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Microscopic Evidence of Flavonoid Accumulation in Specific Stem Tissues of Maytenus senegalensis

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Abstract: The present study was carried out to check the presence of flavonoids in the stem of Maytenus senegalensis by using the microscopic technique. It revealed the presence of flavonoids in the stem by using histochemical tests. This study is helpful in the process of quality control and authentication of the plant in the taxonomy, and for scientific classification.

Keywords: Quality control, Flavonoids, Histochemical analysis, Anatomy of the stem.

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1. Introduction

The pharmaceutical field largely relies on biological fermentation from plant-derived bioactive compound sources for numerous target drugs (1). Secondary metabolites are associated with countless health-promoting effects, particularly due to their action as photoreceptors, feeding deterrents, visual attractors, anti-carcinogenic and anti-oxidative effects, and affecting the regulation of key enzyme functions within cellular processes. They also contain many vital compounds/products across a multitude of pharmaceutical, nutraceutical, cosmetic, and medicinal applications (2,3).

This could ignite the necessity for future research to develop several secondary metabolites. Secondary metabolites accumulate and synthesize multitude of secondary metabolites in vitro cultured cells from parent plants (4). These cultures have the potential to observe at cells or some specific tissues from the plants to synthesise metabolites, which are useful for the in vitro development of new compounds from cheap or low-cost precursors (5).

Several Maytenus species have been reported in the treatment of a variety of human ailments, including asthma, tuberculosis, pneumonia, diarrhoea, and snake bites throughout the world and extensively described in ethnomedical literature (6-7). Maytenus senegalensis (M. senegalensis) is an indigenous shrub belonging to the family Celastraceae that grows to a height of 1-9 m (8) and is characterised by bearing white flowers often with wine-red branches and a fragrant smell. It is found in the Savannah regions of tropical Africa, the central and southern parts of Asia, Europe, Mauritius, Seychelles, and Madagascar. It has a wide distribution occurring in ecologically important habitats such as grasslands, deciduous woodlands, along riverbanks and dried riverbeds, and swamp margins. The pharmacological activity of M. senegalensis is primarily attributed to a group of bioactive compounds, such as alkaloids, terpenoids, tannins, etc., present in the plant (9).

Various components (roots, stem, bark, leaves, and fruits) of this medicinal plant, which are valuable sources of bioactive compounds, have been utilised in many medicinal system traditions for treating numerous infectious and inflammatory diseases. Some species of Maytenus have been historically used in the treatment of various diseases and are still frequently used for treatment. The wide ecological distribution of this medicinal plant in warm tropical and sub-tropical environments is one of the reasons for its long-standing usage in traditional medicine systems (10). The infusion and macerated leaves of M. senegalensis are used traditionally for the treatment of tuberculosis and amoebic dysentery. Whereas a combination of roots and leaves has been reported to treat snake bites and respiratory diseases such as pneumonia (11-12). This study aimed to analyse the anatomy of the stem to confirm the existence of secreting cells or tissues and the presence of flavonoids - histochemical analysis of the plant was performed.

2. Materials and Methods

2.1 Study Design

This study was designed as a laboratory-based observational study aimed at investigating the microscopic accumulation of flavonoids in specific stem tissues of Maytenus senegalensis (M. senegalensis). Histochemical analysis was employed to provide direct evidence of flavonoid localisation within stem tissues.

2.2 Study Area

The research was conducted in the Department of Zoology, D.S. College, Aligarh, Uttar Pradesh. All laboratory analyses, including sample preparation, sectioning, and microscopic observations, were carried out in this department using standard laboratory equipment and reagents.

2.3 Study Participants

Inclusion Criteria:

Fresh, healthy stems of M. senegalensis were collected from naturally growing plants in Chaksu, Rajasthan, India (26.6051° N latitude, 75.94814° E longitude) during December 2023. Only morphologically mature stems without visible disease or mechanical damage were included.

Figure-1: Twig of the aerial part of M. senegalensis

Exclusion Criteria:

Stems showing decay, insect infestation, injury, or abnormal morphology were excluded.

2.4 Sample

Fresh stem samples were collected from different plants to ensure representative sampling. Each sample was handled carefully to prevent contamination or tissue degradation before analysis.

2.5 Procedure

Collected stems were washed under running tap water to remove dust and debris. Thin transverse sections of the stem were obtained using free-hand sectioning. Sections were treated with 5% aqueous

potassium hydroxide to enhance tissue transparency and reveal flavonoid presence. Histochemical detection of flavonoids was performed using standard staining methods, and sections were examined under a bi-concave light microscope at various magnifications. Observations were documented for flavonoid presence, distribution, and intensity within cortex, phloem, xylem, and pith tissues. Photomicrographs were taken for visual documentation.

2.6 Statistical Analysis

Data on flavonoid presence and distribution in different stem tissues were recorded and expressed as frequencies and percentages. Statistical analyses, including descriptive statistics and comparative analysis of flavonoid accumulation across tissue types, were performed using SPSS Version 27.0. Graphical representations and tables were generated to visualize tissue specific accumulation patterns.

3. Result and Discussion

The results showed that the stem had anatomical characteristics and the secreting cells reacted strongly in yellow colour for flavonoids (Figure 2), though there was not a reaction was observed for the other secondary metabolites. Various classes of flavonoids can be present, including flavones, neoflavanoids, flavanols, xanthones, flavanones, isoflavonoids, flavanonols, flavan 3-ols, flavanols or catechins, anthocyanins, aurons, and chalcones (13). It indicates that plants contain flavonoids that have antioxidant constituents as defence mechanisms against oxidative stress, free radical scavengers, and lower the reactivity of the radicals. These characteristics are useful in quality control processes. In the present study, flavonoids showed positive reactions, and they are located in the mesophyll. A collenchyma with approximately 6 layers was found under the epidermis. An open arc vascular system was found in the arms of the petiole. The vascular bundles are closed and conjoint. They lie in parallel rays, in a V shape, surrounded by a parenchyma and sclerenchyma sheath. The vascular system organisation provides mechanical strength and is an important characteristic for species characterisation and differentiation. Pith is made of large, thin-walled parenchymatous cells. The pith will store a large amount of food. The middle pith has a cavity-like structure replacing it (14, 15).

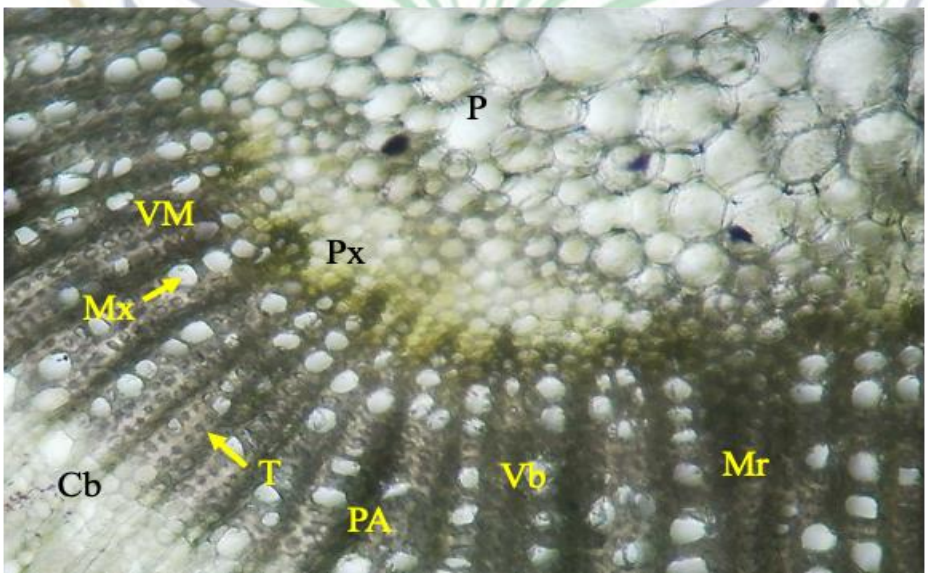

Figure 2 Anatomy of a dicot stem showing the presence of flavonoids in yellow colour. Mr: Medullary rays, Cb: Cambium, Mx: Metaxylem, Px: Protoxylem, Vm: Vessel members, Vb: Vascular bundle, PA: Parenchyma, T: Tracheid, P: Pith. Scale bar: 10 X.

4. Conclusion

The present study revealed the presence of flavonoids in the stem of Maytenus senegalensis. This study is helpful in the process of quality control and authentication of the plant in the taxonomy for the

classification and nomenclature. This study is conducted for the first time in the literature search. This is novel research.

Declaration

We declare that all authors of this manuscript have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

Authors Contribution

RA and YS conceived of' the present idea for the article. HB, VK, and HV performed the literature search, data analysis and wrote the manuscript. RA and YS provided critical feedback and helped to shape the final draft.

Conflict of Interest

The authors announce that they have no conflict of interest.

Ethical Approval

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, and no ethical approval was obtained from the committee concerned.

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