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Original Research Article

Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Stem Extract Using GC-MS Analysis and Pharmacological Potential

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ABSTRACT

Talinum paniculatum (Talinaceae), also known as Javanese ginseng in Indonesia, is a widely recognized medicinal plant. However, the method of fertilization is currently limited to the leaves, and there needs to be more information about the metabolite profile on its stem. Therefore, this study aimed to identify the content of metabolite present in the stem extract of *T. paniculatum* and investigate the potential of an introductory class of metabolites to be utilized. To achieve this, the active compounds of the *T. paniculatum* stem were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with Ethanol Pro Analysis (99.8%) as organic solvents. The profiles of these compounds were identified using the NCBI International Library PubChem branch, NIST Chemistry WebBook branch, SpectraBase branch of WILEY, and FOODB branch of TMIC. The results showed that *T. paniculatum* stem extract possesses a dominating compound with anti-inflammatory properties.

Keywords: antioxidant, bioactivity, GC-MS, metabolite profiling, *Talinum paniculatum*

Introduction

Medicinal plants are sources of bioactive compounds that have long been widely used in traditional medicine. In recent decades, they have become a fundamental ingredient in the health products industry.[1] It is estimated that a considerable percentage, ranging from 70-90%, of the global population relies on herbal medicine to address their health problems.[2] Therefore, it is crucial to conduct extensive studies to investigate and determine the bioactive compounds in traditional and local medicinal plants that can be used to design modern medicines.

Talinum paniculatum (*T. paniculatum*), also known as Javanese ginseng in Indonesia, shares similarities with other renowned traditional medicine such as *Panax ginseng* in East Asia.[3,4] In addition to having good adaptability, this plant has been reported to have various properties, including antioxidants,[5] free radicals,[6] anti-cancer agents,[7] and efficacy in addressing cardiovascular disorders.[6]

Other results showed that *T. paniculatum* is rich in phytochemicals, especially flavonoids, tannins, triterpenes, saponins, polyphenols,[8] and polysaccharides.[9] Saponins, for instance, have demonstrated high efficacy in enhancing spermatozoa viability, motility, and number. They possess anti-inflammatory properties and show potential androgenic effects while promoting cell differentiation through receptor cells, thereby boosting the body's resistance to disease.[7] Flavonoids are known for their antioxidant, anti-cancer, antimicrobial, antipyretic, anti-diabetic, and antihypertensive properties.[3] Tannins, on the other hand, are water-soluble, environmentally friendly, and have antimicrobial and antioxidant activity.[10] Triterpenes have demonstrated the ability to combat gliomas or tumor cell pools in glial cells.[11] Polyphenols, functioning as antioxidant defenses, protect against free radicals.[12] Finally, polysaccharides are biopolymers in plant cell walls that provide nutrients.[13]

T. paniculatum is traditionally consumed by boiling and pounding, as per local practice. The leaves have been used as vegetable ingredients [7,14] and are traditional medicinal materials, particularly in the Grande Duorados region. They have demonstrated significant efficacy in treating skin infections and promoting wound healing, exhibiting potent activity against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus.*[15,16] Additionally, the root of *T. paniculatum* has been the main recipe for Leishmania medicine in Peru, where it is peeled, grated, dried over fire, and applied to ulcers.[17] Recent reports in 2020 highlighted the use of its leaves and roots as material for medicinal baths.[18]

While the roots and leaves of *T. paniculatum* have been extensively utilized as medicinal herbs, information is scarce regarding the phytochemical profile of the stem extract of this plant in Indonesia. This represents the first report on the compound profile of the stem. Therefore, this study aimed to investigate the potential of active compounds contained in the stem of *T. paniculatum* using GC-MS. The potential of the active compounds found is also discussed.

Materials and Method

Plant materials

T. paniculatum was collected from farmers in Bogor, West Java, Indonesia (6°34'38.1 "S 106°53'17.0"E) in September 2022. It was identified by Herbarium Bogoriense, ELSA Botani, BRIN (National Research and Innovation Agency) Indonesia, with voucher number 3079-46085-2. Stem parts were separated and washed using running aqua dest to remove impurities from the plant material. Furthermore, the sample was cut into small pieces of ±4 cm and put into a liquid Nitrogen tube for preservation and transportation to the laboratory.

Extract preparation

The Stem sample was weighed to 50 g using Shimadzu's analytical balance scale and oven-dried for 72 hours at 40ºC. After drying, each sample was mashed into a powder using a blender[19] and macerated with Ethanol Pro Analysis (99.8 %) for 5 days. A total of 10 ml of each extract was fed into different tubes and dried at 60ºC using a Rotary Evaporator Caliper. The resulting solid residue was then re-dissolved using the remaining 200 µL of extract.

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GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using Agilent Technologies 7890 Gas Chromatography with Auto Sampler and 5975 Mass Selective Detector, coupled with the Chemstation data system. This followed the procedures of John Bwire Ochola's study in 2022,[20,21] which was modified by the library of the Research Center for Spice and Medicinal Plants (BALITRO) and equipped with a capillary column (30 m × 0.20 mm I.D × 0.11 µm film thickness). The ethanol plant extract was filtered in split mode through a syringe of 5 µL (8:1). The carrier was helium, which flows at a 1.2 Ml/min speed. The injector temperature is 250°C. Subsequently, the analytes were separated on a column of silica capillaries with dimensions of 30 m × 0.20 mm I.D × 0.11 µm film thickness. The oven was set at an initial temperature of 80°C which was directly increased by 3°C/min to 150°C for 1 minute and raised again by 20°C/min to 280°C for 26 minutes. Finally, the mass spectrum was determined using an ionization energy of 70 eV.

Data analysis

Data analysis was performed using the Agilent MassHunter Qualitative Analysis Software application, which facilitated the identification of active compounds by comparing mass fragments and the standard mass spectrum. To support the analysis, biological activity data were sourced from the international libraries of NCBI (National Center for Biotechnology Information) PubChem branch, NIST (National Institute of Standards and Technology) Chemistry WebBook branch, WILEY SpectraBase branch, and TMIC (The Metabolomics Innovation Centre) FOODB branch. These libraries were also used in Guang-Mei Tang's study conducted in 2022.[22]

Results and Discussion

Profile of Active Compounds of Talinum Paniculatum Stem

The ethanol extract of *T. paniculatum* stem was subjected to GC-MS analysis, as shown in Figure 1, and the results identified 22 active compounds with amounts exceeding 1.00%. The identification process was based on the Retention Time (RT). Furthermore, the dominant compound, accounting for 19.92 %, was (2E)-3,7,11,15-Tetramethyl-2-hexadecane-1-ol, observed at RT 29.40. In contrast, the Ethyl 9-hexadecanoic compound at RT 32.63 constituted only 1.00%. Proportions of 16.34% and 2.71% stigmast-5-en-3-ol compounds were observed at RT 38.82 and RT 39.7, while 0.87% and 4.41% of Oleic Acid compounds were discovered at RT 30.70 and RT 31.58.

The active compounds in *T. paniculatum* stem were identified by their M.F. (Molecular Formula), and M.W. (Molecular Weight) (Table 1), as obtained from the International Library (NCBI; NIST; WILEY). The results were arranged into a profile which becomes a piece of complete information. This discovery proved that GC-MS is an effective tool in identifying various active compounds untargeted in *T. paniculatum*. It has also been used as an analytical tool in the *Panax* ginseng study.[23] The successful compilation of the profile of the active compound showed that they have been identified from living things.

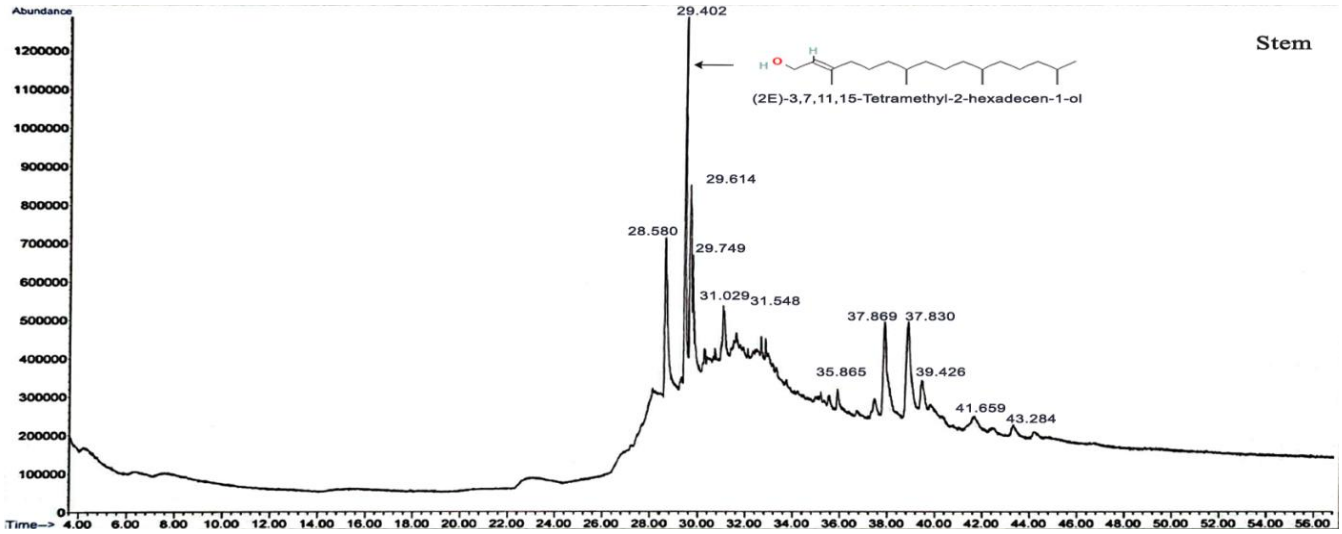

Figure 1: Chromatogram of *Talinum paniculatum* Ethanol Extract

Classification of Active Compounds of Talinum paniculatum Stem

Active compounds are the final product of a metabolic process. The metabolic tissue possessed by each plant has a much more diverse amount than other organisms,[23] with each active compound exhibiting a classification that determined whether it was derived organically or inorganically. The classification provided detailed and essential information regarding their potential application. The active compounds of ethanol extract of *T. paniculatum* stem are pretty diverse at the "Class of Compounds," as presented in Table 2, hence, allowing for different biological roles in the body.

The classification profile of 16 active compounds was obtained from the ethanol extract of *T. paniculatum* stem using a library analysis known as FOODB, which is a branch of The Metabolomics Innovation Centre [(TMIC)]. All the active compounds belonged to the Kingdom of Organic Compounds, indicating that they were produced directly by the plant body. However, they possess a considerable gap as 15 of them were derived from Super Class Lipids and Lipid-Like Molecules, while the remaining was from Super Class Organic Oxygen Compounds. Furthermore, the minority Super Class compound is called Spiro [5.6] dodecane-1,7-dione, characterized by its aromatic ring structure.[24,25]

Active compounds derived from the Super Class Lipids and Lipid Like Molecules were grouped into Classes Fatty Acyls, Prenol Lipids, as well as Steroids and Steroid Derivatives. Fatty Acyls contained acetyl-CoA primary chains with a malonic-CoA (Lipid Maps) group. In contrast, Prenol Lipids were synthesized from a 5-carbon precursor isopentenyl diphosphate (lipid maps), while plant steroids were referred to as phytosterols.[27] Among the identified steroids, there are two distinct properties, namely Ergostane and Stigmastanes Steroids. The difference between these properties lies in the carbon skeleton.[28] Compounds from the Ergostane Steroids group can also be discovered in withanolides, which possessed a carbocyclic skeleton and an enzyme system capable of oxidizing carbon atoms.[29] Stigmastanes, on the other hand, exhibit alkyl-type interactions.[30] When examining the number of compounds present, it is evident that stigmasterol constitutes 15.31% of the total. Stigmast-5-en-3-ol, at 16.34% (RT 38.82), and 2.71% (RT 39.78), demonstrates the dominance of Steroid Stigmastanes over Ergostane Steroid. However, the Campesterol compound only accounts for 2.93% of the total.

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Table 1: Active Compound Profile of *Talinum paniculatum* Stems

No	Metabolites Compound	RT	% of Area	M.F.	M.W. (g/mol)	Library
1	Hexadecanoic acid, ethyl ester	28.58	18.11	$C_{18}H_{36}O_2$	284.48	NCBI
2	Octadecanoic acid	29.30	1.04	$C_{18}H_{36}O_2$	284.48	NCBI
3	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29.40	19.92	$C_{20}H_{40}O$	296,50	NCBI
4	Ethyl (9z,12z)-9,12-octadecadienoate	29.61	13.86	$C_{20}H_{36}O_2$	308,5	WILEY
5	Octadecanoic acid, ethyl ester	29.74	8.70	$C_{20}H_{40}O_2$	312,54	NIST
6	Hexadecanoic acid	30.25	1.50	$C_{16}H_{32}O_2$	256,43	NIST
7	Spiro [5.6] dodecane-1,7-dione	30.35	1.50	$C_{12}H_{18}O_2$	194,27	NCBI
8	Oleic acid	30.70	0.87	$C_{18}H_{34}O_2$	282.46	NCBI
		31.58	4.41			
9	1H-Pyrido [4.3,b] indole, 2,3,4,4a,5,9b-hexahydro-2,8-dimethyl-5-(4-nitrobenzoyl)-, (4ar,9bs)-rel-	31.03	5.80	$C_{18}H_{18}ClNS$	315.9	NCBI
10	n-Hexyltrichlorosilane	31.75	1.02	$C_6H_{13}C_{13}Si$	219,60	NCBI
11	Cholestane, 4,5-epoxy-, (4.alpha.,5.alpha.)-	32.078	0.49	$C_{27}H_{46}O$	386.7	NCBI
12	(6e,10e,14e,18e)-2,6,10,15,19,23-Hexamethyl-2,6,14,18,22-tetracosahexa methexaene	32.44	1.99	$C_{30}H_{50}$	410.71	NIST
13	Ethyl 9-hexadecanoate	32.63	1.00	$C_{18}H_{34}O_2$	282.50	NCBI
14	Squalene	32.81	0.63	$C_{30}H_{50}$	410.71	NCBI
15	2-Nitro-4-(trifluoromethyl)phenol	35.51	0.63	$C_7H_4F_3NO_3$	207.11	NCBI
16	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	35.86	2.12	$C_{29}H_{49}ClO$	449.10	NCBI
17	Campesterol	37.42	2.93	$C_{28}H_{48}O$	400,69	NCBI
18	Stigmasterol	37.53	15.31	$C_{29}H_{48}O$	412,70	NCBI
19	Stigmast-5-en-3-ol	38.82	16.34	$C_{29}H_{50}O$	414.72	NCBI
		39.78	2.71			
20	1,4-Methanoazulen-9-ol, decahydro-1,5,5,8A-tetramethyl-, [1R-(1.α., 3a.β.,)]	39.42	6.14	$C_{15}H_{26}O$	222.37	NCBI
21	18,19-Secolupan-3-ol, (3.β., 17.xi.)-	41.65	4.06	$C_{30}H_{54}O$	430.70	NCBI
22	Olean-12-en-28-oic acid, 3-oxo-, methyl ester	43.28	1.83	$C_{31}H_{48}O_3$	468.71	NIST

Table 2: Classification of Ethanol Extract of *Talinum paniculatum* Stems

Kingdom	Super Class	Class	Sub Class	Compounds
Organic Compounds	Lipids and Lipid-Like Molecules	Fatty Acyls	Fatty Acid and Conjugates	Hexadecanoic acid
		Fatty Acyls	Fatty Acid Esters	Ethyl 9-hexadecanoate
		Fatty Acyls	Fatty Acid Esters	Hexadecanoic acid, ethyl ester
		Fatty Acyls	Fatty Acid Esters	n-Hexyltrichlorosilane
		Fatty Acyls	Fatty Acid Esters	Octadecanoic acid, ethyl ester
		Fatty Acyls	Fatty Acids and Conjugates	Octadecanoic acid
		Fatty Acyls	Fatty Acids and Conjugates	Oleic acid
		Fatty Acyls	Lineolic Acids and Derivatives	Ethyl (9z,12z)-9,12-octadecadienoate

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	Prenol Lipids	Diterpenoids	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol
	Prenol Lipids	Quinone and Hydroquinone Lipids	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol
	Prenol Lipids	Triterpenoids	(6e,10e,14e,18e)-2,6,10,15,19,23-Hexamethyl-2,6,14,18,22-tetracosahexa methexaene
	Prenol Lipids	Triterpenoids	Squalene
	Prenol Lipids	Triterpenoids	Olean-12-en-28-oic acid, 3-oxo-, methyl ester
	Steroids and Steroid Derivates	Ergostane Steroids	Campesterol
	Steroids and Steroid Derivates	Stigmastanes and Derivatives	Stigmast-5-en-3-ol
	Steroids and Steroid Derivates	Stigmastanes and Derivatives	Stigmasterol
Organic Oxygen Compounds	Organooxygen Compounds	Carbonyl Compounds	Spiro [5.6] dodecane-1,7-dione

Biological Activity of the Active Compounds of Talinum paniculatum Stem

Compounds present in plants can exhibit a range of biological activities, which can be either beneficial or detrimental. It is common for a single compound to possess multiple activities. However, medicinal plants classified as dominant often exhibited a variety of activities from each compound contained. The Stem of the ginseng group is quite underutilized. A study reported that *T. triangulare* stem has more robust antioxidant activity than other parts.[6]

(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, as the dominating compound in the stem of *T. paniculatum* exhibited various beneficial properties. It has been reported to possess antimicrobial, antifungal, antibacterial, antiparasitic, antimutagenic, and antioxidant activities.[31] Additionally, studies conducted on *Alnus nitida*,[32] and *Agave tequilana*[33] have indicated that (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol possesses anti-inflammatory properties, and its amount predominantly increases during summer.[34] It was discovered that this compound can synthesize vitamin E.[35]

Anti-inflammatory and antioxidant were the two biological activities dominating the stem of *T. paniculatum*. They were contained in (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, (6e,10e,14e,18e)-2,6,10,15,19,23-Hexamethyl-2,6,14,18,22-tetracosahexa methexaene, 2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecil)-6-chromanol, Ethyl(9z,12z)-9,12-octadecadienoate, Hexadecanoic acid, ethyl ester, Octadecanoic acid, and Squalene. Plant extracts with these two activities have long been recognized as valuable and are believed to possess the ability to reduce the burden of oxidative stress.[36]

Campesterol compounds have been reported to have anti-inflammatory activity.[37] Furthermore, they demonstrated anticholinesterase[38] and anti-cancer properties.[39] This shows that campesterol compounds have similar biological activity with the class they belong to, namely Ergostane Steroids. As reported by Zhabinskii, Ergostane Steroids have antibacterial, anti-inflammatory, and anti-malarial activity.[40] However, Zhang reported that Ethyl 9-hexadecanoic, constituting only 1% of *T. paniculatum* stem, apparently has not been extensively studied for its biological activity. Stem contains compounds that are rarely discovered in living things,[41] such as 18,19-Secolupan-3-ol, (3.β., 17.xi.)-.[42] This study highlighted that the stem of *T. paniculatum* has the potential of being used as a medicinal plant. It uncovered a compound not been explored in terms of its biological activity.

Conclusion

GC-MS successfully identified 20 active compounds from the stem of *T. paniculatum* using pro-analysis ethanol extract (99.8%). These compounds were analyzed using the international library, allowing for the preparation of phytochemical profiles and classifications of active compounds, which showed their beneficial biological activity. The stem bark of this plant exhibited high efficacy as an anti-inflammatory agent, followed by antioxidant properties, with the dominant compound being (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol. Furthermore, the discovery of Ethyl 9-hexadecanoate as a reported compound represented an opportunity for further investigation into its biological activity, as it has not been extensively studied. *T. paniculatum* stem contains compounds rarely found in living things,[41] namely the compound 18,19-Secolupan-3-ol, (3.β., 17.xi.) -. Therefore, the stem of *T. paniculatum* can be used as a medicinal plant due to its biological activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 3: Biological activity of the active compound on the *Talinum paniculatum* stem

No	Metabolite	Biology Activity
1	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial, antifungal, antibacterial, antiparasitic, antimutagenic, antioxidant,[31] anti-inflammatory,[32,33] synthesize vitamin E.[35]
2	(6e,10e,14e,18e)-2,6,10,15,19,23-Hexamethyl-2,6,14,18,22-tetracosahexa methexaene	Antioxidant [43,44] and anti-inflammatory.[44]
3	18,19-Secolupan-3-ol, (3.*β*., 17.xi.)-	Including the triterpenoid framework, which is rarely found in living organisms.[41]
4	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecil)-6-chromanol	Antioxidant,[45] anti-inflammatory.[32]
5	Campesterol	Anticholinesterase,[38] anti-inflammatory,[37] anti-cancer.[39]
6	Ethyl (9z,12z)-9,12-octadecadienoate	Antioxidant,[46] anti-inflammatory.[47]
7	Ethyl 9-hexadecanoate	Not identified.[42]
8	Hexadecanoic acid, ethyl ester	Anti-inflammatory, antioxidant, anti-cancer,[46] and antimicrobial.[48]
9	Hexadecanoic acid	Anti-inflammatory and antioxidant.[49]
10	Octadecanoic acid	Anti-inflammatory[50] and antioxidant.[51,52]
11	Oleic acid	Antibacterial[53], anti-inflammatory, antifungal[54], and anti-cancer.[54,55]
12	Stigmast-5-en-3-ol	Antioxidant, anti-cancer.[24]
13	Stigmasterol	Anti-inflammatory,[56] anti-diabetic,[57] antitumor.[58]
14	Squalene	Antioxidant,[43,44] anti-inflammatory.[44]

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