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BIOACTIVE COMPOUND PROFILE OF MUNTINGIA. CALABURA LEAF EXTRACT WITH DIFFERENT POLARITY SOLVENT

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Abstract

Among the myriad of botanical treasures, M. calabura, colloquially known as the Jamaican cherry or strawberry tree, stands as an intriguing candidate, owing to its extensive historical utilization in folk medicine across diverse cultures. This study aimed to comprehensively characterize the bioactive compound profile of M. calabura leaves by employing a spectrum of solvents with varying polarities for extraction. The choice of solvent during extraction plays a pivotal role in determining both the types and quantities of compounds obtained from plant material. Our methodology encompassed cutting-edge analytical techniques, notably gas chromatography-mass spectrometry (GC-MS), to unravel the intricate chemical composition of the extracts derived from M. calabura leaves. Moreover, we scrutinized the potential influence of solvent polarity on extraction efficiency and subsequent bioactivity of the isolated compounds. Our findings underscore the profound impact of solvent selection on the repertoire of bioactive compounds extracted from M. calabura leaves. Polar solvents exhibited a proclivity for extracting compounds of higher polarity, while non-polar solvents favored the extraction of less polar constituents. The identification of prominent bioactive compounds within these extracts encompassed delta-tocopherol, 7-(γ,γ-dimethylallyloxy)flavanone, Wogonin, Neophytadiene, Octadecanoic acid, and Hexatriacontane, each with its distinctive pharmacological significance. This study thus illuminates the critical role of solvent polarity in optimizing extraction processes and maximizing the therapeutic potential of M. calabura. The insights gleaned from this investigation not only contribute to our understanding of natural product chemistry but also hold the promise of inspiring the development of novel pharmaceutical agents and therapeutic interventions with wide-ranging applications.

Keywords: *Bioactive Compounds, GC-MS, M. Calabura, Solvent Polarity*

Introduction

In the realm of natural products research, the quest for bioactive compounds with potential pharmaceutical and therapeutic applications continues to captivate scientists and researchers alike. Plants, as an abundant source of diverse chemical entities, have long been a focal point in this pursuit. Among these botanical treasures, Muntingia calabura,

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commonly known as the Jamaican cherry or strawberry tree, stands out as a promising candidate due to its extensive traditional use in folk medicine across various cultures (Bandeira et al., 2013).

M. calabura, a member of the Elaeocarpaceae family, is widely distributed in tropical and subtropical regions, and its leaves have been recognized for their medicinal properties. This versatile plant has garnered attention for its potential therapeutic applications, including antimicrobial, anti-inflammatory, antioxidant, and antidiabetic properties. However, the bioactive compounds responsible for these effects have not been comprehensively characterized, and the influence of solvent polarity on their extraction and subsequent bioactivity remains an intriguing and unexplored aspect.

M. calabura, as one of the wild plants, contains various active compounds such as tannins, flavonoids, alkaloids, steroids, and saponins. Several studies have demonstrated the anti-tumor activity of cherry leaves and root extracts. Additionally, other research has indicated that cherry leaves possess insecticidal activity against fruit flies (Andika, Vandervoort, & Wise, 2020).

The present study endeavors to address this gap in knowledge by investigating the bioactive compound profile of M. calabura leaves using a range of solvents with varying polarities. Solvent selection in the extraction process plays a critical role in determining the types and quantities of compounds obtained from plant material (Zhang et al., 2017). By employing state-of-the-art analytical techniques, we aim to elucidate the chemical composition of extracts obtained from M. calabura leaves and assess the potential influence of solvent polarity on the extraction efficiency of various bioactive constituents.

This approach allows us to explore how the choice of solvent, whether polar, nonpolar, or somewhere in between, impacts the extraction of specific classes of compounds from M. calabura leaves. Furthermore, we will investigate the pharmacological significance of these compounds and their potential for pharmaceutical and therapeutic applications, with an emphasis on understanding how solvent polarity may influence bioactivity.

Through this multidisciplinary approach, we hope to shed light on the diverse bioactive compounds hidden within M. calabura leaves and provide valuable insights for researchers, pharmacologists, and herbal medicine enthusiasts interested in harnessing the therapeutic potential of this remarkable plant. The insights gained from this investigation hold the promise of advancing our understanding of natural product chemistry and may pave the way for the development of novel drugs and therapeutic agents.

Research Methods

Simplicia preparation

Healthy leaves were collected from Bantul Regency, Yogyakarta. Leaves were cleaned and sorted to get the healthy leaves only. Leaves were dried and ground resulting in the simplicial.

Plant extraction

M. calabura leaves were extracted by the gradual maceration method. 250ge dried simplicia were extracted with ml of hexane solvent and stood for 48 hours. The solvent was filtered with filter paper. The filtrate was evaporated with a rotary evaporator. The residue was used for the next two solvents with the same method.

GC-MS Analysis

Bioactive content analysis was conducted in the Organic Chemistry Laboratory at the Faculty of Mathematics and Natural Sciences, UGM, Yogyakarta, utilizing GC-MS. A 3 µl portion of the solution was injected into a Shimadzu GCMS-QP2010S gas chromatograph, with an initial temperature set at 100°C and a final temperature of 300°C. The column employed was an Agilent HP 1MS, the detector used was an FID set to 300°C, the injector temperature was 300°C, and helium was used as the carrier gas.

Data Analysis

In the data analysis phase, the outcome of the gas chromatography process yields a compound chromatogram profile. The spectra obtained from the GC-MS results are then compared with library data, namely WILEY229.LIB, which contains spectra databases. This comparison allows for the identification of potential compounds present in the sample.

Results and Discussion

Extraction in this research was carried out using the maceration method. Maceration is one of the simple extraction methods done by soaking the entire herbal material in a solvent. The extraction process with the maceration method begins with the penetration of the solvent into the solid matrix of the herbal material. Subsequently, there is the diffusion of solutes into the solvent used. The solutes are then diffused out of the solid matrix of the herbal material. Thus, solutes containing various metabolite compounds are obtained (Zhang et al., 2017).

Extraction essentially follows the principle of "like dissolves like." The selection of solvents during the extraction process plays a significant role in determining the types of bioactive compounds that will be extracted from the sample. Compounds with high polarity are more likely to be extracted by polar solvents, while non-polar solvents tend to extract low-polarity compounds. Therefore, the choice of extraction solvent influences the types of bioactive compounds that will be extracted from the sample (Lefebvre, Destandau, & Lesellier, 2021).

Table 1 Extraction yield of M. calabura leaves with n-hexane, chloroform, and ethanol solvent

Simplicia weight (gr)	Solvent	Extract weight (gr)	Yield (%)
160	N-hexane	11.29	7.05
	Klorofom	8.73	5.45
	Ethanol	13.23	**8.27**

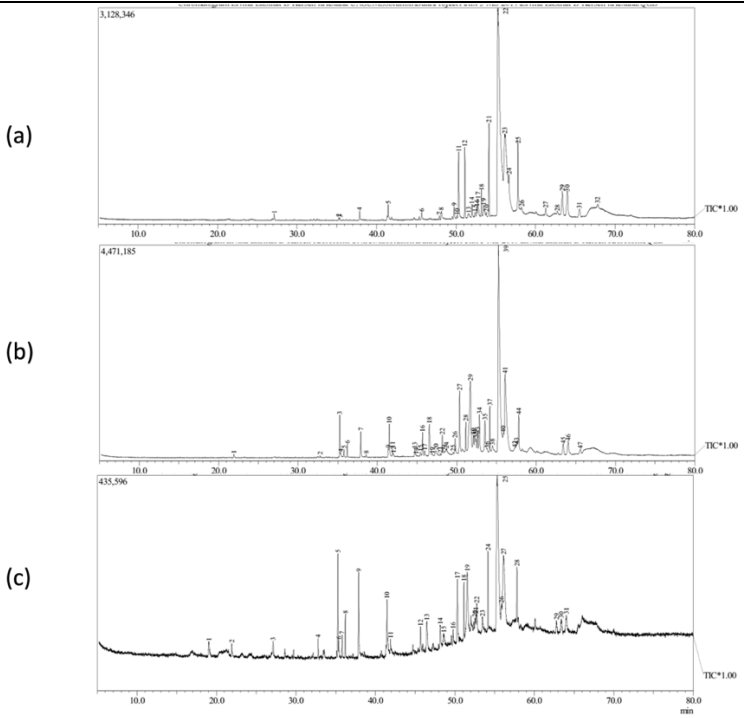

Figure 1 Chromatogram of GC-MS analysis on cherry leaves; n-hexane extract (a), chloroform extract (b), and ethanolic extract (c)

Table 1 shows the yield of cherry leaf extracts with three types of solvents: non-polar (n-hexane), semi-polar (chloroform), and polar (ethanol). Based on these results, it can be observed that cherry leaf extract with a polar solvent has the highest yield compared to the other two types of solvents. Thus, secondary metabolite compounds in cherry leaves are dominated by abundant polar compounds in terms of yield.

The extraction yield of cherry leaves in this study is lower than in previous research. The study by Anindhita and Arsanto (2020) produced cherry leaf extract with a yield of 26.22% using a polar solvent (96% ethanol). Meanwhile, in the study () which used a solvent for extraction, it resulted in a yield of. The difference in yield may be due to several factors, including the influence of the environmental factors of the sample habitat used.

Secondary metabolite compounds are essentially plant responses to various stimuli, including environmental stimuli such as stress, nutritional conditions, and other physicochemical factors. These factors contribute significantly to the biosynthesis of

various bioactive compounds. Different environments will result in the production of different bioactive compounds, thus affecting the extraction yield.

In this study, n-hexane, chloroform, and ethanol solvents were used, so that the detected compounds belong to many groups ranging from polar and nonpolar. Figure 1 shows gas chromatography chromatogram analysis showing 32 detected peaks on the n-hexane extract, 47 on chloroform, and 31 peaks on the ethanol extract. The compounds representing each peak from Figure 1 are presented in Table 2. Based on the results of GC and GC-MS analysis, there is a difference in bioactive compounds profile between different solvents used in extraction.

The results of GC-MS analysis showed 32 peaks in cherry leaf extracts with n-hexane as the solvent. Upon further analysis, five compounds dominated the n-hexane extract, namely delta-tocopherol (45.2%), Tetratetracontane (CAS) n-Tetratetracontan (4.33%), 1-hexagonal (4.00%), Heptacosane (3.9%), and Methyl Commate D (2.68%). Delta-tocopherol belongs to the vitamin E group, a lipophilic antioxidant compound specifically synthesized by plants. Additionally, this compound plays a crucial role in plant signaling system regulation (Ali et al., 2022).

Generally, tocopherol accumulates in plant seeds. However, the presence of genetic and environmental factors can cause fluctuations in tocopherol production and accumulation. Tocopherol can be found in leaf organs in response to ecological factors such as drought, salinity stress, temperature, light, and heavy metal stress (Hasanuzzaman, Nahar, & Fujita, 2014).

Tetratetracontane (CAS) n-Tetratetracontan and Heptacosane belong to the group of volatile organic compounds (VOCs). VOCs are complex secondary metabolites of plants. Amudha (2018) mentioned that tetracontane has antioxidant and cytoprotective activity.

Pratama et al. (2019) mentioned that 1-Hexacosanal belongs to the fatty alcohol group. 1-Hexacosanal has a long-chain fatty aldehyde with 26 carbon atoms. In plants, this compound can be found in various organs such as seeds, fruits, leaves, and flowers, serving as a defense compound against herbivores (Tiku, 2018);(Zahin et al., 2021). It exhibits antitumor characteristics by impeding angiogenesis and metastasis both in laboratory settings and in live organisms. This inhibition is achieved through the suppression of matrix metalloproteinase activity (MMPs) and the prevention of Nf-kB translocation into the nucleus (Figueiredo et al., 2014).

Methyl Commate D is one of the unique compounds found in several plant extracts. Studies and exploration of this compound have been limited. Nevertheless, there have been some reports regarding the potential of this compound with extract activities such as antibacterial, antioxidant, and antimutagenic (Gontijo et al., 2019). The GC-MS analysis results of cherry leaf extract with chloroform as the solvent showed 47 peaks, with the top 5 compounds being delta-tocopherol (32.47%), 7-(γ,γ-dimethylallyloxy)flavanone (3.16%), Wogonin (2.88%), Heneicosane (2.38%), and Octacosane (2.03%).

7-(γ,γ-dimethylallyloxy) flavanone is a chemical compound belonging to the flavonoid group (Fillianty et al., 2021). Flavonoids are a large group of chemical compounds found in plants and serve various biological functions. The name of this compound reflects its molecular structure. It contains γ,γ-dimethylallyloxy groups attached to position 7 of the flavanone nucleus. Flavanone is a type of flavonoid that has the basic structural ring of flavonoids. 7-(γ,γ-dimethylallyloxy)flavanone and other flavonoid compounds can exhibit various biological activities, including antioxidant, anti-inflammatory, and interactions with specific receptors or enzymes in the human and animal body. Therefore, these compounds have the potential as active ingredients in the fields of pharmacy and health (Mahato, Sharma, Sinha, & Cho, 2018).

Wogonin is a flavonoid compound found in plants, first isolated from Scutellaria baicalensis. This compound has been the subject of many studies due to its pharmacological activity potential. Some studies suggest that it has anti-inflammatory, anti-cancer, antioxidant, neuroprotective, antiviral, antidiabetic, and antibacterial effects (Hassanin et al., 2019; Sharifi-Rad et al., 2021).

Heneicosane and Octacosane belong to the group of volatile compounds (Ryu et al., 2020). There is no specific information available regarding the bioactivity of these two compounds. However, Farzaei (2014), mentioned that both types of essential oils obtained from Tragopogon graminifolius have high antimicrobial and antioxidant acitivity. Based on the analysis of bioactive compounds, heneicosane, and some other volatile compounds dominate. Therefore, the presence of volatile compounds becomes a concern regarding their involvement in antimicrobial and antioxidant activities.

The GC-MS analysis results of cherry leaf extract with ethanol as the solvent showed 31 peaks, with the top 5 compounds being delta-tocopherol (28.96%), Wogonin (7.35%), Neophytadiene (4.16%), Octadecanoic acid (4.14%), and Hexatriacontane (3.73%). Neophytadiene belongs to the terpenoid compound group, particularly diterpenes, found as bioactive compounds in plants. This compound has been reported to have biological activities such as antimicrobial, larvicidal, insecticidal, and antidepressant effects (Caceres et al., 2015; Gonzalez-Rivera et al., 2023).

Octadecanoic acid is a volatile compound in plants. This compound possesses the chemical composition C19H38O2, akin to substances such as steroids utilized in lubricants and plasticizers, and also exhibits characteristics associated with fragrances (Asghar, Choudahry, Habib-Ur, & Atta-Ur, 2011). Octadecanoic acid usually known as methyl stearate poss antibacterial activity since it was proven effective for controlling bacteria (Rangel-Sánchez, Castro-Mercado, & García-Pineda, 2014).

Hexatriacontane is a volatile hydrocarbon compound. Hexatriacontane have the chemical formula C36H74. It is a type of alkane and is a straight-chain, saturated hydrocarbon consisting of 36 carbon atoms bonded together with 74 hydrogen atoms. It was reported for its antibacterial and antioxidant activity (Nayak, Roy, Roy, Mitra, & Karak, 2018).

Table 2 Bioactive compound profile of Muntingia calabura leaf with GC-MS analysis

No	R.T	Compound	MF	WF	N-hexane		Chloroform		Ethanolic	
					Peak	Area %	Peak	Area%	Peak	Area%
1	52.052	delta. - tocopherol	C27 H46 O2	402	32, 33	49,74; 11,64	31	35,05		-
2	48.647	Wogonin	C16 H12 O5	284	20	4,56	23	3,03		1,1
3	51.193	Hexatriacontane (CAS) n-Hexatriacontane	C36 H74	507	30	3,07	35	4,24		0,9
4	48.231	Heptacosane	C27H56	380	19	2,32	30	4,62		-
7	54.018	Hexatriacontane (CAS) n-Hexatriacontane	C36 H74	507	35	2,28	-	-		0,93
8	43.556	3-Methyl-2-nitrophenol e	C7H7NO3	153	11	1,39	16	1,34		0,81
9	50.520	CYCLOPENTANE, 1-BENZOYL-2-PHENYL-4-METHYLENE-	C19 H18 O	262	28	1,30	-	-		0,9
10	42.732	2,3-Dimethylhydroquinone	C8H10O2	138	10	1,20	15	1,08		0,7
11	58.376	Olean-12-en-28-al	C30H48O	424	39	1,18	-	-		4,16
12	49.101	1,3,5-TRIPHENYL-IMADAZOLIDIN-2,4-DIONE-5-CARBOXYLIC ACID P-CHLORANILIDE	C28 H20 CL N3 O3	481	22	1,15	25	0,55		0,64
13	45.210	2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)-	C16H14O4	270	14	1,15	17	0,78		0,86
14	46.828	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl- (CAS) Tectochrysin	C16 H12 O4	268	17	1,05	-	-		1,84
15	49.839	Phenol, 4-methyl-2-nitro- (CAS) 2-Nitro-p-cresol	C7 H7 N O3	153	26	1,00	-	-		4,14
16	45.671	3-Methyl-2-nitrophenol	C7H7NO3	153	15	0,92	-	-		3,15
17	49.731	Heptadecane (CAS) n-Heptadecane	C17 H36	240	25	0,88	-	-		0,59
18	53.598	4H-1,3,2-Dioxaborin, 6-ethenyl-2-ethyl-4-methyl-4-(2-methyl propyl)-	C12H21BO2	208	34	0,87	-	-		1,47

19	57.918	1H-Cycloprop[e]azulen-4-ol, decahedron-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-(CAS) Ledol	C15 H26 O	222	38	0,79	-	-	2,66
20	35.035	Octadecanoic acid (CAS) Stearic acid	C18 H36 O2	284	3	0,70	7	04.51	1,33
21	49.258	TRANS-2-PHENYL-1,3-DIOXOLANE-4-METHYL OCTADEC-9,12,15-TRIENOATE	C28 H40 O4	440	23	0,69	-	-	1,21
22	59.412	19-DI-TORULOSOL	C20 H33 D O2	306	40	0,60	-	-	0,93
23	32.374	9-Octadecen-1-ol, (Z)- (CAS) cis-9-Octadecen-1-ol	C18 H36 O	268	1	0,46	-	-	4,21
24	49.934	FARNESOL ISOMER A	C15 H26 O	222	27	0,39	27	0,44	2,31
25	38.570	Oleic Acid	C18H34O2	282	4	0,37	-	-	7,35
26	48.867	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate	C24 H38 O4	390	21	0,32	24	0,29	0,83
27	55.578	Heptacosane (CAS) n-Heptacosane	C27 H56	380	36	0,31	-	-	0,66
28	39.018	Octadecanoic acid	C18H36O2	284	6	0,31	-	-	2,06
29	41.781	1H-Pyrrole-2-carboxylic acid, 5-ethyl-, ethyl ester	C9H13NO2	167	7	0,30	-	-	0,93
30	57.370	Octacosane (CAS) n-Octacosane	C28 H58	394	37	0,28	39	0,87	3,73
31	63.842	Phosphinous chloride, start-butyl isopropyl-100	C7H16ClP	166	41	0,28	-	-	28,96
32	41.985	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	C18H26O3	290	8	0,26	-	-	1,55
33	49.500	dihydro ionone	C13 H22 O	194	24	0,19	-	-	13,91
34	33.312	3-Eicosyne (CAS)	C20 H38	278	2	0,18	6	0,87	3,23
35	45.028	Nonadecane, 2-methyl	C20H42	282	13	0,18	26	2,44	1,06
36	42.473	.delta.-DODECALACTONE	C12 H22 O2	198	9	0,17	-	-	1,25

37	46.658	Eicosane, 2-methyl	C21H44	296	16	0,17	-	-	1,64
38	51.317	Hexadecane, 1-chloro-	C16H33Cl	260	31	0,17	-	-	-
39	50.829	1H-Cycloprop[e]azulen-4-ol, decahedron-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-(CAS) Ledol	C15 H26 O	222	29	0,15	-	-	-
40	38.683	9-Hexadecenoic acid	C16 H30 O2	254	5	0,15	-	-	1,55
41	43.804	2,6-Dimethoxytoluene	C9H12O2	152	12	0,13	-	-	13,91
42	52.590	Octadecane, 1-bromo-	C18H37Br	332	-	-	32	10,86	3,23
43	47.360	Diazene, (4-nitrophenyl)phenyl-, 1-oxide	C12H9N3O3	243	-	-	21	5,05	1,06
44	38.592	13-OXABICYCLO[9.3.1]PENTADECANE	C14 H26 O	210	-	-	26	2,61	1,25
45	32.371	Oxirane, tetradecyl-	C16H32O	240	-	-	3	2,4	1,64
46	38.670	Oxacyclohexadecan-2-one	C15H28O2	240	-	-	10	2,11	-
47	58.346	Lupeol	C30H50O	426	-	-	41	1,5	1,55
48	59.393	Acetamide, N-methyl-N- 4- 4-methoxy-1-hexahydropyridyl -2-butynyl -	C13H22N2O2	238	-	-	42	1,12	13,91
49	57.890	(+)-Aromadendrene	C15 H24	204	-	-	40	1,11	3,23
50	55.566	Tetratetracontane (CAS) n-Tetratetracontane	C44 H90	619	-	-	37	0,91	1,06
51	63.818	Propane, 2- (1,1-dimethyl ethyl)sulfonyl -2-methyl-	C8H18O2S	178	-	-	44	0,82	1,25
52	46.786	2-Methoxybenzyl alcohol	C8H10O2	138	-	-	20	0,72	1,64
53	50.489	Butane, 2,3-dichloro-2-methyl-(CAS) 2,3-Dichloro-2-methyl butane $$ Amylene dichloride	C5 H10 CL2	140	-	-	28	0,54	0,21
54	32.494	5-Ethyl-1-nonene	C11H22	154	-	-	4	0,5	-

55	61.873	Eicosane	C20H42	282	-	-	43	0,50	-
56	54.100	(+) spathulenol	C15 H24 O	220	-	-	36	0,5	-
57	32.899	1-Octadecyne	C18H34	250	-	-	5	0,49	1,55
58	41.773	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, oxime (CAS) 2 HYDROXYIMINOBORNANE	C10 H17 N O	167	-	-	13	0,47	13,91
59	40.681	9,12,15-Octadecatrienal	C18H30O	262	-	-	12	0,42	3,23
60	41.965	9-Octadecenal	C18H34O	266	-	-	14	0,39	1,06
61	37.367	Octadecanal (CAS) Stearaldehyde	C18 H36 O	268	-	-	8	0,31	1,25
62	53.533	Carbamic acid, (.alpha.-methylbenzyl)-, pentyl ester	C14H21NO2	235	-	-	33	0,3	1,64
63	45.657	DIHYDROFURANNO(3,2-F) COUMARANNE	C10 H10 O2	162	-	-	18	0,29	5,05
64	50.800	(-)-ISOLONGIFOLOL	C15 H26 O	222	-	-	29	0,27	2,61
65	53.776	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)- $$ 4,6-Cholestadien-3.beta.-ol, benzoate-	C34H48O2	488	-		34	0,27	2,4
66	46.637	Octadecane (CAS) n-Octadecane	C18 H38	254	-	-	19	0,26	2,11
67	57.113	9-Octadecanone	C18H36O	268	-	-	38	0,26	1,5
68	20.275	EUGENOL	C10 H12 O2	164	-	-	1	0,22	1,12
69	32.224	1-Dodecanol, 3,7,11-trimethyl-	C15H32O	228	-	-	2	0,19	1,11

Conclusion

The choice of extraction solvent significantly influenced the types of bioactive compounds extracted from the leaves. Notably, polar solvents extracted a higher yield of polar compounds, while non-polar solvents favored the extraction of less polar compounds. The results emphasize the diverse bioactive compounds hidden within M. calabura leaves and the influence of solvent polarity on their extraction. These findings provide valuable insights for researchers and pharmacologists interested in harnessing the

therapeutic potential of this remarkable plant. Further research is needed to explore the specific pharmacological activities of these compounds and their potential applications in pharmaceuticals, agriculture, and beyond. Understanding how solvent polarity affects bioactivity is crucial for optimizing extraction processes and maximizing the plant's therapeutic potential.

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