

GCMS Analysis of Bakong Gayo or Gayo Tobacco (*Nicotiana tabacum L*) Leaves Extract and Pharmaceutical Uses

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ABSTRACT

*Tobacco plants are considered more destructive plants than medicinal plants. This research aims to analyze using GCMS (gas chromatography mass spectrometry) the leaves extract of Bakong Gayo or Gayo tobacco (*Nicotiana tabacum L*) and its pharmaceutical uses. Extraction of Gayo tobacco leaves with chloroform solvent obtained 14 phytochemical compounds in the form of alkaloids, terpenoids and fatty acids. The phytochemical compounds in Gayo tobacco can be used as antibacterial, anticancer, anti-depressant, antioxidant, sedative, aromatherapy based on references and other potential to be developed as a medicinal plant.*

Keywords: Gayo tobacco, GCMS, Phytochemical, extraction.

1. INTRODUCTION

Gayo tobacco or Bakong Gayo with the Latin name *Nicotiana tabacum L* is one of the tobacco plants that is widely planted in the Central Aceh Regency area of Aceh Province. People use Gayo tobacco leaves as cigarettes so that they become an agricultural commodity crop in Aceh Province. The aroma of Gayo tobacco is so distinctive that farmers sometimes call it aromatic tobacco ^{1 2}. The distinctive smell of Gayo tobacco when burned makes people think that this plant has the same narcotic content as marijuana. Therefore It must be scientifically proven about the phytochemical content of Gayo tobacco using GCMS ³ and its use in the pharmaceutical sector as a medicinal plant.

Medicinal plants are a source of natural ingredients that have the property of curing disease⁴. 50% of available drugs; including pharmaceutical and cosmetic products on the market including natural products isolated from medicinal plants⁵; which is used in the cosmetic and pharmaceutical industries for new product development. Various kinds of organic compounds can be produced by plants, one of which is Gayo tobacco which is called secondary metabolites⁶. Tobacco is a rich source of antioxidants⁷, antifungal⁸, antidepressant⁹, dan anticancer^{10 11}. Tobacco contains various secondary metabolites and has significant antibacterial activity⁴¹². Phytochemical analysis of tobacco with GCMS was carried out in various extraction solvents^{13 14 15}. The phytochemical content of tobacco has been studied using GCMS, such as alkaloids^{16 17 18}, flavonoid^{7 19}, terpenoid^{20 21} and others.

Several previous studies have investigated the phytochemical content of tobacco from various countries used in medicine, proving the great potential for Gayo tobacco or Bakong Gayo to be developed in the pharmaceutical in Indonesia. The aim of this research is to analyze by GCMS the phytochemical content of Gayo tobacco which will later be useful for investigating the potential for development and utilization as a medicinal ingredient sourced from local wisdom in the Central Aceh province.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The chemical used is chloroform (CHCl₃) pa (Merck- Darmstadt -Germany), dry Gayo tobacco leaves from Dedamar village, Bintang sub-district, Central Aceh Regency, Aceh Province. Grinder (POWTEQ-GT300), test tube, ultrasonicator, vortex and Whatman 42 filter paper.

The instrument used in this research is the Agilent Technologies GCMS (GC system 7890B dan MSD 5977A) - USA

2.2. Research Procedure

Sample preparation of leaves extract

The dried leaf samples were ground then weighed 1 gram and put into a test tube then added 10 ml of chloroform and vortexed for approximately 30 seconds then for 30 minutes the sample was sonicated and centrifuged at 3000 rpm for 5 minutes then filtered using Whatman 42 filter paper.

GCMS analysis

Sample analysis using GCMS Agilent Technologies GC system 7890B and MSD 5977A, column used DB-5MS (30 m x 0.250 mm x 0.25 µm), Helium carrier gas, flow rate 1 mL/min, 1 µL of chloroform leaf extract

injected with split 20:1, initial temperature 100°C, increase 15oC/min to 290oC, hold 5 min until final temperature and Velocity 34.

3. RESULTS AND DISCUSSION

3.1. Analysis of Characterization Results

GCMS analysis of phytochemical contents

In Figure 1 showed the phytochemical content of Gayo tobacco leaf chloroform extract using GCMS. the using mode selection ion monitoring (SIM) based on retention time, molecular weight and chromatogram peak area by comparing with the Willey7 internal library ²².

GCMS analysis of Gayo tobacco extract showed the phytochemical existence of alkaloids, terpenoids and fatty acids ³. The main components of the analysis are listed in table 2. Almost all of the compounds identified have the potential to be used as medicinal ingredients in the pharmaceutical industry. The main compounds of Gayo tobacco are nicotine 41.77%, 4,8,13-duvatriene-1,3-diol 29.89%, methyl linolenate 7.59%, neophytadiene 4.51%, 11E,13Z)-11813- labdadien-8-OL 3.92%, palmitic acid 3.45%, and 4-(2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one 2.05% and subsequent compounds others, namely 3,7,11,15-tetramethyl-2-hexadecen-1-ol 1.52%, 2-hexadecene, 3,7,11,15-tetramethyl-, [R-[R@,R@-(E)]]- 1.38%, 9,12-Octadecadienoic acid (Z,Z)- 1.17%, (4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide 1.03%, phytol 1.03%, quinazoline, 8-methoxy- 0.64% and cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- 0.38% identified in the smaller peak area percentage.

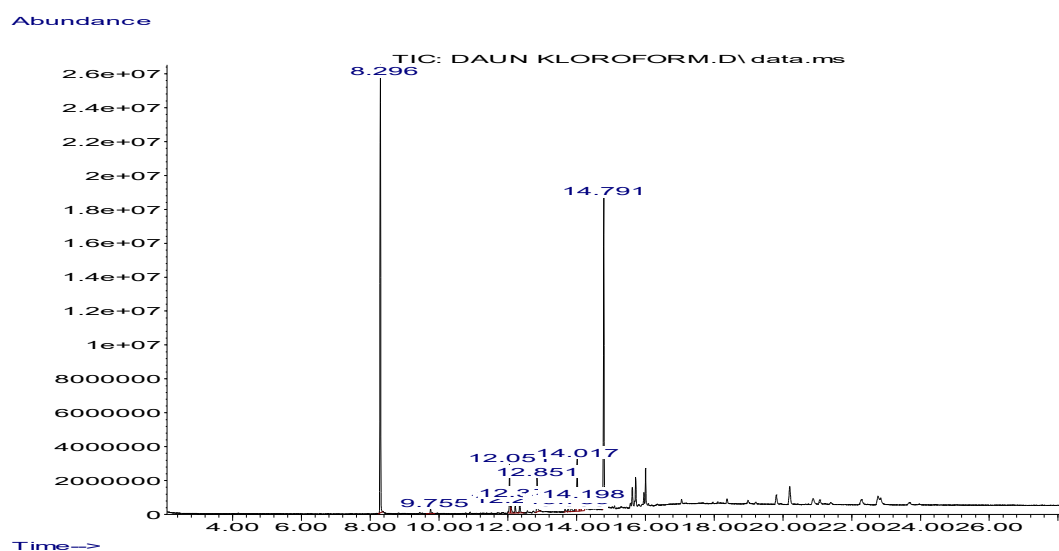


Figure 1. Chloroform extract Chromatogram of Gayo tobacco leaf

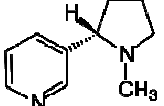
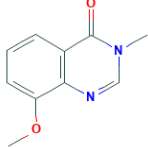
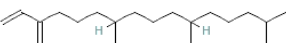
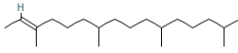
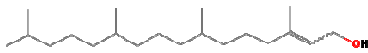

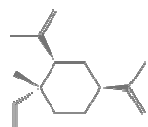
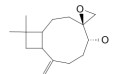
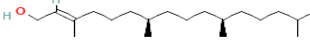
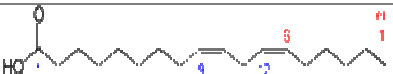
The molecular weight of phytochemical compounds in Gayo tobacco was detected with the highest molecular weight of 306 g/mol compound 4,8,13-duvatriene-1,3-diol and the lowest molecular weight of 162 g/mol, namely nicotine. The phytochemical content of Gayo tobacco in the chloroform extract produces many compounds with different retention times and different molecular weights. This is caused by differences in the polarity of each compound interacting with the stationary phase of GCMS coulumn.

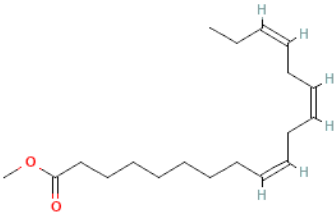
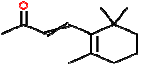
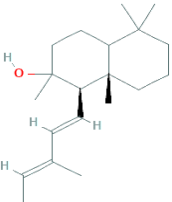
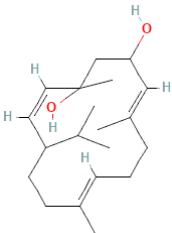
Table 1. GCMS analysis list of Gayo tobacco

Compound	Retention Time (min)	Peak Area (%)	Library /ID	Quality	Molecular Weight (g/mol)
1	8,298	41,77	Nicotine	94	162
2	9,756	0,64	Quinazoline, 8-methoxy-	46	160
3	12,049	4,51	NEOPHYTADIENE	99	278
4	12,096	1,38	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R@,R@-(E)]]-	86	280
5	12,35	1,52	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	87	296
6	12,853	3,45	Palmitic acid	99	256
7	13,668	0,38	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	92	204
8	13,756	1,03	(4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide	72	236
9	13,85	1,03	Phytol	99	296
10	13,969	1,17	9,12-Octadecadienoic acid (Z,Z)-	96	280
11	14,016	7,59	Methyl linolenate	91	292
12	14,109	2,05	4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-buten-2-one	50	192
13	14,197	3,92	(11E,13Z)-11813-LABDADIEN-8-OL	68	290
14	14,794	29,89	4,8,13-Duvatriene-1,3-Diol	91	306

Pharmaceutical uses

Table 2. phytochemical contents in chloroform extract of Gayo tobacco leaves, structure according to Willey's library and pharmaceutical uses.

Compound name	Structure	uses
Nicotine (alkaloid)		As nicotine inhaler by Bolliger et al 2000 ²³
Quinazoline, 8-methoxy- (alkaloid)		As antibacterial agent by German et al 2008 ²⁴
Neophytadiene (diterpene)		sebagai antidepresant, antixiolitic, antikonvulsandan obat penenang by Gonzales et al 2023 ⁹
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R@,R@-(E)]]- (acyclic diterpene alcohol)		As essential oil by rahmiyani et al 2020 ²⁵
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (diterpene alcohol)		as cholinesterase inhibitory activity by Elufiyoe et al 2017 ²⁶
Palmitic acid (fatty acid)		As surfactant in cosmetics by Celm at al 2017 ²⁷
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- (sesquiterpene)		As essential oil by Nour 2022 ²⁸
(4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide (allylic alcohol)		As antioxidant by mulmuyanti 2015 ²⁹
Phytol (diterpene alcohol)		as Anticancer by Handoyo et al 2019 ¹¹
9,12-Octadecadienoic acid (Z,Z)- (fatty acid)		As antioxidant by farag et al 1989

<p>Methyl linolenate (fatty acid)</p>		<p>As aromaterapy componen by Fan et al 2022 ¹</p>
<p>4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-buten-2-one (diterpene alcohol)</p>		
<p>(11E,13Z)-11813-LABDADIEN-8-OL (fatty acid)</p>		<p>as Anticancer by Jozwiak et al 2019 10</p>
<p>4,8,13-Duvatriene-1,3-Diol (diterpene)</p>		<p>As anticancer by Hajjar et al 2022 ³⁰</p>

Apart from nicotine as the largest content, methyl linolenate is also obtained which gives a specific smoke smell when tobacco is burned. Various compounds identified in Gayo tobacco such as alkaloids, terpenoids, saturated and unsaturated fatty acids have the potential to be developed as herbal medicinal ingredients such as antibacterial, anticancer, antioxidant, anti-depressant, sedative, aromatherapy and others from the local wisdom of the Aceh region.

4. CONCLUSION

This research has investigated the chloroform extract of Gayo tobacco leaves and identified 14 phytochemical compounds as alkaloids (nicotine and quinazoline, 8-methoxy-), terpenoids (neophytadiene, 2-hexadecene, 3,7,11,15-tetramethyl-, [R-[R@,R@-(E)]]-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, phytol, (4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide, 4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-buten-2-one and 4,8,13-duvatriene-1,3-Diol) and fatty acid (palmitic acid, (11E,13Z)-11813-labdadien-8-ol, 9,12-Octadecadienoic acid (Z,Z)-, methyl linolenate). The main compounds of Gayo tobacco are nicotine 41.77% and 4,8,13-duvatriene-1,3-diol 29.89%. Several phytochemical compounds have potential applications to be developed in the pharmaceutical uses, such as nicotine as nicotine inhaler, 4,8,13-Duvatriene-1,3-diol,

(11E,13Z)-11813-labdadien-8-ol and phytol as anticancer, neophytadiene as anti-depressant, palmitic acid as surfactant in cosmetics, octadecanoic acid and (4S,5R)-5-hydroxycaryophyll-8(13)-ene-4,12-epoxide as an antioxidant, and methyl linolenate as aromatherapy, 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R@,R@-(E)]]- and cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- as essential oil.

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REFERENCES

1. Fan X, Zi W, Ao J, et al. (2022) Analysis and application evaluation of the flavour-precursor and volatile-aroma-component differences between waste tobacco stems. *Heliyon.*;8(9):e10658.
2. Supiyani, Saisa A ningtiyas. (2023) Phytochemical identification of Gayo tobacco or bakong Gayo (*Nicotiana tabacum* L) and its potency for industrial applications.;6(4):505-512.
3. Das S, Upadhaya P, Barhoi D, Nath Barbhuiya S, Langthasa P, Giri S. (2022) GCMS analysis of sadagura (smokeless tobacco), its enhanced genomic instability causing potential due to arsenic co-exposure, and vitamin-C supplementation as a possible remedial measure: a study involving multiple model test systems. *Drug Chem Toxicol.*;45(1):185-196.
4. Tariq M, Ahmad Z, Shah SA, Gul Z, Khan SA. (2021) Phytochemical Analysis and Antibacterial Activity of *Nicotiana tabacum* and *Nicotiana rustica*. *RADS J Biol Res Appl Sci.*;12(1):60-65.
5. Halberstein RA. (2005) Medicinal plants: Historical and cross-cultural usage patterns. *Ann Epidemiol.*;15(9):686-699.
6. Li M, He X, La Hovary C, et al. A de novo (2022) regulation design shows an effectiveness in altering plant secondary metabolism. *J Adv Res.*;37:43-60.
7. Pottier M, Laterre R, Van Wessem A, et al. (2020) Identification of two new trichome-specific promoters of *Nicotiana tabacum*. *Planta.*;251(3):1-10.
8. Ishizaka N, Tomiyama K, Katsui N, Murai A, Masamune T. (2019) Biological activities of rishitin, an antifungal compound isolated from diseased potato tubers, and its derivatives. *Plant Cell Physiol.*;10(1):183-192.
9. Gonzalez-Rivera ML, Barragan-Galvez JC, Gasca-Martínez D, Hidalgo-Figueroa S, Isiordia-Espinoza M, Alonso-Castro AJ. (2023) In Vivo Neuropharmacological Effects of Neophytadiene. *Molecules.*;28(8):1-12.
10. Józwiak M, Struga M, Roszkowski P, Filipek A, Nowicka G, Olejarz W. (2018) Anticancer effects of

alloxanthoxyletin and fatty acids esters – In vitro study on cancer HTB-140 and A549 cells. *Biomed Pharmacother.*;110 :618-630.

11. Handoyo MOM, Yuliani Y, Purnama ER. (2022) Uji in Silico Senyawa Phytol Hasil Ekstrak Daun Zodia (*Evodiasuaveolens*) sebagai Antikanker. *Berk Ilm Pendidik Biol.*;11(2):368-373.
12. Ameya G, Manilal A, Merdekios B. (2018) In vitro Antibacterial Activity and Phytochemical Analysis of *Nicotiana tabacum* L. Extracted in Different Organic Solvents. *Open Microbiol J.*;11(1):352-359.
13. Anusuya M, Princy V, Nagaveni A, Suganthi M, Poonkodi K, Jayanthi E. (2021) Investigation of Phytochemical Constituents of Tobacco (*Nicotiana Tabacum* L.) Methanol Extract.;10(April):4-5.
14. Ali Alghamdi A. (2021) Phytoconstituents screening and antimicrobial activity of the invasive species *Nicotiana glauca* collected from Al-Baha region of Saudi Arabia. *Saudi J Biol Sci.*;28(3):1544-1547.
15. Schwingel LC, Schwingel GO, Storch N, Barreto F, Bassani VL. (2014) 3-O-Methylquercetin from organic *Nicotiana tabacum* L. trichomes: Influence of the variety, cultivation and extraction parameters. *Ind Crops Prod.*;55:56-62.
16. Sun B, Zhang F, Zhou GJ, et al. (2013) Genetic variation in alkaloid accumulation in leaves of *Nicotiana*. *J Zhejiang Univ Sci B.*;14(12):1100-1109.
17. Hossain AM, Salehuddin SM. Analytical determination of nicotine in tobacco leaves by gas chromatography-mass spectrometry. *Ar(2013)ab J Chem.*;6(3):275-278.
18. Lisko JG, Stanfill SB, Duncan BW, Watson CH. (2015) In Cigarette Filler and Various Tobacco Species.;85(6):3380-3384.
19. Shi H, Xu P, Yu W, et al. (2022) Metabolomic and transcriptomic analysis of roots of tobacco varieties resistant and susceptible to bacterial wilt. *Genomics.*;114(5):110471.
20. Zou X, BK A, Abu-Izneid T, et al. (2021) Current advances of functional phytochemicals in *Nicotiana* plant and related potential value of tobacco processing waste: A review. *Biomed Pharmacother.*;143 (August):112191.
21. Yang PS, Tang SY, Liu CB, et al. (2019) Three new sesquiterpenes from the stems of *Nicotiana tabacum* and their bioactivities. *J Asian Nat Prod Res.*;21(2):109-116.
22. Eswaraiyah G, Peele KA, Krupanidhi S, Indira M, Kumar RB, Venkateswarulu TC. (2020) GC-MS analysis for compound identification in leaf extract of *Lumnitzera racemosa* and evaluation of its in vitro anticancer effect against MCF7 and HeLa cell lines. *J King Saud Univ - Sci.*;32(1):780-783.
23. Bolliger CT, Zellweger JP, Danielsson T, et al. (2000) Smoking reduction with oral nicotine inhalers: Double blind, randomised clinical trial of efficacy and safety. *Br Med J.*;321(7257):329-333.
24. German N, Malik M, Rosen JD, Drlica K, Kerns RJ. (2008) Use of gyrase resistance mutants to guide selection of 8-methoxy-quinazoline-2,4-diones. *Antimicrob Agents Chemother.*;52(11):3915-3921.
25. Rahmiyani I, R TR, Dh N, Yuliana A. (2020) ISOLASI DAN IDENTIFIKASI SENYAWA MINYAK ATSIRI DAUN GAMAL (*Gliricidia sepium* [Jacq] Walp). Published online:134-143.

26. Adesanya SA. **(2017)** Anticholinesterase constituents from the leaves of *Spondias mombin* L. (Anacardiaceae). Published online:107-114.
27. Kelm GR, Wickett RR. **(2017)** *The Role of Fatty Acids in Cosmetic Technology*. Elsevier Inc.
28. Nour AH, Idris AA, Ishag OA, Nour AH, Alara OR. **(2022)** Chemical constituents and cockroach repellent activity of Sudanese *Cyperus rotundus* rhizomes essential oil. *J Res Pharm.*;26(4):920-930.
29. Maimulyanti A, Prihadi AR. **(2016)** Chemical composition of essential oil and hexane extract and antioxidant activity of various extracts of *Acmella uliginosa* (Sw.) Cass flowers from Indonesia. *Agric Nat Resour.*;50(4):264-269.
30. Hajjar S, Jaber A, El Riachi M, Abdel Sater F, Cheble E. **(2022)** GC-MS Analysis of Essential Oil and Anticancer Activities of Extracts From Discarded Leaves of *Nicotiana Tabacum* Linn. *Ankara Univ Eczac Fak Derg.*;46(2):291-307.