applied sciences

Communication

Phytochemical Profiles, Micromorphology, and Elemental Composition of *Gomphocarpus fruticosus* (L.) W.T. Aiton and *Leonotis leonurus* (L.) R.Br., Plants Used for Managing Antidepressant-like Conditions in Folk Medicine

Andisiwe Mnqika [1], Nneka Augustina Akwu [2], Thrineshen Moodley [1], Adeyemi O. Aremu [2,*] and Makhotso Lekhooa [1,*]

[1] DSI/NWU Preclinical Drug Development Platform, Faculty of Health Sciences, North-West University, Private Bag X6001, Potchefstroom 2521, South Africa; 43611753@mynwu.ac.za (A.M.); thrineshen.moodley@nwu.ac.za (T.M.)

[2] Indigenous Knowledge Systems Centre, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2790, South Africa; nneka.akwu@yahoo.com

* Correspondence: oladapo.aremu@nwu.ac.za (A.O.A.); makhotso.lekhooa@nwu.ac.za (M.L.); Tel.: +27-18-389-2573 (A.O.A.); +27-18-299-2270 (M.L.)

Abstract: Medicinal plants have been used to treat mental health-related conditions among different ethic groups. Among the commonly used plants in South Africa are *Gomphocarpus fruticosus* (L.) W.T.Aiton and *Leonotis leonurus* (L.) R.Br. This study aimed at generating the phytochemical profiles, micromorphology, and elemental composition of the leaves of *G. fruticosus* and *L. leonurus* as possible means of explaining the basis for their utilisation for mental health-related conditions in folk medicine and consideration for further development. The plant parts were subjected to successive solvent extractions using an ultrasonic method with dichloromethane (DCM) and were chemically characterised using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). Scanning electron microscopy (SEM) was used to examine the micromorphology of the fresh leaves and energy-dispersive X-ray Spectrometry (EDX) was utilised to perform mineral elemental analyses of *G. fruticosus* and *L. leonurus* using their leaf powder. We identified phytochemicals including rutin and marrubiin, which are known to alleviate depression-like symptoms. Glandular and non-glandular trichomes were present in the plants. A weight (%) of 1.32 and 0.82 for calcium, 1.16 and 1.99 for potassium, and 0.38 and 0.38 magnesium were present in *G. fruticosus* and *L. leonurus*, respectively. These minerals have been linked to mental health stability, with imbalances associated with various disorders. We established the chemical composition that could suggest potential therapeutic effects of these two medicinal plants, offering insights into their uses in folk medicine and potential modern applications in treating mental health issues.

Keywords: Lamiaceae; medicinal plants; mental health; trichomes; marrubiin

check for updates

Citation: Mnqika, A.; Akwu, N.A.; Moodley, T.; Aremu, A.O.; Lekhooa, M. Phytochemical Profiles, Micromorphology, and Elemental Composition of *Gomphocarpus fruticosus* (L.) W.T. Aiton and *Leonotis leonurus* (L.) R.Br., Plants Used for Managing Antidepressant-like Conditions in Folk Medicine. *Appl. Sci.* **2024**, *14*, 11540. https://doi.org/10.3390/app142411540

Academic Editors: Maja Repajić and Ivona Elez Garofulić

Received: 25 October 2024
Revised: 2 December 2024
Accepted: 3 December 2024
Published: 11 December 2024

1. Introduction

The use of medicinal plants to treat different illnesses has been an integral part of indigenous knowledge systems for many centuries [1]. Plants are a rich source of secondary metabolites which are often a source for drug development [2]. Phytochemicals are classified into four major biochemical groups: alkaloids, glycosides, polyphenols, and terpenes [3]. They play a role in reducing the risk of some ailments of the central nervous system and cardiovascular diseases [4,5]. The micromorphology, phytochemical profiles, and antioxidant activity of South African folk medicine plants have shed light on their potential medicinal properties, contributing to the translation of ethnobotanical use for drug development [6,7].

Furthermore, plants such as *Gomphocarpus fruticosus* (L.) W.T. Aiton and *Leonotis leonurus* (L.) R.Br., have been identified as promising for their antidepressant-like effects in folk medicine [8]. The utilisation of traditional complementary and alternative medicine (TCAM) in South Africa, particularly in conjunction with conventional treatments, has been well documented, highlighting the significance of traditional medicine in the country [9]. In recent times, there have been concerted efforts aimed at a comprehensive understanding of the medicinal potential of South African plant species, fostering advancements in both traditional and modern medical practises. Additionally, the economic potential of medicinal plants has been highlighted, emphasising the commercial and pharmacological significance of these traditional remedies for improved livelihoods [10,11].

Results from a previous study by Mnqika et al. [12] showed the extracts of *G. fruticosus* and *L. leonurus* exerted activity towards adenosine receptors, illustrating their potential for neuro activity. On this basis, further characterisation studies such as micromorphology, elemental composition, and phytometabolite profiling are necessary for a comprehensive evaluation of their potential for consideration as alternative antidepressant and neuromodulation effects. In this study, we focused on generating the phytochemical profile for *G. fruticosus* and *L. leonurus* as a means of bridging traditional knowledge with scientific evidence by analysing their chemical and elemental levels to understand the compounds that might contribute to their psychotropic potential. Therefore, the current study delved into the micromorphology, mineral elemental composition, phytochemical groups present, and phytochemical constituents of *G. fruticosus* and *L. leonurus*.

2. Materials and Methods

2.1. Plant Selection, Collection, and Preparation for Screening

Plant selection and collection were conducted as described by Mnqika et al. [12]. Thereafter, a portion of the fresh leaves of *G. fruticosus* and *L. leonurus* were harvested and fixed in 70% ethanol for the plant morphology using a scanning electron microscope (SEM). The other portion of the two plant parts were washed with distilled water and oven-dried at 37 °C for 2 days. The dried plant material was pulverised into powder to screen for mineral elementals using a scanning electron microscope energy-dispersive X-ray spectroscopy (SEM-EDX) (Elecmi, Madrid, Spain). A 1:10 ratio was used to extract the plant material with DCM by using an ultrasonicator (ScienTech, Indore, India). The extraction was repeated, and the extracts were filtered using a Buchner filtration system. This was evaporated using a rotary evaporator and freeze-dried and stored at 4 °C until ready for use. *Leonotis leonurus* was further extracted with other solvents (see Supplementary Materials) and analysed. These extracts were selected based on their high binding affinity to the serotonin reuptake transporter (SERT) and adenosine A_1/A_{2A} receptor assays, as reported in our previously study [12].

2.2. Chemical Fingerprinting Using Ultra-Performance Liquid Chromatography–Mass Spectrometry

UPLC-MS analyses were performed using the Waters Acquity Ultra Performance Liquid Chromatographic system. UPLC separation was achieved on a Kinetex C18 column (150 mm × 2.1 mm, i.d., 1.7 µm particle size, Phenomenex, Torrance, CA, USA) maintained at 40 °C. For the *L. leonurus* extracts, the mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.4 mL/min; a gradient elution was performed as follows: 90% A: 10% B, to 60% A: 40% B for 2 min, to 30% A: 70% B for 10 min, to 5% A: 95% B for 2 min, keeping for 0.5 min and back to the initial ratio for another 0.5 min. The samples were injected in the mobile phase with an injection volume of 1.0 µL (full-loop injection). For detection, mass spectrometry (MS) (G_2QTof) was operated in positive ion electrospray mode. N_2 was used as the desolvation gas. The desolvation temperature was set to 400 °C at a flow rate of 600 L/h, and the source temperature was 100 °C. The data were collected between 100 and 1200 *m*/*z*. For *G. fruticosus* DCM extract, the mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min; a gradient elution was performed as follows: 90% A: 10% B, to 10% A: 90% B for 14 min, keeping for 0.5 min and back to initial ratio for another 0.5 min. The samples were injected in the mobile phase with an injection volume of 1.0 µL (full-loop injection). MS was operated in negative ion electrospray mode. Nitrogen was used as the desolvation gas. The desolvation temperature was set to 400 °C at a flow rate of 600 L/h, and the source temperature was 100 °C. The capillary and cone voltages were set to 2500 and 35 V, respectively. The data were collected between 100 and 1200 m/z.

2.3. Micromorphology Analysis Using Scanning Electron Microscope

The harvested plant parts were cut into small sections approximately 2–3 × 4.0 mm^2 and fixed using freshly prepared 70% ethanol for 15 min and further dehydrated (twice) in 100% ethanol for another 15 min. After dehydration, a critical point drying process was accomplished in a critical point drier (CPD). Thereafter, each sample was mounted on a brass stub with double-sided sticky adhesive carbon tape. The treated samples were made conductive with an automated Emscope TB 500 Module sputter coater through the application of a thin (ca.25 nm) layer of carbon over the samples; thereafter, gold–pallidum was used to further sputter coat the samples. The sample surfaces were examined at varying magnification, with the use of a Quanta FEG250 SEM at an acceleration voltage of 5 kV, and all the examined representative features were captured digitally.

2.4. Elemental Analysis Using a Quanta FEG 250 Scanning Electron Microscope (SEM) Coupled with an Energy-Dispersive X-Ray Spectrometer

The powders (ca. 0.5 mg) of *G. fruticosus* and *L. leonurus* were separately subjected to elemental analysis using a Quanta FEG 250 scanning electron microscope (SEM) (FEI Company, Hillsboro, OR, USA) coupled with an energy-dispersive X-ray spectrometer (EDX. Oxford INCA software system) at 15 kV accelerating voltage [13].

2.5. Data Analysis

The elemental composition was performed in triplicate, and the resulting data were analysed using SPSS 29 for Windows (IBM Corporation, New York, NY, USA). Statistical significance was determined using Student's *t*-test. The results are reported as means ± standard deviation, with a significance level set at $p < 0.05$.

3. Results and Discussion

3.1. Chemical Profiles Based on Ultra-Performance Liquid Chromatography–Mass Spectrometry

Phytometabolites from various plants have shown promise in treating mental health disorders such as depression [5,14]. The efficacy of St. John's wort for major depressive disorder, curcumin and saffron for depression symptoms, and ginkgo for schizophrenia symptoms have been supported by several meta-analyses [15]. Secondary plant metabolites could provide an innovative pathway for developing new drugs by identifying active compounds to address neuromodulation and metabolic diseases, particularly benefiting economically developing countries [16]. The leaves of *L. leonurus* were extracted with DCM and other solvents (Supplementary Figures S1–S3 and Supplementary Tables S1–S3), and *G. fruticosus* leaves were extracted with DCM to obtain fractions. The tentative identification of *L. leonurus* DCM extract and other solvents was conducted using UPLC-MS ESI positive (Tables S1 and S2 in the Supplementary Materials), and UPLC-MS ESI negative mode was used for the tentative identification of *G. fruticosus* DCM extract (Table S3 in Supplementary Materials). The tentative identification of the compounds was achieved by matching MS fragmentation fingerprints from the PubChem database and the accurate mass-generated elemental composition.

As shown in Figure 1, the UPLC-MS revealed the phytochemicals of *G. fruticosus* DCM extract containing rutin (flavonoid), voruscharin (cardenolide), uscharin (cardenolide), afroside (glycoside), and decinnamoyltaxagifine (diterpene). Historically, cardiac glycosides from this plant were isolated in the 1950s [17,18], with a new glycoside and triterpenoids discovered in 2016 [19]. Glycosides are molecules composed of carbohydrates linked to non-carbohydrate molecules [20], and they may influence neuroprotection and synaptic plasticity by modulating proteins including G-coupled proteins, essential proteins for intracellular signalling [21]. These proteins are essential in transmitting intracellular signals through receptors for neurotransmitters, hormones, and neuromodulators [22]. Dysfunctions in glycosides could be associated with neuronal pathway irregularities, potentially linked to mood disorders or suicidal behaviour [21].

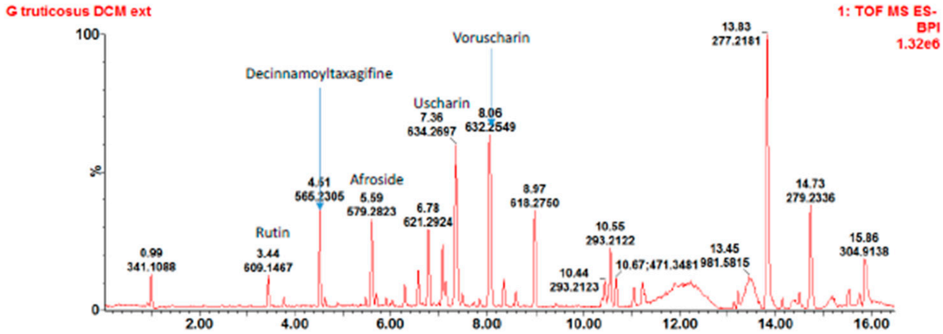

Figure 1. Representative chromatograms obtained for *Gomphocarpus fruticosus* dichloromethane (DCM) extract using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS).

Cardenolides are toxic steroids with historical medicinal use, primarily as Na+/K+ ATPase inhibitors [23]. While their direct link to mental disorders is unclear, some cardenolides affect the central nervous system [24], warranting further research. Flavonoid rutin, from *Schinus molle* L., has demonstrated antidepressant effects by increasing serotonin and noradrenaline levels, as shown in a study by Machado et al. [25], where it reduced immobility time without altering locomotor activity, suggesting its potential role in mood regulation.

The UPLC-MS generated the phytochemical fingerprint of *L. leonurus* DCM (Figure 2) and other solvents namely methanol, acetone and hexane (Supplementary Tables S1–S3), indicating the presence of 6-methoxyluteolin-4′-methyl ether, 9,13-epoxylabda-6(19)-diol dilactone, and marrubiin (diterpenes). Percentages of these compounds varied as indicated by the generated peaks from each of the different solvent extracts. Diterpenes are essential oils widely recognised for their neurobiological activities [26]. While decinnamoyltaxagifine has been identified in plants of the Taxaceae family [27,28], there is currently no evidence supporting its antidepressant properties.

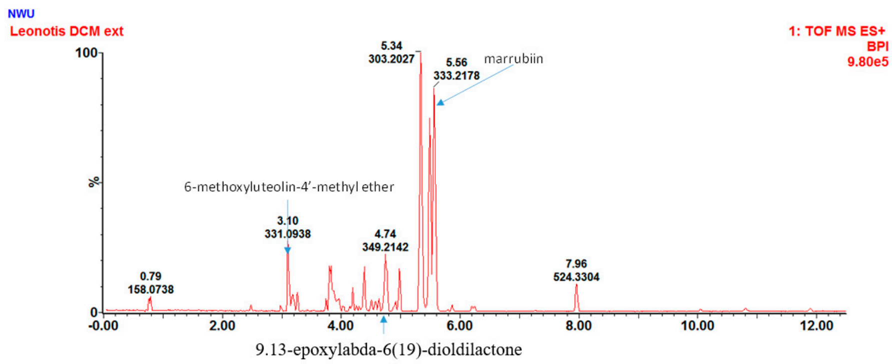

Figure 2. Representative chromatograms obtained for *Leonotis leonurus* dichloromethane (DCM) extract based on ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS).

Conversely, diterpenes, recognised for neurobiological activities [26,29], include ginkgolides, which exhibit antidepressant and anti-anxiety effects through specific neural pathways like NT3 TrkA and Ras-MAPK [30,31]. Based on the use of UPLC-MS, 6-methoxyluteolin-4′-methyl ether, 9,13-epoxylabda-6(19)-diol dilactone, and marrubiin (diterpene) were identified in *L. leonurus* extract (Figure 2). The marker compound, marrubiin, is commonly present in the mint family, Lamiaceae, and it has been previously isolated from *L. leonurus* [32].

Marrubiin has an established pharmacological potential as an antioxidant, anti-inflammatory, calcium channel blocker, antidiabetic, and vasorelaxant [33]. It modulates key neurotransmitters such as GABA and glutamate, inhibits voltage-gated Ca^{2+} channels, and reduces proinflammatory cytokines, which are linked to depression [34]. While the diterpenoid 9.13-epoxylabda-6(19)-diol dilactone from *L. leonurus* lacks studies on antidepressant activity, flavonoids including 6-methoxyluteolin-4′-methyl ether from the same plant family have been isolated and purified from the *L. leonurus* [35,36].

3.2. Micromorphology of Plant Parts

Micromorphological studies of medicinal plants focus on examining the microscopic structure and characteristics of plant tissues. Analysing these specific plants will help ensure the quality and consistency of their medicinal preparations. Psychoactive compounds, such as salvinorin A, are found in the subcuticular space of peltate glandular trichomes (GTs) in *Salvia divinorum* [37]. The variety and distribution of trichome types—peltate, capitate, and non-glandular—differ among plant species and organs, which aids in the identification of medicinal plants [38,39]. The leaves were distinguished by the presence of both glandular and non-glandular trichomes that are present on the leaves of *G. fruticosus* (Figure 3A,B). The non-glandular trichomes exhibited varying lengths (Figure 3A). A similar type of glandular trichomes has been reported in *Asclepias curassavica*, which also possesses antidepressant effect [40].

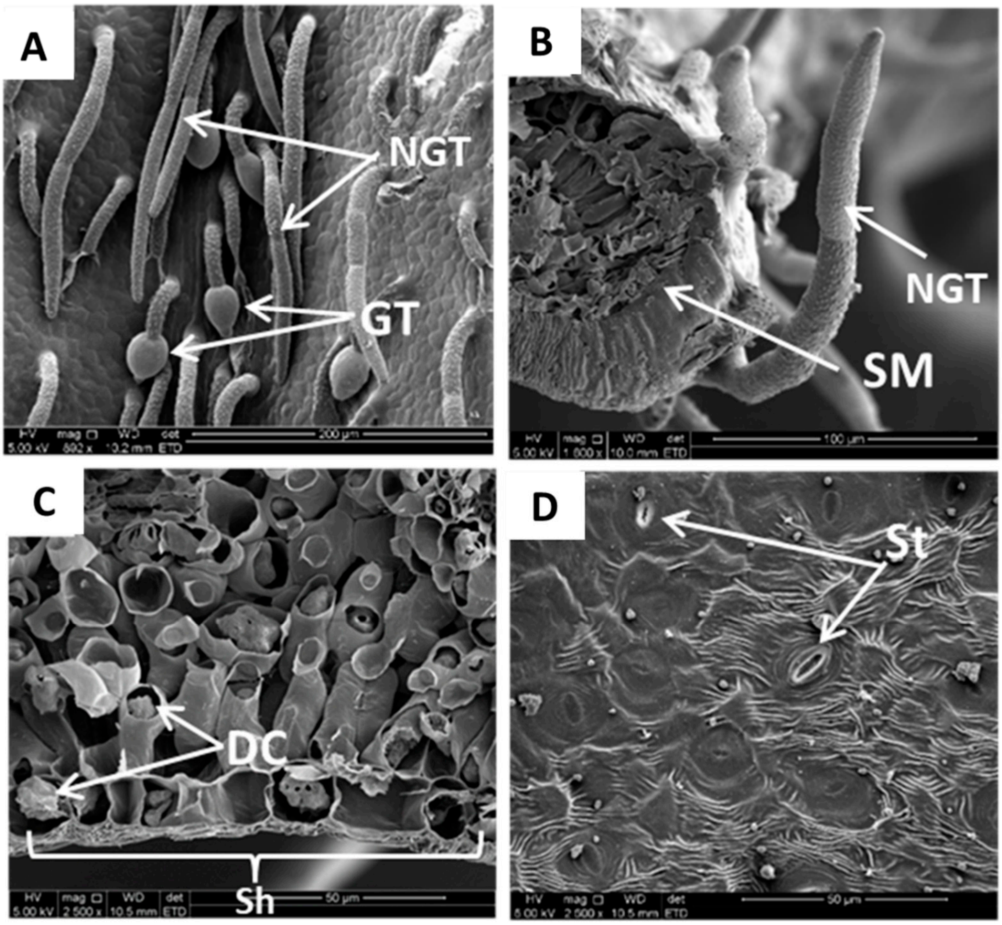

Figure 3. Scanning electron micrographs of *Gomphocarpus fruticosus* leaves. (**A**) Leaf with trichomes, (**B**,**C**) cross section of leaves, and (**D**) multiple stoma/stomata. GT = glandular trichome, NGT = non-glandular trichomes, SM = secretory material, DC = druse crystal, Sh = sheath cell, and St = stoma/stomata.

Flattened and elongated secretory cells were identified by outlining the periphery of the cavity (Figure 3B). These cells, known as sheath cells, serve as a protective layer that encases the secretory cavity [41]. The secretory material within typically contains a variety of compounds, including terpenes, phenolics, and polysaccharides [39]. These phytochemicals likely contribute to the management of depressive disorders by modulating various mechanisms [10]. The milky white latex produced by *G. fruticosus* is secreted by this secretory material and is considered a synapomorphy of the Apocynaceae [42]. Due to the composite nature of latex secretion, often comprising a variety of specialised metabolites, several latex-bearing plants are renowned for their specific substances [43]. Stomatal occurrences were also observed (Figure 3D). Stomata primarily serve two functions: facilitating the uptake of carbon dioxide and restricting water loss through evaporation [44]. Additionally, druse crystals were distributed throughout the mesophyll and phloem of the leaves (Figure 3C). Plants utilise druse crystals to store surplus calcium in the form of calcium oxalate, and this reservoir can be mobilised as necessary during the calcium control process [45].

Microscopy examination of *L. leonurus* leaf surfaces revealed two distinct types of trichomes, namely glandular and non-glandular trichomes (Figure 4A–E). This highlights the presence of dense non-glandular trichomes, suggesting enhanced protection for the plant. These structures act as mechanical barriers, offering defence against excessive water loss and various external factors [46]. Glandular trichomes, evenly distributed across the entire leaf surface, are identified as peltate trichomes, characterised by a large disc-like head and a short stalk (Figure 4D). Each glandular trichome exhibits morphological divisions in three regions: the multicellular head, stalk, and basal cavities [47]. These trichomes are recognised for storing and secreting waxes and phytochemicals, including phenolics, terpenes, flavonoids, and alkaloids. This chemical arsenal provides both chemical and physiological protection against herbivores and pathogens [39]. Ascensão et al. [48] reported similar findings with *L. leonurus* and comparable trichome morphology has been established in other plants within the Lamiaceae family [49] and also in *Cannabis sativa*, which is sometimes used to alleviate psychiatric symptoms, such as anxiety, depression, and mania [50].

The micromorphology of plant, particularly features such as glandular and non-glandular trichomes, affects the production and storage of bioactive compounds [51]. Glandular trichomes, for example, serve as sites for synthesising and storing essential oils, terpenes, and other secondary metabolites [52]. Different trichome types and cellular structures that contribute to the therapeutic characteristics of *G. fruticosus* and *L. leonurus* were identified by micromorphological research. Both glandular and non-glandular trichomes were present on the leaves of *G. fruticosus*; the glandular trichomes were essential for the secretion of psychotropic substances and other phytochemicals, which may help treat depressive illnesses. Similar to *G. fruticosus*, this plant species has non-glandular trichomes that aid in structural defence. The presence of peltate glandular trichomes allowed for the secretion of a wide range of bioactive substances, such as terpenes and flavonoids, while *L. leonurus* demonstrated a high density of non-glandular trichomes that improve defence against environmental stresses.

This work highlights the diversity of trichome distribution and form among these species, emphasising their importance in plant-mediated medicinal effects as well as their adaptive defence mechanisms. For the storage and secretion of psychotropic substances that can reduce the symptoms of anxiety and depression, micromorphological characteristics—such as glandular trichomes in *G. fruticosus*—are essential. In addition to acting as defence mechanisms, these trichomes shield the plants from environmental stresses and herbivory, ensuring a consistent supply of therapeutic chemicals.

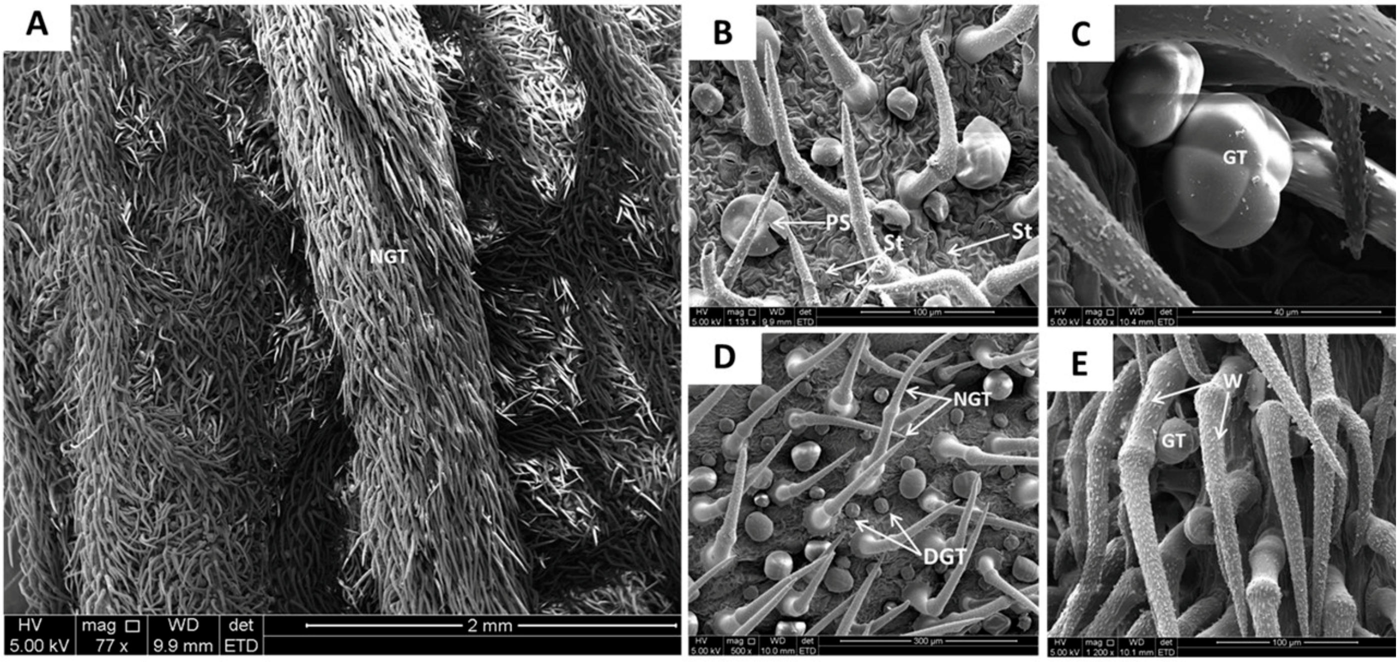

Figure 4. Scanning electron micrographs of *Leonotis leonurus* leaves. (**A**) Abundant non-glandular trichomes were found in the leaves of the plant. (**B**) Stoma/stomata were found with glandular trichomes and non-glandular trichomes. Post-secretory glandular trichomes were also present. (**C**–**E**) Developing and developed glandular trichomes; non-glandular trichomes with cuticular wart. GT = glandular trichome, NGT = non-glandular trichomes, St = stoma/stomata, PS = post-secretory trichome, DGT = developing trichomes, and W = cuticular wart.

3.3. Elemental Composition

Common elements found in these plants include sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), selenium (Se), zinc (Zn), and iron (Fe) [53]. Studies in different regions have identified specific plants used for mental health treatments, such as *Tilia platyphyllos* and *Valeriana officinalis,* in the Catalan linguistic area [54]. Although plants accumulate beneficial major elements, some may contain trace heavy metals at levels exceeding international safety standards [55].

The elemental analysis of *G. fruticosus* leaves and *L. leonurus* leaves using SEM coupled with an energy-dispersive X-ray spectrometer revealed the presence of carbon (C), oxygen (O), magnesium (Mg), silicon (Si), phosphorus (P), chlorine (Cl), potassium (K), and calcium (Ca) (Table 1). The presence of the elements was found to be in decreasing order of C > O > K > Ca > Si > Cl > Mg > P in the leaves of *G. fruticosus* and C > O > K > Ca > Mg = Cl > Si > P in the leaves of *L. Leonurus.* The results indicated an abundance of C, O, K, and Ca in the two studied plants. These elements are crucial for photosynthesis (C and O), for breaking down sugars to obtain energy (C), for cellular respiration (O), for producing grains rich in starch and maintaining turgor (K), and for balancing organic acids within the plant, improving root growth conditions (Ca) [53]. A variety of factors, including climate and soil conditions influence the mineral composition of plants in different regions [56]. The nutritional value of plants is determined by these mineral elements, which are transported from soil to plant and eventually to humans [57]. The presence of specific elements such as calcium, magnesium, and potassium, can directly influence the synthesis and stability of bioactive compounds within the plant [58,59]. These minerals often play a role as cofactors in enzymatic processes essential for metabolite synthesis, such as the production of flavonoids, alkaloids, and terpenes [58].

Table 1. Average weight (%) comparison of *Gomphocarpus fruticosus* and *Leonotis leonurus* elemental composition.

Mineral Elements (% Weight)	Plants	
	Gomphocarpus fruticosus	***Leonotis leonurus***
Carbon (C)	61.28 ± 1.80	58.12 ± 1.33
Oxygen (O)	34.17 ± 1.57	37.86 ± 1.06
Sodium (Na)	0.00	0.00
Potassium (K)	1.16 ± 0.22	1.99 ± 0.10
Calcium (Ca)	1.32 ± 0.29	0.82 ± 0.04
Chlorine (Cl)	0.62 ± 0.06	0.37 ± 0.05
Sulphur (S)	0.32 ± 0.03	0.17 ± 0.04
Silicon (Si)	0.64 ± 0.26	0.16 ± 0.87
Magnesium (Mg)	0.38 ± 0.03	0.37 ± 0.03
Phosphorus (P)	0.13 ± 0.04	0.15 ± 0.02

No significant difference in the quantity of mineral elements between the two studied plants.

Micronutrients such as copper (Cu), selenium (Se), Mg, K, and Ca assist in maintaining a stable mental state, and an imbalance of these minerals is associated with mental disorders [60]. As indicated in the current results, Mg, K, and Ca are present in all the plants selected for the study, and a deficiency in these mineral elements in humans increases the risk of depression via different pathways [60,61]. Mg plays a vital role in maintaining the stability and functioning of the brain and central nervous system [62,63]. The multiple possible mechanisms of magnesium involvement in depression and anxiety involve the blockage of the glutamatergic N-methyl-D-aspartate (NMDA) [64] and its receptors (NMDAR) [65], which modulates the serotoninergic system [66], and alterations in the gut microbiota, leading to a negative impact on the gut–brain axis [67].

For all the present minerals, there was no significant difference. P and Ca are two essential minerals that play a crucial role in maintaining overall physical and mental well-being. Phosphorus governs the proper functioning of the nervous system, muscle contractions, and other vital physiological processes, whereas Ca is paramount for the transmission of nerve impulses, muscle function, and the release of neurotransmitters [68,69]. High concentrations elements may support the growth and density of trichomes, which in turn may act as protective barriers against environmental stressors, preserving the bioactive compounds within [70]. For instance, calcium can contribute to cell wall stability and trichome formation, indirectly supporting the storage and stability of therapeutic compounds [70,71].

The elemental composition of these plants, which includes vital minerals like P, Ca, and Mg, promotes neurological health by supporting vital physiological functions including neurotransmitter activity. Furthermore, the presence of micronutrients such as Cu and Se improves the stability of mental health, and interactions between these minerals and phytochemicals might affect neurotransmitter systems, which helps explain the antidepressant properties of the plants. In general, the medicinal ability of these plants to effectively treat mental health conditions is influenced by both their micromorphology and elemental composition.

4. Conclusions

This study on *G. fruticosus* and *L. leonurus* revealed a complex interplay between phytochemicals, elemental minerals, and trichomes that support the metabolic and medicinal properties of plants. This research data is a preliminary step that bridges ethnobotanical use and modern medicine development. The elements (carbon, oxygen, magnesium, silicon, phosphorus, chlorine, potassium, and calcium) and compounds (6-methoxyluteolin-4′-methyl ether, 9,13-epoxylabda-6(19)-diol dilactone, and marrubiin for *L. Leonurus* and rutin, voruscharin, uscharin, afroside, and decinnamoyltaxagifine (diterpene) for *G. fruticosus*) found in the plant extracts have been previously shown to have neuro activity and neuromodulatory properties. Hence, identification coupled with the chemical and elemental profile of each plant provides empirical data on their potential for further development as alternative therapies for mental health-related diseases including depression. However, further research in disease models needs to be carried out. While this foundational understanding supports their continued use in both folk and modern contexts, further rigorous studies are essential to confirm the efficacy of these phytochemicals. Future research should explore their therapeutic potential to create effective, evidence-based formulations.

Supplementary Materials: The following supporting information can be downloaded at the following website: https://www.mdpi.com/article/10.3390/app142411540/s1. Figure S1: Representative chromatograms obtained for *Leonotis leonurus* methanol extract using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). Figure S2: Representative chromatograms obtained for *Leonotis leonurus* acetone extract using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). Figure S3: Representative chromatograms obtained for *Leonotis leonurus* hexane extract using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). Table S1: Tentative identification of *Leonotis leonurus* extracts using UPLC-MS ESI positive mode; Table S2: Relative percentage (%) of identified peaks for *Leonotis leonurus* extracts; Table S3: Tentative identification of *Gomphocarpus fruticosus* dichloromethane (DCM) extract using UPLC-MS ESI negative mode.

Author Contributions: Conceptualization, A.M., A.O.A. and M.L.; methodology, A.M., N.A.A., A.O.A., T.M. and M.L.; formal analysis, A.M. and N.A.A.; investigation, A.M. and N.A.A.; resources, N.A.A., A.O.A. and M.L.; writing—original draft preparation, A.M.; writing—review and editing, N.A.A., T.M., A.O.A. and M.L.; supervision, A.O.A., T.M. and M.L.; project administration, A.O.A. and M.L.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Research Foundation (NRF, UID 129 870) and partially support from the International Brain Research Organisation (IBRO) and the Welcome Trust Neuroscience Capacity Accelerator for Mental Health programme.

Institutional Review Board Statement: The study protocol was approved by the North-West University Animal Research Ethics Committee (NWU-AnimCareREC) with reference number NWU00780-22-A5.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data related to this study are presented in the manuscript.

Acknowledgments: We thank Alison Young (horticulturist, University of KwaZulu-Natal Botanical Garden) and McMaster Vambe (Mangosuthu University of Technology) for assisting with plant collection. We are grateful to Christina Potgieter (Bews Herbarium, NU) and Prin Naidu (A.P. Goossens Herbarium, North-West University) for assisting with the identification of the plants. We acknowledge the technical support of Anine Jordaan (Laboratory for Electron Microscopy, Chemical Resource Beneficiation (CRB), North-West University). We also thank Weiyang Chen (Department of Pharmaceutical Sciences, Tshwane University of Technology) for assistance with the phytochemical quantification of plant extracts.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Ray, S. Weaving the links: Traditional knowledge into modern science. *Futures* **2023**, *145*, 103081. [CrossRef]
2. Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83*, 770–803. [CrossRef] [PubMed]
3. Breslin, A. The Chemical Composition of Green Plants. Sciencing. Available online: https://www.sciencing.com/chemical-composition-green-plants-8336363/published (accessed on 30 August 2022).
4. Lee, G.; Bae, H. Therapeutic effects of phytochemicals and medicinal herbs on depression. *BioMed Res. Int.* **2017**, *2017*, 6596241. [CrossRef] [PubMed]

5. Rahman, M.H.; Bajgai, J.; Fadriquela, A.; Sharma, S.; Trinh, T.T.; Akter, R.; Jeong, Y.J.; Goh, S.H.; Kim, C.-S.; Lee, K.-J. Therapeutic potential of natural products in treating neurodegenerative disorders and their future prospects and challenges. *Molecules* **2021**, *26*, 5327. [CrossRef]
6. Gebashe, F.; Aremu, A.O.; Gruz, J.; Finnie, J.F.; Van Staden, J. Phytochemical profiles and antioxidant activity of grasses used in South African traditional medicine. *Plants* **2020**, *9*, 371. [CrossRef]
7. Ruiters, A.; Tilney, P.M.; Van Vuuren, S.; Viljoen, A.M.; Kamatou, G.P.; Van Wyk, B.-E. The anatomy, ethnobotany, antimicrobial activity and essential oil composition of southern African species of *Teucrium* (Lamiaceae). *S. Afr. J. Bot.* **2016**, *102*, 175–185. [CrossRef]
8. Bonokwane, M.B.; Lekhooa, M.; Struwig, M.; Aremu, A.O. Antidepressant effects of South African plants: An appraisal of ethnobotanical surveys, ethnopharmacological and phytochemical studies. *Front. Pharmacol.* **2022**, *13*, 895286. [CrossRef]
9. Peltzer, K.; Preez, N.F.-d.; Ramlagan, S.; Fomundam, H. Use of traditional complementary and alternative medicine for HIV patients in KwaZulu-Natal, South Africa. *BMC Public Health* **2008**, *8*, 255. [CrossRef]
10. Faber, R.J.; Laubscher, C.P.; Jimoh, M.O. The importance of *Sceletium tortuosum* (L.) NE brown and its viability as a traditional african medicinal plant. In *Natural Drugs from Plants*; El-Shemy, H.A., Ed.; IntechOpen: London, UK, 2021. [CrossRef]
11. Olatunji, T.L.; Siebert, F.; Adetunji, A.E.; Harvey, B.H.; Gericke, J.; Hamman, J.H.; Van der Kooy, F. *Sceletium tortuosum*: A review on its phytochemistry, pharmacokinetics, biological and clinical activities. *J. Ethnopharmacol.* **2021**, *280*, 114476. [CrossRef]
12. Mnqika, A.; Aremu, A.O.; Janse van Rensburg, H.; Lekhooa, M. Preliminary screening of South African plants for binding affinity to the serotonin reuptake transporter and adenosine A_1/A_{2A} receptors. *Sci. Pharm.* **2023**, *91*, 41. [CrossRef]
13. Marguí, E.; Queralt, I.; Hidalgo, M. Application of X-ray fluorescence spectrometry to determination and quantitation of metals in vegetal material. *TrAC Trends Anal. Chem.* **2009**, *28*, 362–372. [CrossRef]
14. Kumar, A.; Kafeel, M.; Ahmed, K.; Rameez, M.; Mathew, S.R. Phyto-Pharmacological effects of medicinal plants for the treatment of depression. *J. Adv. Zool.* **2024**, *45*, 780–787.
15. Dobrek, L.; Głowacka, K. Depression and its phytopharmacotherapy—A narrative review. *Int. J. Mol. Sci.* **2023**, *24*, 4772. [CrossRef] [PubMed]
16. Mahmoudieh, M.; Naghavi, M.R.; Sobri, Z.M.; Azzeme, A.M.; Abd-Aziz, N.; Abd Rahman, N.M.A.N.; Alitheen, N.B.; Hussin, Y.; Bahmanrokh, G.; Baharum, N.A. Biotechnological approaches in the production of plant secondary metabolites for treating human viral diseases: Prospects and challenges. *Biocatal. Agric. Biotechnol.* **2024**, *59*, 103249. [CrossRef]
17. Watson, R.; Wright, S. The cardiac glycosides of *Gomphocarpus fruticosus* R. Br. II. Gomphoside. *Aust. J. Chem.* **1957**, *10*, 79–84. [CrossRef]
18. Watson, T.; Wright, S. The cardiac glycosides of *Gomphocarpusf ruticosus* R. Br. I. Afroside. *Aust. J. Chem.* **1956**, *9*, 497–511. [CrossRef]
19. Marzouk, A.M.; Osman, S.M.; Gohar, A.A. A new pregnane glycoside from *Gomphocarpus fruticosus* growing in Egypt. *Nat. Prod. Res.* **2016**, *30*, 1060–1067. [CrossRef]
20. Lindhorst, T.K. *Essentials of Carbohydrate Chemistry and Biochemistry*; John Wiley & Sons: Weinheim, Germany, 2007.
21. Serafini, G.; Pompili, M.; Innamorati, M.; Giordano, G.; Tatarelli, R.; Lester, D.; Girardi, P.; Dwivedi, Y. Glycosides, depression and suicidal behaviour: The role of glycoside-linked proteins. *Molecules* **2011**, *16*, 2688–2713. [CrossRef]
22. Neves, S.R.; Ram, P.T.; Iyengar, R. G protein pathways. *Science* **2002**, *296*, 1636–1639. [CrossRef]
23. Yamane, H.; Konno, K.; Sabelis, M.; Takabayashi, J.; Sassa, T.; Oikawa, H. Chemical defence and toxins of plants. In *Comprehensive Natural Products II: Chemistry and Biology*; Mander, L., Lui, H.W., Eds.; Elsevier: Amsterdam, The Netherlands, 2010; Volume 4, p. 671.
24. Sharma, M.; Kumar Delta, A.; Kaushik, P. Phytochemistry and Pharmacology of *Calotropis gigantea*-an update. *Indian J. Biochem. Biophys.* **2022**, *59*, 611–618.
25. Machado, D.G.; Bettio, L.E.; Cunha, M.P.; Santos, A.R.; Pizzolatti, M.G.; Brighente, I.M.; Rodrigues, A.L. Antidepressant-like effect of rutin isolated from the ethanolic extract from *Schinus molle* L. in mice: Evidence for the involvement of the serotonergic and noradrenergic systems. *Eur. J. Pharmacol.* **2008**, *587*, 163–168. [CrossRef] [PubMed]
26. Islam, M.T.; da Silva, C.B.; de Alencar, M.V.O.B.; Paz, M.F.C.J.; Almeida, F.R.d.C.; Melo-Cavalcante, A.A.d.C. Diterpenes: Advances in neurobiological drug research. *Phytother. Res.* **2016**, *30*, 915–928. [CrossRef] [PubMed]
27. Su, J.; Shi, H.-X.; Wang, L.-J.; Guo, R.-X.; Ren, T.-K.; Wu, Y.-B. Chemical constituents of bark of *Taxus chinensis* var. *mairei*. *Zhong Yao Cai = Zhongyaocai = J. Chin. Med. Mater.* **2014**, *37*, 243–251.
28. Li, Y.; Qin, F.; Wang, S.M.; Guo, R.X.; Zhang, Y.F.; Gu, Y.C.; Shi, Q.W. Chemical studies on *Taxus canadensis*. *Chem. Biodivers.* **2013**, *10*, 1729–1753. [CrossRef]
29. Chang, H.-J.; Kim, H.J.; Chun, H.S. Quantitative structure–activity relationship (QSAR) for neuroprotective activity of terpenoids. *Life Sci.* **2007**, *80*, 835–841. [CrossRef]
30. Bai, S.; Zhang, X.; Chen, Z.; Wang, W.; Hu, Q.; Liang, Z.; Shen, P.; Gui, S.; Zeng, L.; Liu, Z. Insight into the metabolic mechanism of Diterpene Ginkgolides on antidepressant effects for attenuating behavioural deficits compared with venlafaxine. *Sci. Rep.* **2017**, *7*, 9591. [CrossRef]
31. Wang, T.; Bai, S.; Wang, W.; Chen, Z.; Chen, J.; Liang, Z.; Qi, X.; Shen, H.; Xie, P. Diterpene ginkgolides exert an antidepressant effect through the NT3-TrkA and Ras-MAPK pathways. *Drug Des. Dev. Ther.* **2020**, *14*, 1279–1294. [CrossRef]

32. Viljoen, A.; Chen, W.; Mulaudzi, N.; Kamatou, G.; Sandasi, M. 15—*Leonotis leonurus*. In *Phytochemical Profiling of Commercially Important South African Plants*; Viljoen, A., Chen, W., Mulaudzi, N., Kamatou, G., Sandasi, M., Eds.; Academic Press: Cambridge, MA, USA, 2022; pp. 147–154.
33. Popoola, O.K.; Elbagory, A.M.; Ameer, F.; Hussein, A.A. Marrubiin. *Molecules* **2013**, *18*, 9049–9060. [CrossRef]
34. Sendrowski, K.; Rusak, M.; Sobaniec, P.; Iłendo, E.; Dąbrowska, M.; Boćkowski, L.; Koput, A.; Sobaniec, W. Study of the protective effect of calcium channel blockers against neuronal damage induced by glutamate in cultured hippocampal neurons. *Pharmacol. Rep.* **2013**, *65*, 730–736. [CrossRef]
35. El-Ansari, M.A.; Aboutabl, E.A.; Farrag, A.R.H.; Sharaf, M.; Hawas, U.W.; Soliman, G.M.; El-Seed, G.S. Phytochemical and pharmacological studies on *Leonotis leonurus*. *Pharm. Biol.* **2009**, *47*, 894–902. [CrossRef]
36. Afolayan, A.J.; Sunmonu, T.O. In vivo studies on antidiabetic plants used in South African herbal medicine. *J. Clin. Biochem. Nutr.* **2010**, *47*, 98–106. [CrossRef] [PubMed]
37. Tang, H.; Jiang, Q.; Liu, H.; Zhang, F.; Liu, Q.; Pu, G.; Li, J.; Wang, L.; Zhang, Y. Glandular trichomes of medicinal plants: Types, separation and purification, biological activities. *Biol. Plant.* **2022**, *66*, 219–227. [CrossRef]
38. Rambau, U.; Naidoo, Y.; Sadashiva, C.T.; Baijnath, H.; Dewir, Y.H.; Magyar-Tábori, K. The foliar anatomy and micromorphology of *Cyphostemma hypoleucum* (Vitaceae). *Plants* **2023**, *12*, 2312. [CrossRef] [PubMed]
39. Akwu, N.A.; Naidoo, Y.; Singh, M. Die anatomie en histochemie van *Grewia lasiocarpa* E. Mey. ex Harv.(Malvaceae). *Suid-Afr. Tydskr. Natuurwetenskap Tegnol.* **2020**, *39*, 91–107. [CrossRef]
40. Demarco, D. Secretory tissues and the morphogenesis and histochemistry of pollinarium in flowers of Asclepiadeae (Apocynaceae). *Int. J. Plant Sci.* **2014**, *175*, 1042–1053. [CrossRef]
41. Leegood, R.C. Roles of the bundle sheath cells in leaves of C_3 plants. *J. Exp. Bot.* **2008**, *59*, 1663–1673. [CrossRef]
42. Nazar, N.; Goyder, D.J.; Clarkson, J.J.; Mahmood, T.; Chase, M.W. The taxonomy and systematics of Apocynaceae: Where we stand in 2012. *Bot. J. Linn. Soc.* **2013**, *171*, 482–490. [CrossRef]
43. Gonçalves, M.P.; Mercadante-Simões, M.O.; Ribeiro, L.M. Ontogeny of anastomosed laticifers in the stem apex of *Hancornia speciosa* (Apocynaceae): A topographic approach. *Protoplasma* **2018**, *255*, 1713–1724. [CrossRef]
44. Clark, J.W.; Harris, B.J.; Hetherington, A.J.; Hurtado-Castano, N.; Brench, R.A.; Casson, S.; Williams, T.A.; Gray, J.E.; Hetherington, A.M. The origin and evolution of stomata. *Curr. Biol.* **2022**, *32*, R539–R553. [CrossRef]
45. Nakata, P.A. Advances in our understanding of calcium oxalate crystal formation and function in plants. *Plant Sci.* **2003**, *164*, 901–909. [CrossRef]
46. Redha, A.; Al-Mansour, N.; Suleman, P.; Afzal, M.; Al-Hasan, R. Leaf traits and histochemistry of trichomes of *Conocarpus lancifolius* a Combretaceae in semi-arid conditions. *Am. J. Plant Sci.* **2011**, *2*, 165–174. [CrossRef]
47. Akwu, N.A.; Naidoo, Y.; Singh, M.; Dewir, Y.H.; Magyar-Tábori, K.; Lekhooa, M.; Aremu, A.O. Development and biomechanics of *Grewia lasiocarpa* E. Mey. Ex Harv. trichomes exudate. *Plants* **2023**, *12*, 2198. [CrossRef] [PubMed]
48. Ascensão, L.; Marques, N.; Pais, M. Glandular trichomes on vegetative and reproductive organs of *Leonotis leonurus* (Lamiaceae). *Ann. Bot.* **1995**, *75*, 619–626. [CrossRef]
49. Więdłocha, M.; Marcinowicz, P.; Krupa, R.; Janoska-Jaździk, M.; Janus, M.; Dębowska, W.; Mosiołek, A.; Waszkiewicz, N.; Szulc, A. Effect of antidepressant treatment on peripheral inflammation markers–A meta-analysis. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2018**, *80*, 217–226. [CrossRef]
50. Livingston, S.J.; Quilichini, T.D.; Booth, J.K.; Wong, D.C.; Rensing, K.H.; Laflamme-Yonkman, J.; Castellarin, S.D.; Bohlmann, J.; Page, J.E.; Samuels, A.L. *Cannabis* glandular trichomes alter morphology and metabolite content during flower maturation. *Plant J.* **2020**, *101*, 37–56. [CrossRef]
51. Shukla, P.; Prasad, A.; Chawda, K.; Saxena, G.; Pandey, K.D.; Chakrabarty, D. Glandular trichomes: Bio-cell factories of plant secondary metabolites. In *In Vitro Propagation and Secondary Metabolite Production from Medicinal Plants: Current Trends (Part 1)*; Anis, M., Khanam, M.N., Eds.; Bentham Science Publishers: Sharjah, United Arab Emirates, 2024; pp. 91–119.
52. Senaratne, S.L. Non-Volatile Secondary Metabolites in Foliar Oil Glands of *Eucalyptus* Species. Doctoral Thesis, University of Melbourne, Parkville, VIC, Australia, 2020, *Unpublished*.
53. Soetan, K.; Olaiya, C.; Oyewole, O. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food Sci.* **2010**, *4*, 200–222.
54. Irún, L.N.; Gras, A.; Parada, M.; Garnatje, T. Plants and mental disorders: The case of *Catalan linguistic* area. *Front. Pharmacol.* **2023**, *14*, 1256225. [CrossRef]
55. Uddin, M.M.; Zakeel, M.C.M.; Zavahir, J.S.; Marikar, F.M.; Jahan, I. Heavy metal accumulation in rice and aquatic plants used as human food: A general review. *Toxics* **2021**, *9*, 360. [CrossRef]
56. Maathuis, F.J.; Diatloff, E. Roles and functions of plant mineral nutrients. *Methods Mol. Biol.* **2013**, *953*, 1–21.
57. Anuradha, V.; Praveena, A.; Sanjayan, K. Nutritive analysis of fresh and dry fruits of *Morinda tinctoria*. *Int. J. Curr. Microbiol. Appl. Sci.* **2013**, *2*, 65–74.
58. Mishra, B.K.; Rastogi, A.; Shukla, S. Regulatory role of mineral elements in the metabolism of medicinal plants. *Med. Aromat. Plant Sci. Biotech.* **2012**, *6*, 1–23.
59. Gerendás, J.; Führs, H. The significance of magnesium for crop quality. *Plant Soil* **2013**, *368*, 101–128. [CrossRef]
60. Shayganfard, M. Are essential trace elements effective in modulation of mental disorders? Update and perspectives. *Biol. Trace Elem. Res.* **2022**, *200*, 1032–1059. [CrossRef] [PubMed]

61. Quan, Z.; Li, H.; Quan, Z.; Qing, H. Appropriate macronutrients or mineral elements are beneficial to improve depression and reduce the risk of depression. *Int. J. Mol. Sci.* **2023**, *24*, 7098. [CrossRef] [PubMed]
62. Botturi, A.; Ciappolino, V.; Delvecchio, G.; Boscutti, A.; Viscardi, B.; Brambilla, P. The role and the effect of magnesium in mental disorders: A systematic review. *Nutrients* **2020**, *12*, 1661. [CrossRef]
63. Veronese, N.; Demurtas, J.; Pesolillo, G.; Celotto, S.; Barnini, T.; Calusi, G.; Caruso, M.G.; Notarnicola, M.; Reddavide, R.; Stubbs, B. Magnesium and health outcomes: An umbrella review of systematic reviews and meta-analyses of observational and intervention studies. *Eur. J. Nutr.* **2020**, *59*, 263–272. [CrossRef]
64. Abumaria, N.; Yin, B.; Zhang, L.; Li, X.-Y.; Chen, T.; Descalzi, G.; Zhao, L.; Ahn, M.; Luo, L.; Ran, C. Effects of elevation of brain magnesium on fear conditioning, fear extinction, and synaptic plasticity in the infralimbic prefrontal cortex and lateral amygdala. *J. Neurosci.* **2011**, *31*, 14871–14881. [CrossRef]
65. Pochwat, B.; Szewczyk, B.; Sowa-Kucma, M.; Siwek, A.; Doboszewska, U.; Piekoszewski, W.; Gruca, P.; Papp, M.; Nowak, G. Antidepressant-like activity of magnesium in the chronic mild stress model in rats: Alterations in the NMDA receptor subunits. *Int. J. Neuropsychopharmacol.* **2014**, *17*, 393–405. [CrossRef]
66. Poleszak, E. Modulation of antidepressant-like activity of magnesium by serotonergic system. *J. Neural Transm.* **2007**, *114*, 1129–1134. [CrossRef]
67. Winther, G.; Jørgensen, B.M.P.; Elfving, B.; Nielsen, D.S.; Kihl, P.; Lund, S.; Sørensen, D.B.; Wegener, G. Dietary magnesium deficiency alters gut microbiota and leads to depressive-like behaviour. *Acta Neuropsychiatr.* **2015**, *27*, 168–176. [CrossRef]
68. Zielińska, M.; Łuszczki, E.; Dereń, K. Dietary nutrient deficiencies and risk of depression (Review Article 2018–2023). *Nutrients* **2023**, *15*, 2433. [CrossRef] [PubMed]
69. Jomova, K.; Makova, M.; Alomar, S.Y.; Alwasel, S.H.; Nepovimova, E.; Kuca, K.; Rhodes, C.J.; Valko, M. Essential metals in health and disease. *Chem.-Biol. Interact.* **2022**, *367*, 110173. [CrossRef] [PubMed]
70. Weigend, M.; Mustafa, A.; Ensikat, H.-J. Calcium phosphate in plant trichomes: The overlooked biomineral. *Planta* **2018**, *247*, 277–285. [CrossRef] [PubMed]
71. Huang, S.-S.; Liao, J.-P.; Kirchoff, B.K. Calcium distribution and function in the glandular trichomes of *Lavandula pinnata* L. *J. Torrey Bot. Soc.* **2010**, *137*, 1–15. [CrossRef]