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Best regards

Abiodun

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Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Stem Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential

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Abstract

Javanese ginseng (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 Javanese ginseng and investigate the potential of an introductory class of metabolites to be utilized. To achieve this, the active compounds of the Javanese ginseng stem were analyzed using the Shidmazu GC-MS tool 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.

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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 antioxidant (5), free radicals (6), anti-cancer agent (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 as well as 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 traditional medicinal materials, particularly in the Grande Duorados region (15). 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, there is a scarcity of information regarding phytochemical profile of stem extract of this plant in Indonesia. This represents the first report on the compound profile of stem. Therefore, this study aimed to investigate the potential of active compounds contained in 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). 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

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 200µL of extract..

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 speed of 1.2 mL/min. 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, 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), M.W. (Molecular Weight), and Chemical Structure (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 *Panax* ginseng study (23). The successful compilation of the active compounds profile showed that they have been identified from living things.

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 (J. Liu et al., 2017), with each active compounds exhibiting a classification that determined whether it was derived from 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 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 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 rings structure (TMIC) and (WILEY) (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 quantity 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.

Biological Activity of the Active Compound of *Talinum paniculatum* Stem

Compounds present in plant 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 variety of activities

from each compound contained. Stem of ginseng group are quite underutilized. A study reported that *T. triangulare* stem have more robust antioxidant activity than other parts (6).

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

Anti-inflammatory and antioxidant were the two biological activities dominating 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 extract 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 (Lorenzi et al., 2019) and anti-cancer properties (39). This shows that campesterol compounds have similar biological activity with the class they belong, namely Ergostane Steroids. As reported by (40), Ergostane Steroids have antibacterial, anti-inflammatory, and anti-malarial activity. However, Zhang et al. (2019) reported that Ethyl 9-hexadecanoic, constituting only 1% in *T. paniculatum* stem, apparently has not been extensively studied for its biological activity. Stem contain compounds that are rarely discovered in living things (42), such as 18,19-Secolupan-3-ol, (3.β., 17.xi.)-. This study highlighted that 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 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. Stem 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 contain compounds rarely found in living things (42), namely the compound 18,19-Secolupan-3-ol, (3.β., 17.xi.) -. Therefore, stem of *T. paniculatum* has the high potential as a medicinal plant due to its biological activity.

Conflict of Interest

The authors declare no conflict of interest.

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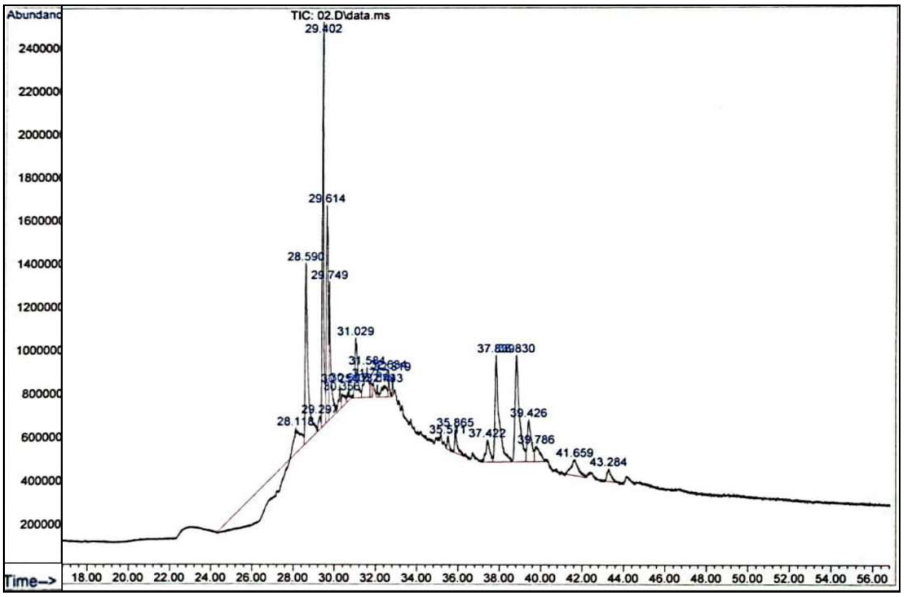


Figure 1. Chromatogram of *Talinum paniculatum* Ethanol Extract

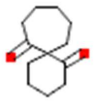
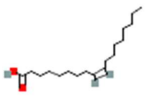
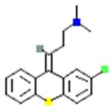
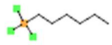
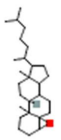
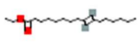
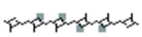
Table 1. Active Compound Profile of *Talinum paniculatum* Stem Ethanol Extract

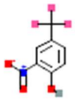
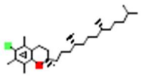
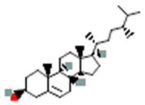
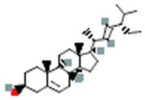
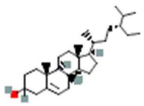
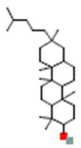
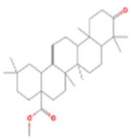
No	Metabolites Compound	RT	% of Area	M.F.	M.W. (g/mol)	Chemical Structure	Library
1	Hexadecanoic acid, ethyl ester	28.58	18,11	$C_{18}H_{36}O_2$	284.48		NCBI
2	Octadecanoic acid	29.30	1,04	$C_{18}H_{36}O_2$	284.48		NCBI
3	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29.40	19,92	$C_{20}H_{40}O$	296,50		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

Commented [AF5]: a high picture quality of the chromatogram should be used in this manuscript

The major peaks should be identified with arrows

Commented [AF6]: All chemical structures should be drawn with ChemDraw software

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 31.58	0,87 4,41	$C_{18}H_{34}O_2$	282.46		NCBI
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	$C_6H_{13}C_{l3}Si$	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 39.78	16,34 2,71	$C_{29}H_{50}O$	414.72		NCBI
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

Library source: (NCBI; NIST; WILEY)

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
		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

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 (45,46) and anti-inflammatory (46).
3	18,19-Secolupan-3-ol, (3.*β*., 17.xi.)-	Including the triterpenoid framework, which is rarely found in living organisms (42)
4	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecil)-6-chromanol	Antioxidant (47), anti-inflammatory (Sajid et al., 2022)
5	Campesterol	Anticholinesterase (Lorenzi et al., 2019), anti-inflammatory (37), anti-cancer (39).
6	Ethyl (9z,12z)-9,12-octadecadienoate	Antioxidant (48), anti-inflammatory (49)
7	Ethyl 9-hexadecanoate	Not identified (41)
8	Hexadecanoic acid, ethyl ester	Anti-inflammatory, antioxidant, anti-cancer (48), and antimicrobial (50).
9	Hexadecanoic acid	Anti-inflammatory and antioxidant (51).
10	Octadecanoic acid	Anti-inflammatory (52) and antioxidant (53,54).
11	Oleic acid	Antibacterial (55), anti-inflammatory, antifungal (56), and anti-cancer (56,57).
12	Stigmast-5-en-3-ol	Antioxidant, anti cancer (TMIC)
13	Stigmasterol	Anti-inflammatory (58), anti-diabetic (59), antitumor (60)
14	Squalene	Antioxidant (45,46), anti-inflammatory (46)

Re: Manuscript Under Peer-Review Process

From: Susilo (susilo@uhamka.ac.id)

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Dear Editor of the Tropical Journal of Natural Product Research

On December 21, 2022, I submitted a manuscript entitled Phytochemical Profiling of Javanese Ginseng (Talinum paniculatum) Leaf Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential. We are still waiting to receive news on the status of the manuscript. Please let me know if any deficiencies exist, and I will fix them.

We convey the details of the author and potential reviewers.
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Journal: Tropical Journal of Natural Product Research www.tjnpr.org

Corresponding Author: SUSILO SUSILO*

Co-authors: FADITA NURUL AINI,

Manuscript No: TJNPR NOV164ARN

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Best regards

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Professor Abiodun Falodun, PhD

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Authors: FADITA NURUL AINI, SUSILO SUSILO*

TJNPR Editorial Decision: accepts with moderate corrections

Congratulations

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam. v11i3.31

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Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential

Fadita Nurul Aini[1] and Susilo[1*]

Abstract

Talinum paniculatum (Family: Talinaceae) or Javanese ginseng (Indonesia) is widely known as a medicinal plant. However, fertilization of *Talinum paniculatum* is still limited to the leaves. Further, information about the metabolite profile on *T. paniculatum* stems has never been reported. Our goal is to identify the content of metabolite compounds in *T. paniculatum* stem extract and investigate the potential of an introductory class of metabolites to be utilized further. The active compounds of *T. paniculatum* stem were analyzed using the Shidmazu GC-MS tool and Ethanol Pro Analysis (99.8%) utilized as organic solvents. Active compound profiles were identified using the NCBI International Library PubChem branch, NIST Chemistry WebBook branch, SpectraBase branch of WILEY, and FOODB branch of TMIC. As a result, GC-MS analysis of *T. paniculatum* stem extract revealed a dominating compound with anti-inflammatory properties.

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

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Introduction

Medicinal plants are a source 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). About 70-90% of people worldwide entrust herbal medicine with solving their health problems (2). Therefore, investigating and determining bioactive compounds from traditional and local medicinal plants require extensive studies that are important to be carried out to design modern medicines.

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Talinum paniculatum, or Javanese ginseng in Indonesia, is one of the herbs widely known to have potential as traditional medicine, such as *Panax ginseng* in East Asia (3,4). In addition to having good adaptability, *T. paniculatum* is reported to have properties as an antioxidant (5), free radicals (6), anti-cancer (7), and overcoming cardiovascular disorders (6).

Other research results reported that *T. paniculatum* is rich in phytochemicals, especially flavonoids, tannins, triterpenes, saponins, polyphenols (8), and polysaccharides (9). Saponin levels in this breed have a high degree of effectiveness in increasing viability, motility, and the number of spermatozoa. Saponins are anti-inflammatory and potentially androgenic, and the body can induce cell differentiation through receptor cells to increase the body's resistance to disease (7). Flavonoids are proven to be antioxidant, anti-cancer, antimicrobial, antipyretic, anti-diabetic, and antihypertensive (3). Tannins are water-soluble, environmentally friendly, and have antimicrobial and antioxidant activity (10). Triterpenes can overcome gliomas or

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tumor cell pools in glial cells (11). Polyphenols as antioxidant defenses against free radicals (12). Polysaccharides are biopolymers in plant cell walls that provide foodstuffs (13).

Based on its local use, *T. paniculatum* is usually consumed by boiling and pounding. Traditionally, *T. paniculatum* leaves have been used as vegetable ingredients (7,14) and traditional medicinal materials such as in the Grande Duorados region (15). *T. paniculatum* leaves are used to treat skin infections and wound healing because they have great activity against *Candida albicans, Escherichia coli*, and *Staphylococcus aureus* (15,16). The root of *T. paniculatum* became the main recipe for Leishmania medicine in Peru by peeling, grated, and drying on the fire, then placing on an ulcer (17). The use of *T. paniculatum* leaves and roots was also a medicinal bath material reported in 2020 (18).

Despite its rich medicinal value shown by more natives using roots and leaves as medicinal herbs, there are limited reports of the phytochemical profile of *T. paniculatum* stem extract in Indonesia. This is the first report to reveal the profile of the compound on the stem of *T. paniculatum* in Indonesia. This study aims to screen the active compounds contained in the stems of *T. paniculatum* using GC-MS. The potential of the active compounds found is also discussed.

Materials and Method

Plant Materials

Talinum paniculatum is collected from farmers in Bogor, West Java, Indonesia (6°34'38.1 "S 106°53'17.0"E). *Talinum paniculatum* was identified by Herbarium Bogoriense, ELSA Botani, BRIN (National Research and Innovation Agency) Indonesia, with voucher number 3079-46085-2. The stem parts were separated and then washed using running aqua dest to remove impurities on the plant material. The sample was cut into small pieces (± 4 cm) and then put into a liquid Nitrogen tube for further taking to the laboratory.

Extract preparation

A stem sample of 50 g (Shimadzu's analytical balance scale) was oven-dried for 72 hours at 40°C. Each dry sample was mashed using a blender until it became a powder (19) and macerated using Ethanol Pro Analysis (99.8 %) for five days. A total of 10 ml of each sample extract was fed into different tubes and dried at 60°C using a Rotary Evaporator Caliper. The solid residue was re-dissolved with the remaining extract of 200 µL.

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector and Chemstation data system, following procedures adopted from John Bwire Ochola's research in 2022 (20,21). 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 through a syringe filter of 5 µL in split mode (8:1). Helium at the flow of 1.2 mL/min was the carrier. The injector temperature is 250°C. Then the analytes are separated on a column of silica capillaries (30 m × 0.20 mm I.D × 0.11 µm film thickness). The oven is set as follows an initial temperature of 80°C which is directly raised by 3°C/min to 150°C and held for 1 minute, then raised by 20°C/min to 280°C and held for 26 minutes. Determination of the mass spectrum using an ionization energy of 70 eV.

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Data Analysis

Data analysis was carried out using the Agilent MassHunter Qualitative Analysis Software application to identify active compounds by comparing mass fragments and the standard mass spectrum. Biological activity data were analyzed using data 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. The library is also used in Guang-Mei Tang's research in 2022 (22).

Results and Discussion

Profile of Active Compounds of Talinum paniculatum Stem Ethanol Extract

The results of the GC-MS chromatogram against ethanol extract of *T. paniculatum* stems (Figure 1) were analyzed to find 22 active compounds that had amounts above 1.00%. The GC-MS chromatogram is based on Retention Time (RT) which indicates the presence of a compound. RT 29.40 shows the compound (2E)-3,7,11,15-Tetramethyl-2-hexadecane-1-ol dominating compound of 19.92%. In contrast to RT 32.63, which is known to be an Ethyl 9-hexadecanoic compound, it only amounts to 1.00%. The discovery of stigmast-5-en-3-ol compounds in RT 38.82 was 16.34%; RT 39.7 was 2.71%, and Oleic Acid compounds in RT 30.70 as much as 0.87% and RT 31.58 as much as 4.41%.

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The active compounds in *T. paniculatum* stems are identified in M.F. (Molecular Formula), M.W. (Molecular Weight), and Chemical Structure (Table 1.) through the International Library (NCBI; NIST; WILEY). The identification results are arranged into a profile which becomes a piece of complete information. This discovery has proven that GC-MS is an effective tool in finding various active compounds untargeted in *T. paniculatum*. GC-MS has also been used as an analytical tool in *Panax ginseng* research (23). The success of compiling the active compound profile shows that the compound has already been identified from living things.

Classification of Active Compounds of Talinum paniculatum Stem Ethanol Extract

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 (J. Liu et al., 2017), and each active compound has a classification that determines whether the compound is derived from organic or inorganic compounds. The classification of active compounds provides detailed information as an essential ingredient before the implementation of the use of active compounds. The active compounds of ethanol extract of *T. paniculatum* stems are pretty diverse at the "Class of Compounds" (Table 2), thus allowing for different biological roles in the body.

The results of the classification profile of 16 active compounds from the ethanol extract of *Talinum paniculatum* stem from a library analysis known as FOODB, a branch of The Metabolomics Innovation Centre (TMIC). The 16 active compounds come from the Kingdom of Organic Compounds, which means they are produced directly by the plant body. However, the compound has a considerable gap because 15 active compounds are derived from Super Class Lipids and Lipid-Like Molecules, and one active compound is from Super Class Organic Oxygen Compounds. The compound that belongs to the minority Super Class is called Spiro [5.6] dodecane-1,7-dione, whose structure is composed of aromatic rings (TMIC) and (WILEY). In

addition, the compound turns out to be Spiro [5.6] dodecane-1,7-dione has a ketones structure (25).

Active compounds derived from the Super Class Lipids and Lipid Like Molecules can be grouped into Class Fatty Acyls, Class Prenol Lipids, and Class Steroids and Steroid Derivatives. Fatty Acyls contains acetyl-CoA primary chains with a malonic-CoA (Lipid Maps).group. In contrast to Prenol Lipids, which are synthesized from a 5-carbon precursor isopentenyl diphosphate (lipid maps). Plant steroids are referred to as phytosterols (27). The steroids found have two different properties: Ergostane Steroids and Stigmastanes Steroids. The difference between the two properties of this steroid lies in its carbon skeleton (28). Compounds from the Ergostane Steroids group are also found in withanolides with a carbocyclic skeleton and an enzyme system capable of oxidizing carbon atoms in steroid nuclei (29). Stigmastanes have alkyl-type interactions (30). If you look at the amount contained, stigmasterol compounds 15.31%, Stigmast-5-en-3-ol 16.34% (RT 38.82), and 2.71% (RT 39.78) show that Steroid Stigmastanes are more dominant than Ergostane Steroid which Campesterol compound only amounts to 2.93%.

Biological activity of the active compound of the *Talinum paniculatum* stem

Compounds found in every plant have beneficial or detrimental biological activities. One compound can have one or more activities. However, plants classified as dominant medicinal plants have a variety of activities from each compound contained. The stems of the ginseng group are quite rarely utilized. However, one study has been reported on *Talinum triangulare* stems that have more robust antioxidant activity than other parts (6).

(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, as the dominating compound on the stem of *T. paniculatum* has an Antimicrobial, antifungal, antibacterial, antiparasitic, antimutagenic, antioxidant (31). In fact, (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol is reported to have anti-inflammatory properties through research on *Alnus nitida* (32), and *Agave tequilana* (33). The compound (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol), whose amount predominantly increases in summer (34), turns out to have the ability to synthesize vitamin E (35). The compound (2E) bioactivity equation-3,7,11,15-Tetramethyl-2-hexadecen-1-ol and Vitamin E is on antioxidant and anti-inflammatory properties.

Anti-inflammatory and antioxidant are the two biological activities dominating the stem of *T. paniculatum*. Compounds containing the two biological activities are (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 that have these two activities have long been considered high-value plants and are certain to have the ability to reduce the burden of oxidative stress (36).

Campesterol compounds are also reported to have anti-inflammatory activity (37). It also acts as an anticholinesterase (Lorenzi et al., 2019) and anti-cancer (39). This shows that campesterol compounds have similar biological activity to their class's activity, namely Ergostane Steroids. As reported by (40), Ergostane Steroids have antibacterial, anti-inflammatory, and anti-malarial activity. However, Zhang et al. (2019) reported that Ethyl 9-hexadecanoic, which amounts to only 1% in *T. paniculatum* stems, apparently has never been studied regarding its biological activity. Even *T. paniculatum* stems contain compounds rarely found in living things (42), namely the compound 18,19-Secolupan-3-ol, (3.β., 17.xi.)-. This

study not only succeeded in proving that the stem of *T. paniculatum* has the potential to be used as a medicinal plant but also found a compound that has never been studied regarding its biological activity.

Conclusion

GC-MS successfully identified 20 active compounds from the stem of *T. paniculatum* using pro-analysis ethanol extract (99.8%). The active compounds were analyzed using the International library so that the successful preparation of phytochemical profiles and classifications of active compounds found beneficial biological activity. Biological activity shows that the stem of *T. paniculatum* has high efficacy as an anti-inflammatory and is followed by antioxidant properties, with the dominant compound being (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol. In addition, the discovery of the reported compound Ethyl 9-hexadecanoate has never been studied regarding its biological activity. Even *T. paniculatum* stems contain compounds rarely found in living things (42), namely the compound 18,19-Secolupan-3-ol, (3.β., 17.xi.) -. Therefore, it is concluded that the stem of *T. paniculatum* has a high potential as a medicinal plant due to its biological activity.

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Revisied of Manuscript Number TJNPR NOV164ARN

From: Susilo (susilo@uhamka.ac.id)

To: editor.tjnpr@gmail.com

Date: Tuesday, June 6, 2023 at 10:07 AM GMT+7

Manuscript Number TJNPR NOV164ARN

Dear Editors,

We have revised the manuscript title "**Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential**" following the Reviewer's comments and the Editor's request.
The corrected manuscript is attached with a certificate of English editing and the results of a plagiarism check.
I hope this result is acceptable to you.
Thank You

Best regard
Susilo

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Re: Revisied of Manuscript Number TJNPR NOV164ARN

From: Editor-in-Chief Tjnpr (editor.tjnpr@gmail.com)

To: susilo@uhamka.ac.id

Date: Saturday, July 8, 2023 at 11:28 PM GMT+7

Received

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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> On Sat, 8 Jul 2023 at 02:20, Susilo <susilo@uhamka.ac.id> wrote:
>
> > Dear Editors,
> >
> > We have corrected a grammar error in the manuscript entitled "**Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential**."
> > I hope this work has been in accordance with your expectations.
> >
> > Thank You

Best regard
Susilo

On Tuesday, June 6, 2023 at 07:56:59 PM GMT+7, Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> wrote:

There are many grammatical errors that should be corrected. Authors should seek the assistance of a native English speaker

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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On Tue, 6 Jun 2023 at 04:07, Susilo <susilo@uhamka.ac.id> wrote:

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Dear Editors,

We have revised the manuscript title "**Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential**" following the Reviewer's comments and the Editor's request.

> > The corrected manuscript is attached with a certificate of English editing and the results of a plagiarism check.
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> > Thank You
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Re: Check galley proof

From: Susilo (susilo@uhamka.ac.id)

To: et.tjnpr@gmail.com

Date: Monday, July 24, 2023 at 04:28 PM GMT+7

Dear Editors,
We are pleased to submit the revised draft of our manuscript "**Phytochemical Profiling of Javanese Ginseng (Talinum paniculatum) Stem Extract Using GC-MS Analysis and Pharmacological Potential**".
We appreciate the time and effort dedicated by the editorial staff and reviewers. We have made revisions to the manuscript based on the suggestions given. Changes to the yellow highlighted manuscript. We declare that all contents of the manuscript are appropriate and correct.
We look forward to your response regarding our submission. Please do not hesitate to contact us if there are any further questions or comments.

Regards,
Susilo

> On Monday, July 24, 2023 at 01:41:00 PM GMT+7, Editorial Team <et.tjnpr@gmail.com> wrote:
>
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>
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>
> Please, as soon as possible (not later than 24 hours) kindly respond with receipt along with any comment or observations. Also respond to the comment(s) where indicated.
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> **All corrections/changes made in the manuscript should be highlighted in yellow ink when submitting the manuscript in the revised form.**
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Ref. No. 05509018024895 DATE:20th May 2023

Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Prof. DR. Hamka, East Jakarta, Indonesia 13830

Dear Susilo,

Provisional Acceptance letter for Article Manuscript Number TJNPR NOV164ARN

Title: Phytochemical Profiling of Javanese Ginseng (*Talinum paniculatum*) Extract by Gas Chromatography (GC/MS) and Its Pharmacological Potential

Authors: FADITA NURUL AINI, SUSILO SUSILO*

I am pleased to inform you that your manuscript sent to the Tropical Journal of Natural Product Research has been reviewed and recommended for publication as a research article.
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Sincerely,

Professor Abiodun Falodun
Editor-in-Chief