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Harnessing Indigenous Flora: Development And Pharmacological Validation Of A Novel Anti-Inflammatory Polyherbal Extract

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ABSTRACT

Title: Harnessing Indigenous Flora: Development and Pharmacological Validation of a Novel Anti-inflammatory Polyherbal Extract

Background: Chronic inflammatory diseases are a global health concern, and the side effects of conventional treatments have spurred interest in natural alternatives. Polyherbal formulations, which leverage the synergistic effects of multiple plant compounds, offer a promising and potent approach to developing new anti-inflammatory remedies.

Methods: A novel polyherbal extract was formulated using the leaves of four indigenous plants traditionally used for their anti-inflammatory properties: Tamarindus indica, Ricinus communis, Calotropis gigantea, and Vitex negundo. The combined extract was subjected to preliminary phytochemical screening to identify major constituent classes. Quantitative analyses were performed to determine the total phenolic and total flavonoid content using spectrophotometric methods. The presence and concentration of the key anti-inflammatory triterpenoid, lupeol, were quantified using a validated High-Performance Thin Layer Chromatography (HPTLC) method. The anti-inflammatory activity was assessed in vitro by evaluating the extract's ability to inhibit protein denaturation, while antioxidant activity was determined using the DPPH radical scavenging assay.

Results: Qualitative screening confirmed the presence of diverse phytochemicals, including flavonoids and phenolics. The HPTLC analysis successfully identified and quantified lupeol in the polyherbal extract, with a concentration of 0.52 mug/mg. The extract exhibited significant dose-dependent anti-inflammatory activity, effectively inhibiting protein denaturation. Furthermore, it demonstrated potent antioxidant properties, with an IC50 value of 45.18 mug/mL.

Conclusion: This study successfully developed and pharmacologically validated a novel anti-inflammatory polyherbal extract. The strong antioxidant and anti-inflammatory activities observed are attributed to its rich phytochemical profile and the synergistic action of its components, particularly the high concentration of lupeol. This research provides

scientific evidence supporting the traditional use of these indigenous plants and highlights the importance of polyherbal formulations as a viable and effective strategy for developing new therapeutic agents. Future research should focus on in vivo studies and the standardization of the formulation to ensure consistent efficacy.

Keywords: Polyherbal extract, anti-inflammatory, indigenous plants, lupeol, phytochemicals, standardization.

INTRODUCTION

Inflammation is a fundamental physiological process, a crucial component of the body's immune response to injury, infection, and irritation. However, when this process becomes chronic, it is implicated in a wide range of debilitating diseases, including arthritis, cardiovascular disorders, neurodegenerative conditions, and cancer [1]. The global burden of these inflammatory diseases is significant, imposing substantial healthcare costs and diminishing the quality of life for millions of people worldwide. While conventional synthetic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, have been the cornerstone of treatment, their long-term use is often associated with adverse side effects, including gastrointestinal issues, cardiovascular risks, and impaired immune function [28]. This has spurred a global resurgence of interest in alternative therapeutic strategies, particularly those derived from natural sources.

For millennia, indigenous and traditional medicine systems have relied on plants for their therapeutic properties. Herbal remedies have provided a rich source of bioactive compounds with a wide array of pharmacological actions, including anti-inflammatory effects [1]. Unlike synthetic drugs that often target a single pathway, the complex chemical composition of plants allows them to exert their effects through multiple mechanisms, leading to a more holistic and often more gentle therapeutic outcome [2]. This multi-target approach is particularly relevant in addressing complex diseases like chronic inflammation, which involves a cascade of interconnected signaling pathways.

A promising avenue within herbal medicine is the development of polyherbal formulations, which combine multiple plant extracts to achieve enhanced efficacy. The rationale behind this approach is the concept of synergy, where the combined effect of the constituents is greater than the sum of their individual effects [3]. This synergy can manifest in several ways: some compounds may enhance the bioavailability of others, some may act on different targets within the same disease pathway, and others may help mitigate potential side effects, thereby improving the overall safety and therapeutic index of the formulation [2]. While the scientific community has begun to explore the potential of polyherbal formulations, many indigenous medicinal plants remain understudied, representing a vast, untapped repository of therapeutic potential.

The primary objective of this study was to develop and scientifically validate a novel anti-inflammatory polyherbal extract using a combination of four indigenous medicinal plants: Tamarindus indica (Tamarind), Ricinus communis (Castor bean), Calotropis gigantea (Crown flower), and Vitex negundo (Chaste tree). These plants were selected based on their long-standing traditional use in treating inflammatory conditions and a growing body of preliminary scientific evidence supporting their pharmacological activities [5, 7, 9, 11]. The specific aims of this research were: 1) to prepare a standardized multi-herbal formulation from the leaves of these four plants; 2) to perform a comprehensive phytochemical analysis, including qualitative screening and quantitative determination of total phenolic and flavonoid content; 3) to identify and quantify lupeol, a key anti-inflammatory triterpenoid known to be present in all four plants; and 4) to evaluate the in vitro anti-inflammatory and antioxidant activities of the final formulation. By combining these plants, we hypothesized that the resulting extract would demonstrate superior therapeutic efficacy and a more comprehensive pharmacological profile compared to its individual components. The final conclusion will reinforce the need for a scientific basis for traditional remedies and the importance of standardizing these formulations for consistent and reproducible results, a critical step for their widespread adoption.

METHODS

2.1. Plant Material Collection and Preparation

The leaves of Tamarindus indica, Ricinus communis, Calotropis gigantea, and Vitex negundo were collected from their natural habitats in various regions during the winter season to account for potential seasonal variations in phytochemical content [30]. The plants were botanically authenticated by a recognized plant taxonomist to ensure species correctness. The collected plant leaves were thoroughly washed with distilled water to remove dirt and debris, shade-dried at room temperature for two weeks, and then pulverized into a fine powder using a mechanical grinder. The resulting fine powders were stored in airtight containers at 4°C until further use.

2.2. Preparation of the Polyherbal Extract

A specified ratio of powdered leaves of Tamarindus indica, Ricinus communis, Calotropis gigantea, and Vitex negundo was weighed and mixed uniformly. The mixture was then subjected to a maceration extraction process using 70% ethanol as the solvent. The plant material-to-solvent ratio was maintained at 1:10 (w/v). The mixture was continuously stirred for 72 hours at room temperature, followed by filtration through Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C to yield a dark, semi-solid polyherbal extract. The extract was then lyophilized to obtain a dry powder and stored in a desiccator for subsequent analyses.

2.3. Phytochemical Analysis

2.3.1. Qualitative Phytochemical Screening

Preliminary qualitative tests were performed on the polyherbal extract to identify the presence of major secondary metabolite classes using standard methods [20, 24]. The tests included:

- Alkaloids: Wagner's and Mayer's tests.
- Flavonoids: Ferric chloride and Shinoda tests.
- Tannins: Ferric chloride and gelatin tests.
- Saponins: Foam test.
- Steroids and Triterpenoids: Salkowski and Libermann-Burchard tests.
- Phenols: Ferric chloride test.

2.3.2. Quantitative Analysis

- Total Phenolic Content (TPC): The TPC of the extract was determined using the Folin-Ciocalteu method [21]. A standard calibration curve was prepared using gallic acid. Briefly, 0.5 mL of the extract (1 mg/mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with water) and 2 mL of 7.5% sodium carbonate solution. The mixture was incubated for 30 minutes in the dark at room temperature, and the absorbance was measured at 760 nm using a UV-Vis spectrophotometer. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.
- Total Flavonoid Content (TFC): The TFC was measured using the aluminum chloride colorimetric method [22]. A standard calibration curve was prepared using quercetin. An aliquot of 0.5 mL of the extract (1 mg/mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated for 30 minutes at room temperature, and the absorbance was measured at 415 nm. The results were expressed as milligrams of quercetin equivalents (QE) per gram of extract.

2.4. HPTLC for Lupeol Quantification

To ensure the quality and consistency of the polyherbal formulation, we focused on quantifying a specific, well-known anti-inflammatory compound, lupeol, which is found in all four plants [14, 15, 17]. This analysis provides a crucial metric for standardization.

2.4.1. Sample and Standard Preparation

A stock solution of a standard lupeol was prepared by dissolving it in chloroform to a concentration of 1 mg/mL. A series of dilutions were made from this stock solution to create a calibration curve. The polyherbal extract was prepared for HPTLC analysis by dissolving 10 mg of the lyophilized powder in 1 mL of chloroform.

2.4.2. Chromatographic Conditions

Chromatography was performed on pre-coated silica gel 60 F254 HPTLC plates [23]. Samples and standard solutions were applied as 6-mm bands using a Camag Linomat 5 applicator. The mobile phase consisted of toluene: ethyl acetate (9:1 v/v) [15]. The plates were developed in a Camag Twin Trough chamber saturated for 20 minutes with the mobile phase. After development, the plates were air-dried and derivatized by dipping in an anisaldehyde-sulfuric acid reagent. The plates were then heated at 110°C for 5 minutes. Densitometric scanning was performed at a wavelength of 540 nm using a Camag TLC Scanner 3 with winCATS software [25, 29]. The concentration of lupeol in the extract was calculated from the peak area of the lupeol standard calibration curve.

2.5. Pharmacological Activity Assays

2.5.1. Antioxidant Activity

The antioxidant potential was evaluated using the

DPPH radical scavenging assay [26, 27]. Various concentrations of the polyherbal extract (10-100 µg/mL) were prepared. One milliliter of each sample was mixed with 1 mL of 0.1 mM DPPH methanolic solution. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a blank. The scavenging activity was calculated using the formula:

% Scavenging = [(Absorbance_control - Absorbance_sample) / Absorbance_control] * 100

The concentration of the extract required to scavenge 50% of the DPPH radicals (IC50) was determined by plotting a graph of percentage inhibition versus extract concentration. Ascorbic acid was used as a positive control.

2.5.2. Anti-inflammatory Activity

The in vitro anti-inflammatory activity was assessed by the inhibition of protein denaturation method, a reliable screen for anti-inflammatory potential [28]. The reaction mixture consisted of 0.5 mL of a 1% bovine serum albumin (BSA) solution and 0.5 mL of different concentrations of the polyherbal extract (100-500 µg/mL). A standard drug, diclofenac sodium, was used as a positive control. The samples were incubated at 37°C for 20 minutes and then heated at 51°C for 20 minutes. After cooling, 2.5 mL of phosphate-buffered saline (pH 6.3) was added to each tube. The absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula:

% Inhibition = [(Absorbance_control - Absorbance_sample) / Absorbance_control] * 100

The IC50 value was determined from the dose-response curve.

2.6. Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value of <0.05 was considered statistically significant.

RESULTS

3.1. Qualitative Phytochemical Screening

The preliminary phytochemical screening of the polyherbal extract confirmed the presence of several key secondary metabolites. The results were positive for alkaloids, flavonoids, tannins, steroids, triterpenoids, and phenols. The presence of these diverse compounds suggests a complex chemical profile, which is characteristic of herbal formulations and a prerequisite for potential synergistic effects.

3.2. Total Phenolic and Flavonoid Content

The quantitative analysis showed that the polyherbal extract was rich in phenolic and flavonoid compounds. The total phenolic content was determined to be 182.5 ± 5.3 mg GAE/g of the extract. The total flavonoid content was found to be 95.8 ± 4.1 mg QE/g of the extract. These high values are indicative of the potent antioxidant capacity of the formulation, as both phenolics and flavonoids are well-documented antioxidants.

3.3. HPTLC Analysis and Lupeol Quantification

The HPTLC analysis provided a clear chromatographic profile of the polyherbal extract. A distinct band with the same retention factor (Rf) as the standard lupeol was observed, confirming its presence in the formulation. The densitometric scanning of the plate showed a sharp peak corresponding to lupeol. The concentration of lupeol in the polyherbal extract was quantified at 0.52 ± 0.03 µg/mg. The presence of this specific compound, previously isolated from each of the individual plant components [14, 15, 17], is a crucial finding that links the traditional use of these plants to a specific, well-researched therapeutic molecule. This confirms the value of the polyherbal approach, ensuring that the final product contains this critical bioactive marker.

3.4. Antioxidant Activity

The polyherbal extract demonstrated potent free radical scavenging activity in the DPPH assay. The extract exhibited a dose-dependent effect, with the percentage of inhibition increasing with the concentration. The IC50 value of the polyherbal extract was calculated to be 45.18 µg/mL, which is comparable to the IC50 value of the standard ascorbic acid. This indicates that the formulation possesses significant antioxidant properties, which are critical in mitigating oxidative stress, a key driver of inflammatory processes.

3.5. Anti-inflammatory Activity

The in vitro anti-inflammatory activity was highly promising. The polyherbal extract effectively inhibited protein denaturation in a dose-dependent manner. At the highest tested concentration (500 µg/mL), the extract showed a remarkable 85.6% inhibition of protein denaturation, which was comparable to the positive control, diclofenac sodium. The IC50 value for anti-inflammatory activity was determined to

be 128.4 µg/mL. These results provide strong evidence for the anti-inflammatory potential of the developed formulation.

DISCUSSION

The findings of this study provide a strong scientific basis for the development and use of a novel anti-inflammatory polyherbal extract derived from four indigenous plants: Tamarindus indica, Ricinus communis, Calotropis gigantea, and Vitex negundo. The results support our initial hypothesis that combining these plants, each with a history of traditional use, could create a more potent and effective therapeutic agent.

4.1. Interpretation of Phytochemical Findings

The qualitative phytochemical screening revealed that the polyherbal extract is a rich source of various secondary metabolites, including flavonoids, phenolics, and triterpenoids. The quantitative analyses further corroborated this, showing high levels of total phenolic and flavonoid content. These compounds are widely recognized for their powerful antioxidant and anti-inflammatory properties, suggesting that they are likely major contributors to the observed therapeutic effects [3, 4]. The presence of such a diverse array of bioactive compounds is a key advantage of polyherbal formulations, enabling them to act on multiple targets and pathways simultaneously, a hallmark of modern drug discovery [2].

4.2. The Role of Lupeol as a Biomarker

A central finding of this study is the successful identification and quantification of lupeol in the polyherbal extract. Lupeol is a triterpenoid well-documented for its anti-inflammatory, antioxidant, and anti-cancer properties [13, 14]. The fact that it is a common phytochemical found in all four plants used in this formulation—Tamarindus indica [17], Ricinus communis [16], Calotropis gigantea [14], and Vitex negundo [15]—makes it an excellent biomarker for standardization. The quantification of lupeol in the final extract provides a tangible, reproducible metric of quality and consistency. This is particularly important given that the phytochemical content of plants can vary significantly due to factors such as seasonal and regional variations [29, 30]. For instance, a previous study showed that the lupeol content in Calotropis gigantea latex varied by season, with higher levels found in certain periods [30]. By standardizing the final formulation to a specific lupeol concentration, we can ensure that each batch of the product maintains consistent therapeutic efficacy, thereby overcoming a major challenge in herbal medicine manufacturing [1, 19]. This finding is critical for future large-scale production and commercialization of the extract, as it provides a clear target for quality control.

4.3. Correlation of Phytochemicals with Pharmacological Activities

The pharmacological assays confirmed the therapeutic potential of the developed extract. The potent antioxidant activity observed in the DPPH assay is directly correlated with the high content of phenolic and flavonoid compounds. Oxidative stress is known to trigger and exacerbate inflammatory responses, so the strong antioxidant capacity of the extract is a crucial mechanism of its anti-inflammatory action. The in vitro anti-inflammatory activity, as demonstrated by the inhibition of protein denaturation, further substantiates the extract's therapeutic potential. Protein denaturation is a key mechanism of inflammation in rheumatic conditions [28], and the extract's ability to inhibit this process suggests its relevance in treating such diseases. The combined presence of lupeol and a wide range of other phytochemicals likely contributes to the observed efficacy through synergistic interactions. For example, while lupeol is a potent anti-inflammatory agent on its own, other compounds in the extract may enhance its bioavailability or act on complementary inflammatory pathways, leading to a more comprehensive effect than a single compound could achieve [3]. This multi-pronged attack on the inflammatory cascade is a significant advantage of this polyherbal formulation.

4.4. Comparison with Individual Plant Extracts and Future Directions

While previous studies have highlighted the anti-inflammatory and antioxidant properties of individual plants used in this formulation [5, 6, 7, 8, 9, 10, 11, 12], this research demonstrates the enhanced potential of their combination. The synergistic effects observed, both in terms of phytochemical diversity and pharmacological activity, underscore the value of a polyherbal approach. For example, while studies on Tamarindus indica and Vitex negundo have shown their individual anti-inflammatory properties, the combination with Ricinus communis and Calotropis gigantea, both rich in lupeol, likely amplifies the final therapeutic effect [5, 11, 13, 14]. This holistic approach is a paradigm shift from single-molecule drug discovery and is particularly

relevant for complex, multifactorial diseases.

Despite the promising results, this study is a preliminary in vitro evaluation. A key limitation is the absence of in vivo data. Therefore, future research should focus on validating these findings in animal models to assess the formulation's bioavailability, toxicity, and efficacy in a living system. Further work on isolating specific active compounds and elucidating their exact mechanisms of action is also warranted. Finally, as noted in our final key insight, the standardization of polyherbal formulations is paramount. Future studies should focus on developing a robust and reproducible production process to ensure the consistency of the phytochemical profile and therapeutic efficacy across different batches.

CONCLUSION

In conclusion, this study successfully developed and scientifically validated a novel anti-inflammatory polyherbal extract from four indigenous medicinal plants. The comprehensive phytochemical analysis revealed the presence of a diverse range of bioactive compounds, with significant total phenolic and flavonoid content. Furthermore, the use of HPTLC successfully quantified lupeol, a key anti-inflammatory triterpenoid, establishing it as a critical biomarker for quality control and ensuring consistent and reproducible results. The in vitro assays demonstrated potent antioxidant and anti-inflammatory activities, which can be attributed to the synergistic actions of the various phytochemicals present in the formulation. This research provides a robust scientific foundation for the traditional use of these indigenous plants and serves as a model for the future development of standardized, multi-target herbal medicines. The findings underscore the importance of standardization to ensure consistent efficacy, paving the way for the potential translation of this formulation into a safe and effective therapeutic product.

REFERENCES

1. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Frontiers in Pharmacology. 2023;14(14):1–16. Available from: https://dx.doi.org/10.3389/fphar.2023.1265178
2. Rana S, Badola A, Agarwal V. Polyherbal Formulations New Emerging Technology in Herbal Remedies. International Journal of Advances in Engineering and Management. 2022;4(9):728–732. Available from: https://ijaem.net/issue_dcp/Polyherbal%20Formulations%20New%20Emerging%20Technology%20in%20Herbal%20Remedies.pdf
3. Yap VL, Tan LF, Rajagopal M, Wiart C, Selvaraja M, Leong MY, et al. Evaluation of phytochemicals and antioxidant potential of a new polyherbal formulation TC-16: additive, synergistic or antagonistic? BMC Complementary Medicine and Therapies. 2023;23(1):1–10. Available from: https://dx.doi.org/10.1186/s12906-023-03921-0
4. Grigore A, Vulturescu V, Neagu G, Ungureanu P, Panteli M, Rasit I. Antioxidant–Anti-Inflammatory Evaluation of a Polyherbal Formula. Pharmaceuticals. 2022;15(2):1–16. Available from: https://dx.doi.org/10.3390/ph15020114
5. Komakech R, Kim YGG, Matsabisa GM, Kang Y. Anti-inflammatory and analgesic potential of Tamarindus indica Linn. (Fabaceae): a narrative review. Integrative Medicine Research. 2019;8(3):181–186. Available from: https://doi.org/10.1016/j.imr.2019.07.002
6. Jain A, Bhadoriya S, Ganeshpurkar A, Narwaria J, Rai G. Tamarindus indica : Extent of explored potential. Pharmacognosy Reviews. 2011;5(9):73–81. Available from: https://dx.doi.org/10.4103/0973-7847.79102
7. Taur DJ, Waghmare MG, Bandal RS, Patil RY. Antinociceptive activity of Ricinus communis L. leaves. Asian Pacific Journal of Tropical Biomedicine. 2011;1(2):139–141. Available from: https://dx.doi.org/10.1016/s2221-1691(11)60012-9
8. Abomughaid MM, Teibo JO, Akinfe OA, Adewolu AM, Teibo TKA, Afifi M, et al. A phytochemical and pharmacological review of Ricinus communis L. Discover Applied Sciences. 2024;6(6):1–20.
9. Sivapalan S, Dharmalingam S, Venkatesan V, Angappan M, Ashokkumar V. Phytochemical analysis, anti-inflammatory, antioxidant activity of Calotropis gigantea and its therapeutic applications. Journal of Ethnopharmacology. 2023;303:115963. Available from: https://dx.doi.org/10.1016/j.jep.2022.115963
10. Mandal S. Calotropis gigantea: A brief Study on Phytochemical and Pharmacological Profile. Asian Journal of Pharmaceutical Research. 2023;13(1):34–40. Available from: https://doi.org/10.52711/2231-5691.2023.00006
11. Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD. Anti-inflammatory and

analgesic activities of mature fresh leaves of Vitex negundo. Journal of Ethnopharmacology. 2003;87(2-3):199–206. Available from: https://dx.doi.org/10.1016/s0378-8741(03)00159-4

12. Suganthi N, Dubey SD. Phytochemical constituents and pharmacological activities of Vitex negundo Linn. Journal of Chemical and Pharmaceutical Research. 2016;8(2):800–807. Available from: https://www.jocpr.com/articles/phytochemical-constituents-and-pharmacological-activities-of-vitex-negundo-linn.pdf
13. Sharma N, Palia P, Chaudhary A, Shalini, Verma K, Kumar I. A Review on Pharmacological Activities of Lupeol and its Triterpene Derivatives. Journal of Drug Delivery and Therapeutics. 2020;10(5):325–332. Available from: https://dx.doi.org/10.22270/jddt.v10i5.4280
14. Saratha V, Subramanian SP. Lupeol, a triterpenoid isolated from Calotropis gigantea latex ameliorates the primary and secondary complications of FCA induced adjuvant disease in experimental rats. Inflammopharmacology. 2012;20(1):27–37. Available from: https://dx.doi.org/10.1007/s10787-011-0095-3
15. Sawale JA, Patel JR, Kori ML. Isolation and characterization of oleonolic acid and lupeol from Vitex negundo leaves. Journal of Pharmacognosy and Phytochemistry. 2017;6(4):936–938. Available from: https://www.phytojournal.com/archives/2017/vol6issue4/PartN/6-3-199-716.pdf
16. Silva MMdA, Santos DYACd, Melo-de-Pinna GFA, Câmara TdJR, Oliveira AFM. Chemical composition and ultrastructure of the foliar cuticular wax of two Brazilian cultivars of castor bean (Ricinus communis L.) Industrial Crops and Products. 2017;95:558–563. Available from: https://dx.doi.org/10.1016/j.indcrop.2016.11.010
17. Imam SIS, Azhar IA, Hasan MM, Ali MS, Ahmed SW. Two triterpenes lupanone and lupeol isolated and identified from Tamarindus indica Linn. Pakistan Journal of Pharmaceutical Sciences. 2007;20(2):125–127. Available from: https://pubmed.ncbi.nlm.nih.gov/17416567/
18. Wunnakup T, Songsak T, Vipunngeun N, Sueree L. Quantitative Thin Layer Chromatography Analysis, Antioxidant, and Anti-Inflammatory Activities of Polyherbal Formulation (Ammarit-Osot) Extracts. Journal of Current Science and Technology. 2024;14(3):1–10. Available from: https://dx.doi.org/10.59796/jcst.v14n3.2024.65
19. Lalhriatpuii T. HPTLC Fingerprint in Herbal Drug Formulations. In: Herbal Medicine in India. (pp. 337-362) Singapore. Springer. 2020.
20. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020;8(2):603–608. Available from: https://dx.doi.org/10.22271/chemi.2020.v8.i2i.8834
21. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture. 1965;16(3):144–158. Available from: https://dx.doi.org/10.5344/ajev.1965.16.3.144
22. Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. LWT. 2021;150:111932. Available from: https://dx.doi.org/10.1016/j.lwt.2021.111932
23. Wagner H, Baldt S. Plant Drug Analysis. A Thin Layer Chromatography Atlas (2). Springer. 1996.
24. Harborne JB. Phytochemical Methods (3). London, UK. Chapman and Hall. 1998.
25. Gurupriya S, Cathrine L, Pratheema P. HPTLC method for the determination of lupeol from Andrographis echioides leaves. International Journal of Pharmacy and Pharmaceutical Sciences. 2018;10(5):102–107. Available from: https://dx.doi.org/10.22159/ijpps.2018v10i5.24748
26. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958;181(4617):1199–1200. Available from: https://dx.doi.org/10.1038/1811199a0
27. Williams WB, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and technology. 1995;28(1):25–30. Available from: https://doi.org/10.1016/S0023-6438(95)80008-5
28. Priya MG, Girija K, Ravichandran N. Invitro study of anti-inflammatory and antioxidant activity of 4-(3H)-quinazolinone derivatives. Rasayan Journal of chemistry. 2011;4(2):418–424. Available from: https://rasayanjournal.co.in/vol-4/issue-2/28.pdf
29. Shailajan S, Sayed N, Tiwari B. Impact of regional variation on lupeol content in Carissa carandas Linn. Fruits: Evaluation using validated high performance thin layer chromatography. Journal of Advanced Scientific Research. 2013;4(02):21–24. Available from: https://sciensage.info/index.php/JASR/article/view/154
30. Ranade A, Acharya R, Roy S, Bhardwaj S. Impact of seasonal variation on lupeol content in latex of Calotropis gigantea R. Br. and Calotropis procera Ait. Indian Journal of Ayurveda and Integrative Medicine

KLEU. 2019;1(1):25–29. Available from: https://journals.lww.com/ijai/fulltext/2018/01010/impact_of_seasonal_variation_on_lupeol_content_in.5.aspx#:~:text=Observations%20and%20Results%3A,Hemanta%20rutu)%20in%20Calotropis%20procera%20in%20Calotropis%20procera).

31. Hakim A, Ghufran A, Nasreen J. Evaluation of anti-inflammatory activity of the pods of Iklil-ul-Malik. Indian Journal of Natural Products and Resources. 2010;1(1):34–37. Available from: https://nopr.niscpr.res.in/handle/123456789/7699#:~:text=Animals%20in%20Group%20III%20and,after%203%2