https://doi.org/10.48047/AFJBS.6.7.2024.2664-2676

Evaluation of *Vitex negundo* Linn. in parasitized and unparasitized form for Anti-inflammatory activity

Trushal V. Chorage[1]*, Ravindra Y. Patil[2]

1. Department of Pharmacognosy, PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, – 412 301 (India)
2. Department of Pharmacognosy, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune – 411 014 (India)

Corresponding Address:

Mr. Trushal V. Chorage
Department of Pharmacognosy,
PDEA's Seth Govind Raghunath Sable College of Pharmacy,
Saswad, Pune – 412 301
E-mail: trushalchorage83@gmail.com
Contact No.: 9921196235

Article History
Volume 6, Issue 7, April 2024
Received:3 June 2024
Accepted: 20 June 2024
Published: 27June 2024
doi: 10.48047/AFJBS.6.7.
2024.2664- 2676

ABSTRACT

Medicinal plants have therapeutic potential and are used worldwide to treat various diseases. Vitex negundo Linn.; family Lamiaceae has been used for the treatment of Internally alternative, aromatic, bitter and vermifuge anodyne, tonic, febrifuge, expectorant and diuretic, skin diseases, pruritus, helminthiasis, rheumatism and puerperal fever.

This study was designed to investigate the phytochemical and anti-inflammatory activity of methanolic extract of Vitex negundo Linn. *in parasitized and unparasitized form (VNMpar and VNMunpar). The anti-inflammatory activity of methanolic extract of Vitex negundo* Linn. *in parasitized and unparasitized form was evaluated by inflammatory models of carrageenan induced paw edema in rats and acetic acid induced vascular permeability in Mice.*

The result that preliminary phytochemical analysis showed presence of flavonoids, triterpenoids, tannins, phenolic compounds along with primary metabolites. Methanolic extract of Vitex negundo Linn. in parasitized form significantly decreased carrageenan-induced paw edema and acetic acid-induced capillary permeability. Paw edema is dramatically reduced at 3, 4, and 5 h after carrageenan injection by VNMpar at 400 mg/kg along with vascular permeability too. In conclusion, the findings suggested that methanolic extract of Vitex negundo Linn. in parasitized form produced potential anti-inflammatory activity than unparasitized form which support the claim for its traditional use in the treatment of various diseases.

Keywords: *Vitex negundo*, Anti-inflammatory activity, paw edema, vascular permeability

INTRODUCTION

Since nature provides us with medications made of plants and herbs that can treat incurable diseases with little side effects, it can be thought of as a storehouse of cures for human ailments. It is possible to change the chemical or biological activity of natural products to develop medications with significant therapeutic potential, even though these items may contain molecules with minimal or no action on their own.[1]

For many years, the only access to basic healthcare in rural or village areas has been through the use of medicinal plants.[2] India is native to a vast array of medicinal plants, and traditional Indian medical systems such as Siddha, Ayurveda, and Unani use a number of plant-derived extracts to

treat different diseases. Not many of them have been studied scientifically. Because of their many pharmacological properties, which include inflammatory, antipyretic, and analgesic actions, plant-derived natural substances like flavonoids, terpenes, glycosides, and alkaloids have experienced a marked increase in interest in recent years.[3]

There are over 250 species of trees and shrubs in the genus Vitex, most of which are found in tropical and subtropical climates. India is native to about 14 species, some of which are said to have particular commercial and therapeutic uses. *Vitex negundo* Linn. also known as Nirgundi, is a plant which belongs to a member of the Lamiaceae family.[4] The majority of Lamiaceae members are annual or perennial herbs. Although molecular research suggests that several of the woody species that were formerly included in Verbenaceae actually belong in Lamiaceae.

Vitex negundo typically reaches a height of three to nine feet, however with proper care, it can reach up to 25 feet. This species is found throughout the world in America, Europe, Indo-Malaysia, West Indies, Asia and India. It can be found throughout most of the substantial portion of India.[5]

Numerous potentially beneficial compounds, including phytosteroids, iridoids, flavonoids, and diterpenoids, are found in the Vitex negundo species. Analgesic, anti-inflammatory, antibacterial, antioxidant, hepatoprotective, antihistamine, and antiasthmatic properties are found in the majority of these species.[6,7]

It's interesting to observe that a single species of plant is used to cure a wide range of disorders in traditional and folk medicine. The plant's major usage is in treating female reproductive diseases, including as hyperprolactinemia, menopause, and premenstrual syndrome. Every single part of the plant, from the root to the fruit, has a wide range of secondary metabolites which allow the plant an unprecedented range of therapeutic applications.[8]

The current study is designed to investigate anti-inflammatory properties of methanol extract of *Vitex negundo* Linn. (in parasitized and unparasitized form i.e. VNMpar and VNMunpar) in comparison to a number of experimental models in rats and mice in order to verify its ethno medicinal uses. In parasitized form, *Cuscuta reflexa* roxb. is grown as a parasite on host i. e. on *Vitex negundo* Linn.

When tissue is injured by physical trauma, toxic chemicals or microorganisms, inflammation is the body's natural defensive reaction. It is the body's reaction to get rid of the irritants, inactivate or destroy the invasive organisms and prepare the tissue for healing.

Uncontrolled or excessive inflammation, despite acting as a defense mechanism, can cause or worsen a variety of illnesses. It is commonly known that inflammation generates free radicals and reactive oxygen species, which can set off a series of events that impede healing. Moreover, it has been demonstrated that the inflammatory process generates significant amounts of reactive oxygen species (ROS). These ROS have been shown to shed light on the pathophysiology of many chronic illnesses viz. cancer, cardiovascular disease, rheumatoid arthritis and neurological disorders. Consequently, anti-inflammatory and antioxidant medications play a significant role in prevention as well as treatment of number of human diseases. Unfortunately, there are a lot of side effects associated with anti-inflammatory drugs now on the market, like NSAIDs (non-steroidal anti-inflammatory drugs), which limits their use. Therefore, creating strong anti-inflammatory medications with fewer side effects is crucial. Therefore, plants or substances with antioxidant qualities may aid in the reduction of inflammatory disorders by absorbing reactive oxygen species and free radicals during the inflammatory process.[9]

MATERIALS AND METHODS

Herbal preparation and extraction

Stems and small branches of *Vitex negundo* Linn., family Lamiaceae (in parasitized and unparasitized form) were collected from Wai, Satara region, western ghat, Maharashtra. Plant sample was authenticated from Joint Director, BSI, (Western Regional Centre) Pune, India (No.: BSI/WRC/IDEN.CER./2020/H3) and the departmental library kept the voucher specimen (Specimen No. TVC-1) for further references. The coarsely ground, shade-dried stems and small branches of both parasitized and unparasitized form were first treated with petroleum ether for defatting at 60–80°C for 4 hours followed by extraction for 10–12 hours using methanol in a soxhlet apparatus. Yield of dry residue of 19.50 % w/w and 18.80 % w/w was obtained in case of VNMpar and VMNunpar respectively by filtering the extract through Whatman Filter Paper (Grade. 1) and concentrating it by rotary evaporator at a lower pressure until it will get solidify.

Drugs and Chemicals

Following chemicals were utilized in the present study: Thermosil Fine Chem Industries (Pune, India) provided the carrageenan, methanol, and indomethacin. We bought acetic acid, Evans blue, and Na-CMC from Shivaji Scientific Supplier (Pune, India).

Animal Preparation

The present study includes animals such as mature Wistar Albino Rats (200–220 g) as well as Swiss Albino Mice (20–22 g), were procured from Global Bioresearch Solution Pvt. Ltd., Pune, India. In a 12-hour light-dark cycle environment with relative humidity (50±5%) and a steady temperature (22±1◦C), they were kept within standard lab cages. Throughout the study, all the animals had unlimited access to food and water. Experiment protocol was approved by the CPCSEA committee of Jayawant Shikshan Prasarak Mandal's Rajarshi Shahu College of Pharmacy and Research, Tathwade - 411033 (Ethics approval: IAEC/2023/03).

Anti - inflammatory activity

1. Carrageenan Induced Paw Edema in Rats:

Six groups of rats were prepared: Group 1: Control (Inflammation control, 0.5 % CMC-Na); Group : 2 Indomethacin treated (10 mg/kg, dispersed in 0.5 % CMC-Na); Groups 3 and 4 : received an oral VNMpar treatment at 200 and 400 mg/kg, respectively; Groups 5 and 6 : received an oral VNMunpar treatment at 200 and 400 mg/kg, respectively 30 minutes before to the carrageenan injection. Each rat was given a sub-plantar dose of 100 µL of 1%w/v Carrageenan in its right hind paw. A varnier caliper was used to measure the volume of the rat paw at 0, 1, 2, 3, 4, and 5 hours. The edema thickness was calculated using the millimeter difference between the contralateral and inoculated footpads at the same evaluation time. [10, 11]

2. Acetic Acid Induced Vascular Permeability in Mice:

The current model represents, mice of both sex divided in six different groups consisting of six animals in each. Group 1 represents control group, Group II: Indomethacin (10 mg per kg, positive control group), Group 3, 4: VNMpar (200 and 400 mg per kg) and Group 5, 6: VNMunpar (200 and 400 mg/kg) were administered via oral gavage. Following a 60-minute gavage, 20 ml/kg body weight of 0.6% (v/v) acetic acid was applied immediately (i.p.), followed by 10 ml per kg bodyweight of 2% Evans blue prepared in normal saline solution administered to tail vein. The mice were cervical dislocated and sacrificed 20 minutes after the acetic acid was given.

Later, 10 ml saline solution was used to rinse peritoneal cavity. After collecting, washing solutions into a collecting tube, they were centrifuged for five min at 1000 × g for 5 min. The capillary permeability of the exudates was indicated by the absorbance of Evans blue at 590 nm, which was determined in the supernatant using spectrometry. An oral dose of VNMpar, VNMunpar and an hour prior to the injection of acetic acid, indomethacin was administered.[12]

Statistical Analysis

Data will have presented as the means±SEM (standard error of mean) (n = 6). Significant differences between prepared group (means) will be processed by one-way ANOVA followed by Dunnett's t-test for multiple comparisons test. Data were defined as the statistical significance when ****$p < 0.0001$, ***$p < 0.001$, etc. Statistical analysis was generated with help of Graph Pad Prism 10

RESULTS AND DISCUSSION:

The process through which living tissues respond to stimuli produced by inflammatory substances, such as heat, physical trauma, microbial infections, and harmful chemical irritations, is known as inflammation. Certain clinical symptoms, such as redness, heat, swelling, and pain, as well as compromised physiological processes, can be brought on by the cells' response to inflammation. Numerous diseases, such as cancer, stroke, and arthritis, have pathological processes that involve inflammation.[13]

Results obtained after performing preliminary phytochemical investigation confirmed existence of carbohydrates, phytosterols, tannins, triterpenoids, steroids and flavonoids.

Natural products contain bioactive compounds that hold potential for the development of novel pharmaceutical treatments.[14] The initial phytochemical screening may help identify plant bioactive components, which may result in the creation of new medications.[15] Many plants contains tannins, phenolic components, such as flavonoids, phenolic acids etc. are the main source of their antioxidant action. Studies have indicated that the aforementioned plant-derived chemicals possess anti-inflammatory, anti-atherosclerotic and anti-carcinogenic properties.[16] The search for new anti-inflammatory drugs to treat inflammation as soon as possible has received a lot of attention nowadays.[17]

The model known as paw-edema induced by carrageenan induction is often used to evaluate acute anti-inflammatory medications. Via the vascular endothelium, which expresses adhesion molecules and encourages inflammatory cell migration into wounded tissue, cell recruitment at the injury site causes inflammation. When administered to rats, it causes inflammation in a biphasic pattern that may be split into early and late phases. During the nearly hour-long early phase, pre-synthesised inflammatory mediators such as histamine, serotonin, and bradykinins are released. After the first hour, early phase mediators initiate the late phase, which is characterized by neutrophil infiltration and increased prostaglandin synthesis by cyclooxygenases (COX).[18] Phospholipids are the starting point for the cyclooxygenase and lipoxygenase pathways, which produce prostaglandins and leukotrienes. Vasodilation is induced by prostaglandins, particularly PGE 2 and LTB 4, and is a common sign of inflammation.[19] Two important inflammatory mediators can be blocked significantly by VNMpar which lowers the volume of provocative paw volume.

Table 1: Effect of VNMpar and VNMunpar extracts on Carrageenan induced paw edema in rat.

Sr. No.	Group	Parameter (Paw edema at mm)					
		0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
1.	Control	1.16 ± 0.05	1.42 ± 0.02	1.51 ± 0.01	1.61 ± 0.01	1.59 ± 0.01	1.51 ± 0.06
2.	Indomethacin	1.13 ± 0.21	1.18**** ± 0.16	1.31**** ± 0.05	1.34**** ± 0.03	1.23**** ± 0.03	1.25**** ± 0.06
3.	VNMpar 200 mg/kg	1.19 ± 0.08	1.32 ± 0.04	1.47* ± 0.01	1.57** ± 0.01	1.54*** ± 0.03	1.41*** ± 0.01
4.	VNMpar 400 mg/kg	1.19 ± 0.06	1.30 *± 0.06	1.46 **± 0.02	1.56***± 0.02	1.53**** ± 0.01	1.39**** ± 0.04
5.	VNMunpar 200 mg/kg	1.15 ± 0.03	1.40 ± 0.02	1.47* ± 0.01	1.57** ± 0.02	1.55** ± 0.01	1.43** ± 0.00
6.	VNMunpar 400 mg/kg	1.12 ± 0.12	1.30* ± 0.02	1.46** ± 0.01	1.57** ± 0.01	1.54*** ± 0.01	1.41*** ± 0.02

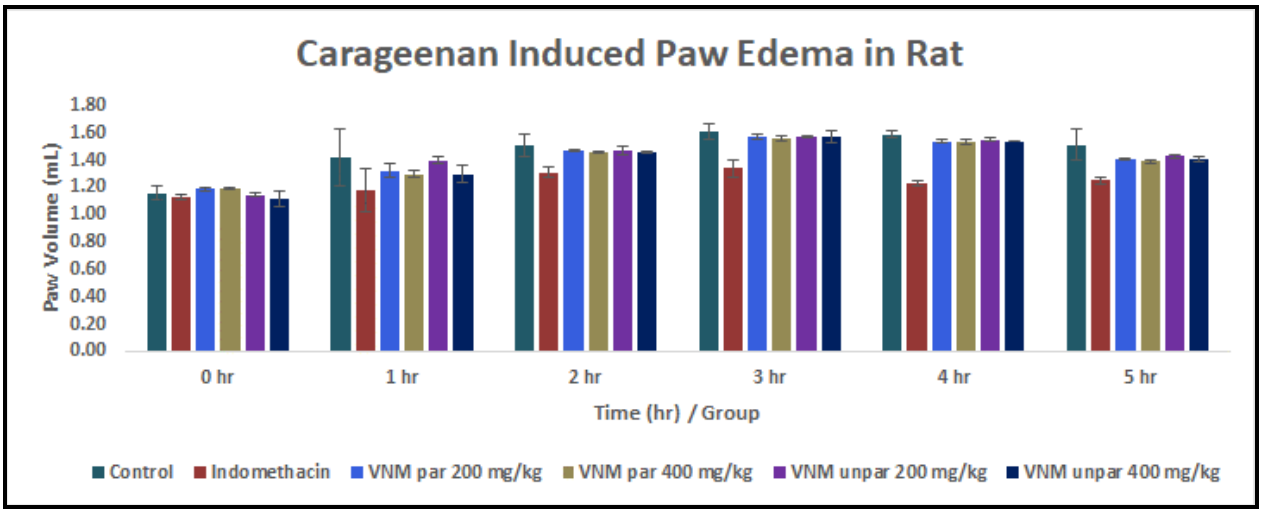

Figure 1: Effect of VNMpar and VNMunpar extracts on Carrageenan induced paw edema in rat

Pretreatment of VNMpar at 400 mg/kg results in dramatically reduction of rat paw volume (****=$p<0.0001$) at 4 and 5 hour's time interval while Indomethacin (10 mg per kg, p.o) as well greatly decreased (**** = $p<0.0001$) paw volume (Fig. 1).

A fundamental and early model of acute inflammation used to evaluate potential anti-inflammatory medicines is vascular permeability induced by acetic acid in mice. It was believed that acetic acid produced mice's nociception and muscle constriction by indirectly promoting prostaglandin and associated mediator synthesis in the peritoneal cavity.[20]

Moreover, phospholipase A2 and PLA2, fibrinolysin, histamine and kinin are inflammatory mediators that may exacerbate edema. By stimulating vasodilation and raising vascular permeability, these mediators cause edema.[21]

Acetic acid (10ml/kg) was injected intraperitoneally. VNMpar extract (200, 400 mg / kg, per oral) dose dependently greatly decreased (**** = $p<0.001$) acetic acid-induced plasma exudation in mice as compared to VNMunpar extract. Indomethacin (10 mg / kg, p.o) also clearly, greatly inhibited (****=$p<0.001$) the exudation (Fig. 2). The mice receiving VNMpar and VNMunpar extract treatment did not develop stomach ulcers or hemorrhage after receiving inflammatory medication.

Table 2: Effect of VNMpar and VNMunpar extracts on Acetic acid induced vascular permeability in Mice

Sr. No.	Group	Parameter (Concⁿ. of dye)
1.	Control	3.27 ± 0.0.05
2.	Indomethacin	2.01**** ± 0.12
3.	VNMpar 200 mg/kg	2.79*** ± 0.10
4.	VNMpar 400 mg/kg	2.53**** ± 0.45
5.	VNMunpar 200 mg/kg	2.90** ± 0.05
6.	VNMunpar 400 mg/kg	2.88** ± 0.05

n=6, Values are expressed as Mean±S.E.M.
*= p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001 when compared to control group. Statistically analyzed by One Way ANOVA followed by Dunnett test.

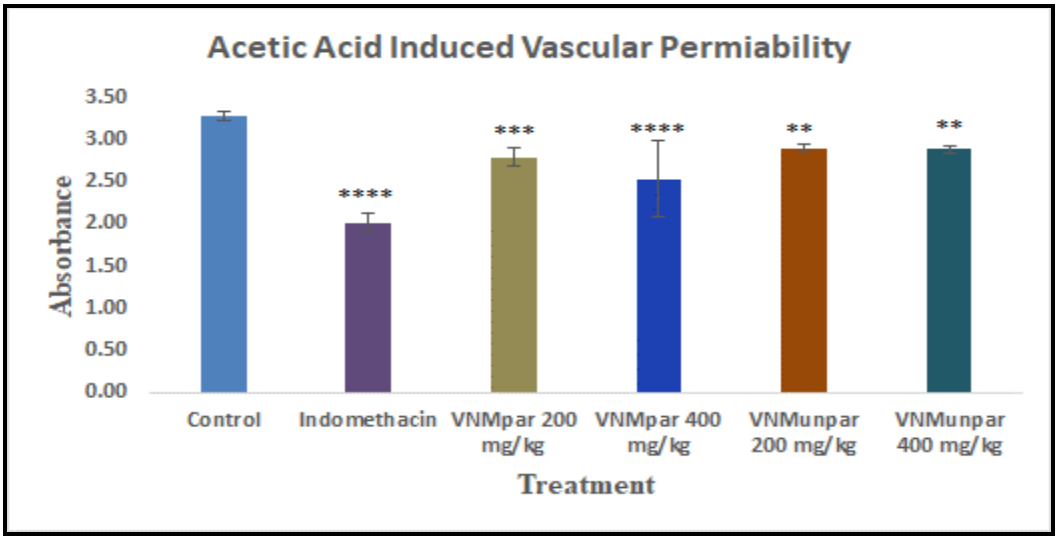

Figure 2: Effect of VNMpar and VNMunpar extracts on Acetic acid induced vascular permeability in Mice

In this model, the VNMpar extract reduced inflammation and the enhanced vascular permeability brought on by acetic acid. These results suggest that by inhibiting the activity of inflammatory mediators, VNMpar extract may have an anti-inflammatory impact. A few phytochemicals viz. flavonoids, polyphenolic compounds and triterpenes, have been demonstrated for having anti-

inflammatory and antioxidant qualities in animal models. The VNMpar extract contains phytochemicals like flavonoids, polyphenols, glycosides, saponins, and reducing sugars.

Because of this, anti-inflammatory properties of VNMpar extract may be associated with availability of various phytochemicals, particularly flavonoids and polyphenols. It has been demonstrated that flavonoids have potent anti-inflammatory properties, making them useful for the treatment of chronic inflammatory illnesses.[22,23]

CONCLUSION

The outcome of this study is the Evaluation of *Vitex negundo* Linn. in parasitized and unparasitized form for Anti-inflammatory activity. VNMpar and VMNunpar extracts were evaluated in vivo against vascular permeability induced by acetic acid in mice and rat paw edema induced by carrageenan. This provides scientific support for its long-standing applications in the treatment of inflammatory conditions. The findings demonstrate the high number of secondary metabolites present in the VNMpar.

In order to promote healthy living, society will benefit from additional research on the isolation and identification of phytochemical constituents from plant extracts for biological activity, toxicity effect, and pharmacological in-vivo research.

CONFLICT OF INTEREST

No conflicts of interest have been reported by the authors.

REFERENCES:

1. Fernandes, Z. (2018). In Vitro Anti Inflammatory Activity on the Ethanolic bark extract of *Persea americana* M. *Research Journal of Pharmacy and Technology*. 11(12): 5517-5519. doi: 10.5958/0974-360X.2018.01004.1

2. Santos, J.B.X., Passos, J.G.R. and Gomes, J.A.S. (2022). Topical gel containing phenolic-rich extract from Ipomoea pes-capre leaf (Convolvulaceae) has anti-inflammatory, wound healing, and antiophidic properties. *Biomedicine & Pharmacotherapy*. (149): 112921. doi: https://doi.org/10.1016/j.biopha.2022.112921

3. Sasikala, V., Saravanan, S. and Parimelazhagan T. (2011). Analgesic and anti-inflammatory activities of Passiflora foetida L. *Asian Pacific Journal of Tropical Medicine*. 4(8):600-603. doi: 10.1016/S1995-7645(11)60155-7

4. Lubna, A., Mohd, M. and Showkat, R. M. (2014). Comparative assessment of extraction methods and quantitative estimation of luteolin in the leaves of Vitex negundo Linn. by HPLC. *Asian Pac J Trop Med*. Sep:7S1:S289-93. doi: 10.1016/S1995-7645(14)60248-0.

5. Kambham, V. (2012). Vitex negundo: Medicinal values, biological activities, toxicity studies and phytopharmacological actions. *International Journal of Pharmaceutical and Phytopharmacological Research*. 2(2):126–133.

6. Meena, A.K., Niranjan, U.S. and Rao, M.M. (2011). A review of the important chemical constituents and medicinal uses of *Vitex* genus. *Asian Journal of Traditional Medicines*. 6(2):54–60.

7. Vishwanathan, S.A. and Basavaraj, R. (2010). A review on *Vitex negundo* L.-a medicinally important plant. *Eur. J. Biol. Sci*. April-June 3(1), 30–42.

8. Meena, A.K., Perumal, A. and Kumar. N. (2022) Studies on physicochemical, phytochemicals, chromatographic profiling & estimation and *in-silico* study of Negundoside in roots & small branches of *Vitex Negundo* plant. *Phytomedicine Plus*. (2) 100205. https://doi.org/10.1016/j.phyplu.2021.100205.

9. Anokwah, D., Kwatia, E.A. and Amponsah, I.K. (2022). Evaluation of the anti-inflammatory and antioxidant potential of the stem bark extract and some constituents of Aidia genipiflora (DC.) dandy (rubiaceae). *Heliyon*. Aug 13;8(8):e10082.
doi: 10.1016/j.heliyon.2022.e10082.

10. Mohankumar, R., Prakash, S.E. and Irfan, N. (2022). Mohanraj S, Kumarappan C. Evaluation of analgesic, anti-inflammatory, and antipyretic activities of Ziziphus mauritania Lam leaves in animal models. *Pharmacological Research-Modern Chinese Medicine*. (4):100153 https://doi.org/10.1016/j.prmcm.2022.100153

11. Teles, A.M., Xavier. G.S. and Moreira, W.F. (2022). Talaromyces purpurogenus from a marine-polluted environment inhibits pro-inflammatory markers in LPS-stimulated RAW 264.7 cells and in carrageenan-induced paw edema. *Journal of King Saud University – Science*. June 4(34):102021 https://doi.org/10.1016/j.jksus.2022.102021

12. Zhang, Y., Shu, Z. and Yin, L. (2015). Anti-inflammatory and antinociceptive activities of non-alkaloids fractions from Aconitum flavum in vivo. *Revista Brasileira de Farmacognosia*., 1(25):47-52

13. Jain, A., Anitha, R. and Rajeshkumar, S. (2019). Anti-inflammatory activity of Silver nanoparticles synthesized using Cumin oil. *Research J. Pharm. and Tech.* 12(6):2790-2793. doi: 10.5958/0974-360X.2019.00469.4

14. Ganga, B., Wadud, A. and Jahan, N. (2021) Makbul SAA, Anti-inflammatory and analgesic activity of Habbe Gule Aakh, A polyherbal Unani formulation in animal models. *J. Ayurveda Integr. Med*. Jan – Mar 12(1):9–12. doi: 10.1016/j.jaim.2018.01.001

15. Shinde, P.R., Gujrani, P.V. and Gupta, A.R. (2021). Exploration of pharmacognostic, phytochemical and antibacterial potential of Rhoeo discolor Hance. *J. Pharmacogn. Phytochem.* 10(1):1625–1630.

16. Hapidin, H., Hashim, N.M. and Kasiram, M.Z. (2022). Abdullah H. The effects of polyphenol, tannic acid, or tannic acid in combination with pamidronate on human osteoblast cell line metabolism. *Molecules*. 27 (2):451. https://doi.org/10.3390/molecules27020451

17. Ghias, M., Shah, S.W.A. and Al-Joufi, F.A. (2022). In vivo antistress effects of synthetic flavonoids in mice: behavioral and biochemical approach. *Molecules*. 27 (4):1402. https://doi.org/10.3390/molecules27041402

18. Ayertey, F., Ofori-Attah, E. and Antwi, S. (2021). Anti-inflammatory activity and mechanism of action of ethanolic leaf extract of Morinda lucida Benth. *Journal of Traditional and Complementary Medicine*. May 11(3):249-258
https://doi.org/10.1016/j.jtcme.2020.07.001

19. Santos, E.S., Abrantes Coelho, G.L. and Saraiva Fontes Loula, Y,K. (2022). Hypoglycemic, hypolipidemic, and anti-inflammatory effects of beta-pinene in diabetic rats, *Evid.-Base. Complem. Alternat. Med.* :1–8 https://doi.org/10.1155/2022/8173307

20. Wang, Y., Yu, C. and Pan, Y. (2012). A novel synthetic mono-carbonyl analogue of curcumin, A13, exhibits anti-inflammatory effects in vivo by inhibition of inflammatory mediators. *Inflammation*. April 35 (2):594–604 doi: 10.1007/s10753-011-9350-4

21. Li, Y.C., Xian, Y.F. and Ip, S.P. (2011). Anti-inflammatory activity of patchouli alcohol isolated from Pogostemonis Herba inanimal models. *Fitoterapia*., Dec 82(8):1295–1301. doi: 10.1016/j.fitote.2011.09.003

22. Aquila, S., Giner, R. M. and Recio, M.C. (2009). Spegazzini ED, Ríos JL. Anti-inflammatory activity of flavonoids from Cayaponia tayuya roots. *J Ethnopharmacol*. Jan 121(2):333-337. doi: 10.1016/j.jep.2008.11.002

23. Pan, M.H., Ching-Shu Lai, C.S. and Ho, C.T. (2010). Anti-inflammatory activity of natural dietary flavonoids. *Food Funct.* Oct 1(1):15-31. doi: 10.1039/c0fo00103a.