ORIGINAL ARTICLE

GC-MS Analysis Reveals Unique Chemical Composition of *Blumea balsamifera* (L.) DC in *Ie-Jue* Geothermal Area

Nur Balqis Maulydia[1], Khairan Khairan[2,3], Trina Ekawati Tallei[4], Ethiene Castellucci Estevam[5], Mohsina Patwekar[6], Fazlin Mohd Fauzi[7] and Rinaldi Idroes[2,3,*]

[1]Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; [2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala Banda Aceh 23111, Indonesia; [3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; [4]Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, Indonesia; [5]Instituto do Câncer do Estado de São Paulo, Brazil; [6]Department of Pharmacology, Luqman College of Pharmacy, Karnataka 585102, India; [7]Faculty of Pharmacy, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42 300 Bandar Puncak Alam, Selangor, Malaysia

* Correspondence: rinaldi.idroes@usk.ac.id

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Abstract

Blumea balsamifera (L.) DC. or *Sembung* is a flowering plant belonging to the genus *Blumea* of the family *Asteraceae*. Many pharmacological activities of this plant show potential in human therapy. In this study, an investigation was conducted on the ethanolic extract of *B. balsamifera* collected from a geothermal area known as Ie-Jue, in Aceh Province, Indonesia. The results showed that the ethanolic extract of *B. balsamifera* contained secondary metabolites of flavonoids and tannins. The chemical constituents of the ethanolic extracts of *B. balsamifera* were further analyzed using gas chromatography-mass spectrometry (GC-MS). The results showed that the active compound of this plant is proximadiol ($C_{15}H_{28}O_2$), which constitutes 41.76% of the total relative area. The presence of these compounds indicates that *Blumea balsamifera* plants positively contain active secondary metabolites of the terpenoid class that are predicted to have a therapeutic role against 17β-HSD Inhibitors. This research underscores the exciting potential of the Ie-Jue geothermal area as a promising reservoir of flora due to the adaptability of plants to geothermal areas.

Introduction

Geothermal areas have a significant influence on microbial and plant ecosystems by providing unique ecological conditions that facilitate growth and adaptation [1–3]. Aceh Province, Indonesia is known to have several reported geothermal manifestations, especially on Mount Seulawah Agam [4,5]. *Ie-Jue* is one of the manifestations in the up-flow zone of the Mount Seulawah Agam geothermal system, with hot springs reaching 80-102 °C and ambient air temperatures reaching 40-45°C [6]. The *Ie-Jue* manifestation is in the northern coastal hills of Aceh Besar District, Aceh Province located at coordinates 5°30'24''N-95°37'45''E, at an elevation of 254 meters above sea level [7]. Exploration of plant-based medicine from geothermal areas is still limited. Geothermal manifestations not only provide benefits in the energy field but can also extend their benefits to other fields, including medicine [8–10]. Plants originating from the *Ie-Jue* area are known to have the potential as broad-spectrum antibacterial and antifungal agents, for example against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* [11,12].

Indonesia is renowned for its rich plant biodiversity. The utilization of medicinal plants in disease prevention is undertaken through an empirical approach [13–17]. Among the botanical specimens prevalent in the *Ie-Jue* area is *Blumea balsamifera* (L.) DC [18]. This plant, belonging to Asteraceae family, and commonly referred to as *sambong/sembung*, has been utilized for medicinal practices across various Southeast Asian countries for an extensive period [19]. Over time, *B. balsamifera* has served as an important component in diverse traditional medicine formulations [20]. The botanical specimen of this plant encompasses a diverse array of chemical compounds, comprising both volatile and non-volatile substances [21]. Essential oils derived from *B. balsamifera* in China have been reported to feature significant concentration of 1,8-cinoele (20.98%), borneol (11.99%) and camphor (8.06%) [22]. The myriad compounds within this plant have contributed to its extensive use as a complementary therapy in multiple nations [21,23].

This study pursues a dual objective: (i) to identify the secondary metabolite compounds within *B. balsamifera* specimen from the *Ie-Jue* geothermal area, Aceh Province-Indonesia, employing phytochemical analysis and gas chromatography-mass spectrometry (GC-MS), and (ii) to conduct a comprehensive assessment of their potential as pharmaceutical candidate. The results of this investigation offer insights into the compound composition of *B. balsamifera* in the *Ie-Jue* areas, positioning them as prospective candidates for medicinal application.

Materials and Methods

Sampling and Plant Preparations

The leaves of *B. balsamifera* were collected from the geothermal locale of *Ie-Jue*, situated at coordinates 5°30'24''N and 95°37'45''E. Subsequently, the leaves were subjected to thorough washing, followed by air-drying at room temperature. The dried leaves were subsequently finely powdered. To extract the plant's compounds, a maceration process spanning three days was conducted, employing a weight-to-volume ratio (w:v) of 1:10. An ethanol extract was derived from this process. Subsequently, the extract was subjected to dehydration through the application of a rotary evaporator (Butchi Rotavapor®, Switzerland) to yield the crude extract.

Phytochemical analysis

A qualitative phytochemical screening was performed to assess the presence of six distinct classes of secondary metabolites, specifically flavonoids, tannins, saponins, terpenoids, steroids, and alkaloids [14,24]. Furthermore, gas chromatography-mass spectrometry (GC-MS) was employed, utilizing the TRACE 1310 GC coupled with a single quadrupole (iSQ) 7000 MS equipped with the TraceGOLD TG-35MS column [2].

Biological Activity Predictions

Data on the simplified molecular input line-entry system (SMILES) were collected to evaluate the chemical structures of metabolite compounds [25]. Subsequently, the of metabolite compounds present in the ethanolic extracts of B. balsamifera was predicted using the PASS web server [26].

Results and Discussion

Blumea balsamifera, a member of the Asteraceae family, is a well-known constituent of one of the largest assemblages of flowering plants, the Asteraceae or Compositae family[27]. The classification of the samples is detailed in Table 1. Based on observations made at the sampling point, it is eviden that *B. balsamifera* in the *Ie-Jue* geothermal area thrives in soils with elevated temperatures, reaching as high as 72-86°C (Figure 1). This shows the remarkable adaptability of *B. balsamifera* plants to grow in extreme environmental conditions.

Phytochemical analysis of the ethanolic extract of *B. balsamifera* showed the presence of flavonoids and tannins (Table 2). The absence of other compound classes may be attributed to the distinctive geothermal soil conditions in which the plant thrives, characterized by notably elevated temperatures in comparison to typical environmental circumstances. Notably, in a separate study conducted on *B. balsamifera* from another city in Aceh Province, it was observed that when employing three distinct extraction solvents, the plant exhibited a diverse array of secondary metabolites classes, including steroids, saponins, phenolics, and tannins [28].

Table 1. Classification of plant.

Rank	Scientific Name
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Blumea
Species	*Blumea balsamifera* (L.) DC.

Figure 1. *B. balsamifera* in *Ie-Jue* area.

Table 2. Phytochemical analysis of ethanolic extract of *B. balsamifera* leaves.

No	Secondary metabolite	Ethanolic extract of *B. balsamifera* leaves
1	Alkaloids	-
2	Flavonoids	+
3	Steroids	-
4	Saponins	-
5	Tannins	+
6	Triterpenoids	-

(–) absent; (+) present

The chemical composition of *B. balsamifera* was ascertained through the application of GC-MS analysis. In the ethanolic extract of it leaves, a total of twenty-two compounds were identified. The findings of this analysis notably highlight the prevalence of compounds exhibiting a retention time of 29.69 minutes, with the most prominent peak reaching a relative abundance of 41.76% (Figure 2).

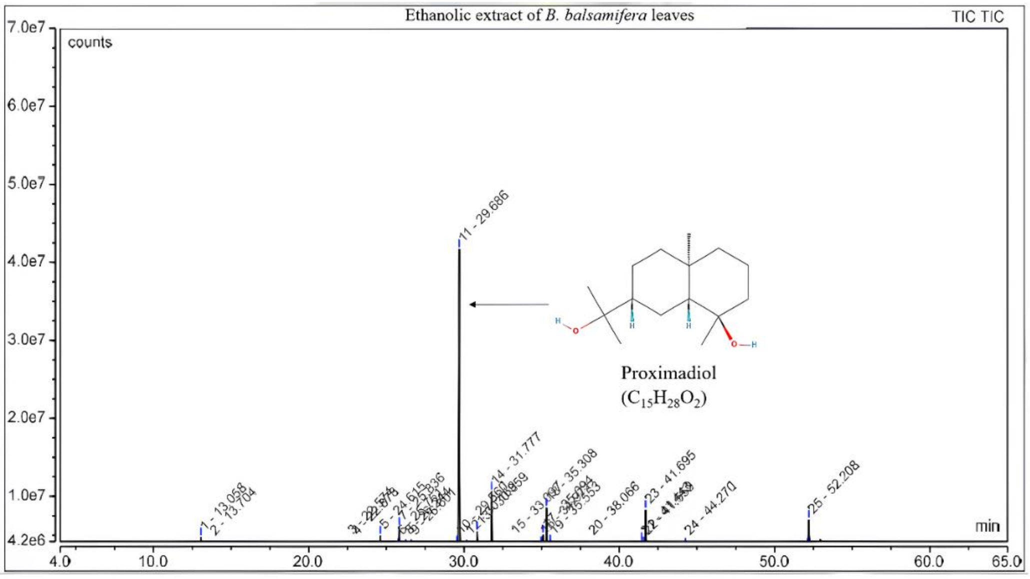


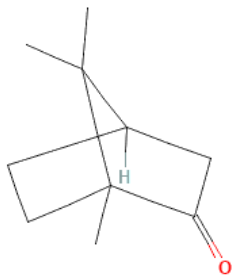
Figure 2. Chromatogram of ethanolic extract *B. balsamifera* leaves.

Table 3. Metabolite compounds of ethanolic extract *B. balsamifera* leaves.

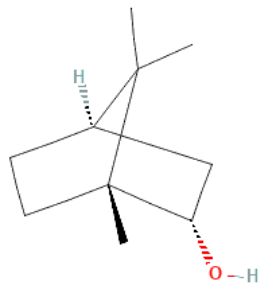
No	Retention time (Min)	Relative area (%)	SI	Compound names
1	13.06	2.67	819	Camphor
2	13.70	0.77	706	endo-Borneol
3	22.57	0.74	714	3-Methyl-thiophene-2-carboxamide
4	22.87	0.65	687	2,4-Di-tert-butylphenol
5	24.61	2.65	833	Caryophyllene oxide
6	25.76	1.18	784	trans-Z-a-Bisabolene epoxide
7	25.84	4.10	867	11,11-Dimethyl-4,8dimethylenebicyclo[7.2.0]undecan-3-ol
8	26.24	0.65	797	ß-Acorenol
9	26.60	1.26	819	trans-Z-a-Bisabolene epoxide
10	29.56	1.51	770	cis-Z-a-Bisabolene epoxide
11	29.69	41.76	831	Proximadiol
12	30.17	0.98	734	Aspidospermidin-17-ol, 1-acetyl-19,21epoxy-15,16-dimethoxy
13	30.86	4.19	701	Dihydroartemisinin, 6-deshydro-5deshydroxy-3-desoxy
14	31.78	8.54	877	Hexadecanoic acid, methyl ester
15	33.10	0.89	705	E-11-Hexadecenoic acid, ethyl ester
16	34.97	1.44	850	Methyl 9-cis,11-trans-octadecadienoate
17	35.09	3.12	808	Cyclopropaneoctanoic acid, 2-[[2-[(2ethylcyclopropyl)methyl]cyclopropyl]methyl] methyl ester
18	35.31	6.84	874	Phytol
19	35.55	1.91	805	Heptadecanoic acid, 10-methyl-, methyl ester
20	38.07	0.63	677	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-
21	41.70	5.89	639	Cyclopentanone, 2-acetyl-3,3-dimethyl-2-(3oxo-1-butenyl)-,
22	52.21	4.46	755	Stigmasterol

B. balsamifera is characterized by a robust aromatic fragrance, notably redolent of compounds such as camphor and borneol. In this study, an analysis of metabolite compounds within B. balsamifera from the *Ie-Jue* region revealed the presence of camphor and endo-borneol (Figure

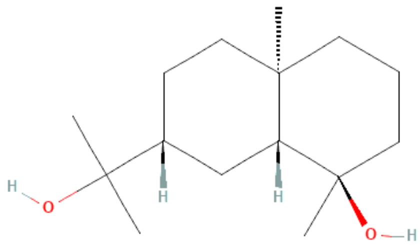
3a and 3b), with relative area percentages 2.67% and 0.77% (Table 3). Notably, the chromatographic profile exhibited its highest peak at a retention time of 29.69 minutes, primarily corresponding to proximadiol, with a relative area accounting for 41.76%. Furthermore, within the steroid class, stigmasterol was identified, representing 4.46% of the composition (Figure 3c and 3d). Proximadiol, also known as cryptomeridiol, is a naturally occurring compound originating from *B. balsamifera* [23]. It has the chemical formula of $C_{15}H_{28}O_2$, a molecular weight of 240.38 g/mol, and is classified as a sesquiterpene alcohol [25]. Proximadiol has been previously reported in the *Cymbopogon proxymus* [29].



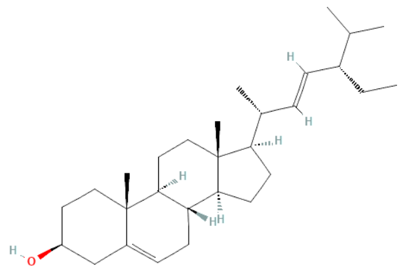
A
Camphor
($C_{10}H_{16}O$)



B
Endo-borneol
($C_{10}H_{18}O$)



C
Proximadiol
($C_{15}H_{28}O_2$)



D
Stigmasterol
($C_{29}H_{48}O$)

Figure 3. Metabolite active from *B. balsamifera leaves* in *le-Jue* area. A. Champor, B. Endo-borneol, C. Proximadiol and D. Stigmasterol.

Biological Activity Prediction

The Table 4 presents the biological activity predictions of compounds found in *B. balsamifera* leaves. The PASS results are presented as "active" (Pa) and "inactive" (Pi) outputs, where these probabilities range from 0 to 1, with values closer to 1 indicating higher activity or inactivity [26]. In this study, four compounds were identified as active in inhibiting testosterone 17beta-dehydrogenase (NADP+), namely camphor (Pa: 0.936), endo-borneol (Pa: 0.962), proximadiol (Pa: 0.821) and stigmasterol (Pa: 0.915) (Table 4). 17beta-hydroxysteroid dehydrogenases (17beta-HSDs) are significant contributors to the modulation of steroid hormones, specifically estrogens and androgens [30]. Inhibitors targeting 17β-HSDs have proven to be valuable instruments for investigating the involvement of these enzymes in specific biological systems or for therapeutic purposes. The presence of active compounds from the ethanolic extract of *B. balsamifera* suggests their potential utility as an inhibitors of 17β-HSDs. These inhibitors are particularly effective in preventing the synthesis of active hydroxysteroids, which are known to promote estrogen-sensitive conditions such as breast, ovarian, and endometrial cancers, as well as androgen-sensitive conditions such as prostate cancer, benign prostatic hyperplasia, acne, and hirsutism [30].

Table 4. Biological activity of metabolite compounds.

Camphor		
Pa	**Pi**	**Activity name**
0.936	0.004	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.922	0.004	Respiratory analeptic
0.907	0.004	CYP2J substrate
Endo-borneol		
Pa	**Pi**	**Activity name**
0.962	0.002	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.954	0.002	Acylcarnitine hydrolase inhibitor
0.947	0.002	Alkylacetylglycerophosphatase inhibitor
0.947	0.003	Alkenylglycerophosphocholine hydrolase inhibitor
0.942	0.003	CYP2J substrate
0.918	0.003	Cardiovascular analeptic
Proximadiol		
Pa	**Pi**	**Activity name**
0.814	0.012	Acylcarnitine hydrolase inhibitor
0.821	0.020	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.782	0.011	Alkylacetylglycerophosphatase inhibitor
0.774	0.005	Cardiovascular analeptic
0.776	0.008	Anti inflammatory
Stigmasterol		
Pa	**Pi**	**Activity name**
0.970	0.002	Antihypercholesterolemic
0.965	0.001	Cholesterol antagonist
0.933	0.001	Oxidoreductase inhibitor
0.915	0.005	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.913	0.004	Prostaglandin-E2 9-reductase inhibitor

Conclusions

The GC-MS analysis of *B, balsamifera* L. (DC), commonly known as *sembung*, from the *Ie-Jue* geothermal area has revealed a prevalence of sesquiterpene compounds, particularly proximadiol. The presence of these compounds suggests promising therapeutic potential. This finding underscores the potential utility of *B. balsamifera* as a complementary therapeutic agent within the context of Aceh Province, Indonesia. The compounds identified in this study may offer valuable insights for further research into the medicinal applications of this plant in the region.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: All the authors declare that there are no conflicts of interest.

References

[1] Abubakar A, Yusuf H, Syukri M, Nasution R, Yusuf M, Idroes R. Heavy metals contamination in geothermal medicinal plant extract (Chromolaena odorata Linn). Global Journal of Environmental Science and Management 2023;9:995–1004. https://doi.org/10.22035/gjesm.2023.04.22.

[2] Maulydia NB, Idroes R, Khairan K, Tallei TE, Mohd Fauzi F. Ecotoxicological insight of phytochemicals, toxicological informatics, and heavy metal concentration in Tridax procumbens L. in geothermal areas. Global

Journal of Environmental Science and Management 2024;10:369–84. https://doi.org/10.22034/gjesm.2024.01.23.
[3] Idroes GM, Syahnur S, Majid MSA, Idroes R, Kusumo F, Hardi I. Unveiling the Carbon Footprint: Biomass vs. Geothermal Energy in Indonesia. Ekonomikalia Journal of Economics 2023;1:10–8. https://doi.org/10.60084/eje.v1i1.47.
[4] Idroes R, Yusuf M, Saiful S, Alatas M, Subhan S, Lala A, et al. Geochemistry Exploration and Geothermometry Application in the North Zone of Seulawah Agam, Aceh Besar District, Indonesia. Energies 2019;12:4442. https://doi.org/10.3390/en12234442.
[5] Bahri RA, Noviandy TR, Suhendra R, Idroes GM, Yanis M, Yandri E, et al. Utilization of Drone with Thermal Camera in Mapping Digital Elevation Model for Ie Seu'um Geothermal Manifestation Exploration Security. Leuser Journal of Environmental Studies 2023;1:25–33. https://doi.org/10.60084/ljes.v1i1.40.
[6] Idroes R, Yusuf M, Alatas M, Subhan, Lala A, Muhammad, et al. Geochemistry of Sulphate spring in the Ie Jue geothermal areas at Aceh Besar district, Indonesia. IOP Conference Series: Materials Science and Engineering 2019;523. https://doi.org/10.1088/1757-899X/523/1/012012.
[7] Aprianto A, Maulana A, Noviandy TR, Lala A, Yusuf M, Marwan M, et al. Exploring Geothermal Manifestations in Ie Jue , Indonesia : Enhancing Safety with Unmanned Aerial Vehicle. Leuser Journal of Environmental Studies 2023;1:47–54. https://doi.org/10.60084/ljes.v1i2.75.
[8] Maulydia NB, Khairan K, Noviandy TR. Prediction of Pharmacokinetic Parameters from Ethanolic Extract Mane Leaves (Vitex pinnata L.) in Geothermal Manifestation of Seulawah Agam Ie-Seu'um, Aceh. Malacca Pharmaceutics 2023;1:16–21. https://doi.org/10.60084/mp.v1i1.33.
[9] Nuraskin C, Marlina, Idroes R, Soraya C, Djufri. Identification of secondary metabolite of laban leaf extract (Vitex pinnata l) from geothermal areas and non-geothermal of agam mountains in Aceh Besar, Aceh province, Indonesia. Rasayan Journal of Chemistry 2020;13:18–23. https://doi.org/10.31788/RJC.2020.1315434.
[10] Ningsih DS, Celik I, Abas AH, Bachtiar BM, Kemala P, Idroes GM, et al. A Review of the Ethno-dentistry Activities of Calotropis gigantea. Malacca Pharmaceutics 2023;1:8–15.
[11] Idroes R, Khairan, Fakri F. Skrining Aktivitas Tumbuhan yang berpotensi sebagai bahan antimikroba di kawasan ie Jue (upflow geotermal Zone) Aceh besar. Banda Aceh: Unsyiah Press; 2017.
[12] Fakri F, Harahap SP, Muhni A, Khairan K, Hewindati YT, Idroes GM. Antimicrobial Properties of Medicinal Plants in the Lower Area of Ie Seu-um Geothermal Outflow, Indonesia. Malacca Pharmaceutics 2023;1:55–61. https://doi.org/10.60084/mp.v1i2.44.
[13] Imelda E, Khairan K, Lubis RR, Kemala P, Zulfiani U, Rahayu S, et al. Anticataract activity of ethanolic extract from Hippobroma longiflora (L.) G.Don leaves: Ex vivo investigation. Journal of Pharmacy & Pharmacognosy Research 2023;11:833–40. https://doi.org/10.56499/jppres23.1691_11.5.833.
[14] Rachmawati R, Idroes R, Suhartono E, Maulydia NB, Darusman D. In Silico and In Vitro Analysis of Tacca Tubers (Tacca leontopetaloides) from Banyak Island, Aceh Singkil Regency, Indonesia, as Antihypercholesterolemia Agents. Molecules 2022;27. https://doi.org/10.3390/molecules27238605.
[15] Abas AH, Tallei TE, Idroes R, Fatimawali F. Ficus minahassae (Teijsm. & de Vriese) Miq.: A Fig Full of Health Benefits from North Sulawesi, Indonesia: A Mini Review. Malacca Pharmaceutics 2023;1:1–7. https://doi.org/https://doi.org/10.60084/mp.v1i1.24.
[16] Khairan K, Arini M, Idroes R, Awang K, Jacob C. Antibacterial Activity of n-Hexane Dragon's Blood Resin Extract (Daemonorops draco wild Blume) from Bener Meriah, Aceh Province, Indonesia. Malacca Pharmaceutics 2023;1:22–9. https://doi.org/https://doi.org/10.60084/mp.v1i1.29.
[17] Dolongtelide JI, Fatimawali F, Tallei TE, Suoth EJ, Simbala HEI, Antasionasti I, et al. In Vitro Antioxidant Activity of Chrysanthemum indicum Flowers Extract and Its Fraction. Malacca Pharmaceutics 2023;1:43–7. https://doi.org/10.60084/mp.v1i2.26.
[18] Idroes R, Khairan K, Fakri F. Screening of Potential Plant Activities as Antimicrobial Materials in the Ie Jue Area (Upflow Geothermal Zone) Aceh Besar. Banda Aceh: Syiah Kuala Universiti Press; 2017.
[19] Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X, et al. Blumea balsamifera- A phytochemical and pharmacological review. Molecules 2014;19:9453–77. https://doi.org/10.3390/molecules19079453.
[20] Widhiantara IG, Jawi IM. Phytochemical composition and health properties of Sembung plant (Blumea balsamifera): A review. Veterinary World 2021;14:1185–96. https://doi.org/www.doi.org/10.14202/vetworld.2021.1185-1196.
[21] Wang J, He H, Zhou Z, Bai L, She X, He L, et al. Chemical constituents and bioactivities of Blumea balsamifera (Sembung): a systematic review. Food Science and Technology (Brazil) 2023;43. https://doi.org/10.1590/fst.132322.

[22] Chu SS, Du SS, Liu ZL. Fumigant Compounds from Essential Oil of Chinese Blumea balsamifera Leaves againts the Maize Weevil (Sitophilus zeamais). Journal of Chemistry 2012;2013.
[23] Shirota O, Oribello JM, Sekita S, Satake M. Sesquiterpenes from Blumea balsamifera. Journal of Natural Products 2011;74:470–6. https://doi.org/10.1021/np100646n.
[24] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 1987.
[25] Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic Acids Research 2023;51:D1373–80. https://doi.org/10.1093/nar/gkac956.
[26] Filimonov DA, Lagunin AA, Gloriozova TA, Rudik A V., Druzhilovskii DS, Pogodin P V., et al. Prediction of the biological activity spectra of organic compounds using the pass online web resource. Chemistry of Heterocyclic Compounds 2014;50:444–57. https://doi.org/10.1007/s10593-014-1496-1.
[27] Rolnik A, Olas B. The plants of the asteraceae family as agents in the protection of human health. International Journal of Molecular Sciences 2021;22:1–10. https://doi.org/10.3390/ijms22063009.
[28] Masyudi, Hanafiah M, Rinidar, Usman S, Marlina. Phytochemical screening and GC-MS analysis of bioactive compounds of Blumea balsamifera leaf extracts from South Aceh, Indonesia. Biodiversitas 2022;23:1346–54. https://doi.org/10.13057/biodiv/d230319.
[29] El-Askary HI, Meselhy MR, Galal AM. Sesquiterpenes from Cymbopogon proximus. Molecules 2003;8:670–7. https://doi.org/10.3390/80900670.
[30] Poirier D. Inhibitors of 17B-Hydroxysteroid Dehydrogenases. Current Medicinal Chemistry 2012;10:453–77. https://doi.org/10.2174/0929867033368222.