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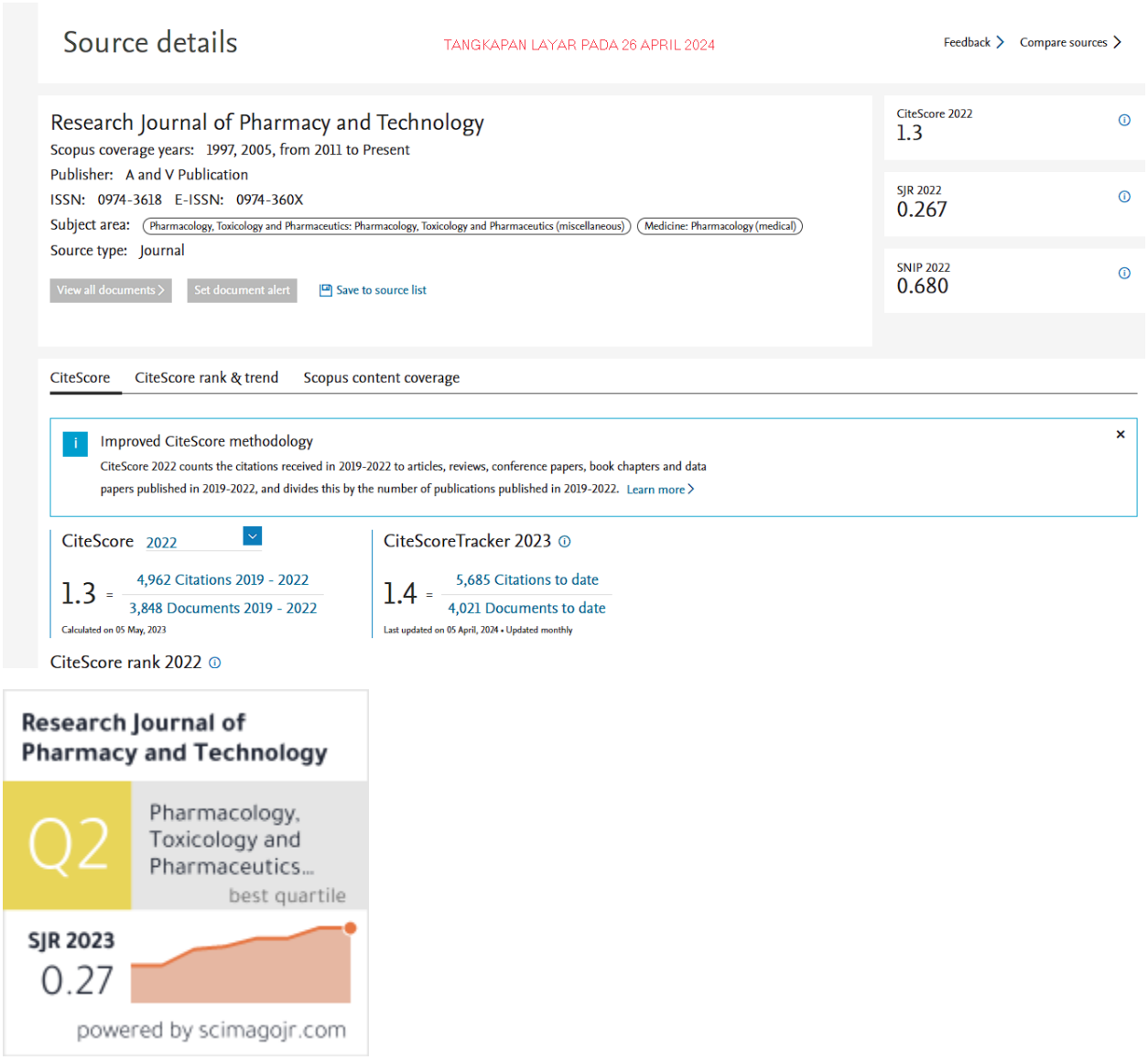

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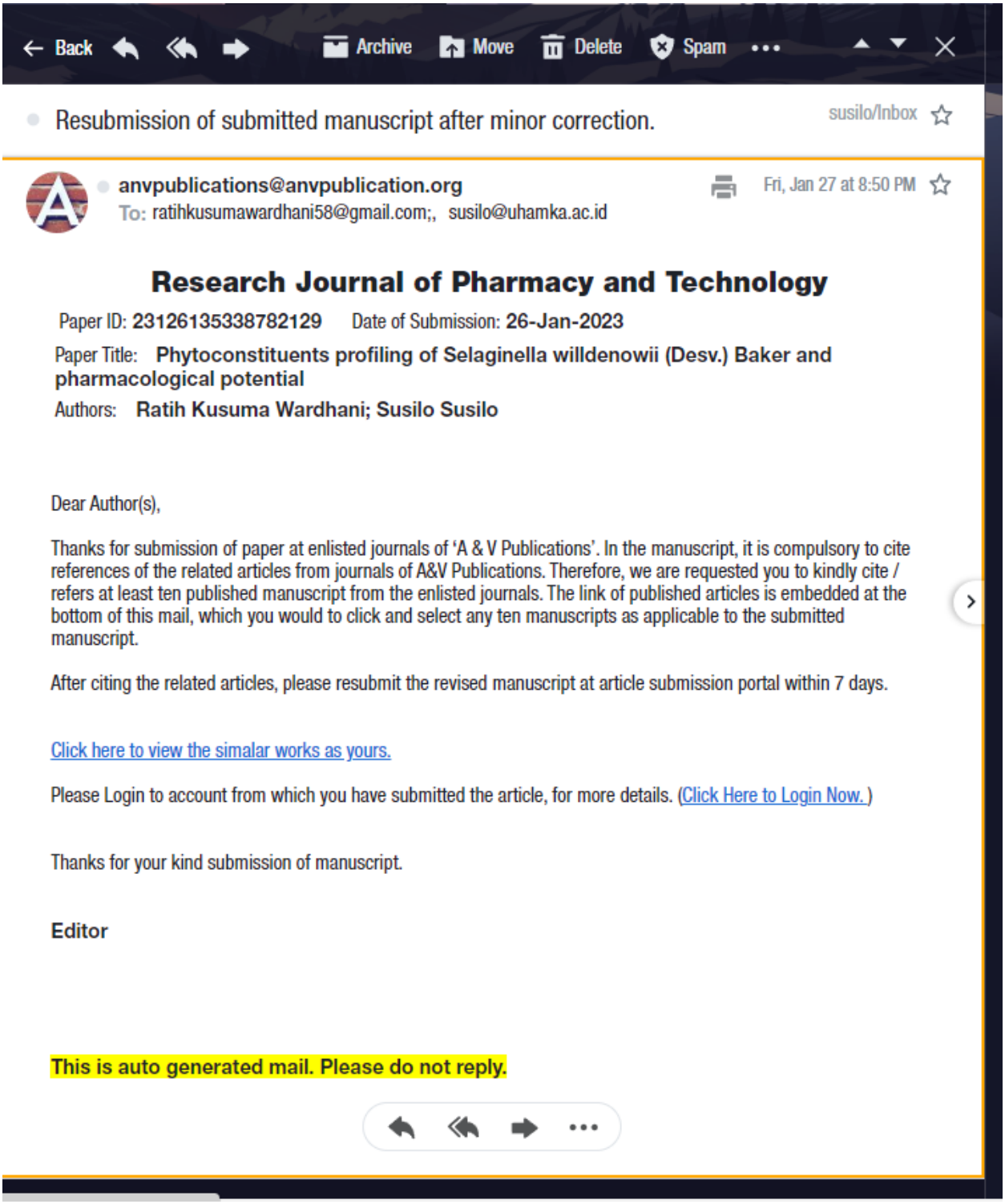

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Paper ID: **23126135338782129** Date of Submission: **26-Jan-2023**

Paper Title: **Phytoconstituents profiling of Selaginella willdenowii (Desv.) Baker and pharmacological potential**

Authors: **Ratih Kusuma Wardhani; Susilo Susilo**

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Author(s) Name:	Ratih Kusuma Wardhani; Susilo Susilo		
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Journal:	Research Journal of Pharmacy and Technology		
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Chromatography (GC/MS) and Its Pharmacological Potential

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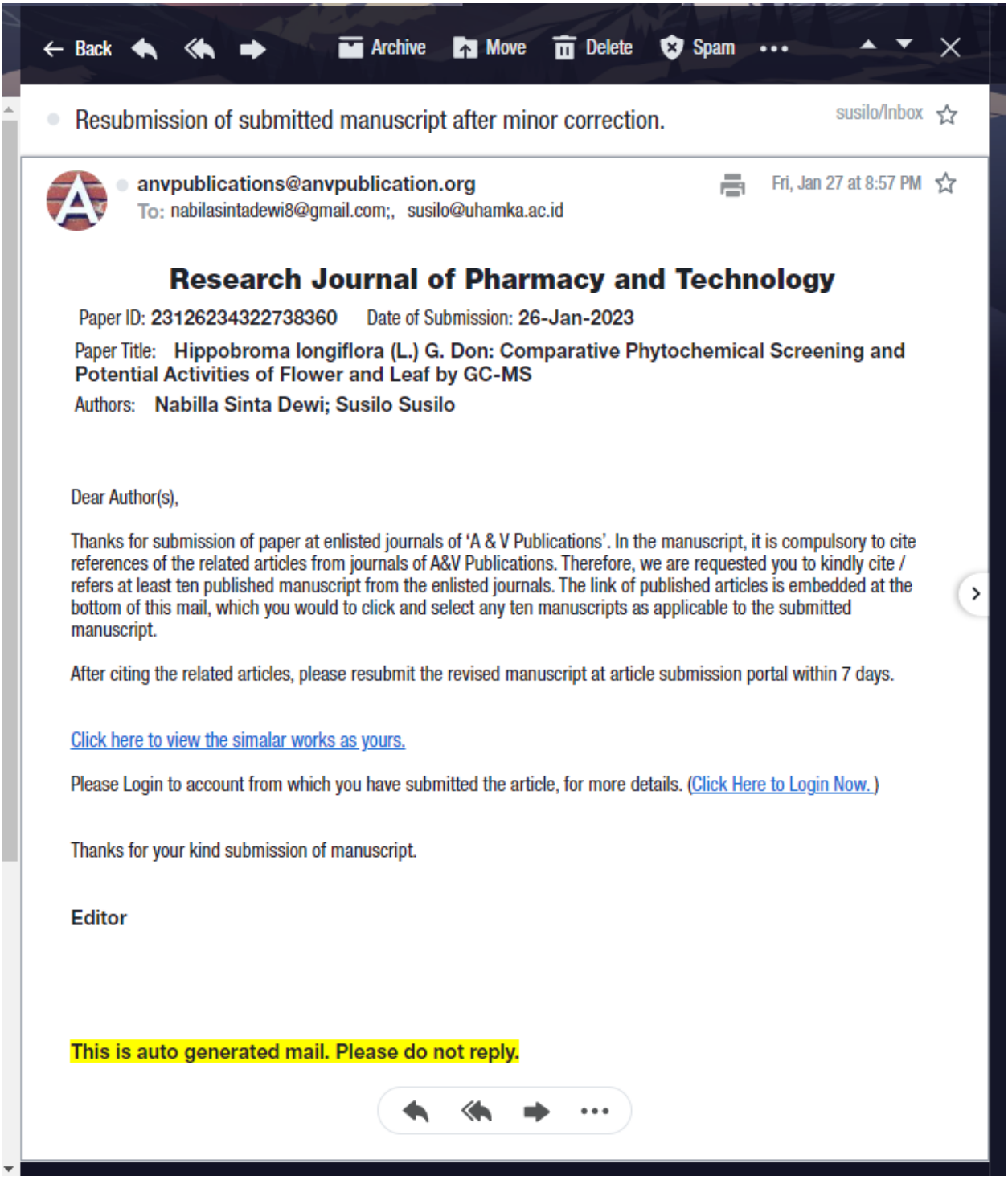

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Author(s) Name: Nabilla Sinta Dewi; Susilo Susilo

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Author(s) Name: Nabilla Sinta Dewi; Susilo Susilo

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Article Number (original Submitted manuscript No)	23126135338782129
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2	Left	1	8	Bakerfor	Baker for
2	Left	3	1	Bakerwas	Baker was
2	Right	1	1	foranalyzing	for analyzing
3	1	1	1	Figure 1	New Figure 1 attached

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4	Left	2	3	Baker(Table 1)	Baker (Table 1)
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4	Right	1	10	Bakersampleswere	Baker samples were
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5	Left	1	4	Bakerexhibits	Baker exhibits
5	Left	1	11	Bakerleaves	Baker leaves
5	Right	2	2	Bakerstems	Baker stems
6	Left	2	2	Bakeris	Baker is
6	Left	2	14	Bakeris	Baker is
6	Left	3	1	Baker(leaves	Baker (leaves

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2. Is the paper acceptable
   ~~a. In the present form~~
   b. After revision
3. Comments:

The manuscript entitled ''Phytoconstituents profiling of *Selaginella willdenowii* (Desv.) Baker and pharmacological potential'' has a novelty that should be considered for publication. The manuscript title is appropriate, informative, concise, clear, and sufficiently reflects the content. Abstract writing has been carried out comprehensively and presents essential information from the research.

The introduction is exceptionally well written, but there are a few comments that might make the text better.

1. Please recheck the grammar, especially the use of Tense.

2. IF there is a relevant scientific study about *Selaginella willdenowii*, it will add to this article's state of the art (This is my humble suggestion).

The methods section is self-explanatory and detailed.

The results section is clear.

Discussion section

The statements presented are reasonable. Some of the exfoliated compounds are attributed to the pharmacological effects of legal sources.

Type of Manuscript:
Research

Phytoconstituents profiling of *Selaginella willdenowii* (Desv.) Baker and pharmacological potential

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ABSTRACT

Selaginella willdenowii is a terrestrial herb with a high source of antioxidants. However, the phytoconstituents of these plants have not been reported. Therefore, we explored the metabolite in the leaf, stem, and root of *S. willdenowii* and also investigated the potential of its bioactive compounds. Analysis of the phytoconstituents of *S. willdenowii* ethanol extract was performed with GC-MS. We identified 69 metabolites that appear to be 16 categories of compound classes. 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne, Stigmasterol, Hexadecanoic, and acid methyl ester are four compounds consistently present in each part of the *S. willdenowii*. Known pharmacological properties of phytocompounds found can be used as anticancer drugs, antioxidants, anti-inflammatory, antitumor, and antimicrobial. The identified phytoconstituents provide the foundation for utilizing *S. willdenowii* as a future ethnomedical, nutraceutical, and phytopharmaceutical source.

Keywords: antioxidants; Pharmacology; GC-MS; natural product; *Selaginella willdenowii*

INTRODUCTION

Selaginella is distributed throughout the continent except for the Antarctic continent, which is estimated to have 700-800 species [1–4]. The growth forms of this genus are herbaceous, creeping, climbing, prostrate, upright, epiphytic, and rosette shapes [5]. The stem is branched dichotomous, with a rhinophores-positively gravitropic rooting structure [6]. Its distribution in tropical rainforests, deserts, alpines, and arctic habitats such as *Selaginella doederleinii*, *Selaginella tamariscina*, *Selaginella pulvinata*, *Selaginella sinensis*, and *Selaginella bryopteris* [7].

In pharmacology, members of Selaginella have the potential to cure a variety of diseases. For example, *Selaginella tamariscina* (P.Beauv.) introduced the Chinese Pharmacopoeia for its effectiveness in improving blood circulation since its 1953rd edition [8]. *Selaginella doederleinii* and *Selaginella sinensis* (Desv.) have anti-inflammatory, antibacterial, antiviral, immune-stimulating, antitumor, analgesic, antispasmodic biological properties, and antispasmodic [9,10]. *Selaginella trichoclada* is a traditional Chinese medicine (TCM) for treating dysentery, jaundice and coughing with lung heat [11,12].

The Selaginella family is a plant rich in bioflavonoids, aglycone flavonoids, alkaloids, lignins, polyphenol compounds selaginellin, diterpenoids, terpenoids, and steroid glycosides [1,11,13,14]. To date, about 80 bioflavonoids have been found from the genus Selaginella including Brivaracetam (BRV) related to C-C; amentoflavone, robustaflavone, taiwaniaflavone, sumaflavone, 2′,8"-biapigenin, and C-O-C related Brivaracetam (BRV); ochnaflavone, delicaflavone, hinokiflavone, and isocryptomerin [15,16]. Some ~~of these~~ can act as pharmacological antibacterial, anti-inflammatory, and potential anticancer molecules involving many factors, including apoptosis induction, angiogenic cascade retardation, and metastasis [3,9,16–19]. Despite the many reports on the bioactivity of this plant, the complete profile of the phytoconstituents is still essential to decipher.

Recent reports mention that *S. willdenowii,* a medicinal herb, has a high source of antioxidants [20,21]. Looking at its toxicity value, an *S. willdenowii* concentration of 50% cannot exert toxic effects on juvenile carp [22]. To complete the metabolite data, an analysis was performed on the roots, stems, and leaves of *S. willdenowii* for the first time.

MATERIAL AND METHODS

Sample

All fresh plant parts of *S. willdenowii* (leaves, roots, and stems) were obtained from the edge of the forest near Cibadak, Sukamakmur, Bogor, Indonesia (6°35'44.0"S 106°57'24.0"E) in mid-August 2022. Samples are taken directly and stored in the Coolerbox to be taken to the laboratory for further analysis. Sample authentication was carried out at the Bogoriensi Herbarium Laboratory, BRIN (National Research and Innovation Agency), Indonesia, and the collection were stored with specimen voucher number BO-1560831.

Extract preparation

Every part of *S. willdenowii* was separated and washed using running equadest water to remove dirt. 50 g of samples were oven-dried for 14 hours at 33 ^{o}C [23]. The dry sample of each part is mashed with a blender machine until it becomes powder (40 mesh) following the previous study [24]. Each part was macerated with ethanol solvent (99.8 % p.a.) for five days. With the Rotary Evaporator (BUCHI), each extract (10 ml) was put into Ependoft and dried at 60 ^{o}C. Finally, 200 µL of the solid residue solution was used for GC-MS.

GC-MS Analysis

Gas Chromatography (Agilent Technologies 7890) and 5975 Mass Selective Detector and Chemstation data system were implemented. following the procedures of the Spice and Medicinal Plants Research Institute (BALITRO). Briefly, the ethanol extract of each portion was filtered through a 5 µL syringe filter in split mode (8:1). The helium gas was set at 1.2 mL/min and the injector at 250°C. Then, the analyte is separated into a silica capillary column. The oven program and determination of the mass spectrum follow the previous method [24].

Data Analysis

Data analysis and constituent identification were performed by comparing the mass fragments and standard mass spectra in Agilent MassHunter Qualitative Analysis Software. International library databases such as PubChem, FOODB, Chemistry WebBook, and SpectraBase are used to study the potential of compounds [25].

RESULT

GC–MS is still a powerful analytical tool in the analysis of phytochemicals, natural products, foods, and metabolomics. Identification of metabolites based on GC-MS can be carried out perfectly because it has sensitive detection, fast work, and efficiency in separating the complexity of phytoconstituents [26,27]. Analysis of many plant compounds has been well done with GC-MS, for example *Cinnamomum malabatrum* [28], *Diospyros virginiana* [29], *Tephrosia villosa* [30], *Achnatherum inebrians* [31], *Azima tetracantha* [32], *Terminalia catappa* [33], *Citrus medica* [34], and many more. The phytoconstituents of the leaves, stems, and roots of *S. willdenowii* (Desv.) Baker was well confirmed by Gas Chromatogram (**Fig. 1**). Compounds present with varying retention times, molecular weights, and peak areas. Interestingly, there are new compounds whose activities are unknown based on chemical library data. There were 22 compounds detected in the extract on the leaves that had a percentage of more than 1%, for the most compounds were Phytol (peak area: 14.98%), Glycerin (peak area: 14.95%), 2,6,10-Trimetyl, 14-Ethylene-14-Pentadecne (peak area: 8.84%), 9,12,15-Octadecatrienoic Acid, Cyclopropane Carboxamide, 2-Cyclopropylethyl-2-Methyl-N-(1-Cyclopropylethyl)- (peak area: 5.09%), Ethyl Ester (peak area: 8.12%), and Hexadecanoic Acid, Methyl Ester (peak area: 4,87%). A complete list of compounds can be seen in **Table 1**.

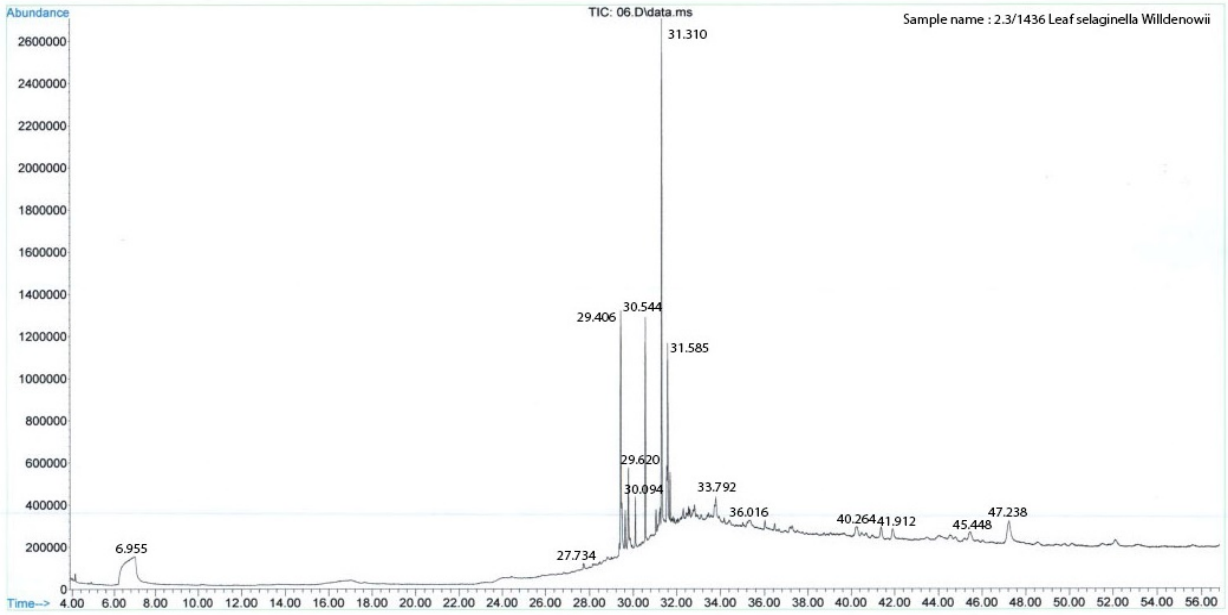

Figure 1. Chromatogram analysis of GC-MS secondary metabolites obtained from Selaginella willdenowii leaves (Desv.) Baker

Phytol belongs to the class of prenol lipids, with the subclass of diterpenoids with the highest % of the area. The most common group of sugar alcohols found in leaves is glycerin with the subclass carbohydrates and carbohydrate conjugates. The compounds 9,12,15-Octadecatrienoic Acid, 2-Cyclopropylethyl-2-Methyl-N-(1-Cyclopropylethyl)-, and Ethyl Ester and Cyclopropane Carboxamide have a reasonably high percentage and these two compounds are not found in other parts.

24 different compounds were present in the stem extract. The main phytochemical compounds include Stigmast-5-En-3-Ol (peak area: 9.96%), Stigmasterol (peak area: 9.53%), 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne (peak area: 8.35%), Hexadecanoic Acid, Ethyl Ester (peak area: 7.67%), and Linoleic Acid Ethyl Ester (peak area: 7.22%%). Of the five most common compounds, Linoleic Acid Ethyl Ester is not found in other parts. Some compounds are only present in the stem, such as Formamide, N-Methoxy- (peak area: 6.3%), 4,4-Dimethylcholest-7-En-3-One (peak

acre: 2.79%), 13-Docosenamide, (Z)-, (peak area: 2.55%), and N-Ethyl-N-.Beta., . Beta., . Beta.-D3-Ethylacetamide (peak area: 2.44%). On the stem found, quite a lot of compounds have not been reported.

Table 1. Identified similar phytocompounds from *Selaginella willdenowii* (Desv.) Baker

No.	Compund	Leaf		Stem		Root	
		RT	% of Area	RT	% of Area	RT	% of Area
1	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(All-E)-	-	-	36,447	3,42	36,04	19,83
2	2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne	29,406	8,84	29,40	8,35	29,40	6,73
		29,765	4,38	29,758	3,97	29,765	3,11
3	2-Methyl-Z,Z-3,13-Octadecadienol	31,896	1,20	-	-	36,04	1,26
		32,551	2,22	-	-	-	-
4	2-Propenoic Acid, 3-(4-Methoxyphenyl)-, 2-Ethylhexyl Ester	-	-	32,544	2,46	32,544	1,29
5	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	29,62	1,67	29,613	1,29	-	-
6	Ergost-5-En-3-Ol	-	-	44,535	3,85	44,5	1,18
7	Hexadecanoic Acid, Methyl Ester	30,096	1,78	30,082	1,36	30,089	2,22
		30,544	4,87	-	-	-	-
8	Hexadecanoic Acid, Ethyl Ester	-	-	30,537	7,67	30,537	5,14
9	Octadecanoic Acid, Ethyl Ester	-	-	31,682	2,42	31,689	2,72
10	Oleic Acid	32,454	1,63	-	-	32,82	22,2
		33,792	3,23	-	-	-	-
12	Phytol	31,31	14,98	31,296	6,91	-	-
12	Stigmast-5-En-3-Ol	-	-	47,224	9,96	40,218	1,58
		-	-	-	-	47,176	5,35
13	Stigmastan-3, 5-Diene	41,37	1,00	-	-	41,342	1,25
14	Stigmasterol	45,445	1,27	45,438	9,53	45,404	3,85
15	Trans-13-Octadecenoic Acid, Methyl Ester	31,227	2,76	-	-	31,22	5,49
16	Vitamen E	-	-	41,88	2,41	41,88	4,8

Ethanol extract of *S. willdenowii* root is found in 23 compounds. In this note, compounds **1** (peak area: 19,83%) is the main phytochemicals by quantity. The highest triterpenoids are found at the root, with a significant percentage. 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(All-E)- is the main compound in all parts *of S. willdenowii* which has a retention time of 36.04 to exit the column to the detector meaning it has a high enough boiling point and a large enough molecular weight. In addition, some compounds are present at the root that is not found in other parts, such as 2-[4 (E)-Formylcyclohex-(E)-YL]-3,5,6-Trimethyl)-1,4-Benzoquinone (peak area, 6,80%), (2E)-2,7,11,15-Tetramethyl-2-Hexadecen-1-Ol (peak area: 6,10%), and (9E)-9-Octadecanoic acid (peak area 6,01%).

DISCUSSION

From the metabolite profile, 16 equations of compound variants with different percentages of the roots, stems, and leaves of *S. willdenowii* (**Table 1**) were obtained. Compounds **1**, **8**, and **14** compounds are consistently present in every part of the plant. If we look at the compounds **2**, the percentage on leaves, stems, and roots is almost the same, but the highest percentage is in the leaves. At relatively the same time retention, hexadecanoic acid compounds, and ethyl esters, were found in the stem with the highest percentage. A significant percentage is found in the stem, as much as 9.53%, namely stigmasterol compounds. Some compounds found only in the two parts of the *S. willdenowii* sample are laced with different percentages of area and significance.

Generally, the reliability of medicinal plant use is evaluated by linking phytochemical compounds with their biological activity [35,36]. In this study, GC-MS analysis of the stems, leaves,and roots of *S. willdenowii* showed the presence of 69 phytocompounds presenting pharmacological activities isolated from leaf, stem, and root extracts (**Table 2**), varying the concentration of these molecules in each plant specimen.

Table 2. Biological activities of *Sellaginella wildenowii*

Plant part	Metabolite compounds	Biological activities
Leaf	Glycerin	Increase body fluids, osmotic laxatives, lubricants or [37]
Leaf, stem, root	2,6,10-Trimetyl, 14-Ethylene-14-Pentadecne	Not Found
Leaf, stem	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Anti-inflammatory, anticancer, antieczemic, Anti-inflammatory, Hypocholesterolemic, Hepatoprotective, Nematicide Insectifuge, [38,39]
Leaf, stem, root	Hexadecanoic Acid, Methyl Ester	anti-inflammatory and anticancer, treating type 2 diabetes, ulcerative colitis, psoriasis, and rheumatoid arthritis [36,40]
Leaf	Pyrrolo [1,2-A] Pyrazine, 1,4-Dimethyl-	Antibacterial, antimicrobial and anticancer [41,42]
Leaf, root	Trans-13-Octadecenoic Acid, Methyl Ester	Anti-inflammatory and cancer prevention [36]
Leaf, stem	Phytol	Anticancer, antioxidant, anti-inflammatory, antitumor, antimicrobial, diuretic, and chemopreventive and used in vaccine formulations [36,43]
Leaf	9,12,15-Octadecatrienoic Acid, Ethyl Ester	Cell survival and antiplasmodical [39,44,45]
Leaf	Heptadecanoic Acid, 15-Methyl-,Ethyl Ester	Antibacterial, antimycobacterial, and antioxidant activity [45,46]
Leaf	12-methyl-E,E-2,13-Octadecadien-1-Ol	Not Found
Leaf , root	2-Methyl-Z,Z-3,13-Octadecadienol	Not Found
Leaf	1,3-Cyclohexadecanedione,6-Nitro	Not Found
Leaf, root	Oleic Acid	Antitumor, antidiabetic and anticancer [47–50]
Leaf	Cyclopropane Carboxamide, 2-Cyclopropylethyl-2-Methyl-N-(1-Cyclopropylethyl)-	Not Found
Leaf	17-(1,5-Dimethyl-Hexyl)-10,13-Dimethyl-4-Vinyl-Hexadecahydro-Cyclopenta [A] Phenanthren-3-Ol	Not Found
Leaf, root	Stigmastan-3, 5-Diene	Not Found
Leaf, stem, root	Stigmasterol	Anti-inflammatory [51,52]
Leaf	.Beta. – Sitosterol	Anticancer potential [38,44]
Stem	Methanecarbothiolic Acid	Not Found
Stem	Formamide, N-Methoxy-	Not Found
Stem	Azetidine, 2-Methyl-	Anti-inflammatory [17,53]
Stem	N-Ethyl-N-.Beta., .Beta., .Beta.-D3-Ethylacetamide	Not Found
Stem, root	Hexadecanoic Acid, Ethyl Ester	Antibacterial, antimycobacterial, and low antioxidant activity [45,46]
Stem	Oxirane, 2-Decyl-3-(5-Methylhexyl)-, Cis-	Not Found
Stem	Linoleic Acid Ethyl Ester	Anti-inflammatory [54,55]
Stem, root	Octadecanoic Acid, Ethyl Ester	Antibacterial, antimycobacterial, and low antioxidant activity [45,46]
Stem	1-Nonadecene	Antimicrobial and antioxidant [15]
Stem, root	2-Propenoic Acid, 3-(4-Methoxyphenyl)-, 2-Ethylhexyl Ester	Not Found
Stem	Cyclopropaneoctanal, 2-Octyl-	Not Found
Stem	1-Docosene	Not Found
Stem	13-Docosenamide, (Z)-	Antifungal and antibacterial [56]
Stem, root	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(All-E)-	Antibacterial, antioxidant, antitumor, anticancer, immunostimulant and lipoxygenase inhibitor (Zayed et al., 2019)
Stem, root	Vitamin E	Antioxidant, anti-inflammatory and anti-fibroblastic [57,58]
Stem, root	Ergost-5-En-3-Ol	Anti-inflammatory, anti-diabetic and antioxidant [59,60]
Stem, root	Stigmast-5-En-3-Ol	Anticancer, antitumor, and anti-diabetic [61,62]
Stem	4,4-Dimethylcholest-7-En-3-One	Not Found
Root	6,6-Dimethyl-4-Cycloocten-1-One 6,6-Dimethyl-Cyclooct-4-Enone	Not Found
Root	Trans-13-Octadecenoic Acid, Methyl Ester	Anti-inflammatory and anti-cancer [36]
Root	(2E)-2,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Not Found
Root	(9E)-9-Octadecanoic Acid	Antibacterial, antimycobacterial, and low antioxidant activity [45,46]
Root	1-Eicosene	Anticancer, antifungal and antioxidant [63,64]
Root	2-[4 (E)-Formylcyclohex-(E)-YL]-3,5,6-Trimethyl)-1,4-Benzoquinone	Not Found
Root	3,7,11,Trimethyl-Dodeca-2,4,6,10-Tetraenal	Not Found
Root	Octacosane	Anti-diabetic and antibacterial [14,65]

The leaves of *S. willdenowii* may promote some pharmacological effects due to the interaction between plant molecules and organic systems. The effects that *S. willdenowii* exhibits include the main phytol compounds that have anticancer, antioxidant, diuretic, antitumor, antimicrobial, and anti-inflammatory properties [36,43]. Diterpenoid

derivatives such as Phytol [66], which acts as a precursor of vitamin E in plants [67]. Phytol can cause oxidative cell death of opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa*. Thus *S. willdenowii* leaves can be used as an important anti-bacterial agent that causes nosocomial infections [68]. Glycerin is the second most common compound that can increase body fluids, osmotic laxatives, and lubricants [68]. Literature studies reveal Hexadecanoic Acid, Methyl Ester acts as an anti-inflammatory and cancer prevention and treats type 2 diabetes, ulcerative colitis, rheumatoid arthritis, and psoriasis [40]. The presence of phytocomponents in the leaves can be used as anti-inflammatory and antioxidants, as explained in previous reports [51,52], antibacterial [41,42], antitumor [47,49,50], and anticancer [36,40]. Uniquely, some compounds still have not been reported, which can be further studied to determine their potential.

The potential for important biological activity in *S. willdenowii* stems is dominated by Stigmast-5-En-3-ol which can inhibit total cholesterol, Low-Density Lipoprotein (LDL), and triglycerides, and Stigmasterol can increase High-Density Lipoprotein (HDL) [61], providing significant antihyperlipidemic and antitumor activity [51]. Stigmasterol belongs to the group of sterols [69] with the primary function of maintaining the shape of cell membranes [70] and can be used as oleogelators leading to the formation of lipid structures in plant organelles [71]. For the human body, Stigmasterol acts as an anti-inflammatory [72], antidiabetic [73], lowering cholesterol [74], antitumor [75].

The main compound Squalene on the root *S. willdenowii* is pharmacological potential in protecting the liver, fighting fatigue, antioxidants, anticancer, lowering cardiovascular diseases, and boosting the immune system [76], and antibacterial [77]. This phytocomponent is a natural triterpene hydrocarbon with great potential as an adjuvant to induce an immune response [78]. Squalene-based adjuvant MF59 compounds have been used in human influenza vaccines [79]. The compound (9E)-9-Octadecanoic acid acts as an antibacterial. There is proven inhibition in three strains of *Salmonella sp.*, *Staphylococcus aureus*, and *Escherichia coli* in vitro [80]. In closing, we believe *S. willdenowii* is one of the sources of natural products that have important constituents in pharmacology.

CONCLUSION

S. willdenowii (leaves, stems and roots) is an important source of phytoconstituents in pharmacology. GC-MS analysis revealed that various main compounds in leaves, such as phytol (14.98%) have a lot of potential to be developed. Stigmast-5-en-3β-ol and Stigmasterol which are dominant in stems can be used as a source of diabetes drugs. The triterpenoids group in roots has pharmacological potential in protecting the liver, fighting fatigue, antioxidants, anticancer, and boosting the immune system. To the best of our knowledge, these GC-MS results provide the most complete metabolite distribution data from *S. willdenowii*. However, our present results are the first stage in the identification of the biochemical components of the natural product *S. willdenowii*. Future studies need to be expanded for the development of the pharmaceutical and bioceutical industries.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

1. Zhou, X. M. *et al.* Plastome structure, evolution, and phylogeny of Selaginella. *Mol. Phylogenet. Evol.* **169**, 107410 (2022).
2. Zhou, X. M. & Zhang, L. B. A classification of Selaginella (Selaginellaceae) based on molecular (chloroplast and nuclear), macromorphological, and spore features. *Taxon* **64**, 1117–1140 (2015).
3. Thamnarak, W., Eurtivong, C., Pollawatn, R., Ruchirawat, S. & Thasana, N. Two new nor-lignans, siamensinols A and B, from Selaginella siamensis Hieron. and their biological activities. *Nat. Prod. Res.* (2021) doi:10.1080/14786419.2021.2022664/SUPPL_FILE/GNPL_A_2022664_SM1929.DOCX.
4. Rasdianah Aziz, I., Restu Puji Raharjeng, A., Susilo & Nasution, J. Ethnobotany of traditional wedding: A

comparison of plants used by Bugis, Palembang, Sundanese and Karo ethnic in Indonesia. *J. Phys. Conf. Ser.* **1175**, (2019).
5. Risnawati, R., Meitiyani & Susilo. The effect of adding Kepok Banana peels (Musa paradisiaca) to powder media on the growth of white oyster mushrooms (Pleurotus ostreatus). *IOP Conf. Ser. Earth Environ. Sci.* **755**, (2021).
6. Adame-González, A. B., Muñíz-DL, M. E. & Valencia-A., S. Comparative leaf morphology and anatomy of six Selaginella species (Selaginellaceae, subgen. Rupestrae) with notes on xerophytic adaptations. *Flora* **260**, 151482 (2019).
7. Jermy, A. C. Selaginellaceae. *Pteridophytes and Gymnosperms* 39–45 (1990) doi:10.1007/978-3-662-02604-5_11.
8. Xu, K. P. *et al.* Two new selaginellin derivatives from Selaginella tamariscina (Beauv.) Spring. *http://remote-lib.ui.ac.id:2131/10.1080/10286020.2011.558840* **13**, 356–360 (2011).
9. Demehin, A. A. *et al.* Siamenflavones A-C, three undescribed biflavonoids from Selaginella siamensis Hieron. and biflavonoids from spike mosses as EGFR inhibitor. *Phytochemistry* **203**, 113374 (2022).
10. Li, G. *et al.* Aqueous two-phase extraction of polysaccharides from Selaginella doederleinii and their bioactivity study. *Process Biochem.* **118**, 274–282 (2022).
11. Xie, Y. *et al.* Trichocladabiflavone A, a chalcone-flavonone type biflavonoid from Selaginella trichoclada Alsto. *Nat. Prod. Res.* **36**, 1797–1802 (2022).
12. Akbar, B. *et al.* Antifertility Effect of the Ethanol Extract of Centella asiatica L. Urban Against the White Rat (Rattus norvegicus L.) in the Early Post-Implantation. *J. Phys. Conf. Ser.* **1114**, (2018).
13. Kunert, O. *et al.* Two Novel Spirostene Glycosides from Selaginella chrysocaulos and their Chemotaxonomic Significance. *https://doi.org/10.1177/1934578X1501000624* **10**, (2015).
14. Wei, Q. & Liu, R. jie. Flower colour and essential oil compositions, antibacterial activities inLagerstroemia indica L. *Nat. Prod. Res.* **36**, 2145–2148 (2022).
15. Heng, Y. W., Ban, J. J., Khoo, K. S. & Sit, N. W. Biological activities and phytochemical content of the rhizome hairs of Cibotium barometz (Cibotiaceae). *Ind. Crops Prod.* **153**, 112612 (2020).
16. Zou, Z. X. *et al.* Two new biflavonoids from Selaginella doederleinii. *Phytochem. Lett.* **40**, 126–129 (2020).
17. Yang, J. W., Yang, S. J., Na, J. M., Hahn, H. G. & Cho, S. W. 3-(Naphthalen-2-yl(propoxy)methyl)azetidine hydrochloride attenuates NLRP3 inflammasome-mediated signaling pathway in lipopolysaccharide-stimulated BV2 microglial cells. *Biochem. Biophys. Res. Commun.* **495**, 151–156 (2018).
18. Yao, C. P. *et al.* New adenine analogues and a pyrrole alkaloid from Selaginella delicatula. *Nat. Prod. Res.* **33**, 1985–1991 (2019).
19. Bhattacharya, R. & Naitam, P. Green Anticancer Drugs-An Review. *Res. J. Pharmacogn. Phytochem.* **11**, 231 (2019).
20. Wong, T. C. F., Kimia, J. I., Sains, F., Tunku, U. & Rahman, A. Sifat antioksidan dari ekstrak air dari Selaginella willdenowii. **6**, 1289–1296 (2012).
21. Tsun-Thai Chai. Antioxidant properties of aqueous extracts of Selaginella willdenowii. *J. Med. Plants Res.* **6**, 1289–1296 (2012).
22. Rahmani, A., L.Endang Widiastuti1, Kanedi1, M. & Susanto1, G. N. TOXICITY TEST OF Selaginella willdenowii EXTRACT ON SURVIVAL OF COMMON CARP JUVENILE (Cyprinus sp.). **2**, 139 (2014).
23. Balachandar, R., Karmegam, N. & Subbaiya, R. Extraction, separation and characterization of bioactive compounds produced by streptomyces isolated from vermicast soil. *Res. J. Pharm. Technol.* **11**, 4569–4574 (2018).
24. Nabila, N. & Susilo, S. A Comparative Metabolite Analysis of Pandanus Amaryllifolius Leaves from Different Growth Stages using GC-MS and Their Biological. *Eur. Chem. Bull.* **11**, 22–38 (2022).
25. Tang, G.-M. *et al.* Comparative Analysis of Volatile Constituents in Root Tuber and Rhizome of Curcuma longa L. Using Fingerprints and Chemometrics Approaches on Gas Chromatography–Mass Spectrometry. *Molecules* **27**, 3196 (2022).
26. Thakur, P. *et al.* A Review on GC-MS Hyphenated Technique. *Asian J. Pharm. Anal.* **11**, 285–292 (2021).
27. Reddy, M. Y. *et al.* The Quantitative Determination of Process Related Genotoxic Impurities in Esomeprazole Magnesium by GC-MS. *Asian J. Pharm. Anal.* **4**, 898–901 (2011).
28. Aravind, R., Bindu, A. R., Bindu, K. & Alexeyena, V. GC-Ms analysis of the bark essential oil of cinnamomum malabatrum (burman. f) blume. *Res. J. Pharm. Technol.* **7**, 754–759 (2014).
29. Priya, S., Nethaji, S. & Sindhuja, B. GC-MS analysis of some bioactive constituents of diospyros Virginiana. *Res. J. Pharm. Technol.* **7**, 429–432 (2014).
30. Rajabudeen, E., Ganthi, A. & Subramanian, M. GC-MS Analysis of the Methanol Extract of Tephrosia

villosa (L.) Pers. *Asian J. Res. Chem.* **5**, 1331–1334 (2012).
31. Zahi, M. R., Liang, H., Khan, A. & Yuan, Q. Identification of Essential Oil Components in Chinese Endemic Plant Achnatherum inebrians. *Asian J. Res. Chem.* **7**, 576–579 (2014).
32. Jose, B. E. & Selvam, P. P. Identification of Phytochemical Constituents in the Leaf Extracts of Azima tetracantha Lam using Gas Chromatography-Mass Spectrometry (GC-MS) analysis and Antioxidant Activity. *Asian J. Res. Chem.* **11**, 857 (2018).
33. Krishnaveni, M., Krishna Kumari, G., Ragina Banu, C. & Kalaivani, M. Phytochemical analysis of Terminalia catappa stem using GC-MS/MS. *Res. J. Pharm. Technol.* **8**, 1281–1283 (2015).
34. Pandian, R. S. & Noora, A. T. GC-MS analysis of phytochemical compounds present in the leaves of Citrus medica. L. *Res. J. Pharm. Technol.* **12**, 1823–1826 (2019).
35. Saxena, M. *et al.* Phytochemical screening and in-vitro antioxidant activity isolated bioactive compounds from Tridax procumbens Linn. *Pakistan J. Biol. Sci. PJBS* **16**, 1971–1977 (2013).
36. Krishnamoorthy, K. & Subramaniam, P. Phytochemical Profiling of Leaf, Stem, and Tuber Parts of Solena amplexicaulis (Lam.) Gandhi Using GC-MS . *Int. Sch. Res. Not.* **2014**, 1–13 (2014).
37. Koehler, K., Thevis, M. & Schaenzer, W. Meta-analysis: Effects of glycerol administration on plasma volume, haemoglobin, and haematocrit. *Drug Test. Anal.* **5**, 896–899 (2013).
38. Alzurfi, S. K. L., Abdali, S. A., Aattaby, E. A. S., Rabeea, M. A. A. & Al-Haidarey, M. J. S. Identification of lipid compounds in the plant of Ceratophyllum demersum using two different solvents. *Mater. Today Proc.* **60**, 1596–1605 (2022).
39. Ms, R. & Pushpa, K. Phytochemical Screening and GC-MS Analysis of Leaf Extract of Pergularia daemia (Forssk) Chiov. *Asian J. Plant Sci. Res.* (2017).
40. Alzurfi, S. K. L., Abdali, S. A., Aattaby, E. A. S., Rabeea, M. A. A. & Al-Haidarey, M. J. S. Identification of lipid compounds in the plant of Ceratophyllum demersum using two different solvents. (2021) doi:10.1016/j.matpr.2021.12.127.
41. Mangrolia, U. & Osborne, W. J. Staphylococcus xylosus VITURAJ10: Pyrrolo [1,2α] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (PPDHMP) producing, potential probiotic strain with antibacterial and anticancer activity. *Microb. Pathog.* **147**, 104259 (2020).
42. Abdulrahman, I., Jamal, M. T., Pugazhendi, A., Dhavamani, J. & Satheesh, S. Antibiofilm activity of secondary metabolites from bacterial endophytes of Red Sea soft corals. *Int. Biodeterior. Biodegradation* **173**, 105462 (2022).
43. Prabhadevi, V., Sahaya, S. S., Johnson, M., Venkatramani, B. & Janakiraman, N. Phytochemical studies on Allamanda cathartica L. using GC–MS. *Asian Pac. J. Trop. Biomed.* **2**, S550–S554 (2012).
44. Agustikawati, N., Andayani, Y. & Suhendra, D. Uji Aktivitas Antioksidan Dan Penapisan Fitokimia Dari Ekstrak Daun Pakoasi Dan Kluwih Sebagai Sumber Antioksidan Alami. *J. Penelit. Pendidik. IPA* **3**, (2017).
45. Ahmad, I. *et al.* GC–MS profiling, phytochemical and biological investigation of aerial parts of Leucophyllum frutescens (Berl.) I.M. Johnst. (Cenizo). *South African J. Bot.* **148**, 200–209 (2022).
46. A Elaiyaraja and G Chandramohan. Comparative phytochemical profile of Indoneesiella echioides (L.) Nees leaves using GC-MS A Elaiyaraja and G Chandramohan. *J. Pharmacogn. Phytochem.* (2016).
47. Ali, H., Yesmin, R., Satter, M. A., Habib, R. & Yeasmin, T. Antioxidant and antineoplastic activities of methanolic extract of KaempferAli, H., Yesmin, R., Satter, M. A., Habib, R., & Yeasmin, T. (2018). Antioxidant and antineoplastic activities of methanolic extract of Kaempferia galanga Linn. Rhizome against Ehr. *J. King Saud Univ. - Sci.* **30**, 386–392 (2018).
48. Carrillo, C., Cavia, D. M. & Alonso-Torre, S. R. Antitumor effect of oleic acid; mechanisms of action. A review. *Nutr Hosp* **27**, 1860–1865 (2012).
49. Priore, P. *et al.* Oleic acid and hydroxytyrosol inhibit cholesterol and fatty acid synthesis in C6 glioma cells. *Oxid. Med. Cell. Longev.* **2017**, (2017).
50. Lattibeaudiere, K. G. & Alexander-Lindo, R. L. Oleic Acid and Succinic Acid Synergistically Mitigate Symptoms of Type 2 Diabetes in Streptozotocin-Induced Diabetic Rats. *Int. J. Endocrinol.* **2022**, (2022).
51. Jie, F. *et al.* Stigmasterol attenuates inflammatory response of microglia via NF-κB and NLRP3 signaling by AMPK activation. *Biomed. Pharmacother.* **153**, 113317 (2022).
52. Khan, M. A., Sarwar, A. H. M. G., Rahat, R., Ahmed, R. S. & Umar, S. Stigmasterol protects rats from collagen induced arthritis by inhibiting proinflammatory cytokines. *Int. Immunopharmacol.* **85**, 106642 (2020).
53. Drouillat, B., Wright, K., Marrot, J. & Couty, F. Practical preparation of enantiopure 2-methyl-azetidine-2-carboxylic acid; a γ-turn promoter. *Tetrahedron: Asymmetry* **23**, 690–696 (2012).
54. Kolar, M. J. *et al.* Linoleic acid esters of hydroxy linoleic acids are anti-inflammatory lipids found in plants

and mammals. *J. Biol. Chem.* **294**, 10698–10707 (2019).
55. Simopoulos, A. P. Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr.* **70**, 560s-569s (1999).
56. dos Reis, C. M. *et al.* Antifungal and antibacterial activity of extracts produced from Diaporthe schini. *J. Biotechnol.* **294**, 30–37 (2019).
57. Montalvo, G. *et al.* Immune gene expression and antioxidant response to vitamin E enriched diets for males Litopenaeus vannamei breeder (Boone, 1931). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **268**, 111187 (2022).
58. Sudirman, T. *et al.* Vitamin E administration as preventive measures for peritoneal/intra-abdominal adhesions: A systematic review and meta-analysis. *Ann. Med. Surg.* **80**, 104225 (2022).
59. Zahid, M., Arif, M., Rahman, M. A., Singh, K. & Mujahid, M. Solvent Extraction and Gas Chromatography–Mass Spectrometry Analysis of Annona squamosa L. Seeds for Determination of Bioactives, Fatty Acid/Fatty Oil Composition, and Antioxidant Activity. *https://remote-lib.ui.ac.id:2075/10.1080/19390211.2017.1366388* **15**, 613–623 (2017).
60. Tan, D. C. *et al.* Comparative study of the antidiabetic potential of Paederia foetida twig extracts and compounds from two different locations in Malaysia. *https://remote-lib.ui.ac.id:2075/10.1080/13880209.2019.1610462* **57**, 345–354 (2019).
61. Fernando, I. P. S. *et al.* Apoptotic and antiproliferative effects of Stigmast-5-en-3-ol from Dendronephthya gigantea on human leukemia HL-60 and human breast cancer MCF-7 cells. *Toxicol. Vitr.* **52**, 297–305 (2018).
62. Iyer, D. & Patil, U. K. Efficacy of Stigmast–5–en–3β–ol Isolated from Salvadora persica L. as Antihyperlipidemic and Anti–tumor agent: Evidence from animal studies. *Asian Pacific J. Trop. Dis.* **2**, S849–S855 (2012).
63. Sahin Yaglioglu, A., Yaglioglu, M. S., Tosyalıoglu, N., Adem, S. & Demirtas, I. Chemical profiling, in vitro biological activities and Pearson correlation between chemical profiling and anticancer activities of four Abies species from Turkey. *South African J. Bot.* (2022) doi:10.1016/J.SAJB.2022.08.005.
64. Harada, H. *et al.* Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer Res.* **22**, 2587–2590 (2002).
65. Okokon, J. E. *et al.* In vivo antihyperglycaemic and antihyperlipidemic activities and chemical constituents of Solanum anomalum. *Biomed. Pharmacother.* **151**, 113153 (2022).
66. Wu, Y.-Q. *et al.* Exogenous GbHMGS1 Overexpression Improves the Contents of Three Terpenoids in Transgenic Populus. *Forests* **12**, 1–14 (2021).
67. Mekinić, I. G. *et al.* Seasonal changes in essential oil constituents of cystoseira compressa: First report. *Molecules* **26**, (2021).
68. Lee, W., Woo, E. R. & Lee, D. G. Phytol has antibacterial property by inducing oxidative stress response in Pseudomonas aeruginosa. *http://remote-lib.ui.ac.id:2131/10.1080/10715762.2016.1241395* **50**, 1309–1318 (2016).
69. Weremczuk-Jeżyna, I., Hnatuszko-Konka, K., Lebelt, L. & Grzegorczyk-Karolak, I. The protective function and modification of secondary metabolite accumulation in response to light stress in dracocephalum forrestii shoots. *Int. J. Mol. Sci.* **22**, (2021).
70. Aboobucker, S. I. & Suza, W. P. Why do plants convert sitosterol to stigmasterol? *Front. Plant Sci.* **10**, (2019).
71. Tang, C. *et al.* Structure and Properties of Organogels Prepared from Rapeseed Oil with Stigmasterol. *Foods* **11**, 939 (2022).
72. Navarro, A., De las Heras, B. & Villar, A. Anti-inflammatory and immunomodulating properties of a sterol fraction from Sideritis foetens CLEM. *Biol. Pharm. Bull.* **24**, 470–473 (2001).
73. Wang, J. *et al.* Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. *Food Nutr. Res.* **61**, (2017).
74. Prasad, M. *et al.* A Comprehensive Review on Therapeutic Perspectives of Phytosterols in Insulin Resistance: A Mechanistic Approach. *Molecules* **27**, 1–17 (2022).
75. Gao, Z., Maloney, D. J., Dedkova, L. M. & Hecht, S. M. Inhibitors of DNA polymerase β: Activity and mechanism. *Bioorganic Med. Chem.* **16**, 4331–4340 (2008).
76. Gohil, N., Bhattacharjee, G., Khambhati, K., Braddick, D. & Singh, V. Engineering strategies in microorganisms for the enhanced production of squalene: Advances, challenges and opportunities. *Front. Bioeng. Biotechnol.* **7**, 1–24 (2019).
77. Peng, W. *et al.* Characteristics of antibacterial molecular activities in poplar wood extractives. *Saudi J. Biol.*

Sci. **24**, 399–404 (2017).
78. Azmi, L. *et al.* Effect of squalene in surgically induced gastro-oesophageal reflux disease on rats. *Res. J. Pharmacol. Pharmacodyn.* **9**, 1 (2017).
79. Chae, G. E., Kim, D. W. & Jin, H. E. Development of Squalene-Based Oil-in-Water Emulsion Adjuvants Using a Self-Emulsifying Drug Delivery System for Enhanced Antigen-Specific Antibody Titers. *Int. J. Nanomedicine* **17**, 6221–6231 (2022).
80. Pu, Z. hui *et al.* Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. *Agric. Sci. China* **9**, 1236–1240 (2010).

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Phytoconstituents profiling of *Selaginella willdenowii* (Desv.) Baker and pharmacological potential

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ABSTRACT

Selaginella willdenowii (Desv.) Baker is a terrestrial herb with a high source of antioxidants. However, the phytoconstituents of these plants have not been reported. Therefore, we explored the metabolite in the leaves, stems, and roots of *S. willdenowii* (Desv.) Baker investigated its bioactive compounds' potential. Analysis of the phytoconstituents of *S. willdenowii* (Desv.) Baker ethanol extract was performed with Gas Chromatography-Mass Spectrometry (GCMS). We identified 69 metabolites that appear to be 16 categories of compound classes. 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne, Stigmasterol, Hexadecanoic, and acid methyl ester are four compounds consistently present in each part of the *S. willdenowii* (Desv.) Baker. Known pharmacological properties of phytocompounds found can be used as anticancer drugs, antioxidants, anti-inflammatory, antitumor, and antimicrobial. The identified phytoconstituents provide the foundation for utilizing *S. willdenowii* (Desv.) Baker is a future ethnomedical, nutraceutical, and phytopharmaceutical source.

Keywords: antioxidants; Pharmacology; GC-MS; natural product; *Selaginella willdenowii* (Desv.) Baker

INTRODUCTION

Selaginella is distributed throughout the continent except for the Antarctic continent, which is estimated to have 700-800 species [1–4]. The growth forms of this genus are herbaceous, creeping, climbing, prostrate, upright, epiphytic, and rosette shapes [5]. The stem is branched dichotomous, with a rhinophores-positively gravitropic rooting structure [6]. Its distribution in tropical rainforests, deserts, alpines, and arctic habitats such as *Selaginella doederleinii*, *Selaginella tamariscina*, *Selaginella pulvinata*, *Selaginella sinensis*, and *Selaginella bryopteris* [7].

In pharmacology, members of Selaginella have the potential to cure a variety of diseases. For example, *Selaginella tamariscina* (P.Beauv.) introduced the Chinese Pharmacopoeia for its effectiveness in improving blood circulation since its 1953rd edition [8]. *Selaginella doederleinii* and *Selaginella sinensis* (Desv.) has anti-inflammatory, antibacterial, antiviral, immune-stimulating, antitumor, analgesic, antispasmodic biological properties, and antispasmodic [9,10]. *Selaginella trichoclada* is a Traditional Chinese Medicine (TCM) for treating dysentery, jaundice, and coughing with lung heat [11,12].

The Selaginella family is a plant rich in bioflavonoids, aglycone flavonoids, alkaloids, lignins, polyphenol compounds selaginellin, diterpenoids, terpenoids, and steroid glycosides [1,11,13,14]. To date, about 80 bioflavonoids have been found from the genus Selaginella including Brivaracetam (BRV) related to C-C; amentoflavone, robustaflavone, taiwaniaflavone, sumaflavone, 2′,8"-biapigenin, and C-O-C related Brivaracetam (BRV); ochnaflavone, delicaflavone, hinokiflavone, and isocryptomerin [15,16]. Some can act as pharmacological antibacterial, anti-inflammatory, and potential anticancer molecules involving many factors, including apoptosis induction, angiogenic cascade retardation, and metastasis [3,9,16–19]. Despite the many reports on the bioactivity of this plant, the complete profile of the phytoconstituents is still essential to decipher.

Recent reports mention that *S. willdenowii* (Desv.) Baker, a medicinal herb, has a high source of antioxidants [20,21]. Looking at its toxicity value, an *S. willdenowii* (Desv.) Baker concentration of 50% cannot exert toxic effects on juvenile carp [22]. To complete the metabolite data, this study aims to analyze the metabolite profile of the roots, stems, and leaves of *S. willdenowii* (Desv.) Baker for the first time.

MATERIAL AND METHODS

Sample

All fresh plant parts of *S. willdenowii* (Desv.) Baker (leaves, roots, and stems) were obtained from the edge of the forest near Cibadak, Sukamakmur, Bogor, Indonesia (6°35'44.0"S 106°57'24.0"E) in mid-August 2022. Samples were taken directly and stored in the coolerbox to be taken to the laboratory for further analysis. Sample authentication was carried out at the Bogoriensi Herbarium Laboratory, BRIN (National Research and Innovation Agency), Indonesia, and the collection were stored with specimen voucher number BO-1560831.

Extract preparation

Every part of *S. willdenowii* (Desv.) Baker was separated and washed using running equadest water to remove dirt. 50 g of samples were oven-dried for 14 hours at 33 ºC [23]. The dry sample of each part was mashed with a blender machine until it became powder (40 mesh) following the previous study [24]. Each part was macerated with ethanol solvent (99.8 % p.a.) for five days. With the Rotary Evaporator (BUCHI), each extract (10 ml) was put into Ependoft and dried at 60 ºC. Finally, 200 µL of the solid residue solution was used for GC-MS.

GC-MS Analysis

Gas Chromatography (Agilent Technologies 7890) and 5975 Mass Selective Detector and Chemstation data system were implemented. following the procedures of the Spice and Medicinal Plants Research Institute (BALITRO). Briefly, the ethanol extract of each portion was filtered through a 5 µL syringe filter in split mode (8:1). The helium gas was set at 1.2 mL/min and the injector at 250°C. Then, the analyte is separated into a silica capillary column. The oven program and determination of the mass spectrum follow the previous method [24].

Data Analysis

Data analysis and constituent identification were performed by comparing the mass fragments and standard mass spectra in Agilent MassHunter Qualitative Analysis Software. International library databases such as PubChem, FOODB, Chemistry WebBook, and SpectraBase are used to study the potential of compounds [25].

RESULT

GC–MS is still a powerful analytical tool for analyzing phytochemicals, natural products, foods, and metabolomics. Identification of metabolites based on GC-MS can be carried out perfectly because it has sensitive detection, fast work, and efficiency in separating the complexity of phytoconstituents [26,27]. Analysis of many plant compounds has been well done with GC-MS, for example, *Cinnamomum malabatrum* [28], *Diospyros virginiana* [29], *Tephrosia villosa* [30], *Achnatherum inebrians* [31], *Azima tetracantha* [32], *Terminalia catappa* [33], *Citrus medica* [34], and many more. The phytoconstituents of the leaves, stems, and roots of *S. willdenowii* (Desv.) Baker was well confirmed by Gas Chromatogram (**Fig. 1**). Compounds present with varying retention times, molecular weights, and peak areas. Interestingly, there are new compounds whose activities are unknown based on chemical library data. There were 22 compounds detected in the extract on the leaves that had a percentage of more than 1%, for the most compounds were Phytol (peak area: 14.98%), Glycerin (peak area: 14.95%), 2,6,10-Trimetyl, 14-Ethylene-14-Pentadecne (peak area: 8.84%), 9,12,15-Octadecatrienoic Acid, Cyclopropane Carboxamide, 2-Cyclopropylethyl-2-Methyl-N-(1-Cyclopropylethyl)- (peak area: 5.09%), Ethyl Ester (peak area: 8.12%), and Hexadecanoic Acid, Methyl Ester (peak area: 4,87%). A complete list of compounds can be seen in **Table 1**.

Phytol belongs to the class of prenol lipids, with the subclass of diterpenoids with the highest % of the area. The most common group of sugar alcohols found in leaves is glycerin with the subclass carbohydrates and carbohydrate conjugates. The compounds 9,12,15-Octadecatrienoic Acid, 2-Cyclopropylethyl-2-Methyl-N-(1-Cyclopropylethyl)-, and Ethyl Ester and Cyclopropane Carboxamide have a reasonably high percentage and these two compounds are not found in other parts.

24 different compounds were present in the stem extract. The main phytochemical compounds include Stigmast-5-En-3-Ol (peak area: 9.96%), Stigmasterol (peak area: 9.53%), 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne (peak area: 8.35%), Hexadecanoic Acid, Ethyl Ester (peak area: 7.67%), and Linoleic Acid Ethyl Ester (peak area: 7.22%%). Of the five most common compounds, Linoleic Acid Ethyl Ester is not found in other parts. Some compounds are only present in the stem, such as Formamide, N-Methoxy- (peak area: 6.3%), 4,4-Dimethylcholest-7-En-3-One (peak acre: 2.79%), 13-Docosenamide, (Z)-, (peak area: 2.55%), and N-Ethyl-N-.Beta., . Beta., . Beta.-D3-Ethylacetamide (peak area: 2.44%). On the stem found, quite a lot of compounds have not been reported.

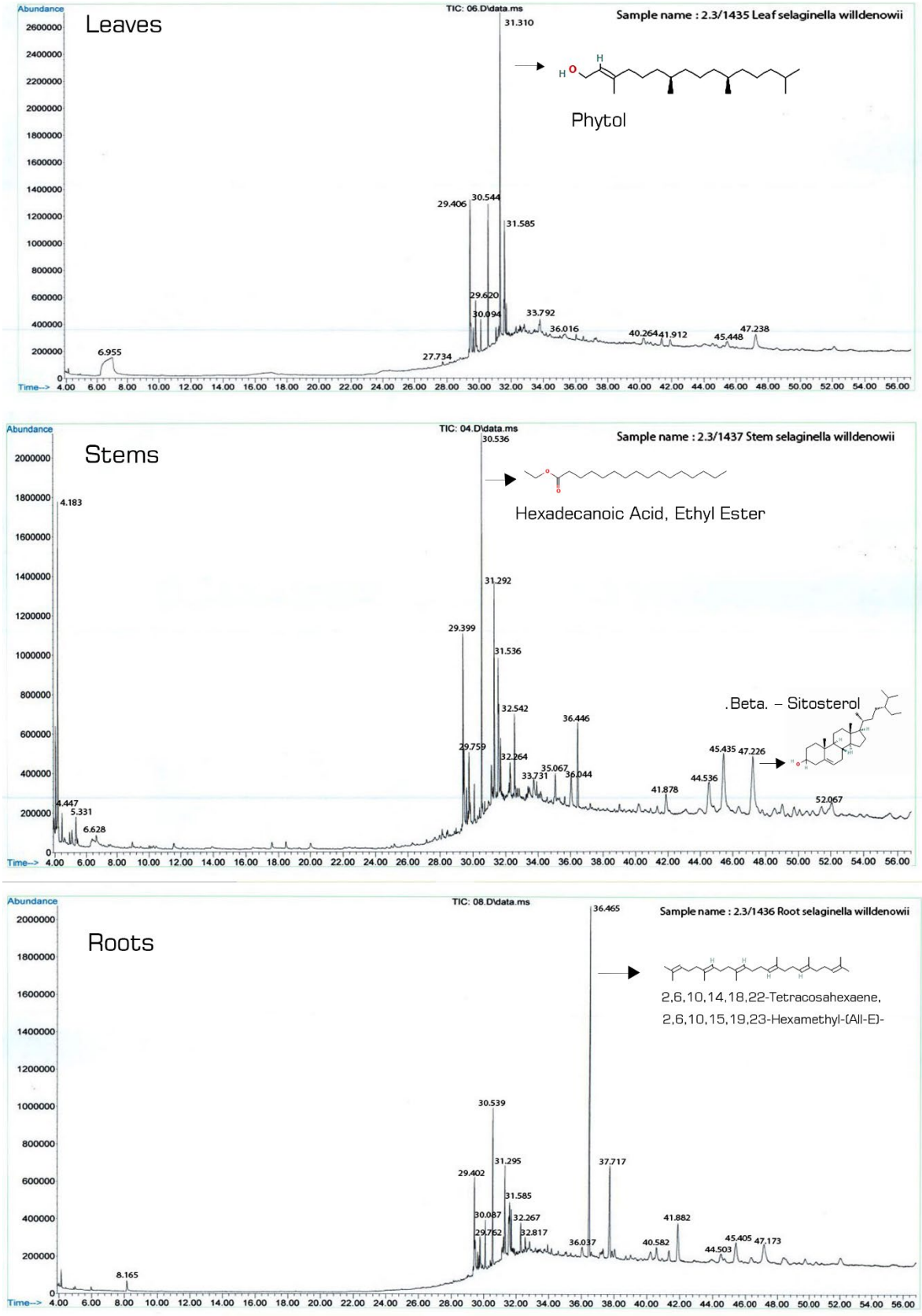

Figure 1. Chromatogram analysis of GC-MS secondary metabolites obtained from *Selaginella willdenowii* (Desv.) Baker

Table 1. Identified similar phytocompounds from *Selaginella willdenowii* (Desv.) Baker

No.	Compund	leaves		Stem		Root	
		RT	% of Area	RT	% of Area	RT	% of Area
1	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(All-E)-	-	-	36,447	3,42	36,04	19,83
2	2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne	29,406	8,84	29,40	8,35	29,40	6,73
		29,765	4,38	29,758	3,97	29,765	3,11
3	2-Methyl-Z,Z-3,13-Octadecadienol	31,896	1,20	-	-	36,04	1,26
		32,551	2,22	-	-	-	-
4	2-Propenoic Acid, 3-(4-Methoxyphenyl)-, 2-Ethylhexyl Ester	-	-	32,544	2,46	32,544	1,29
5	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	29,62	1,67	29,613	1,29	-	-
6	Ergost-5-En-3-Ol	-	-	44,535	3,85	44,5	1,18
7	Hexadecanoic Acid, Methyl Ester	30,096	1,78	30,082	1,36	30,089	2,22
		30,544	4,87	-	-	-	-
8	Hexadecanoic Acid, Ethyl Ester	-	-	30,537	7,67	30,537	5,14
9	Octadecanoic Acid, Ethyl Ester	-	-	31,682	2,42	31,689	2,72
10	Oleic Acid	32,454	1,63	-	-	32,82	22,2
		33,792	3,23	-	-	-	-
12	Phytol	31,31	14,98	31,296	6,91	-	-
12	Stigmast-5-En-3-Ol	-	-	47,224	9,96	40,218	1,58
		-	-	-	-	47,176	5,35
13	Stigmastan-3, 5-Diene	41,37	1,00	-	-	41,342	1,25
14	Stigmasterol	45,445	1,27	45,438	9,53	45,404	3,85
15	Trans-13-Octadecenoic Acid, Methyl Ester	31,227	2,76	-	-	31,22	5,49
16	Vitamen E	-	-	41,88	2,41	41,88	4,8

Ethanol extract of *S. willdenowii* (Desv.) Baker root is found in 23 compounds. In this note, compounds **1** (peak area: 19,83%) is the main phytochemicals by quantity. The highest triterpenoids are found at the root, with a significant percentage. 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(All-E)- is the main compound in all parts *of S. willdenowii* (Desv.) Baker which has a retention time of 36.04 to exit the column to the detector meaning it has a high enough boiling point and a large enough molecular weight. In addition, some compounds are present at the root that is not found in other parts, such as 2-[4 (E)-Formylcyclohex-(E)-YL]-3,5,6-Trimethyl)-1,4-Benzoquinone (peak area, 6,80%), (2E)-2,7,11,15-Tetramethyl-2-Hexadecen-1-Ol (peak area: 6,10%), and (9E)-9-Octadecanoic acid (peak area 6,01%).

DISCUSSION

From the metabolite profile, 16 equations of compound variants with different percentages of the roots, stems, and leaves of *S. willdenowii* (Desv.) Baker (**Table 1**) were obtained. Compounds **1**, **8**, and **14** compounds are consistently present in every part of the plant. If we look at the compounds **2**, the percentage on leaves, stems, and roots is almost the same, but the highest percentage is in the leaves. At relatively the same time retention, hexadecanoic acid compounds, and ethyl esters, were found in the stem with the highest percentage. A significant percentage is found in the stem, as much as 9.53%, namely stigmasterol compounds. Some compounds found only in the two parts of the *S. willdenowii* (Desv.) Baker samples were laced with different percentages of area and significance.

Generally, the reliability of medicinal plant use is evaluated by linking phytochemical compounds with their biological activity [35,36]. In this study, GC-MS analysis of the stems, leaves, and roots of *S. willdenowii* (Desv.) Baker showed the presence of 69 phytocompounds presenting pharmacological activities isolated from leaf, stem, and root extracts (**Table 2**), varying the concentration of these molecules in each plant specimen.

Table 2. Biological activities of *Sellaginella wildenowii* (Desv.) Baker

Plant part	Metabolite compounds	Biological activities
Leaf	Glycerin	Increase body fluids, osmotic laxatives, lubricants or [37]
Leaf, stem, root	2,6,10-Trimetyl, 14-Ethylene-14-Pentadecne	Not Found
Leaf, stem	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Anti-inflammatory, anticancer, antieczemic, Anti-inflammatory, Hypocholesterolemic, Hepatoprotective, Nematicide Insectifuge, [38,39]
Leaf, stem, root	Hexadecanoic Acid, Methyl Ester	anti-inflammatory and anticancer, treating type 2 diabetes, ulcerative colitis, psoriasis, and rheumatoid arthritis [36,40]
Leaf	Pyrrolo [1,2-A] Pyrazine, 1,4-Dimethyl-	Antibacterial, antimicrobial and anticancer [41,42]
Leaf, root	Trans-13-Octadecenoic Acid, Methyl Ester	Anti-inflammatory and cancer prevention [36]
Leaf, stem	Phytol	Anticancer, antioxidant, anti-inflammatory, antitumor, antimicrobial, diuretic, and chemopreventive and used in vaccine formulations [36,43]
Leaf	9,12,15-Octadecatrienoic Acid, Ethyl Ester	Cell survival and antiplasmodical [39,44,45]
Leaf	Heptadecanoic Acid, 15-Methyl-,Ethyl Ester	Antibacterial, antimycobacterial, and antioxidant activity [45,46]
Leaf	12-methyl-E,E-2,13-Octadecadien-1-Ol	Not Found
Leaf , root	2-Methyl-Z,Z-3,13-Octadecadienol	Not Found
Leaf	1,3-Cyclohexadecanedione,6-Nitro	Not Found
Leaf, root	Oleic Acid	Antitumor, antidiabetic and anticancer [47–50]
Leaf	Cyclopropane Carboxamide, 2-Cyclopropylethyl-2-Methyl-N-(1-Cyclopropylethyl)-	Not Found
Leaf	17-(1,5-Dimethyl-Hexyl)-10,13-Dimethyl-4-Vinyl-Hexadecahydro-Cyclopenta [A] Phenanthren-3-Ol	Not Found
Leaf, root	Stigmastan-3, 5-Diene	Not Found
Leaf, stem, root	Stigmasterol	Anti-inflammatory [51,52]
Leaf	.Beta. – Sitosterol	Anticancer potential [38,44]
Stem	Methanecarbothiolic Acid	Not Found
Stem	Formamide, N-Methoxy-	Not Found
Stem	Azetidine, 2-Methyl-	Anti-inflammatory [17,53]
Stem	N-Ethyl-N-.Beta., .Beta., .Beta.-D3-Ethylacetamide	Not Found
Stem, root	Hexadecanoic Acid, Ethyl Ester	Antibacterial, antimycobacterial, and low antioxidant activity [45,46]
Stem	Oxirane, 2-Decyl-3-(5-Methylhexyl)-, Cis-	Not Found
Stem	Linoleic Acid Ethyl Ester	Anti-inflammatory [54,55]
Stem, root	Octadecanoic Acid, Ethyl Ester	Antibacterial, antimycobacterial, and low antioxidant activity [45,46]
Stem	1-Nonadecene	Antimicrobial and antioxidant [15]
Stem, root	2-Propenoic Acid, 3-(4-Methoxyphenyl)-, 2-Ethylhexyl Ester	Not Found
Stem	Cyclopropaneoctanal, 2-Octyl-	Not Found
Stem	1-Docosene	Not Found
Stem	13-Docosenamide, (Z)-	Antifungal and antibacterial [56]
Stem, root	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(All-E)-	Antibacterial, antioxidant, antitumor, anticancer, immunostimulant and lipoxygenase inhibitor (Zayed et al., 2019)
Stem, root	Vitamin E	Antioxidant, anti-inflammatory and anti-fibroblastic [57,58]
Stem, root	Ergost-5-En-3-Ol	Anti-inflammatory, anti-diabetic and antioxidant [59,60]
Stem, root	Stigmast-5-En-3-Ol	Anticancer, antitumor, and anti-diabetic [61,62]
Stem	4,4-Dimethylcholest-7-En-3-One	Not Found
Root	6,6-Dimethyl-4-Cycloocten-1-One 6,6-Dimethyl-Cyclooct-4-Enone	Not Found
Root	Trans-13-Octadecenoic Acid, Methyl Ester	Anti-inflammatory and anti-cancer [36]
Root	(2E)-2,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Not Found
Root	(9E)-9-Octadecanoic Acid	Antibacterial, antimycobacterial, and low antioxidant activity [45,46]
Root	1-Eicosene	Anticancer, antifungal and antioxidant [63,64]
Root	2-[4 (E)-Formylcyclohex-(E)-YL]-3,5,6-Trimethyl)-1,4-Benzoquinone	Not Found
Root	3,7,11,Trimethyl-Dodeca-2,4,6,10-Tetraenal	Not Found
Root	Octacosane	Anti-diabetic and antibacterial [14,65]

The leaves of *S. willdenowii* (Desv.) Baker may promote some pharmacological effects due to the interaction between plant molecules and organic systems. The effects that *S. willdenowii* (Desv.) Baker exhibits include the main phytol compounds that have anticancer, antioxidant, diuretic, antitumor, antimicrobial, and anti-inflammatory properties [36,43]. Diterpenoid derivatives such as Phytol [66], which acts as a precursor of vitamin E in plants [67]. Phytol can cause oxidative cell death of opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa*. Thus *S. willdenowii* (Desv.) Baker

leaves can be used as an important anti-bacterial agent that causes nosocomial infections [68]. Glycerin is the second most common compound that can increase body fluids, osmotic laxatives, and lubricants [68]. Literature studies reveal Hexadecanoic Acid, Methyl Ester acts as an anti-inflammatory and cancer prevention and treats type 2 diabetes, ulcerative colitis, rheumatoid arthritis, and psoriasis [40]. The presence of phytocomponents in the leaves can be used as anti-inflammatory and antioxidants, as explained in previous reports [51,52], antibacterial [41,42], antitumor [47,49,50], and anticancer [36,40]. Uniquely, some compounds still have not been reported, which can be further studied to determine their potential.

The potential for important biological activity in *S. willdenowii* (Desv.) Baker stems is dominated by Stigmast-5-En-3-ol which can inhibit total cholesterol, Low-Density Lipoprotein (LDL), and triglycerides, and Stigmasterol can increase High-Density Lipoprotein (HDL) [61], providing significant antihyperlipidemic and antitumor activity [51]. Stigmasterol belongs to the group of sterols [69] with the primary function of maintaining the shape of cell membranes [70] and can be used as oleogelators leading to the formation of lipid structures in plant organelles [71]. For the human body, Stigmasterol acts as an anti-inflammatory [72], antidiabetic [73], lowering cholesterol [74], antitumor [75].

The main compound Squalene on the root *S. willdenowii* (Desv.) Baker is pharmacological potential in protecting the liver, fighting fatigue, antioxidants, anticancer, lowering cardiovascular diseases, and boosting the immune system [76], and antibacterial [77]. This phytocomponent is a natural triterpene hydrocarbon with great potential as an adjuvant to induce an immune response [78]. Squalene-based adjuvant MF59 compounds have been used in human influenza vaccines [79]. The compound (9E)-9-Octadecanoic acid acts as an antibacterial. There is proven inhibition in three strains of *Salmonella sp.*, *Staphylococcus aureus*, and *Escherichia coli* in vitro [80]. In closing, we believe *S. willdenowii* (Desv.) Baker is one of the sources of natural products that have important constituents in pharmacology.

CONCLUSION

S. willdenowii (Desv.) Baker (leaves, stems and roots) is an important source of phytoconstituents in pharmacology. GC-MS analysis revealed that various main compounds in leaves, such as phytol (14.98%) have a lot of potential to be developed. Stigmast-5-en-3β-ol and Stigmasterol which are dominant in stems can be used as a source of diabetes drugs. The triterpenoids group in roots has pharmacological potential in protecting the liver, fighting fatigue, antioxidants, anticancer, and boosting the immune system. The results of this study also show that 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne is a compound that is always present in all parts of the plant where its bioactivity is unknown. To the best of our knowledge, these GC-MS results provide the most complete metabolite distribution data from *S. willdenowii* (Desv.) Baker. However, our present results are the first stage in the identification of the biochemical components of the natural product *S. willdenowii* (Desv.) Baker. Future studies need to be expanded for the development of the pharmaceutical and bioceutical industries.

CONFLICT OF INTERESTS

The authors declare that no competing interests.

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REFERENCES

1. Zhou XM, Zhao J, Yang JJ, Péchon T, Zhang L, He XR, Zhang RB. Plastome structure, evolution, and phylogeny of Selaginella. Mol. Phylogenet. Evol. 2022; 169: 107410. doi: 10.1016/J.YMPEV.2022.107410
2. Zhou XM, Zhang LB. A classification of Selaginella (Selaginellaceae) based on molecular (chloroplast and nuclear), macromorphological, and spore features. Taxon. 2015; 64: 1117–1140. doi: 10.12705/646.2
3. Thamnarak W, Eurtivong C, Pollawatn R, Ruchirawat S, Thasana, N. Two new nor-lignans, siamensinols A and B, from Selaginella siamensis Hieron. and their biological activities. Nat. Prod. Res. 2021: 36(21): 5591-5599. doi: 10.1080/14786419.2021.2022664.

4. Aziz Ir, Raharjeng PR, Susilo, Nasution J. Ethnobotany of traditional wedding: A comparison of plants used by Bugis, Palembang, Sundanese and Karo ethnic in Indonesia. J. Phys. Conf. Ser. 2019; 1175. doi: 10.1088/1742-6596/1175/1/012005
5. Risnawati R, Meitiyani, Susilo. The effect of adding Kepok Banana peels (*Musa paradisiaca*) to powder media on the growth of white oyster mushrooms (*Pleurotus ostreatus*). IOP Conf. Ser. Earth Environ. Sci. 2021; 755. doi: 10.1088/1755-1315/755/1/012066
6. Adame-González AB, Muñíz-DL ME, Valencia-A S. Comparative leaf morphology and anatomy of six Selaginella species (Selaginellaceae, subgen. Rupestrae) with notes on xerophytic adaptations. Flora. 2019; 260: 151482. doi: 10.1016/j.flora.2019.151482
7. Jermy AC. Selaginellaceae. Pteridophytes and Gymnosperms. 1990; 39–45. doi: 10.1007/978-3-662-02604-5_11.
8. Xu KP, Zou H, Li FS, Xiang HL, Zou ZX, Long HP, Li J, Luo YL, Li YJ, Tan GS. Two new selaginellin derivatives from Selaginella tamariscina (Beauv.) Spring. J Asian Nat Prod Res. 2011; 13: 356–360. doi: 10.1080/10286020.2011.558840
9. Demehin AA, Thamnarak W, Lamtha T, Chatwichien J, Eurtivong C, Choowongkomon K, Chainok K, Ruchirawat S. Thasana N. Siamenflavones A-C, three undescribed biflavonoids from *Selaginella siamensis* Hieron. and biflavonoids from spike mosses as EGFR inhibitor. Phytochemistry. 2022; **203**, 113374. doi: 10.1016/j.phytochem.2022.113374
10. Li G, Ma X, Jiang Y, Li W, Wang Y, Liu L, Sun C. Xiao S, Kuang J, Wang G. Aqueous two-phase extraction of polysaccharides from *Selaginella doederleinii* and their bioactivity study. Process Biochem. 2022; 118: 274–282. doi: 10.1016/j.procbio.2022.04.024
11. Xie Y, Yao XC, Tan LH, Long HP, Xu PS, Li J, Tan GS. Trichocladabiflavone A, a chalcone-flavonone type biflavonoid from *Selaginella trichoclada* Alsto. Nat. Prod. Res. 2022; 36: 1797–1802. doi: 10.1080/14786419.2020.1817920
12. Akbar B, Susilo, Nissa RA, Ritonga RF, Lestari S, Astuti Y, Parwito. Antifertility Effect of the Ethanol Extract of *Centella asiatica* L. Urban Against the White Rat (*Rattus norvegicus* L.) in the Early Post-Implantation. J. Phys. Conf. Ser. 2018; 1114. doi: 10.1088/1742-6596/1114/1/012002
13. Kunert O., Swamy RC., Kumar BR, Rao AVNA, Nandi OI, Schuehly W. Two Novel Spirostene Glycosides from *Selaginella chrysocaulos* and their Chemotaxonomic Significance. Natural Product Communications. 2015; 10(6). doi: 10.1177/1934578X1501000624
14. Wei Q, Liu R. Flower colour and essential oil compositions, antibacterial activities in *Lagerstroemia indica* L. Nat. Prod. Res. 2022 Apr; 36(8): 2145–2148. doi: 10.1080/14786419.2020.1843034
15. Heng YW, Ban J J, Khoo K S, Sit NW. Biological activities and phytochemical content of the rhizome hairs of *Cibotium barometz* (Cibotiaceae). Ind. Crops Prod. 2020; 153(1): 112612. doi: 10.1016/j.indcrop.2020.112612
16. Zou, Z. X. *et al.* Two new biflavonoids from Selaginella doederleinii. *Phytochem. Lett.* **40**, 126–129 (2020).
17. Yang, J. W., Yang, S. J., Na, J. M., Hahn, H. G. & Cho, S. W. 3-(Naphthalen-2-yl(propoxy)methyl)azetidine hydrochloride attenuates NLRP3 inflammasome-mediated signaling pathway in lipopolysaccharide-stimulated BV2 microglial cells. Biochem. Biophys. Res. Commun. 2018; 495: 151–156. doi: 10.1016/j.bbrc.2017.10.131
18. Yao CP, Zou ZX, Zhang Y, Li J, Cheng F, Xu PS, Zhou G, Li XM, Xu KP. Tan GS *et al.* New adenine analogues and a pyrrole alkaloid from *Selaginella delicatula*. Nat. Prod. Res. 2019; 33: 1985–1991. doi: 10.1080/14786419.2018.1482892
19. Bhattacharya, R. & Naitam, P. Green Anticancer Drugs-An Review. Res. J. Pharmacogn. Phytochem. 2019; 11(4): 231. doi: 10.5958/0975-4385.2019.00040.2
20. Wong CF, Chai TT. Antioxidant properties of aqueous extracts of *Selaginella willdenowii*. Journal of Medicinal Plants Research. 2012; 6(7): 1289–1296. doi: 10.5897/JMPR11.1376
21. Tsun-Thai Chai. Antioxidant properties of aqueous extracts of *Selaginella willdenowii*. *J. Med. Plants Res.* 2012; 6: 1289–1296.
22. Rahmani, A., L.Endang Widiastuti1, Kanedi1, M. & Susanto1, G. N. Toxicity test of *Selaginella willdenowii* extract on survival of common carp juvenile (*Cyprinus* sp.). 2014; 2: 139. doi: 10.23960/j-bekh.v2i1.2220
23. Balachandar R, Karmegam N. Subbaiya R. Extraction, separation and characterization of bioactive compounds produced by streptomyces isolated from vermicast soil. Res. J. Pharm. Technol. 2018; 11(10): 4569–4574. doi: 10.5958/0974-360X.2018.00836.3
24. Nabila N, Susilo, S. A Comparative Metabolite Analysis of *Pandanus amaryllifolius* Leaves from Different Growth Stages using GC-MS and Their Biological. Eur. Chem. Bull. 2022; 11: 22–38.

25. Tang GM, Shi YT, Gao W, Li MN, Li P, Yang H. Comparative Analysis of Volatile Constituents in Root Tuber and Rhizome of *Curcuma longa* L. Using Fingerprints and Chemometrics Approaches on Gas Chromatography–Mass Spectrometry. Molecules. 2022 May 17; 27(10): 3196. doi: 10.3390/molecules27103196.
26. Thakur P, Thakur U, Kaushal P, Ankalgi AD, Kumar P, Kapoor A, Ashawat MS. A Review on GC-MS Hyphenated Technique. Asian J. Pharm. Anal. 2021; 11(4): 285–292. doi: 10.52711/2231-5675.2021.00049
27. Reddy MY, Ramesh V, Reddy CK, Venugopal N, Saravanana G, Suryanarayana MV, Sunder BS. *et al.* The Quantitative Determination of Process Related Genotoxic Impurities in Esomeprazole Magnesium by GC-MS. Asian J. Pharm. Anal. 2011; 4(6): 898–901.
28. Aravind R, Bindu AR, Bindu K, Alexeyena V. GC-Ms analysis of the bark essential oil of cinnamomum malabatrum (burman. f) blume. Res. J. Pharm. Technol. 2014; 7(7): 754–759.
29. Priya S, Nethaji S, Sindhuja B. GC-MS analysis of some bioactive constituents of diospyros Virginiana. Res. J. Pharm. Technol. 2014; 7(4): 429–432.
30. Rajabudeen E, Ganthi A, Subramanian M. GC-MS Analysis of the Methanol Extract of *Tephrosia villosa* (L.) Pers. Asian J. Res. Chem. 2012; 5(11):1331–1334.
31. Zahi MR, Liang H, Khan A, Yuan Q. Identification of Essential Oil Components in Chinese Endemic Plant Achnatherum inebrians. Asian J. Res. Chem. 2014; 7(6):576–579.
32. Jose BE, Selvam PP. Identification of Phytochemical Constituents in the Leaf Extracts of *Azima tetracantha* Lam using Gas Chromatography-Mass Spectrometry (GC-MS) analysis and Antioxidant Activity. Asian J. Res. Chem. 2018; 11(4):857. doi: 10.5958/2321-5836.2019.00004.1
33. Krishnaveni M, Kumari KG, Banu, CR, Kalaivani M. Phytochemical analysis of *Terminalia catappa* stem using GC-MS/MS. Res. J. Pharm. Technol. 2015; 8(9):1281–1283. doi: 10.5958/0974-360X.2015.00232.2
34. Pandian RS, Noora AT. GC-MS analysis of phytochemical compounds present in the leaves of *Citrus medica*. L. Res. J. Pharm. Technol. 2019; 12(4):1823–1826. doi: 10.5958/0974-360X.2019.00304.4
35. Saxena M, Mir AH, Sharma M, Malla MY, Qureshi S, Mir MI, Chaturvedi Y. Phytochemical screening and in-vitro antioxidant activity isolated bioactive compounds from *Tridax procumbens* Linn. Pakistan J. Biol. Sci. PJBS. 2013; 16(24):1971–1977. doi: 10.3923/pjbs.2013.1971.1977
36. Krishnamoorthy K, Subramaniam P. Phytochemical Profiling of Leaf, Stem, and Tuber Parts of *Solena amplexicaulis* (Lam.) Gandhi Using GC-MS . Int. Sch. Res. Not. 2014; 567409. doi: 10.1155/2014/567409
37. Koehler K, Thevis M, Schaenzer W. Meta-analysis: Effects of glycerol administration on plasma volume, haemoglobin, and haematocrit. Drug Test Anal. 2013; 5(11):896–899.
38. Alzurfi SKL, Abdali SA, Aattaby EAS, Rabeea MAA, Al-Haidarey MJS. Identification of lipid compounds in the plant of *Ceratophyllum demersum* using two different solvents. Mater. Today Proc. 2022; 60(3):1596–1605. doi: 10.1016/j.matpr.2021.12.127
39. Rukshana MS, Dos A, Pushpa K. Phytochemical Screening and GC-MS Analysis of Leaf Extract of *Pergularia daemia* (Forssk) Chiov. Asian J. Plant Sci. Res. 2017; 7(1):9-15.
40. Alzurfi SKL, Abdali SA, Aattaby EAS, Rabeea MAA, Al-Haidarey MJS. Identification of lipid compounds in the plant of *Ceratophyllum demersum* using two different solvents. 2022; 60(3):1596–1605. doi:10.1016/j.matpr.2021.12.127.
41. Mangrolia U, Osborne WJ. Staphylococcus xylosus VITURAJ10: Pyrrolo [1,2α] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (PPDHMP) producing, potential probiotic strain with antibacterial and anticancer activity. Microb. Pathog. 2020; 147: 104259. doi: 10.1016/j.micpath.2020.104259
42. Abdulrahman I, Jamal MT, Pugazhendi A, Dhavamani J, Satheesh S. Antibiofilm activity of secondary metabolites from bacterial endophytes of Red Sea soft corals. Int. Biodeterior. Biodegradation. 2022; 173:105462. doi: 10.1016/j.ibiod.2022.105462
43. Prabhadevi V, Sahaya SS, Johnson M, Venkatramani B, Janakiraman N. Phytochemical studies on Allamanda cathartica L. using GC–MS. Asian Pac. J. Trop. Biomed. 2012; 2(2): S550–S554. doi: 10.1016/S2221-1691(12)60272-X
44. Agustikawati N, Andayani Y, Suhendra D. Antioxidant Activity Test and Phytochemical Screening of Pakoasi and Kluwih Leaf Extracts as Natural Antioxidant Sources. J. Penelit. Pendidik. IPA. (2017; 3(2): 60-68. doi: 10.29303/jppipa.v3i2.93
45. Ahmad I, Ahmed S, Akkol EK, Rao H, Shahzad MN, Shaukat U, Basit A, Fatima M. GC–MS profiling, phytochemical and biological investigation of aerial parts of *Leucophyllum frutescens* (Berl.) I.M. Johnst. (Cenizo). South African J. Bot. 2022; 148:200–209. doi: 10.1016/j.sajb.2022.04.038
46. Elaiyaraja A, Chandramohan G. Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. J. Pharmacogn. Phytochem. 2016; 5(6): 158-171.

47. Ali H, Yesmin R, Satter MA, Habib R, Yeasmin T. Antioxidant and antineoplastic activities of methanolic extract of *Kaempferia galanga* Linn. Rhizome against Ehr. J. King Saud Univ. - Sci. 2018; 30(3): 386–392. doi: 10.1016/j.jksus.2017.05.009
48. Carrillo C, Cavia DM, Alonso-Torre SR. Antitumor effect of oleic acid; mechanisms of action. A review. *Nutr Hosp* **27**, 1860–1865 (2012). doi: 10.3305/nh.2012.27.6.6010
49. Priore, P. *et al.* Oleic acid and hydroxytyrosol inhibit cholesterol and fatty acid synthesis in C6 glioma cells. *Oxid. Med. Cell. Longev.* 2017; 29435099. doi: 10.1155/2017/9076052
50. Lattibeaudiere KG, Alexander-Lindo RL. Oleic Acid and Succinic Acid Synergistically Mitigate Symptoms of Type 2 Diabetes in Streptozotocin-Induced Diabetic Rats. Int. J. Endocrinol. 2022 Feb 27; 2022:8744964. doi: 10.1155/2022/8744964
51. Jie F, Yang X, Yang B, Liu Y, Wu L, Lu B. Stigmasterol attenuates inflammatory response of microglia via NF-κB and NLRP3 signaling by AMPK activation. Biomed. Pharmacother. 2022 Sep; 153:113317. doi: 10.1016/j.biopha.2022.113317
52. Khan MA, Sarwar AHMG, Rahat R, Ahmed RS, Umar, S. Stigmasterol protects rats from collagen induced arthritis by inhibiting proinflammatory cytokines. Int. Immunopharmacol. 2020 Aug; 85:106642. doi: 10.1016/j.intimp.2020.106642
53. Drouillat B, Wright K, Marrot J, Couty F. Practical preparation of enantiopure 2-methyl-azetidine-2-carboxylic acid; a γ-turn promoter. Tetrahedron: Asymmetry. 2012; 23(9):690–696. doi: 10.1016/j.tetasy.2012.05.006
54. Kolar MJ, Konduri S, Chang T, Wang H, McNerlin C, Ohlsson L, Härröd M. *et al.* Linoleic acid esters of hydroxy linoleic acids are anti-inflammatory lipids found in plants and mammals. J. Biol. Chem. 2019 Jul 5; 294(27):10698-10707. doi: 10.1074/jbc.RA118.006956
55. Simopoulos AP. Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. 1999 Sep; 70(3 Suppl):560S-569S. doi: 10.1093/ajcn/70.3.560s
56. Dos Reis CM, da Rosa BV, da Rosa GP, do Carmo G, Morandini LMB, Ugalde GA, Kuhn KR *et al.* Antifungal and antibacterial activity of extracts produced from Diaporthe schini. J. Biotechnol. 2019 Mar 20; 294:30-37. doi: 10.1016/j.jbiotec.2019.01.022
57. Montalvo G, Campos S, Arenas M, Barreto A, Escalante K, Cuzon G, Gaxiola G.. Immune gene expression and antioxidant response to vitamin E enriched diets for males *Litopenaeus vannamei* breeder (Boone, 1931). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2022 Jun; 268:111187. doi: 10.1016/j.cbpa.2022.111187.
58. Sudirman T, Hatta M, Prihantono P, Bukhari A, Tedjasaputra TR, Lie H. Vitamin E administration as preventive measures for peritoneal/intra-abdominal adhesions: A systematic review and meta-analysis. Ann. Med. Surg. 2022; 80: 104225. doi: 10.1016/j.amsu.2022.104225
59. Zahid M, Arif, M, Rahman MA, Singh K, Mujahid M. Solvent Extraction and Gas Chromatography–Mass Spectrometry Analysis of *Annona squamosa* L. Seeds for Determination of Bioactives, Fatty Acid/Fatty Oil Composition, and Antioxidant Activity. J Diet Suppl. 2018 Sep 3; 15(5):613-623. doi: 10.1080/19390211.2017.1366388.
60. Tan DC, Idris KI, Kassim NK, Lim PC, Ismail IS, Hamid M, Ng RC. Comparative study of the antidiabetic potential of *Paederia foetida* twig extracts and compounds from two different locations in Malaysia. Pharm Biol. 2019 Dec; 57(1):345-354. doi: 10.1080/13880209.2019.1610462
61. Fernando IPS, Sanjeewa KKA, Ann YS, Ko CI, Lee SH, Lee WW, Jeon YJ. Apoptotic and antiproliferative effects of Stigmast-5-en-3-ol from *Dendronephthya gigantea* on human leukemia HL-60 and human breast cancer MCF-7 cells. Toxicol In Vitro. 2018 Oct; 52:297-305. doi: 10.1016/j.tiv.2018.07.007
62. Iyer D, Patil UK. Efficacy of Stigmast–5–en–3β–ol Isolated from *Salvadora persica* L. as Antihyperlipidemic and Anti–tumor agent: Evidence from animal studies. Asian Pacific J. Trop. Dis. 2012; 2(2): S849–S855. doi: 10.1016/S2222-1808(12)60278-3
63. Yaglioglu AS, Yaglioglu MS, Tosyalıoglu N, Adem S, Demirtas I. Chemical profiling, in vitro biological activities and Pearson correlation between chemical profiling and anticancer activities of four *Abies species* from Turkey. South African J. Bot. Chem Biodivers. 2023 Mar; 20(3):e202201142. doi: 10.1002/cbdv.202201142.
64. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002 Sep-Oct; 22(5):2587-90.
65. Okokon JE, Etuk IC, Thomas PS, Drijfhout FP, Claridge TDW. In vivo antihyperglycaemic and antihyperlipidemic activities and chemical constituents of *Solanum anomalum*. Biomed Pharmacother. 2022 Jul;151:113153. doi: 10.1016/j.biopha.2022.113153

66. Wu YQ,Wang, Xin Y, Huang SJ, Wang GB, Xu LA.. Exogenous GbHMGS1 Overexpression Improves the Contents of Three Terpenoids in Transgenic Populus. Forests. 2021; 12(5): 1–14. https://doi.org/10.3390/f12050595
67. Mekinić IG, Čagalj M, Tabanelli G, Montanari C, Barbieri F, Skroza D, Šimat V. Seasonal changes in essential oil constituents of cystoseira compressa: First report. Molecules. 2021 Nov 2; 26(21):6649. doi: 10.3390/molecules26216649
68. Lee W, Woo ER, Lee DG. Phytol has antibacterial property by inducing oxidative stress response in *Pseudomonas aeruginosa*. Free Radic Res. 2016 Dec; 50(12):1309-1318. doi: 10.1080/10715762.2016.1241395
69. Weremczuk-Jeżyna, I, Hnatuszko-Konka K, Lebelt L, Grzegorczyk-Karolak I. The protective function and modification of secondary metabolite accumulation in response to light stress in dracocephalum forrestii shoots. Int J Mol Sci. 2021 Jul 26; 22(15):7965. doi: 10.3390/ijms22157965.
70. Aboobucker SI, Suza WP. Why do plants convert sitosterol to stigmasterol? Front. Plant Sci. 2019; **10**: 354. doi: 10.3389/fpls.2019.00354.
71. Tang C, Wan Z, Chen Y, Tang Y, Fan W, Cao Y, Song M, Qin J, Xiao H, Guo S, Tang Z. Structure and Properties of Organogels Prepared from Rapeseed Oil with Stigmasterol. *Foods* 2022 Apr; 11(7): 939. doi: 10.3390/foods11070939
72. Navarro A, De las Heras B, Villar A. Anti-inflammatory and immunomodulating properties of a sterol fraction from Sideritis foetens CLEM. Biol Pharm Bull. 2001 May; 24(5):470-3. doi: 10.1248/bpb.24.470
73. Wang J, Huang M, Yang J, Ma X, Zheng S, Deng S, Huang Y, Yang X, Zhao P. Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food Nutr Res. 2017 Aug 23; 61(1):1364117. doi: 10.1080/16546628.2017.1364117.
74. Prasad M, Jayaraman S, Eladl MA, El-Sherbiny M, Abdelrahman MME, Veeraraghavan VP, Vengadassalapathy S, Umapathy VR, Hussain SFJ, Krishnamoorthy K, Sekar D, Palanisamy CP, Mohan SK, Rajagopal P. A Comprehensive Review on Therapeutic Perspectives of Phytosterols in Insulin Resistance: A Mechanistic Approach. Molecules. 2022 Feb 28;27(5):1595. doi: 10.3390/molecules27051595.
75. Gao Z, Maloney DJ, Dedkova LM, Hecht SM. Inhibitors of DNA polymerase β: Activity and mechanism. Bioorg Med Chem. 2008 Apr 15; 16(8):4331-40. doi: 10.1016/j.bmc.2008.02.071.
76. Gohil N, Bhattacharjee G, Khambhati K, Braddick D, Singh V. Engineering strategies in microorganisms for the enhanced production of squalene: Advances, challenges and opportunities. *Front. Bioeng. Biotechnol.* 2019; 7: 1–24. doi: 10.3389/fbioe.2019.00050
77. Peng W, Li D, Zhang M, Ge S, Mo B, Shasha Li S, Ohkoshi M. Characteristics of antibacterial molecular activities in poplar wood extractives. Saudi J. Biol. Sci. (2017; 24(2):399–404. doi: 10.1016/j.sjbs.2015.10.026
78. Lubna Azmi L, Gupta SS, Shukla I, Kant P, Sidhu OP, Rao CV. Effect of squalene in surgically induced gastro-oesophageal reflux disease on rats. Res. J. Pharmacol. Pharmacodyn. 2017; **9(1):** 1-9. doi: 10.5958/2321-5836.2017.00001.5
79. Chae GE, Kim DW, Jin HE. Development of Squalene-Based Oil-in-Water Emulsion Adjuvants Using a Self-Emulsifying Drug Delivery System for Enhanced Antigen-Specific Antibody Titers. Int J Nanomedicine. 2022 Dec 9; 17:6221-6231. doi: 10.2147/IJN.S379950
80. Pu ZH, Zhang YQ, Yin ZQ, Xu J, Jia RY, Lu Y, Yang F. Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. Agric. Sci. China. 2010; 9(8):1236–1240. doi: 10.1016/S1671-2927(09)60212-1