it inactive. Based on the theory that substances with anti-inflammatory properties may be able to prevent or reduce egg albumin, the egg albumin denaturation assay assesses a drug's or compound's ability to do so. Protein denaturation is thought to be one of the causes of inflammation. NSAIDs simultaneously inhibit the COX enzyme and stop protein denaturation. In controlled experimental settings, the test sample at varying concentrations can be incubated with egg albumin solution to observe the reactions. The absorbance can then be measured to determine the percentage inhibition.

Protein denaturation mechanism is brought by some unpredictable and changes or modifications of hydrophobic, electrostatic, disulphide and hydrogen bondings. In some arthritic, cancer, and diabetes conditions there is involvement of protein denaturation and so production of autoantigens. So that it causes declining of inflammatory activity because of protein denaturation. NSAIDs drug types used as reference drugs in this study. By inhibiting the activity of the cyclooxygenase enzyme, NSAIDs reduce inflammation. Ibuprofen is one of the propionic acid derivatives and a nonselective nonsteroidal anti-inflammatory drug (NSAID) used for analgesic, anti-inflammatory, and antipyretic. The denaturation technique of egg albumin offers a less expensive alternative for evaluating the anti-inflammatory properties of herbal medicine; further research should be done to validate this method. Concentration dependent manner of protein denaturation was observed with ibuprofen results. Literature suggested that Aomhatai Deethae et al examined the anti-inflammatory properties of extracts from the leaves and stem bark of *Millingtonia hortensis* Linn. and used the GC-MS method to identify the bioactive components. According to this aqeous leaf extract showed anti-inflammatory activity by attenuating NO generation. In this research aqeous leaf extract invitro activity was assessed [11].

Table 1. Phytochemical screening of the *Millingtonia hortensis* leaf aqeous extract

S. No.	Phytochemical Constituent	Reagent used	Observation
1.	Alkaloids	Dragandroff's reagent	+ve
		Mayer's reagent	+ve
		Hager's reagent	+ve
2.	Carbohydrates	Molish's reagent	+ve
3.	Flavonoids	Alkaline reagent test	+ve
4.		Lead acetate test	+ve
5.	Proteins and amino acids	Ninhydrin test	-ve
6.	Saponins	Foam test	+ve
7.	Tannins	Ferric chloride test	+ve
8.	Phenols	Ferric chloride test	+ve
9.	Glycosides	Ferric chloride and glacial acetic acid	-ve

Table 2. Inhibition percentage of protein denaturation of *M. hortensis* leaf aqueous extract

S. No.	Concentration (µg/ml)	% Rate of Inhibition
1.	0.01	10.21±0.40
2.	0.1	17.14 ±0.55
3.	1	20.21 ±0.32
4.	10	23.21 ±0.14
5.	100	24.12 ±0.21

Table 3. Inhibition percentage of protein denaturation of reference drug (Ibuprofen)

S. No.	Concentration (µg/ml)	% Rate of Inhibition
1.	0.01	14.20 ±0.21
2.	0.1	1 2.02±0.35
3.	1	11.35±0.12
4.	10	10.21 ±0.01
5.	100	12.10 ±0.03

Table 4. Statistical analysis of %protein denaturation of *M.hortensis* vs ibuprofen using t test one tailed

Table Analyzed	**Data**
Column A vs Column B data	*M.hortensis* vs Ibuprofen data
Unpaired t test	
P value	0.0136
P value summary	*
Are means signif. Different at (P < 0.05)	Yes
One- or two-tailed P value?	One-tailed test
T and df	t=2.699 and df=8
How big is the difference? Was found	
Mean ± SEM of column A value	18.98 ± 2.511 N=5
Mean ± SEM of column B value	11.98 ± 0.6509 N=5
Difference between means was	7.002 ± 2.594
95% confidence interval was	1.020 to 12.98
R^2 analysed	0.4766
F test done to compare variances	
F,DFn, Dfd was	14.88, 4, 4
P value was	0.0228
P value summary	*
Are variances significantly different? Was found	Yes

Figure 1. Leaves of *Millingtonia hortensis*

Figure 2. *Millingtonia hortensis* leaf aqueous extract

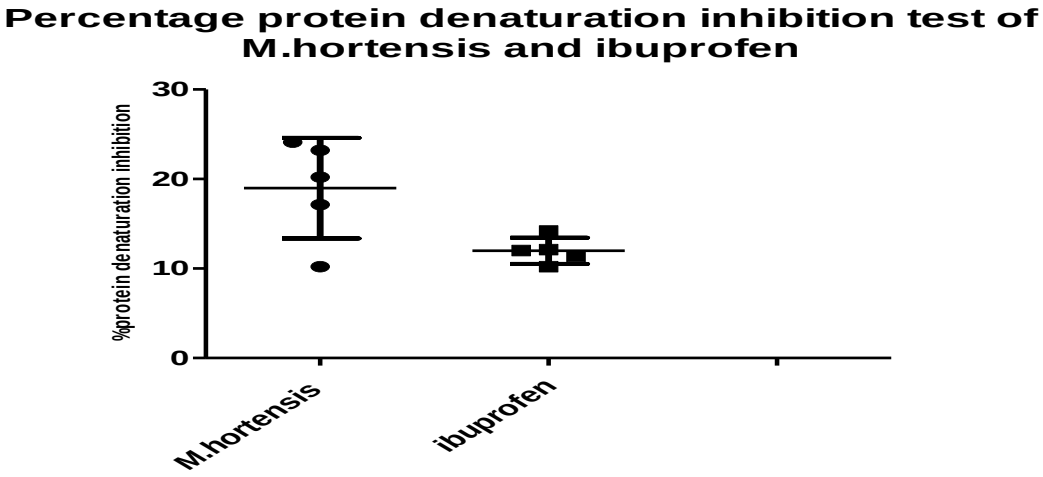

Figure 3: % protein denaturation inhibition of *Millingtonia hortensis* vs Ibuprofen

From the previous findings utilised by S Dharmadeva et al studied utilizing the albumin denaturation method, *Ficus racemosa L.* bark exhibits anti-inflammatory properties in vitro. According to Damle M et al. flavonoids exhibit anti-inflammatory property [12]. In the present investigation anti-inflammatory activity of the sample might be due to the phytochemical active principles present in the aqueous extract. According to Anish Babu VB et al. flavonoids present in the plants of bigoniaceae family are responsible for anti-inflammatory activity [14]. A vast array of secondary metabolites, including saponins, tannins, flavonoids, quinines, alkaloids, reducing sugars, glycosides, carbohydrates, quercetin, kaempferol, sitosterols, iridoids, terpenes, steroids, and coumarins, are found in members of the Bignoniaceae family (Solomon et al.,) [15-24]. It is possible to conclude that flavanoids, saponins, or carbohydrates alone or in combination may explain this invitro anti-inflammatory activity by combining the evidence from the literature with phytochemical screening.

3.2. Statistical analysis

Statistically analyzed data of %protein denaturation inhibition values of *Millingtonia hortensis* leaf aqueous extract vs ibuprofen exhibited a significant difference ($p=0.0136$) when tested at $P < 0.05$. This means prepared various proportions of plant extract is significant enough to produce the anti-inflammatory activity compared to ibuprofen reference drug.

Conclusion

The study unequivocally shows that the aqueous extracts of *Millingtonia hortensis* significantly and similarly inhibited the denaturation of BSA in vitro compared to Ibuprofen, the reference anti-inflammatory drug. Therefore, it can be said that these extracts have a strong anti-inflammatory effect and further investigations have to be carried out at phytoconstituent level.

Acknowledgement

We would like to convey gratitude to the Narayana Pharmacy College management for providing facilities to conduct this novel work.

Reference

[1] E. Blatter, W.S. Millard. (1954). Some Beautiful Indian Trees, The Bombay Natural History Society, Mumbai. 6: 106.

[2] J.S. Gamble. (1957). Flora of the Presidency of Madras, Botanical Survey of India, Calcutta. 9. 699.

[3] S. Tansuwanwong, H. Yamamoto, K. Imai, U. Vinitketkumnuen. (2009) Antiproliferation and apoptosis on RKO colon cancer by Millingtonia hortensis. Plant Foods for Human Nutrition. 64: 11-7.

[4] C. Sittiwet. (2009). Anti-microbial activities of Millingtonia hortensis Linn. Flowers essential oil. Journal of Pharmacology and Toxicology. 4(1): 41-4.

[5] A.C. Cardoso Reis, G.M. Valente, B.D. Silva, C.L. Brito Magalhães, M. Kohlhoff, G.C. Brandão. (2023). Anti-arboviral activity and chemical characterization of hispidulin and ethanolic extracts from *Millingtonia hortensis Lf* and *Oroxylum indicum (L.) Kurz* (Bignoniaceae). Natural Product Research. 37(4): 613-7.

[6] S. Hegde, L.D. Souza. (1995). In vitro propagation of an ornamental tree Millingtonia hortensis L. f. Gartenbauwissenschaft, 60(6): 258-261.

[7] pA. Kavitha, M. Kensa. (2016) Pharmacognostic Studies on Millingtonia Hortensis L. Kongunadu. Research Journal. 3(2): 52-8.

[8] A. Jetty, D.S. Iyengar. (2000) Antimicrobial activity of Millingtonia hortensis leaf extract. Pharmaceutical biology. 38(2): 157-60.

[9] S. Dharmadeva, LS. Galgamuwa, C. Prasadinie, N. Kumarasinghe. (2019). *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. Ayu. 39(4), 239-242.

[10] A. Janaki, P.K. Kaleena, D. Elumalai, P. Hemalatha, M. Babu, K. Velu, R. Sudha. (2017). Phytochemical screening, antioxidant and antibacterial activities of Millingtonia hortensis (L). International Journal of Current Pharmaceutical Research. 9(5): 162-7.

[11] A. Deethae, T. Sritapunya, S. Khetnaoanurak, W. Jinwan. (2023). Evaluation of antibacterial, antiinflammatory activities and GC-MS profiling of Millingtonia hortensis Linn. leaf and stem bark extracts. Asian Journal of Scientific and Technological Reports. 28: 72.

[12] M. Damle. (2014). Glycyrrhiza glabra (Liquorice)-a potent medicinal herb. International Journal of Herbal Medicine. 2: 132-6.

[13] A. Vinchurkar, A. Valsange, L. Dama, S. Sonawane, N. Gaikwad, P. Mane, S.B. Dama. (2014). Evaluation of in-vitro anti-inflammatory activity of crude Lawsonia inermis leaf extract using egg albumin denaturation assay. Trends in Biotechnology Research. 8(33): 44.

[14] V.B. AB, A. VT, B. Thomas B. (2015). Ethno-pharmacological potentialities of some ornamental plants in the family Bignoniaceae. Journal of Medicinal Plants. 3: 135-40.

[15] J. Solomon, J. Joselin, T.S. Brinith, A. Florence. Phytochemical evaluation of Bignoniaceae flowers. (2013). Journal of Chemical and Pharmaceutical Research. 5(4): 106-111.

[16] R. Peeriga, K.P. Adarapu, A. Kurama, N. Mohammed, L.R. Atmakuri, D. Kumar. (2022). Insilico Investigation on Phytoconstituents in Pamburus missionis S. for Antioxidant Activity. Pharmacognosy Research. 14(3): 246-250.

[17] R. Peeriga, C.K. Bonnth. (2016). Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of Myxopyrum Smilacifolium B. Pharmacognosy Journal. 8(2): 159-164.

[18] K.B. Chandrasekhar, R. Peeriga. (2017). Evaluation of the antiarthritic activity of ethanolic leaf extract of Myxopyrum smilacifolium Blume. International Journal of Research in Medical Sciences. 3(10): 1-12.

[19] R. Peeriga, K.B. Chandrasekhar. Antiarthritic activity of leaf extracts of Pamburus missionis Swingle. (2017). International Journal of Pharmaceutical Science and Research. 8(2): 1-5.

[20] P. Raveesha, B.P. Kumar, G.E. Raju, R. Narayanan, J. Mohammed, Y.L. Chakradhar. (2023). Findings of Steroids, Coumarins and Saponins in Ipomea sagittifolia (Burn. f.)., Leaf Extract by HPTLC Fingerprints. International Journal of Chemical and Biochemical Sciences. 24(4): 132-140.

[21] P. Raveesha, Afsar Shaik, S.A. Mohamed Shiek Arabath, M. Latha, S.M. Narayana. (2023). Assessment of Steroids in ethanolic stem extract of Morning glory by High Performance Thin Layer Chromatography. International Journal of Chemical and Biochemical Sciences. 23(3): 361-366.

[22] P. Raveesha, S.A. M.S. Arabath, A.N. Rao, Y. Ankireddy, K.V.B. Ganesh. (2023). Exploring the Coumarins from Stem Extract of Ipomea Sagittiofolia Burm. f. by High-Performance Thin Layer Chromatography. International Journal of Chemical and Biochemical Sciences. 23(3): 268-273.

[23] P. Raveesha, S.K. Aminabee, G. Manasa, S. Tejaswini, C. Mounika, P.B. Rekha, K.V.B. Ganesh. (2023). Analyzing Phenols and Triterpenoids in Dechaschistia Crotonifolia Leaves Using HPTLC Fingerprinting. Tuijin Jishu/Journal of Propulsion Technology. 44(3): 605-612.

[24] P. Raveesha, A.K. Priyanka, K. Akhila, M. Naseemunnisa, A.L. Rao, D. Kumar. (2022). Insilico Investigation on Phytoconstituents in Pamburus missionis S. for Antioxidant Activity. Pharmacognosy Research. 14(3): 246-250.

[25] Raveesha Peeriga, Lakshmana Rao Atmakuri, Sindhu Sri Reddy, Sireesha Kancherla, Pravallika Gunturu, Alekhya Kodali, Sri Akhil Yenduri, Jhansi Chinthalapudi, Mohansai Maragani, Praneeth Varma et al. (2022). Computational Study of Phytoconstituents in Myxopyrum Smilacifolium Blume against Inflammatory Mediator TNF-α. Journal of Drug and Alcohol Research. 11 (12): 1-5.