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MORPHOLOGICAL ALTERATION IN EUCALYPTUS PELLITA LEAVES FOLLOWING ESSENTIAL OIL EXTRACTION

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ABSTRACT

Conventional distillation of essential oils can induce physical changes in plant tissues. Therefore, it is crucial to conduct a morphological analysis of the Eucalyptus pellita leaf surface after extraction to assess the extent of structural alteration and its possible correlation with the efficiency of essential oil release. This study aimed to characterize and compare morphological alterations in the leaf surface of Eucalyptus pellita leaves using two prevalent methods: steam distillation (SD) and hydrodistillation (HD). Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) were employed to analyze untreated and post-extraction leaves. SEM observations showed that untreated leaves exhibited intact, rounded oil glands and a rough cuticular surface. Post-extraction, both methods cause significant microstructural damage with a distinct pattern. Leaves from post-SD displayed a smoother, more homogeneous surface due to partial melting of the cuticular wax layer, accompanied by moderate tissue disruption. Conversely, HD induced more severe damage, characterized by the formation of distinct pores and cavities resulting from epidermal degradation and rupture of oil glands. EDX analysis indicated a decrease in nitrogen content and an increase in oxygen concentration after extraction, suggesting oxidative reactions during the distillation. Both extraction methods altered the surface morphology of Eucalyptus pellita leaves; however, hydrodistillation resulted in more pronounced cell wall degradation than steam distillation. This study provides novel evidence that differentiates the microstructural physical impacts of post-extraction Eucalyptus pellita leaves. The morphological analysis provides a basis for evaluating and selecting extraction methods based on their tissue-altering effects.

Keywords: Eucalyptus Pellita, SEM, Leaf Morphology, Oil Glands, Cuticular.

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INTRODUCTION

Eucalyptus pellita, a significant member of the Myrtaceae family, grows extensively across Indonesia, particularly on the island of Sumatra. As a fast-growing timber species, this plant has high economic value, not only for the pulp and solid wood industries, but also as a source of essential oil.[1,2] Essential oil from Eucalyptus pellita leaves, rich in 1,8-cineole, α-pinene, and limonene, displays strong bioactivity.[3] These compounds function as active ingredients in the pharmaceutical, cosmetic, aromatherapy, and agrochemical industries.[3,4] Conventional extraction methods, such as steam distillation and hydrodistillation, have been used to obtain essential oil from Eucalyptus leaves. The principle of both methods is the separation of oil based on differences in boiling points, heat, water, and vapour, ruptures of the oil storage structure in plant tissues. Although efficient on a production scale, the application of heat and pressure during the distillation process caused physical, morphological, and microscopic structural

changes in the leaves.[5,6] These microstructural changes not only affect the yield and extraction rate but can also serve as an indicator of the thermal stress experienced by the raw material. The morphology of the leaf surface, particularly the cuticle, epicuticular wax layer, stomata, and epidermal tissue, plays an important role in tissue protection and secondary metabolite storage.[7,8] Previous studies have reported on the yield and chemical composition of E. pellita essential oil.[9-12] However, scientific reports on the physical impact of the extraction process on leaf surface morphology at the microscopic level remain limited. Understanding post-process structural alterations is important for evaluating the efficiency of extraction methods, optimizing process parameters, and even identifying the potential use of extracted leaf waste as a derivative raw material. Scanning Electron Microscopy (SEM) is an effective characterization technique for observing topographic and surface morphological changes in biological materials at high resolution. It is widely used to evaluate microstructural changes due to physical and thermal treatments.[13] Several SEM-based studies reported that distillation and heating processes can cause cuticle degradation, wax layer melting, and epidermal cell damage in Eucalyptus leaves and other aromatic plant species.[14,15] Hydro-distilled leaves typically display more pronounced morphological damage, characterized by a substantial loss of the cuticle layer and a rougher, more irregular leaf surface resulting from extended direct exposure to hot water.[6] These morphological differences indicate that the distillation method directly influences the degree of degradation of the leaf surface structure and the mechanism of essential oil release. Therefore, SEM analysis of E. pellita leaves under untreated conditions(control) and after steam distillation and hydrodistillation treatments is important to compare changes in leaf surface morphology, evaluate the degree of microstructural damage caused by the extraction process, and relate this to the mechanism of essential oil release during distillation.

EXPERIMENTAL

Material and Methods

The raw material used was 5-year-old Eucalyptus pellita leaves from PT Musi Hutan Persada, Muaraenim Regency, Province of South Sumatra, Indonesia. The leaves were distilled using steam and hydrodistillation methods with the following equipment: a steam distillation glass apparatus, a Clevenger apparatus, an oil separator, a scanning electron microscope (SEM), a thermometer, and a hotplate.

General Procedure

The leaf samples were divided into three groups, namely, (1) Control: fresh leaves without treatment, (2) SD (steam distillation), and (3) HD (hydro distillation). The methods used to extract essential oil from E. pellita were steam distillation and hydrodistillation. For the extraction treatment, 150 grams of fresh leaves were placed in a distillation flask. The SD process was carried out by passing saturated steam into a flask containing the leaves at a leaf: solvent ratio of 1:11.6 (g/g). The HD process was carried out by soaking the leaves in distilled water at a leaf: water ratio of 1:5.4 (g/g). Both extraction processes were carried out for 5 hours at 100°C. After extraction, the leaves were dried at room temperature before SEM.

Detection Method

The leaves were analyzed using SEM-EDX with the AxiaChemiSEM device, which combines SEM imaging with integrated EDX elemental analysis. Axia ChemiSEM collects EDX data in the background during SEM imaging. The SEM analysis was conducted at the SEM Laboratory of the Faculty of Engineering, Universitas Sriwijaya. Images were captured at 1000x magnification on the surface and at 550x magnification from two cross sections.

RESULTS AND DISCUSSION

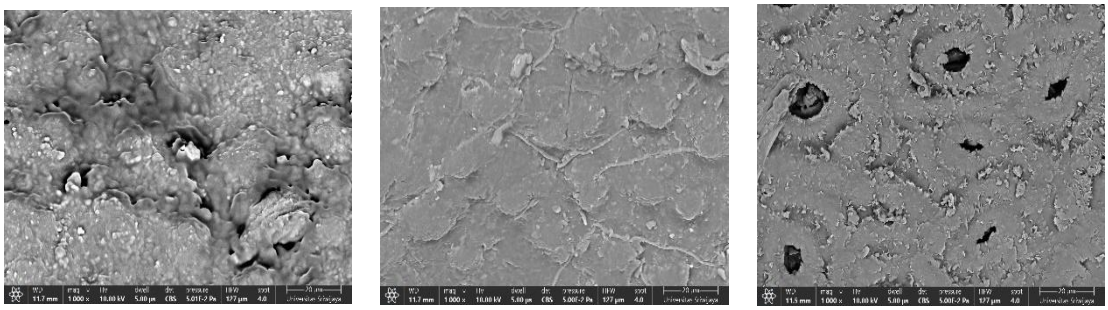
The characteristics of Eucalyptus pellita leaves before and after extraction using steam distillation and hydrodistillation are distinctive, with physical and chemical properties that differ. Visually, the leaves are fresh green with a rough surface due to oil glands that appear as transparent spots.[16] The texture is stiff and thick due to the high lignin and cellulose content. The aroma is strong and distinctive, dominated by the compound 1,8-cineole (eucalyptol), which is the main component of essential oils. The visual appearance of untreated leaves is shown in Fig.-1(a). After extraction, Eucalyptus pellita leaves undergo significant changes. The color of the leaves turns brown due to chlorophyll degradation and phenolic

compound oxidation, as well as cuticle degradation, which accelerates leaf pigment oxidation.[8] The texture becomes softer and more brittle due to damage to the cellular structure, including the destruction of secretory glands and the plasmolysis of the cell walls. The leaf surface becomes duller and rougher due to the melting and oxidation of the cuticle wax. The aroma also decreases drastically because the volatile compounds have been extracted. Chemically, Eucalyptus leaf essential oil is dominated by volatile compounds, especially 1,8-cineole. At the same time, non-volatile components such as tannins and coarse fibres are not extracted by steam distillation or hydrodistillation, so that even though most of the volatile oil is extracted, the non-volatile structure remains in the leaf residue.[17] Once the cuticle is damaged, volatile compounds are more easily extracted because cell permeability increases.[7] The condition of the leaves after extraction using steam distillation is shown in Fig.-1(b), and hydro distillation in Fig.-1(c).

(a) (b) (c)

Fig.-1: Visual of Eucalyptus pellita Leaves; **(**a) Control, (b) After Steam Distillation, (c) After Hydrodistillation

The leaves of E. pellita control are coated with lignin, which plays an important role in protection and performs various physiological functions.[15,18] In addition, the cuticle also contributes to the growth and differentiation of plant organs. In control leaves, the cuticle contains lipid components[14] including cutin and cuticular waxes, which are composed of hydrophobic compounds such as long-chain fatty acids, alcohols, esters, and alkanes. It is this lipid content that gives the leaf surface its hydrophobic properties. Figure-2(a) shows a control leaf with SEM image observation at 1000x magnification. The leaf surface shows a tightly packed epidermal cell structure, covered by a relatively rough, uneven surface, indicating a high level of cuticular wax. This wax layer provides natural protection for the leaves against water loss, microbial attack, and other environmental factors. Morphologically, the layer appears to cover the epidermal cells so that the pores or openings on the leaf surface are not clearly visible. Previous studies have reported that the epidermal cells of Eucalyptus species generally have straight anticlinal walls. [14,19,20] Based on observations of the anticlinal walls in the control leaves, they are not clearly defined in Fig.-2(a) due to limitations in SEM visualization of internal structures. The visible cell surface tends to be smooth rather than wavy, indicating that the anticlinal walls are straight, as reported in previous studies on Eucalyptus species.



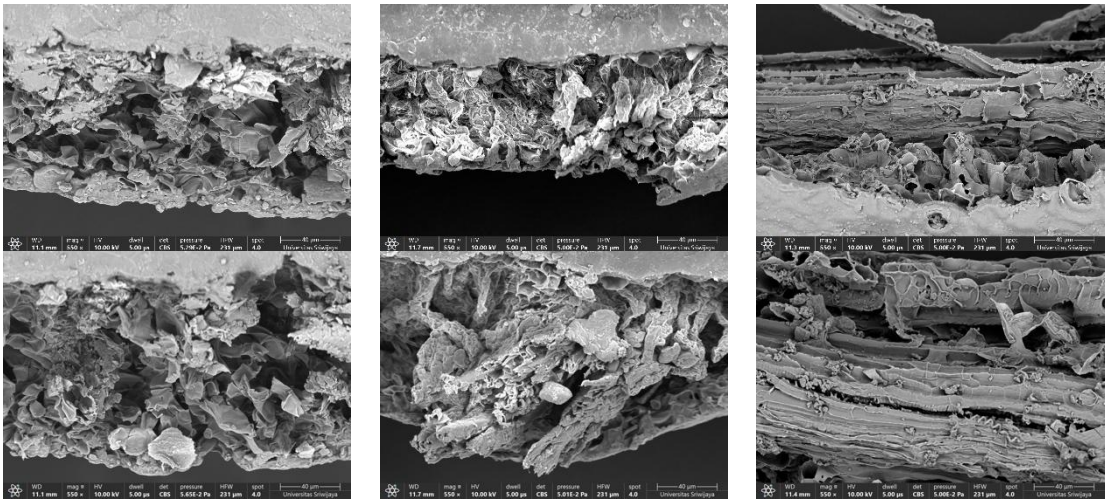
(a) (b) (c)

Fig.-2: SEM Surface Image of Eucalyptus pellita Leaves: (a) Control, (b) After Steam Distillation, (c) After Hydrodistillation

After undergoing steam distillation (Fig.-2b), the leaf surface showed significant morphological changes. The surface texture appears smoother and more homogeneous than that of fresh leaves, indicating melting and removal of some of the wax layer due to exposure to hot steam. The high temperature during the steam distillation process softens lipid components, including cuticular wax and some volatile compounds, and causes them to be distributed or detached from the leaf surface. In leaves extracted using hydrodistillation (Fig.-2c), the morphological changes on the surface are more drastic. The results of this observation are consistent with those of[21], which showed the presence of cavities on the surfaces of Eucalyptus leaves after extraction. The leaf surface appears more porous, with distinct cavities or holes. This condition indicates that direct contact with hot water for a specific period not only dissolves the wax layer but also severely damages the leaf epidermis. The water vapor pressure within the leaf tissue during hydrodistillation causes the oil glands to rupture and release volatile compounds, leaving pores or depressions on the leaf surface.

After the extraction using the hydrodistillation method, the wax layer has decreased, as evidenced by its fading color. The wax layer on the leaf surface is part of the cuticle, which is the outer layer formed by the excretion of lipid compounds such as cutin and wax by epidermal cells. After the distillation process, this wax layer undergoes degradation because the lipid components and volatile compounds that form it can dissolve or evaporate along with the water vapor. In addition, high temperatures can also cause protein denaturation and damage to the epidermal cell walls, disrupting the structure and integrity of the cuticle.[22] As a result, the leaf surface, which initially appeared shiny and intact, becomes dull, uneven, and appears faded when observed microscopically. This change reflects a loss of some of the leaf's natural protection during extraction. The loss of the epidermis and cuticle layers on the leaf surface after extraction indicates that secondary metabolites, especially essential oils, have begun to evaporate or be extracted. Biologically, these compounds are stored in or near the epidermal tissue, such as in subepidermal cavities or secretory trichomes. Figure-3 shows the SEM cross-section observation results of Eucalyptus pellita leaves, including control leaves (a), leaves after steam distillation (b), and leaves after hydrodistillation (c). In the control leaves (Fig.-3a), the leaf tissue structure still appears compact and well-organised. The epidermal layer appears relatively intact and fused with the underlying tissue, while the mesophyll tissue shows a dense cell arrangement with limited intercellular spaces. This condition indicates the natural state of the leaves before extraction, with the glands or cavities that store essential oils still closed and trapped within the leaf tissue.

After undergoing steam distillation (Fig.-3b), the leaf cross-section shows morphological changes. The mesophyll tissue structure appears to have begun to open and become irregular, marked by increased intercellular spaces and microcracks.



(a) (b) (c)

Fig.-3: SEM Cross-Section Image of Eucalyptus pellita Leaves: (a) Control, (b) After Steam Distillation, (c) After Hydrodistillation

Exposure to hot steam causes tissue expansion due to steam pressure from within the cells, thereby facilitating the release of essential oils from the oil glands.[23] However, in the image, the main structure remains relatively intact, indicating that steam distillation tends to cause moderate tissue damage rather than destroying the entire leaf matrix.

In leaves extracted using hydrodistillation (Fig.-3c), the internal structural changes are more significant. The cross-section shows that the tissue layers are more separated, folded, and partially collapsed. The intercellular spaces are larger and irregular, indicating more severe tissue damage from prolonged direct contact with hot water. Water vapor pressure within leaf tissue during hydrodistillation can rupture oil glands and degrade cell structure, resulting in higher internal porosity than in steam distillation. Extraction not only causes a reduction in active compound content but also weakens the natural protective structure of the leaves because many cuticle-forming compounds, such as wax, are also carried away. In Energy-Dispersive X-ray Spectroscopy (EDX) analysis, this is demonstrated by a decrease in carbon and nitrogen compounds following the extraction process. Compared to C-O bonds, which have a high bond dissociation energy and therefore require less energy to break, C-C and C-N chemical bonds break more easily at high temperatures. The bond energy of C-O is 358 kJ/mol, whereas those of C-C and C-N are 346 and 305 kJ/mol, respectively. According to the EDX analysis results shown in Table-1, nitrogen atoms decreased (for HD) and disappeared (for SD) during the extraction process. The low C-N bond energy, which is lower than C-C and C-O EDS before extraction, was another factor contributing to this.

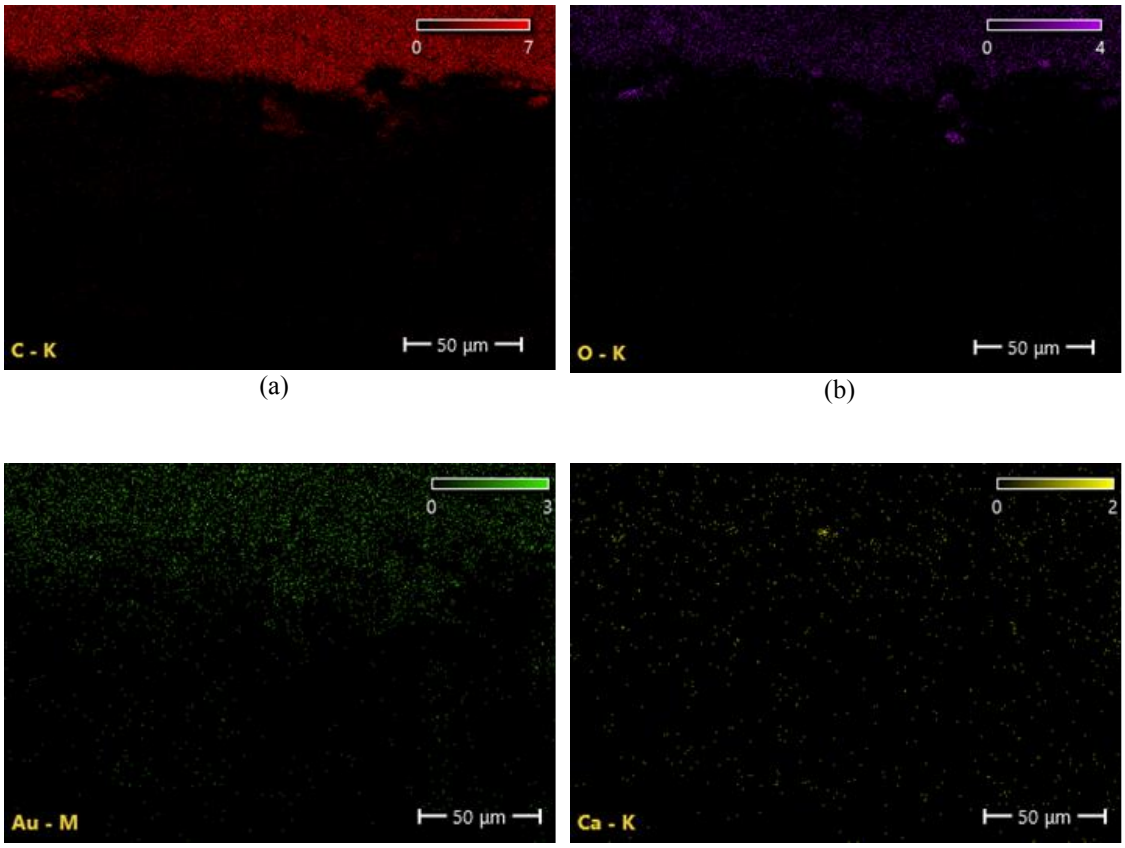
Table-1: Elemental Composition of Eucalyptus pellita Leaf Surface Based on SEM-EDX Analysis

Element	Leaves Control		Leaves SD		Leaves HD	
	At. %	Wt. %	At. %	Wt. %	At. %	Wt. %
C	68.6	46.1	67.5	47.5	52.6	31.6
N	5.4	4.3	-	-	3.7	2.6
O	21.7	19.4	29.0	27.1	39.9	32.0
Na	0.2	0.2	-	-	-	-
Si	0.4	0.6	-	-	-	-
K	0.4	0.9	-	-	-	-
Ca	0.7	1.5	0.7	1.6	0.2	0.5
Br	0.2	0.7	-	-	-	-
Au	2.4	26.3	1.7	19.1	3.4	33.1
Mg	-	-	0.3	0.4	0.2	0.2
Al	-	-	0.4	0.6	-	-
Ta	-	-	0.4	3.7	-	-

Based on Table-1, carbon (C) and oxygen (O) dominate the composition of leaves in all samples. In fresh leaves, the carbon content is relatively high (68.6 at. %; 46.1 wt.%), reflecting the presence of organic compounds such as cellulose, hemicellulose, lignin, and a carbon-rich cuticle wax layer. After steam and hydrodistillation, the carbon content decreased, especially in hydrodistillation (52.6 At. %; 31.6 wt.%). This decrease indicates the release of nonpolar organic compounds, particularly wax and essential oil components, due to heating and contact with steam or hot water. The oxygen (O) content increased significantly after extraction. In fresh leaves, oxygen was recorded at 21.7 At. % (19.4 Wt.%), then increased to 29.0 At. % (27.1 Wt.%) in steam distillation and to 39.9 At. % (32.0 wt.%) in hydrodistillation. This increase indicates that after the removal of the wax layer and hydrophobic compounds, the leaf surface became richer in polar groups containing oxygen, such as hydroxyl and ether groups in cell wall polysaccharides (cellulose and hemicellulose). The increase in O atoms during steam distillation and hydrodistillation indicates oxidation during the extraction process, which causes the leaves to turn brown, as shown in Fig.-1, which aligns with the research carried out.[24] Brown chemicals, such as quinones or oxidised polymer complexes, are created by the oxidation of phenolic compounds and chlorophyll. Enzymes like lipoxygenase, peroxidase, and oxidase are involved in the process.[25] This response is typical of plant tissues exposed to thermal or chemical stress, when natural antioxidant compounds can no longer resist the oxidation rate. Nitrogen (N) was detected in fresh leaves and hydro-distilled leaves, but not in steam-distilled leaves. Nitrogen is an essential element and a significant

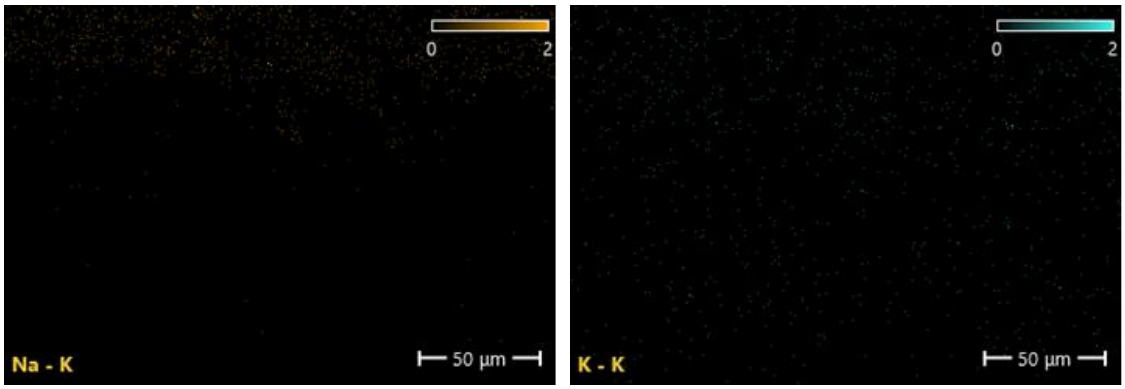
component of proteins, nucleic acids, phospholipids, and chlorophyll pigments in plant tissues, which play a central role in primary leaf metabolism.[26] The presence of N detected by EDX on the surface of the control leaves likely reflects residual proteins/primary metabolites in the leaf tissue of Eucalyptus pellita. The decrease or undetectability of nitrogen after extraction suggests the possible degradation or release of nitrogen compounds during heating of the distillation process. Small levels of mineral elements such as Na, Si, K, Ca, Br, Mg, Al, and Ta were detected and showed changes between treatments. These elements are natural plant minerals from leaf tissue or inorganic residues left behind after organic compounds are released. The presence of Au in all samples was mainly due to the SEM sample preparation process, namely, gold coating to improve the surface conductivity. This element is not representative of the original composition of the leaves and hence was not investigated as a chemical component of the material.[27] Reported that nitrogen (N) was detected relatively homogeneously distributed across all cell types, consistent with its role in protein and chlorophyll formation. In contrast, phosphorus (P) and potassium (K) accumulated in spongy parenchyma tissue and some palisade parenchyma. Magnesium (Mg) was mainly distributed in the dermal layer and mesophyll tissue. Sodium (Na) was mostly found in the mesophyll and showed limited accumulation in the dermal tissue, especially in young leaves. The equilibrium of sodium was maintained by the chloride (Cl), which was also found in the spongy parenchyma and dermal tissue. However, based on the results of this study, phosphorus (P), potassium (K), and sodium (Na) were only identified in fresh leaves and not after extraction. Whereas magnesium (Mg) was only discovered after distillation.

Atoms detected in leaf samples after the extraction procedure that were not present in control leaf samples (Mg, Al, Ta) are considered to be foreign to the leaf tissue and to have originated from external sources during the treatment process. The presence of elements such as aluminium (Al) and tantalum (Ta), which were not detected in the control leaf analysis but were identified in the post-extraction EDX results, indicates contamination or impurities entering the system.



(a) (b)

(c) (d)



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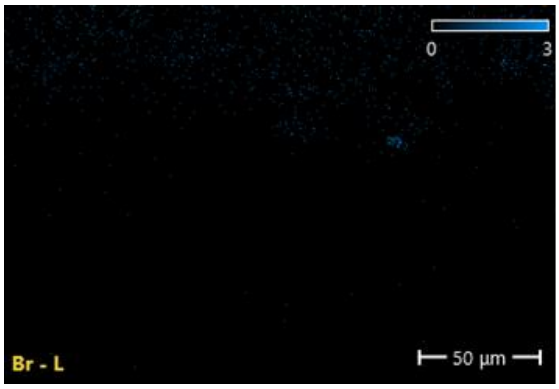

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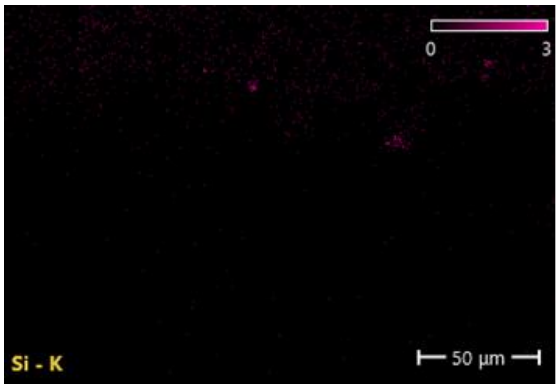

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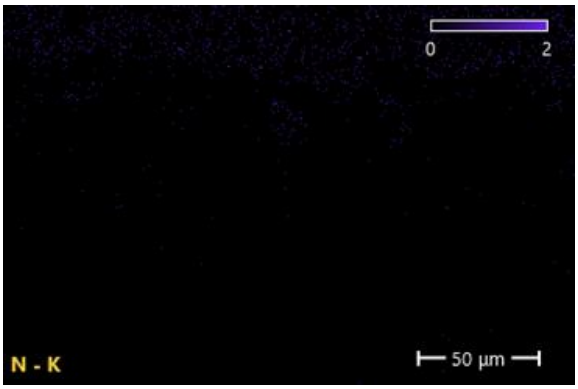

(i)

Fig.-4: Mapping of EDX Elements on a Cross-Section of a Control Leaf. (a) BSE (Backscattered Electron) image; (b–i) Element Distribution Map Based on C-K, O-K, Ca-K, Na-K, K-K, Br-L, Si-K, N-K, and Au-M Emissions in Pseudo-Colour Representation. The Analysis Was Performed with an Acceleration Voltage of 10 kV, an Acquisition Time of 307 Seconds, and a Chamber Pressure of 5.29×10^{-2} Pa. The Map Resolution was 768 × 512 Pixels

Heavy metals, such as Tantalum (Ta) and aluminum (Al), are rarely found in plant tissue but are often used in electronic equipment components and holders. Mapping of EDX elements on a cross-section of the leaves shown in Figure-4 for the leaf from the control, Figure-5 for the leaf from SD, and Fig.-6 for the leaf from HD.

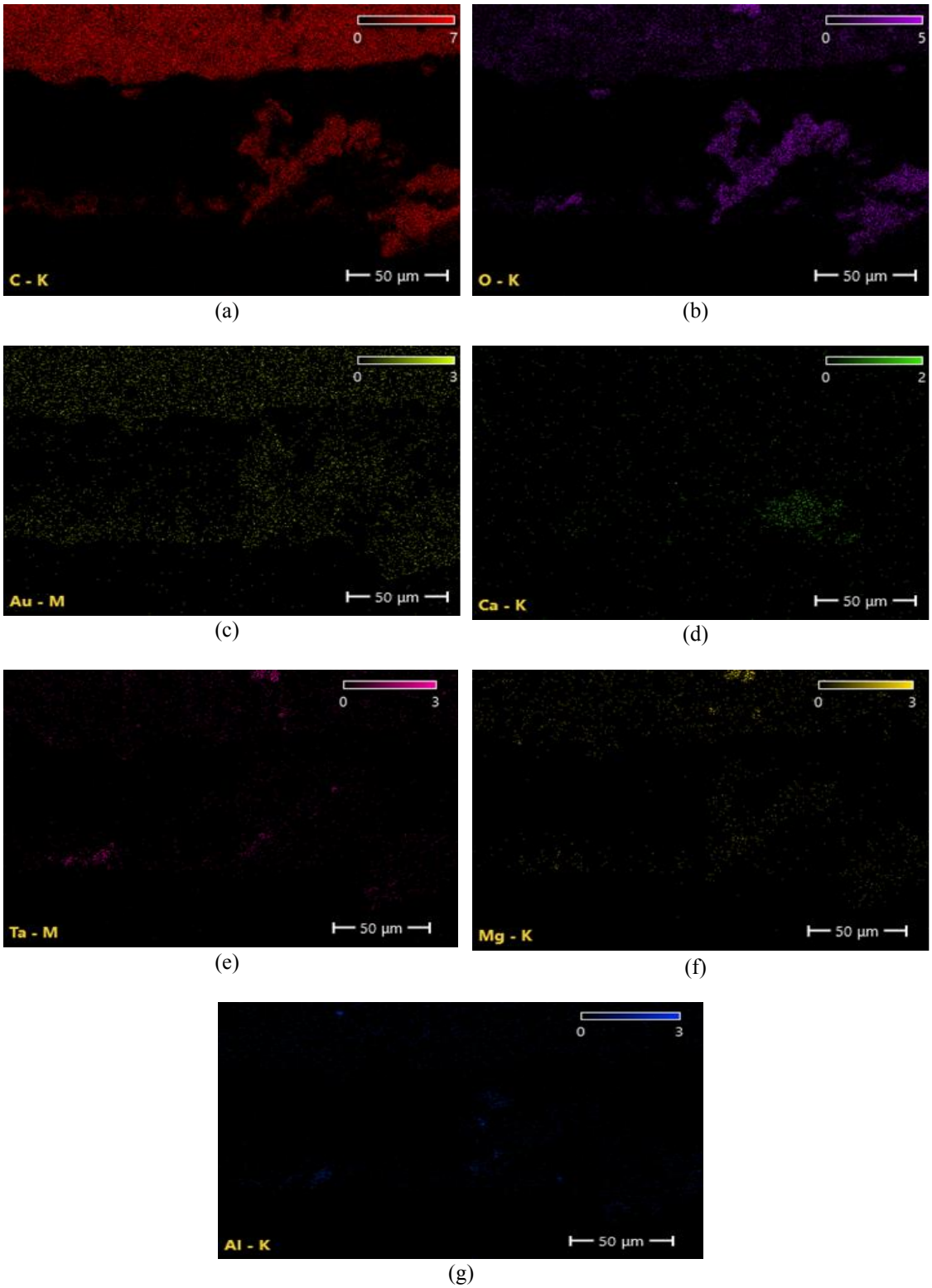

Fig.-5: Mapping of EDX Elements on a Cross-Section of Leaves Obtained by Steam Distillation. Images Were Obtained Using Electrons with an Acceleration Voltage of 10 kV and a Total Acquisition Time of 306 Seconds. (a) Back-Scattered Electron (BSE) Image; (b–g) Pseudocolour Maps of C-K, O-K, Au-M, Ca-K, Ta-M, Mg-K, and Al-K Element Distribution. Mapping Was Performed at a Resolution of 768 × 512 Pixels and a Chamber Pressure of 5.00×10^{-2} Pa

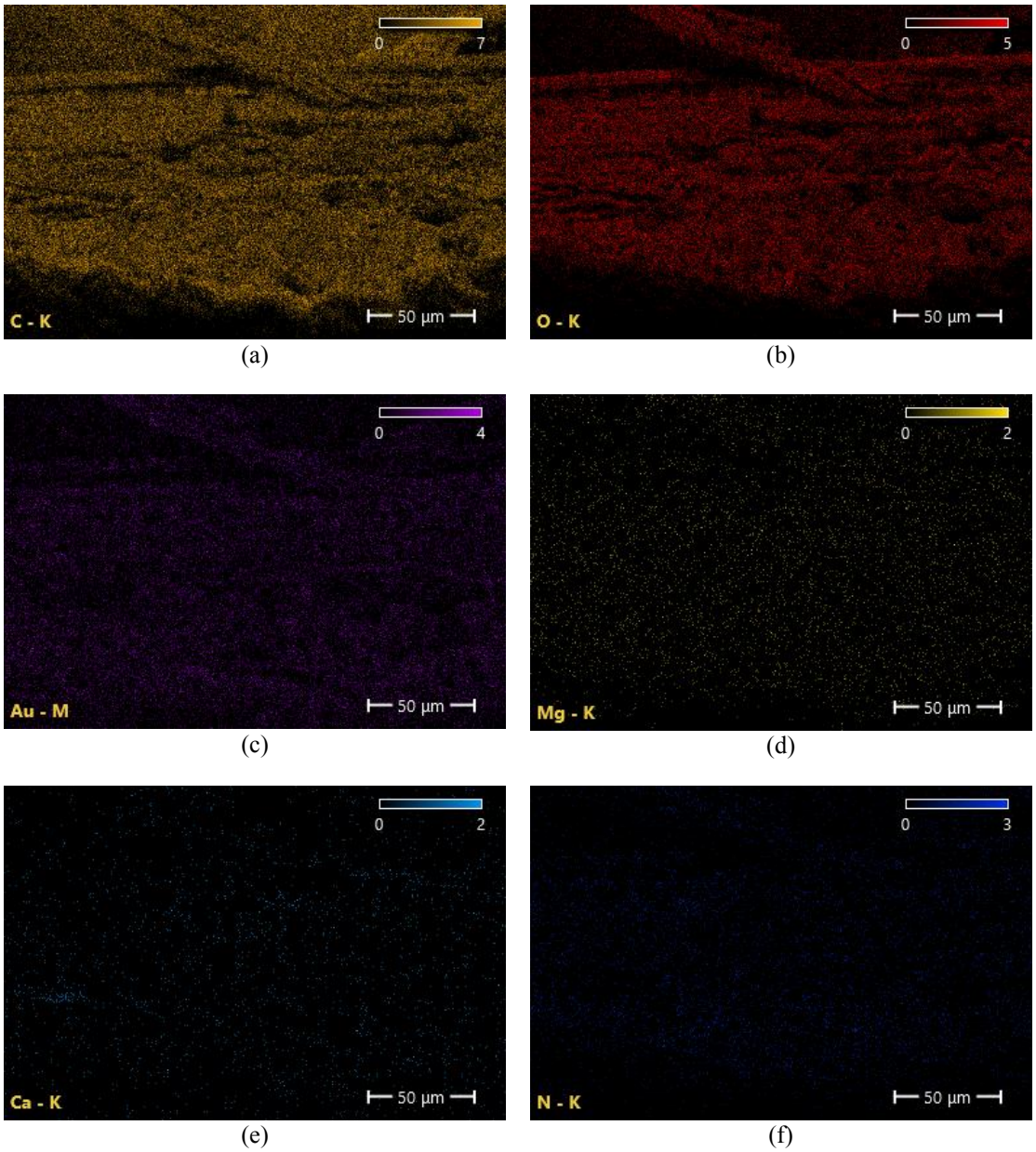

Fig.-6: Mapping of EDX Elements on a Cross-Section of a Leaf Obtained by Hydrodistillation. The Image Was Obtained Using Electrons with an Acceleration Voltage of 10 kV and a Total Acquisition Time of 306 Seconds. (a) Back-Scattered Electron (BSE) Image; (b–f) Pseudo colour Maps of C-K, O-K, Au-M, Mg-K, Ca-K, and N-K Element Distribution. Mapping was performed at a Resolution of 768 × 512 Pixels and a Chamber Pressure of 5.00 × 10^{-2} Pa

CONCLUSION

The extraction of essential oils using steam distillation and hydrodistillation methods has been shown to cause morphological and chemical composition changes in the surface of Eucalyptus pellita leaves. SEM analysis showed that both extraction procedures caused damage to the leaf microstructure with different levels of severity. Steam distillation caused partial melting of the cuticle wax layer and moderate tissue damage. Conversely, hydrodistillation caused more severe degradation, marked by pores and cavities formation from damage to the epidermal layer and rupture of the oil glands. EDX analysis revealed changes in the elemental composition of the leaf surface after extraction, particularly a decrease in nitrogen and an increase in oxygen, suggesting oxidation reactions during distillation. The differences in

morphological damage patterns and chemical element changes indicated that the mechanism and energy intensity of the hydrodistillation process are higher than those of steam distillation.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest in our manuscript.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-15: Life on Land*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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