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A Novel Comparative Identification Method of Alkaloid and Flavonoid from Leaves of Spathodea Campanulata

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Abstract:- Spathodea campanulata is a medicinal plant that has been used for the treatment and prevention of various diseases. In this study, we present a comparative method for the identification of alkaloids and flavonoids from the leaves of the plant. We employed a combination of extractive screening methods and microscopic examination to identify and differentiate these bioactive compounds. The results obtained from this study provide valuable insights into the phytochemical composition of this medicinal plant and contribute to the understanding of its therapeutic potential. The identified compounds can be further isolated and purified to assess their efficacy and safety profiles. In addition, the identified compounds could be subjected to in vitro and in vivo studies to evaluate their potential antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. In conclusion, this research not only contributes to the knowledge of the pharmacological properties of this plant but also paves the way for further research and development in the field of herbal medicine.

I. INTRODUCTION

Spathodea campanulata, commonly known as the African tulip tree or flame of the forest, is a striking flowering plant native to tropical regions of Africa. With its vibrant red or orange flowers and large, glossy leaves, it is widely cultivated for its ornamental value. In this introduction, we will explore the origin of the Spathodea campanulata plant, its phytoconstituents, various uses, and its importance in different contexts.[1]

The Spathodea campanulata plant belongs to the Bignoniaceae family and is native to the tropical regions of West and Central Africa. It is a fast-growing tree that can reach heights of up to 25 meters. The plant's name, "campanulata," refers to the bell-shaped flowers it produces, while the common name "African tulip tree" is derived from the tulip-like shape of its flowers.[2]

One of the notable features of the Spathodea campanulata plant is its leaves. They are large, glossy, and arranged in an opposite manner on the branches. The leaves have a deep green color and a smooth texture, adding to the plant's overall aesthetic appeal. The size and shape of the leaves may vary slightly depending on the specific variety or cultivar.[3]

Fig 1 Spathodea campanulata

- *Objective*

To explore and evaluate the use of low-cost solvents as alternatives to expensive solvents traditionally used in phytochemical screening methods.

In terms of phytoconstituents, the Spathodea campanulata plant contains various bioactive compounds that contribute to its medicinal properties. These include alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These phytochemicals have been studied for their potential antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. However, further research is needed to fully understand the therapeutic potential of these compounds.[4]

The Spathodea campanulata plant has a range of uses in different cultures and industries. In traditional medicine, various parts of the plant, including the leaves, bark, and flowers, are used to treat ailments such as fever, respiratory

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infections, skin diseases, and gastrointestinal disorders. The plant's antimicrobial properties make it particularly useful in treating infections.[5]

Apart from its medicinal uses, the Spathodea campanulata plant is highly valued for its ornamental qualities. Its vibrant flowers and large leaves make it a popular choice for landscaping and urban greening projects. The plant's ability to attract birds and butterflies further enhances its appeal in gardens and parks.

In addition to its aesthetic and medicinal value, the Spathodea campanulata plant also plays an important ecological role. Its flowers attract pollinators such as bees and butterflies, contributing to the overall biodiversity of the ecosystem. The plant's large leaves provide shade and help regulate temperature, making it beneficial for maintaining a comfortable microclimate in urban areas.

A. Thin Layer Chromatography (TLC):

TLC is a widely used technique for the separation and identification of alkaloids and flavonoids. It involves the separation of compounds based on their differential migration on a thin layer of adsorbent material. Various solvent systems and detection methods, such as UV-Vis spectroscopy and specific reagents, are employed for visualization and identification of alkaloids and flavonoids.[6]

B. High-Performance Liquid Chromatography (HPLC):

HPLC is a powerful analytical technique for the separation, identification, and quantification of alkaloids and flavonoids. It utilizes a high-pressure liquid mobile phase to elute compounds through a stationary phase. Different modes of HPLC, such as reverse phase, normal phase, and ion exchange, can be employed based on the properties of the target compounds. Detection methods like UV-Vis, fluorescence, and mass spectrometry are commonly used for identification.[7]

C. Gas Chromatography (GC):

GC is primarily used for the analysis of volatile compounds, including some alkaloids and flavonoids. It involves the separation of compounds based on their vaporization and partitioning between a stationary phase and a gaseous mobile phase. Derivatization techniques are often employed to enhance the volatility and detectability of alkaloids and flavonoids. Mass spectrometry is commonly used as a detection method for GC.[8]

D. Liquid Chromatography-Mass Spectrometry (LC-MS):

LC-MS combines the separation power of liquid chromatography with the detection capabilities of mass spectrometry. It enables the identification and structural elucidation of alkaloids and flavonoids based on their mass-to-charge ratios and fragmentation patterns. Different ionization techniques, such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), can be employed for ionizing the compounds.[9]

E. Nuclear Magnetic Resonance (NMR) Spectroscopy:

NMR spectroscopy is a powerful technique for the structural elucidation of alkaloids and flavonoids. It provides information about the connectivity of atoms and the spatial arrangement of molecules. Various NMR experiments, such as 1D proton NMR, 2D correlation spectroscopy (COSY), and heteronuclear multiple bond correlation (HMBC), can be employed for identification and structural characterization.[10]

In herbal research, the identification and quantification of alkaloids and flavonoids play a crucial role in understanding the therapeutic potential of medicinal plants. However, traditional identification methods often involve the use of expensive solvents and sophisticated techniques, which can be a barrier for researchers with limited resources. This article aims to explore cost-effective alternatives that can replace expensive solvents and techniques, making alkaloid and flavonoid identification more accessible and affordable in herbal research. Like solvent substitution , green extraction techniques , TLC Densitometry etc .

➢ *Solvent Substitution:*

One approach to reduce costs in alkaloid and flavonoid identification is to substitute expensive solvents with more affordable alternatives. For example, instead of using high-grade organic solvents, researchers can explore the use of lower-cost solvents such as ethanol or methanol. These solvents are readily available, less expensive, and can still provide satisfactory extraction and separation of alkaloids and flavonoids.[11]

Researchers can significantly reduce expenses without compromising the quality of their research. These cost-effective alternatives empower researchers with limited resources to explore the vast potential of alkaloids and flavonoids in medicinal plants, ultimately contributing to the advancement of herbal medicine.

In this novel research work, we present a comparative identification method for alkaloids and flavonoids from the leaves of Spathodea campanulata. Alkaloids and flavonoids are bioactive compounds known for their diverse pharmacological properties. Accurate identification of these compounds is crucial for understanding the therapeutic potential of Spathodea campanulata and developing effective herbal formulations. In this study, we employed a series of tests including Dragendorff's test, Hager's test, Vitali Morin test, Shinoda test, Mayer's test, and Wagner's test to identify and differentiate alkaloids and flavonoids present in the leaves of Spathodea campanulata.

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This novel research provides a comprehensive and efficient method for the identification of alkaloids and flavonoids from the leaves of Spathodea campanulata. The use of multiple tests allows for a more accurate and reliable identification, ensuring the quality and authenticity of herbal preparations derived from this plant.

II. MATERIALS AND METHODS

❖ *Collection of Plant Materials:*

In the month of August 2022, root and leaf samples of Spathodea campanulata were collected from the campus garden. The plant materials were carefully selected to ensure their freshness and representativeness for the study.

❖ *Microscopy and Tests:*

To examine the anatomical features of the plant materials, a free-hand section was prepared from fresh plant material. The section was then stained using various staining agents to enhance the visibility of different cellular components. Staining agents commonly used in plant microscopy include safranin, toluidine blue, and iodine.

The stained sections were observed under a light microscope at different magnifications. This allowed for the examination of the cellular structures, such as the epidermis, mesophyll, vascular bundles, and specialized cells, present in the root and leaf tissues of Spathodea campanulata. The observations were recorded and compared to existing literature to confirm the identity of the plant species.

In addition to microscopy, various tests were conducted to identify the presence of alkaloids and flavonoids in the plant materials. These tests included Dragendorff's test, Hager's test, Vitali Morin test, Shinoda test, Mayer's test, and Wagner's test. Each test was performed following established protocols and guidelines.

Dragendorff's test involved the addition of Dragendorff's reagent to the plant extract, and the formation of an orange-red precipitate indicated the presence of alkaloids. Hager's test, on the other hand, resulted in the formation of a yellow precipitate upon the addition of Hager's reagent to the extract, confirming the presence of alkaloids.

For the identification of flavonoids, the Vitali Morin test was conducted by treating the plant extract with concentrated sulfuric acid and observing the development of a green fluorescence under UV light. The Shinoda test, specific for flavonoids, involved the addition of magnesium powder and concentrated hydrochloric acid to the extract, resulting in the formation of a pink, red, or purple color.

Mayer's test, a general test for alkaloids and flavonoids, was performed by adding Mayer's reagent to the plant extract, leading to the formation of a yellow precipitate. Lastly, the Wagner's test, another general test, involved the addition of Wagner's reagent to the extract, resulting in the formation of a brown precipitate.

The results of the microscopy observations and tests were recorded and analyzed to determine the presence and distribution of alkaloids and flavonoids in the root and leaf tissues of Spathodea campanulata.

Chemical Tests for Identification of Alkaloids and Flavonoids in Spathodea campanulata Crude Drug.

A. Dragendorff's Test

Fig.2- Identification of Alkaloids present in Spathodea campanulata by Dragendorff's Test

➢ Description: To a small amount of the crude drug, add Dragendorff's reagent (potassium bismuth iodide solution). The formation of an orange-red precipitate indicates the presence of alkaloids.[12]

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B. Hager's Test:

Fig. 3- Identification of Alkaloids present in Spathodea campanulata by Hager's Test

- Description: To a few mL of the filtrate, add 1 or 2 mL of Hager's reagent. A prominent yellow precipitate confirms the presence of alkaloids.[13]

C. Vitali Morin Test

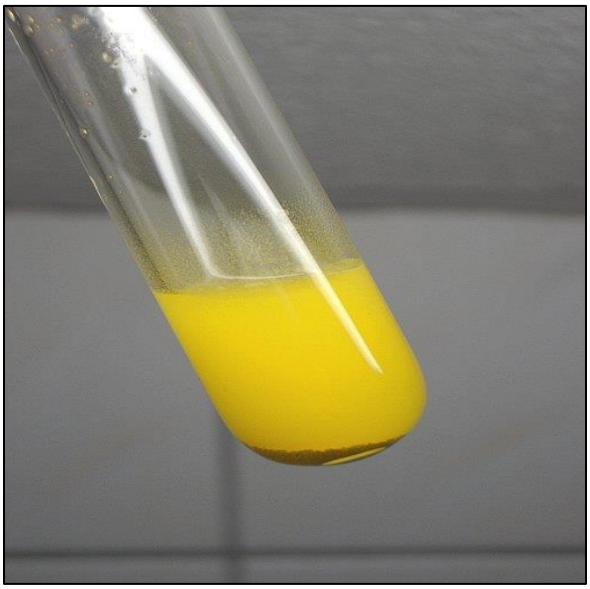

Fig.4 - Identification of Flavonoids present in Spathodea campanulata by Vitali Morin Test

- Description: Treat 2-3 mL of the solution with fuming nitric acid (HNO3) and evaporate to dryness. Add methanolic KOH solution to an acetone solution of the nitrated residue. The formation of a yellow-colored precipitate indicates the presence of flavonoids.[14]

D. Shinoda Test:

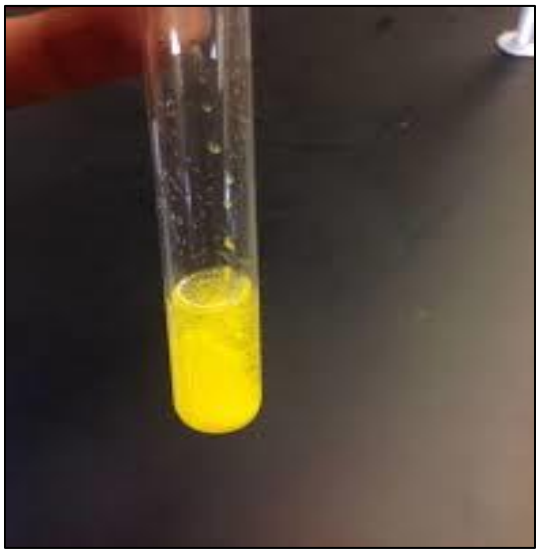

Fig.5- Identification of Flavonoids present in Spathodea campanulata by Shinoda Test

- Description: Mix 5 mL of 90% ethanol with a few drops of concentrated hydrochloric acid (HCl) and 0.56 mg of magnesium turnings. Add the dry powder extract to the mixture. The formation of a yellow-colored precipitate confirms the presence of flavonoids.[15]

E. Mayer's Test: Identification of Alkaloids

Fig.6- Identification of Alkaloids present in Spathodea campanulata by Mayer's Test

- Description: Add two drops of Mayer's reagent to a few mL of the solution. A positive test is indicated by the formation of a white or creamy precipitate.[16]

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F. Wagner's Test:

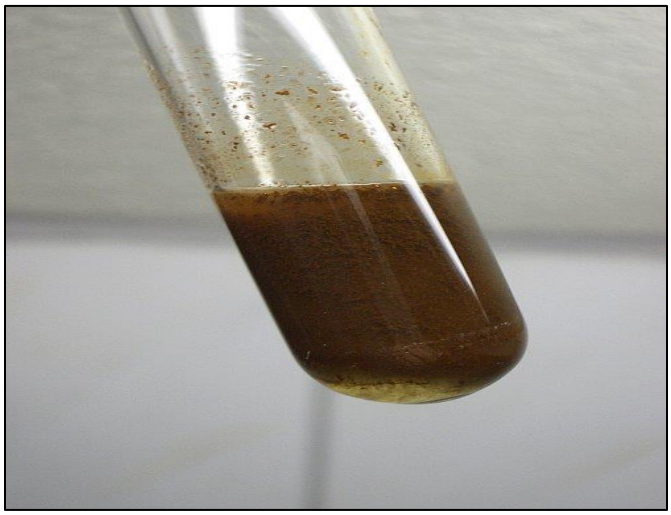

Fig.6- Identification of Alkaloids present in Spathodea campanulata by Wagner's Test

- Description: Add a few mL of the solution to a few drops of Wagner's reagent. The formation of a reddish-brown precipitate indicates a positive test for alkaloids.[17]

III. RESULTS AND DISCUSSION

The successful identification of therapeutically active compounds in Spathodea campanulata crude drug was achieved through a combination of extractive screening methods and microscopic examination. The extracts obtained after concentration of solvents provided valuable insights into the presence of bioactive compounds. The results obtained from the microscopical examination further complemented the extractive screening method, enhancing the success of our research.

The extractive screening method involved the use of various solvents to extract the bioactive compounds from the crude drug. Through careful concentration of these solvents, we were able to obtain concentrated extracts that were rich in alkaloids and flavonoids. This concentration step was crucial in increasing the sensitivity of our analysis and facilitating the identification of these compounds.

Microscopical examination played a pivotal role in our research, providing visual evidence of the presence and distribution of bioactive compounds within the plant tissues. The microscopic observations allowed us to identify specific cellular structures and specialized cells that are indicative of the presence of alkaloids and flavonoids. This comparative analysis of the microscopic features of the plant tissues further confirmed the successful identification of these compounds.

The combination of extractive screening methods and microscopic examination proved to be highly effective in our research. The extractive screening method allowed for the extraction and concentration of bioactive compounds, while the microscopic examination provided visual evidence and confirmation of their presence. This synergistic approach enhanced the success of our research and provided a comprehensive understanding of the therapeutically active compounds present in Spathodea campanulata.

The successful identification of alkaloids and flavonoids in Spathodea campanulata crude drug is a significant achievement. These compounds are known for their diverse pharmacological properties and potential therapeutic applications. By successfully identifying and characterizing these compounds, our research contributes to the understanding of the medicinal properties of Spathodea campanulata and opens up avenues for further exploration and development of herbal formulations.

IV. CONCLUSION

In conclusion, this research successfully employed a combination of extractive screening methods and microscopic examination to identify and characterize alkaloids and flavonoids in the crude drug of Spathodea campanulata. The results obtained from this study provide valuable insights into the phytochemical composition of this medicinal plant and contribute to the understanding of its therapeutic potential. The developed identification methods, including Dragendorff's test, Hager's test, Vitali Morin test, Shinoda test, Mayer's test, and Wagner's test, proved to be effective in distinguishing these bioactive compounds. The successful identification of alkaloids and flavonoids in Spathodea campanulata crude drug lays the foundation for further research and development of herbal formulations derived from this plant.

V. FUTURE SCOPE

This research opens up several avenues for future exploration and development in the field of herbal medicine. Firstly, further studies can be conducted to investigate the specific bioactive compounds identified in Spathodea campanulata and their individual pharmacological activities. This will help in understanding the mechanisms of action and potential therapeutic applications of these compounds.

Additionally, the identified alkaloids and flavonoids can be further isolated and purified to assess their efficacy and safety profiles. This will enable the development of standardized herbal formulations with optimized dosages and improved therapeutic outcomes.

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Moreover, the developed identification methods can be further optimized and validated to enhance their accuracy and reliability. This will ensure consistent and reproducible results, facilitating quality control and standardization of herbal preparations derived from Spathodea campanulata.

REFERENCES

[1]. C. E. Anarado, C. J. O. Anarado, N. L. Umedum and Q. M. Ogbodo. Asian Journal of Applied Chemistry Research, Comparative Phytochemical and Anti-microbial Studies of Leaf, Stem, Root of Spathodea,companulata V6i130148. 18 June 2020.

[2]. Sowjanya Pulipati, Sk. Hapsana Parveen, R. Kiran Babu, G.Vagdevi, Srinivasa Babu. P, Journal of Pharmacognosy and Phytochemistry. Pharmacognostical and Physicochemical Standardization of Leaves of Spathodea Campanulata P. Beauv,Volume 2 Issue 2,2013.

[3]. Anita Wagh, Santosh Butle, Dipak Raut, Future Journal of Pharmaceutical Sciences. Isolation, identification, and cytotoxicity Evaluation of phytochemicals from Chloroform extract of Spathodea Campanulata,2021.

[4]. Sandhya E,Sankari M,Dr Meena A, International Journal of Modern Pharmaceutical Research, ANTI-ANGIOGENESIS AND ANTI-CANCER ACTIVITY OF THE ETHANOLIC EXTRACT OF SPATHODEA CAMPANULATA ROOT BARK, Volume 5, Issue 5. 2021.

[5]. Akharaiyi Fred Coolborn, Boboye Bolatito, Akpambang Victoria Omolara, F. C. Adetuyi. International Journal of Biochemistry Research & Review, Phytochemical and Antioxidant Effect of Spathodea campanulata leaf Extracts, 4th May 2015.

[6]. Jack Silver, Journal of chemical education,Let Us Teach Proper Thin Layer Chromatography Technique,October 22, 2020.

[7]. Jyoti Vastrad, Giridhar Gouder,Asian Journal of Chemistry,Evaluation of phenolic compounds and development of chromatographic profiles in Spathodea campanulata inflorescence by HPTLC, January 2016.

[8]. Gurleen Kaur, Sahil Sharma,INTERNATIONAL JOURNAL OF INFORMATION AND COMPUTING SCIENCE,Gas Chromatography – A Brief Review, July 2018.

[9]. Stefani N. Thomas, Deborah French,William A. Clarke,Nature Reviews Methods Primers 2,Liquid chromatography–tandem mass spectrometry for clinical diagnostics,08 December 2022.

[10]. J.R. Everett,Encyclopedia of Analytical Science (Second Edition),NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY APPLICATIONS | Pharmaceutical,2005.

[11]. kasz Swiatek,Elwira Sieniawska,Kouadio Ibrahime Sinan,Gokhan Zengin,ORCID,Abdullahi Ibrahim Uba,Kouadio Bene,Magdalena Maciejewska-Turska,Barbara Rajtar,MaIgorzata Polz-Dacewicz,Abdurrahman Aktumsek, MDPI currently publishes 421 peer-reviewed journals,Bridging the Chemical Profiles and Biological Effects of Spathodea campanulata Extracts: A New Contribution on the Road from Natural Treasure to Pharmacy Shelves, 22 July 2022.

[12]. John daintith,A Dictionary of Chemistry (6 ed.),Dragendorff test,2008.

[13]. Katarzyna Godlewska,PaweI Pacyga,Antoni Szumny,Anna Szymczycha-Madeja,Maja Wełna,Izabela Michalak,MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations, Methods for Rapid Screening of Biologically ActiveCompounds Present in Plant-Based Extracts,20 October 2022.

[14]. A.N.Panche, A.D Diwan, S.R.Chandra, journal of nutrition science,Flavonoids,29 December 2016.

[15]. Sri Adelila Sari,Mellya Ernita,M. Nasir Mara,Muhammad Rudi AR,Indonesian Journal of Chemical,Science and Technology,State University of Medan,Identification of Active Compounds on Muntingia calabura L.Leaves using Different Polarity Solvents, 28 Jan 2020.

[16]. Jonathan Law and Richard Rennie,A Dictionary of Chemistry (8 ed.),Mayer's test,2020.

[17]. Yakubu Rufai,Scholars Journal of Agriculture and Veterinary Sciences,Comparative Phyto-Constituents Analysis from the Root Bark and Root Core Extractives of Cassia ferruginea (Schrad D. C) Plant,July 2016.