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Gas chromatography-mass spectrometry (GC-MS) analysis of phytocompounds isolated from *Hydnophytum formicarum* Jack tuber extracts.

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Abstract

Hydnophytum formicarum Jack is an epiphyte also known as "pokok sarang semut" or "pokok kepala beruk". It is a traditional herbal remedy for hepatitis, rheumatism, diarrhoea, and lung cancer. There is a lack of information about the presence of phytochemicals in this plant, particularly those from Malaysia, and different extraction protocols indicate the presence of different chemicals. For this study, *H. formicarum tuber* were extracted using hexane, methanol, and ethyl acetate and the chemical compounds were identified using GC-MS. The extracts of *H. formicarum* tubers yielded 28 different compounds. The tuber of *H. formicarum* contains a wide range of chemical compounds with potential medicinal values.

1. INTRODUCTION

The tropical rainforest is home to a diverse range of medicinal plants with great therapeutic potential and abundant bioactive metabolites such as alkaloids, essential oils, and flavonoids (Prachayasittikul et al., 2012). Over 80% of the population in underdeveloped countries cannot afford drugs, according to the World Health Organization, and must rely on traditional cures, particularly those derived from plants (Elumalai et al., 2012). *Hydnophytum formicarum* has a smooth, irregularly lobed tuber that ranges in size from 10–55 cm in diameter and contains multiple tunnels and apertures for ant inhabitation and is critically endangered in Singapore (Tan et al., 2008). In Thailand, it is known as Hua-Roi-Roo, while in Malaysia, it is known as "pokok kepala beruk" or "pokok sarang semut." Its tuber extracts are reported to contain twenty-two elements (Be, Al, Ca, Cr, Mn, Fe, Zn, Ba, P, Li, Sr, Rb, Hg, Tl, In, Pb, Cd, As, Cs, Na, K and Mg) (Prachayasittikul et al., 2012). The plant extract of *H. formicarum* was found to be an antioxidant (Prachayasittikul et al., 2008; Prachayasittikul et al., 2012; Sababathy et al., 2020), antimicrobial (Prachayasittikul et al., 2008; Hertiani and Pratiwi, 2015), anticancer (Senawong et al., 2013; Nugraha et al., 2019; Sababathy et al., 2020;), neuroprotective (Gay et al., 2018). This plant has traditionally been used to treat hepatitis, rheumatism, and diarrhoea (Ueda et al., 2002). It has antiproliferative action against human tumour cell lines (HeLa, A549), cervical cancer, colon cancer, and T-cell leukaemia, as well as antifungal activity (Ueda et al., 2002; Senawong et al., 2013; Musman et al., 2015). Phytochemical screening of *H. formicarum* tuber and leaves revealed the presence of alkaloid, terpenoid, and glycoside (Elya et al., 2012; Musman et al., 2015). In this study, the chemical compounds found in *H. formicarum* tuber extracts were identified using gas chromatography-mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1. Sample preparation

Tubers of *H. formicarum* Jack were collected from Gunung Stong State Park, Dabong, Kelantan, Malaysia. 5.46° N, 101.79° E (Approval No. JH/100/22/54). The species identification and conformation was done by Forest Research Intitute of Malaysia. Specimen (voucher number is KTK 026/21, Herbarium Unit, Universiti Malaysia Kelantan).

2.2. Sample extraction

H. formicarum tubers (Figure 1) were collected and dusted with a brush before being cut into small pieces. Parts that could have been contaminated were removed. It was then air-dried until the weight was reduced to between

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80 and 90%. Using a mechanical grinder, the air-dried tubers were pulverised to powder. 60 g of *H. formicarum* powder was macerated separately for 5 days in 240 mL of solvents (methanol, ethyl acetate, and hexane) (Maimulyanti and Prihadi, 2016). The extractant was filtered using Whatman filter paper No. 41. A rotary evaporator was then used to concentrate the filtrate. The weight of the crude extract obtained were hexane (3.51 g), methanol (9.35 g), and ethyl acetate (2.54 g).

Figure 1: Epiphytic *Hydnophytum formicarum* Jack.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS was used to identify the chemical compounds in the *H. formicarum* extracts (hexane, ethyl acetate, and methanol). Identification of the components was accomplished by comparing their mass spectra to those stored in the mass spectral library. The GC-MS analyses were conducted on the following Agilent Technologies (Wilmington, Delaware, USA) equipment: 7890B GC System; 5977A MSD, HP-5MS Ultra Inert with fused silica (30m x 0.25mm x 0.25m film thickness); split-less mode; total flow, 24 mL/min; septum purge flow, 3 mL/min; and gas saver set to 20 mL/min after 3 minutes. The oven temperature was programmed to rise from 100°C. Then 4°C/min for 10 minutes up to 260°C, for a total run time of 51 minutes. The multispectral acquisition via scan, parameters were as follows: electron multiplier voltage (EMV), mode, relative, relative voltage, 0V(V); scan parameters were as follows: low, 50.00; high mass, 500.00; threshold, 150; and the MS zones were as follows: MS source, 230 °C; S quadrupole, 150 °C; mass selective detector transfer line, 275 °C. The National Institute of Standards and Technology (NIST) library was compared to the MS Search Program v.2.0f software for compound identification.

2.4. Identification of compounds

The GC-MS results were interpreted using the National Institute of Standards and Technology (NIST) database, which contains over 62, 000 patterns. The known component's spectrum was compared to the known component's spectrum stored in the NIST library. The component of the test materials name, retention time, molecular weight, and chemical formula were determined.

3. RESULT AND DISCUSSION

GC-MS analysis revealed a variety of phytochemical compositions in the various *H. formicarum* solvents. The presence of phytochemical compounds such as phenol, monoterpenoid, benzofurans, ester, lignin, sterol, phytosterols, siloxane, and dicarboxylic acid was determined through the analysis. The GC-MS chromatogram of the hexane extract of *H. formicarum* is shown in Figure 2. It demonstrated the presence of six major peaks corresponding to six chemical compounds, which are also depicted in Table 1. The active principles are listed in Table 1 along with their peak, retention time (RT), and molecular formula. Phenol (91.18 %), Bisphenol A (89.8 %), Benzofuranone (83.27 %), Carveol (72.84 %), Phthalic acid (70.6 %), and 2-Furanmethanol (67.85 %). The hexane extract's primary constituent was phenols (91.18 %). Plant phenolics are involved in a variety of processes, including defence against ultraviolet radiation and aggression from pathogens, parasites, and predators, as well as contributing to the colour of plants (Dai and Mumper, 2010). They are found in every organ of the plant and thus form an integral part of the human diet. The antioxidant properties of phenolic compounds have increased interest in them over the last decade. Their ability to scavenge free radicals contributes to the prevention of chronic and oxidative stress-related diseases such as cancer, cardiovascular, and neurodegenerative diseases. Different types of phenolic compounds exist. They can be classified into sixteen classes based on their carbon chain. Bisphenol A (89.8 %) and Benzofuran (83.27 %) are the next two compounds. Benzofuran compounds are found in abundance. Recent studies have revealed that benzofuran compounds exhibit a range of biological activities, including anti-tumor, antibacterial, anti-oxidative, and anti-viral activity (Nevagi et al., 2015).

Ethyl acetate solvent was used for maceration and extraction processes because of medium polarity and minimum cell toxicity. Ethyl acetate biphasic actions extract both polar and non-polar compounds of *H. formicarum* where the peaks of the compounds were revealed in the GC-MS chromatography (Figure 3). On comparison of mass spectra of the constituents with NIST library,11 phytochemical compounds were identified, and their RT, formula, and percentage of the quality were listed in Table 2. Chemicals compounds identified were Phenol (94.8%), 4,4'-(Hexafluoroisopropylidene) diphenol (93.16%), Stigmasterol (88.94%), Campesterol (88.8%), Clionasterol (87.65%), Spinasterone (81.28%), [(2*S*)-2-[(2*R*)-4-hexadecanoyloxy-3-hydroxy-5-oxo-2*H*-furan-2-yl]-2-hydroxyethyl] hexadecanoate, a-Homocholest-4a-en-3-one (76.29%), 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis(acetyloxy)-

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1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-,[1aR (1a.α.,1b.β., 2.β.,4a.β.,7a.α.,7b.α.,8.α.,9.β.,9a.α.)] (70.52%), 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris (acetyloxy)-3-[(acetyloxy)methyl]-2-chloro 1,1a,1b,2,3,4,4a,7a 7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-,[1aR-(1a.α.,1b.β.,2.α.,3.β.,4a.β.,7a.α.,7b.α.,8.α (69.33%) , and 2,5-Octadecadiynoic acid (69.33%). Four of the compounds extracted belong to the sterol group. The two prevailing compounds detected were phenolic compounds such as phenols and 4,4'-(Hexafluoroisopropylidene) diphenol. 4,4'-(Hexafluoroisopropylidene) diphenol also known as Bisphenol AF. This chemical compound is a derivative of bisphenol A. Phytosterol was also identified which is a steroid derived from plants. Phytosterols naturally occur in small amounts and may act in cancer prevention ("The Metabolomics Innovation Centre (TMIC)," n.d.). One of such phytosterol is stigmasterol which is derived from sitosterol by the action of sterol C-22 desaturases. Stigmasterol, also known as Stigmasterin, is an unsaturated plant sterol present in various medicinal plants. The analysis also determines another form of steroid derived from plants known as campesterol. Both campesterol and stigmasterol are chemically similar to cholesterol, hence the chromatograph and the extract results may be similar (Kaur et al., 2011).

GC-MS chromatogram analysis of the methanol extract of *H. formicarum* (Figure 4) showed 13 peaks which indicating the presence of thirteen phytochemical compounds. Methanol was used for solvent extraction due to its good penetration to the cell content and commonly used to extract chemicals compounds. The compounds that detected by the analysis was listed in Table 3. The phytocompounds were Brucine (100%), Phenol (88.83%), 2-Pentadecanone (86.74%), Ethane (83.98%), Palmitoleic acid Caryophyllene (83.27%), Cholestan-3-ol (75.87%), 9,10-Anthracenedione, cis-13-Octadecenoic acid (70.6%), acetate (ester) (73.2%), Octasiloxane v, 1H-Cyclopropa[3,4] benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-,9,9a diacetate,[1aR(1a.α.,1b.β.,4a.β.,5β.,7a.α .,7b.α.,8. α.,9.β,9a.α)]-(68.68%), Prednisolone acetate (68.58%), and 1,4-Methanoazulene-9-methanol, decahydro -4,8,8-trimethyl-,[1S-(1.α,3a.β.,4. α,8a.,9R*)]-(67.85%). The most prevailing polar component was brucine. Brucine is usually used as an anti-inflammatory and analgesic drug to relieve arthritis, traumatic pain, and anti-tumor (Lu et al., 2020). Phenol is also one of the major chemicals extracted by methanol due to its polar properties. A compound called 2-Pentadecanone was also extracted. 2-Pentadecanone is also known as fema 3724, belongs to the class of organic compounds known as ketones. 2-Pentadecanone is a very hydrophobic molecule, and relatively neutral. The identified compounds illustrate various pharmacological and biological activities are ascertained as in Table 4 and the chemical structure of the compounds were illustrated in Figure 5.

Table 1: Chemical compositions of hexane extract from *H. formicarum* tubers.

No	Chemical Composition	Retention Time (min)	Quality (%)	Formula	Chemical Class
1	Carveol	7.693	72.84	$C_{10}H_{16}O$	Phenol lipids Monoterpenoid
2	Benzofuranone	13.318	83.27	C_6H_6O	Benzofurans
3	Phthalic acid	15.017	70.6	$C_{23}H_{32}O_4$	Dicarboxylic acid
4	2 Furanmethanol	18.033	67.85	$C_{15}H_{26}O_2$	heteroaromatic compounds
5	Bisphenol A	25.597	89.8	$C_{15}H_{16}O_2$	Phenol
6	Phenol	29.597	91.18	C_6H_6O	Phenol

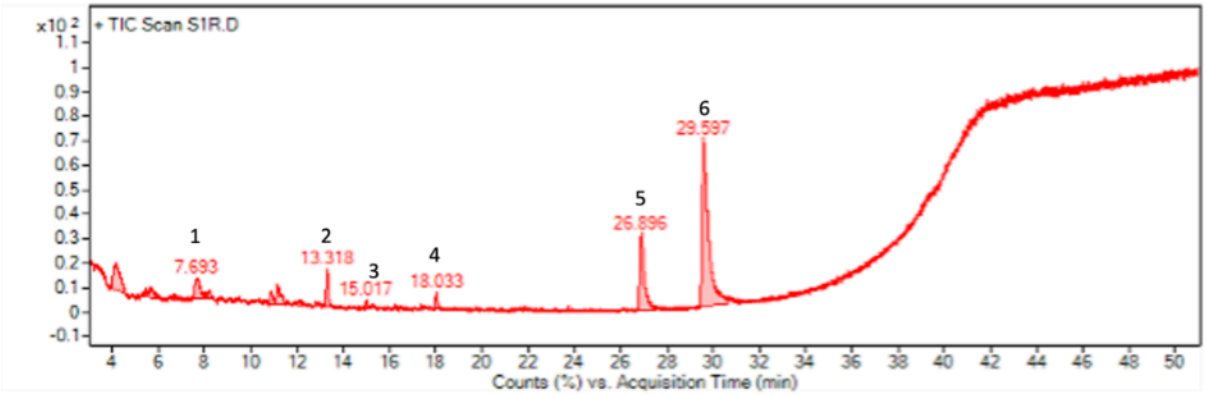

Figure 2: Chromatogram of hexane extract of *H. formicarum* tubers

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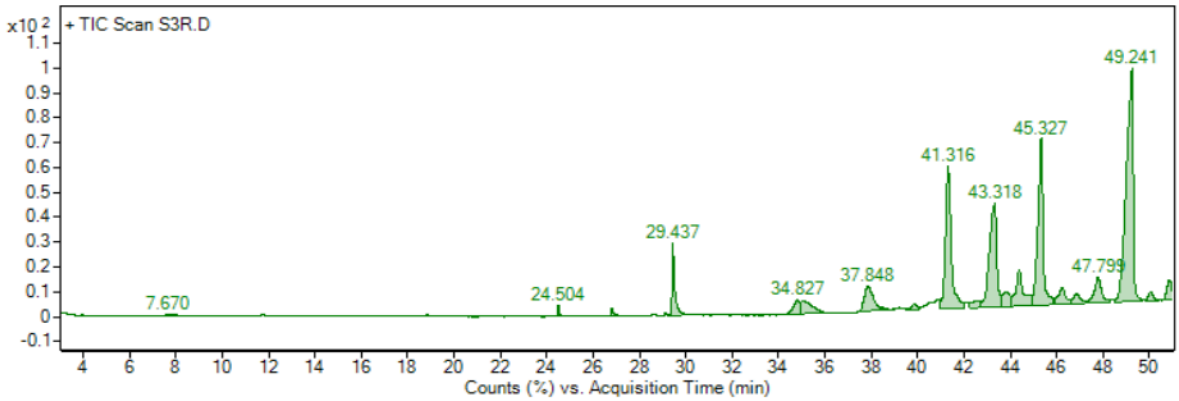

Figure 3: Chromatogram of ethyl acetate extract of *H. formicarum* tubers.

Table 2: Chemical compositions of ethyl acetate extract from *H. formicarum* tubers.

No	Chemical Composition	Retention Time (min)	Quality (%)	Formula	Chemical Class
1	2,5-Octadecadiynoic acid, methyl ester	7.47	69.2	$C_{19}H_{30}O_2$	Ester
2	[(2*S*)-2-[(2*R*)-4-hexadecanoyloxy-3-hydroxy-5-oxo-2*H*-furan-2-yl]-2-hydroxyethyl] hexadecanoate	24.504	79.84	$C_{18}H_{30}O_7$	Lignin
3	4,4'-(Hexafluoroisopropylidene)diphenol	26.437	93.16	$C_{15}H_{10}F_6O_2$	Phenol
4	Phenol	29.385	94.8	C_6H_5OH	Phenol
5	Campesterol	34.827	88.8	$C_{28}H_{48}O$	Sterol
6	Stigmasterol	37.848	88.94	$C_{29}H_{48}O$	Phytosterols
7	Clionasterol	41.316	87.65	$C_{29}H_{50}O$	Sterol
8	a-Homocholest-4a-en-3-one	43.318	76.29	$C_{28}H_{46}O$	Sterol
9	Spinasterone	45.327	81.28	$C_{29}H_{46}O$	Sterol
10	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1a.α.,1b.β.,2.α.,3.β.,4a.β.,7a.α.,7b.α.,8.α	47.799	69.33	$C_{28}H_{36}O_{11}$	Ester
11	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9a-bis(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, [1aR-(1a.α.,1b.β.,2.β.,4a.β.,7a.α.,7b.α.,8.α.,9.β.,9a.α.)]-	49.241	70.52	$C_{24}H_{32}O_9$	Ester

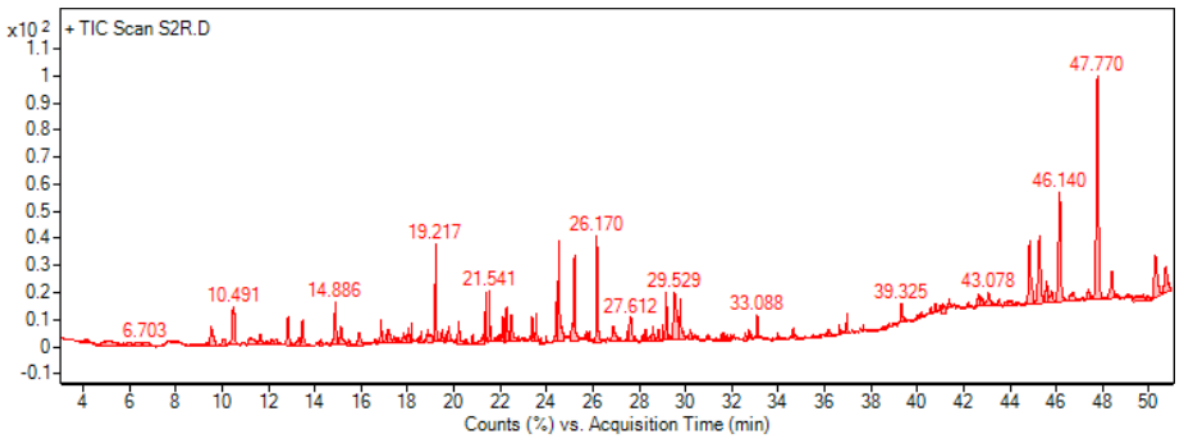

Figure 4: Chromatogram of methanol extract of *H. formicarum* tubers.

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Table 3: Chemical compositions of methanol extract from *H. formicarum* tubers.

No	Chemical Composition	Retention Time (min)	Quality (%)	Formula	Chemical Class
1	Cholestan-3-ol	6.703	75.87	$C_{27}H_{48}O$	Phenol lipids monoterpenoid
2	Palmitoleic acid Caryophyllene	10.491	83.27	$C_{16}H_{30}O_2$	Benzofurans
3	cis-13-Octadecenoic acid	14.886	70.6	$C_{18}H_{34}O_2$	Dicarboxylic acid
4	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1. α,3a.β,4.α,8a. β,9R*)]-	19.217	67.85	$C_{15}H_{26}O$	heteroaromatic compounds
5	2-Pentadecanone	21.541	86.74	$C_{15}H_{30}O$	Phenol
6	Ethane	26.170	83.98	C_2H_6 or CH_3CH_3	Phenol
7	9,10-Anthracenedione	27.612	74.86	$C_{14}H_8O_2$	Phenol
8	Phenol	29.529	88.83	C_6H_5OH or C_6H_6O	Ester
9	acetate (ester)	33.088	73.2	$C_2H_3O_2^-$	Ester
10	Prednisolone Acetate	39.325	68.58	$C_{23}H_{30}O_6$	Sterol/Ester
11	Octasiloxane	43.078	71.76	O_7Si_8	Siloxane
12	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1a. α,1b. β,4a.β,5.β,7a. α,7b.α,8.α,9. β,9a. α)]-	46.140	68.68	$C_{26}H_{36}O_8$	Ester
13	Brucine	47.770	100	$C_{23}H_{26}N_2O_4$	Monoterpenoid

Table 4: Bioactivity of phytocompounds identified in the extract of *H. formicarum* by GC-MS.

No.	Name of the compound	Quality (%)	Synonyms	Pharmacological/ Biological Activity	References
1	Brucine	100	10,11-Dimethoxystrychnine, Brucinum	anti-tumor, anti-inflammatory, analgesic, paralysis effect on sensory nerve endings, inhibit tumor angiogenesis, bone metastasis, inhibit the growth and migration of colorectal cancer cells.	Lu et al. (2020)
2	Phenol	94.8	Hydroxybenzene, Phenic acid	antioxidant, cancer prevention properties, treatment of focal spasticity, anti-infective agents, disinfectants, sclerosing solutions, antimicrobial	Visveshwari et al. (2018)
3	4,4'-(Hexafluoroisopropylidene)diphenol	93.16	Bisphenol AF, 2,2-Bis(4-hydroxyphenyl)hexafluoropropane, Hexafluorobisphenol A	endocrine disruptors	"Bisphenol AF" (2021)
4	Bisphenol A	89.8	4,4'-(1-methylethylidene) bis-; 2,2-bis(4-hydroxyphenyl) propane	an environmental contaminant, a xenobiotic and endocrine disruptor	"PubChem Compound Summary for CID 6623, Bisphenol A" (2021)
5	Stigmasterol	88.94	Stigmasterin	antimicrobial, anticancer, diuretic, anti-inflammatory, antioxidant)	Dinesh et al. (2018)

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6	Campesterol	88.8	Campesterin, Campest-5-en-3beta-ol	cholesterol-lowering, anticarcinogenic effects, antiangiogenic effects, anti-cancer, anti-inflammatory, antibacterial and antifungal activities.	Choi et al. (2007)
7	Clionasterol	87.65	γ-Sitosterol; Stigmast-5-en-3-ol	antihyperglycemic activity; antidiabetic activity)	Balamurugan et al. (2011)
8	2-Pentadecanone	86.74	Pentadecan-2-one; Methyl tridecyl ketone	antibacterial, anti-inflammatory, skin wound healing effect, gastroprotective effect, antioxidant, and its protein modulating effect under stressed conditions wound healing and anti-bacterial activities	Kamran et al. (2019);Cheng et al. (2018)
9	Ethane	83.98	Bimethyl; Dimethyl; Ethyl hydride; Methyl methane	organic hydrocarbon or naturally found compound of carbon and hydrogen.	"Ethane" (2021)
10	Benzofuranone	83.27	Benzofuran-2(3H)-one; 2-Coumaronone	antioxidant activity, antimicrobial agents, Antibacterial and antifungal	Nevagi et al. (2015)
11	Palmitoleic acid	83.27	Palmitolinoleic acid; cis-9-Hexadecenoic acid, (Z)-Hexadec-9- enoic acid;	anti-inflammatory activity, antimicrobial activity	Astudillo et al. (2018)
	Caryophyllene		Beta-caryophyllene; (-)-trans-Caryophyllene	non-steroidal anti-inflammatory drug, a fragrance, a metabolite and an insect attractant, flavouring agent or adjuvant	"PubChem Compound Summary for CID 5281515, beta-Caryophyllene" (2021)
12	Spinasterone	81.28	alpha-Spinasterone	antiproliferative action against CACO-2 cells	Ragasa and Arenal (2014)
13	[(2S)-2-[(2R)-4-hexadecanoyloxy-3-hydroxy-5-oxo-2H-furan-2-yl]-2-hydroxyethyl] hexadecanoate	79.84	L-Ascorbyl 2,6-Dipalmitate, l-(+)-Ascorbic acid 2,6-dihexadecanoate, ascorbic acid dipalmitate	allelopathic activity	Yang et al. (2016)
14	a-Homocholest-4a-en-3-one	76.29	1-(1,5-Dimethylhexyl)-10a,12a-dimethyl-2,3,3a,3b,4,5,7,9,10,10a,10b,11,12,12a-tetradecahydrocyclohepta[a]cyclopenta[f]naphthalen-8(1H)-one	Not reported.	
15	Cholestan-3-ol	75.87	cholestane-3-ol; Dihydrocholestenol	antimicrobial, anti-inflammatory, anticancer, diuretic Antiasthma, antiarthritic	Thanga et al. (2012)
16	9,10-Anthracenedione	74.86	Anthraquinon; anthracene-9,10-dione	phytotoxic, antibacterial, antiviral, anticancer, antitumor, algicide, antifungal, enzyme inhibiting, immunostimulant, antiplatelet aggregation, cytotoxic, and antiplasmodium activities	Masi and Evidente (2020)
17	Acetate (ester)	73.2	Acetate; Acetic Acid Esters	human metabolite	
18	Carveol	72.84	p-Mentha-6,8-dien-2-ol	antibacterial, antioxidant capacity, antimicrobial compound on gram-positive and gram-negative pathogenic bacteria.	Ambrosio et al. (2021)
19	Octasiloxane	71.76	-	antimicrobial, antioxidant	Dinesh et al. (2018)

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20	Phthalic acid	70.6	1,2-benzenedicarboxylic acid, Pathalic acid	allelopathic, antimicrobial, insecticidal activity	Huang et al. (2021)
21	cis-13-Octadecenoic acid	70.6	13Z-octadecenoic acid	Therapeutic uses in medicine, surgery	Arora et al. (2017)
22	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9a-bis(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, [1aR-(1a.α,1b.β,2.β,4a.β,7a.α,7b.α.,8.α,9.β,9a.α)]-	70.52	9a-(Acetyloxy)-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1a,1b,2,4a,5,7a,7b,8,9,9a-decahydro-1H-cyclopropa[3,4]benzo[1,2-E]azulen-9-yl acetate	Not reported.	
23	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1a.α,1b.β,2.α,3.β,4a.β,7a.α,7b.α,8.α	69.33	9a-(Acetyloxy)-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1a,1b,2,4a,5,7a,7b,8,9,9a-decahydro-1H-cyclopropa[3,4]benzo[1,2-E]azulen-9-yl acetate	Not reported.	
24	2,5-Octadecadiynoic acid, methyl ester	69.2	2,5-Octadecadiytioic acid, methyl ester	anti-inflammatory	Hameed et al. (2016)
25	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1a.α,1b.β,4a.β,5.β,7a.α,7b.α.,8.α,9.β,9a.α.)]-	68.68	[1aR-(1a.alpha.,1b.β,4a.β,5.β,7a.alpha.,7b.alpha.,8.alpha.,9.β,9a.alpha.)]- 9a-(Acetyloxy)-5,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-1H-cyclopropa[3,4]benzo[1,2-E]azulen-9-yl acetate ("PubChem Compound Summary for CID 537626," 2021)	antiallergic, antibacterial, antihistaminic, anti-inflammatory, hepatoregenerative, antiulcer	Abdelhamid et al. (2015)
26	Prednisolone Acetate	68.58	21-(acetyloxy)-11,17-dihydroxy-, Prednisolone 21-acetate	inhibiting pro-inflammatory signals, and promoting anti-inflammatory signals, and immunomodulating properties	"PubChem Compound Summary for CID 5834, Prednisolone acetate" (2021)
27	2 Furanmethanol	67.85	Furylcarbinol; 2-(Hydroxymethyl) furan, 2-furylcarbinol or furfural alcohol	Antioxidative activity inhibited hexanal oxidation	Osada and Shibamoto (2006)
28	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1.α,3a. β,4. α,8a. β,9R*)]-	67.85	Isolongifolol, 1,4-Methanoazulene-9-methanol	organic synthesis for the preparation of dilongifolylborane, a chiral hydroborating agent ("longifolene 1,4-methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-," 2021).	"PubChem Compound Summary for CID 572865, (-)-Isolongifolol." (2024)

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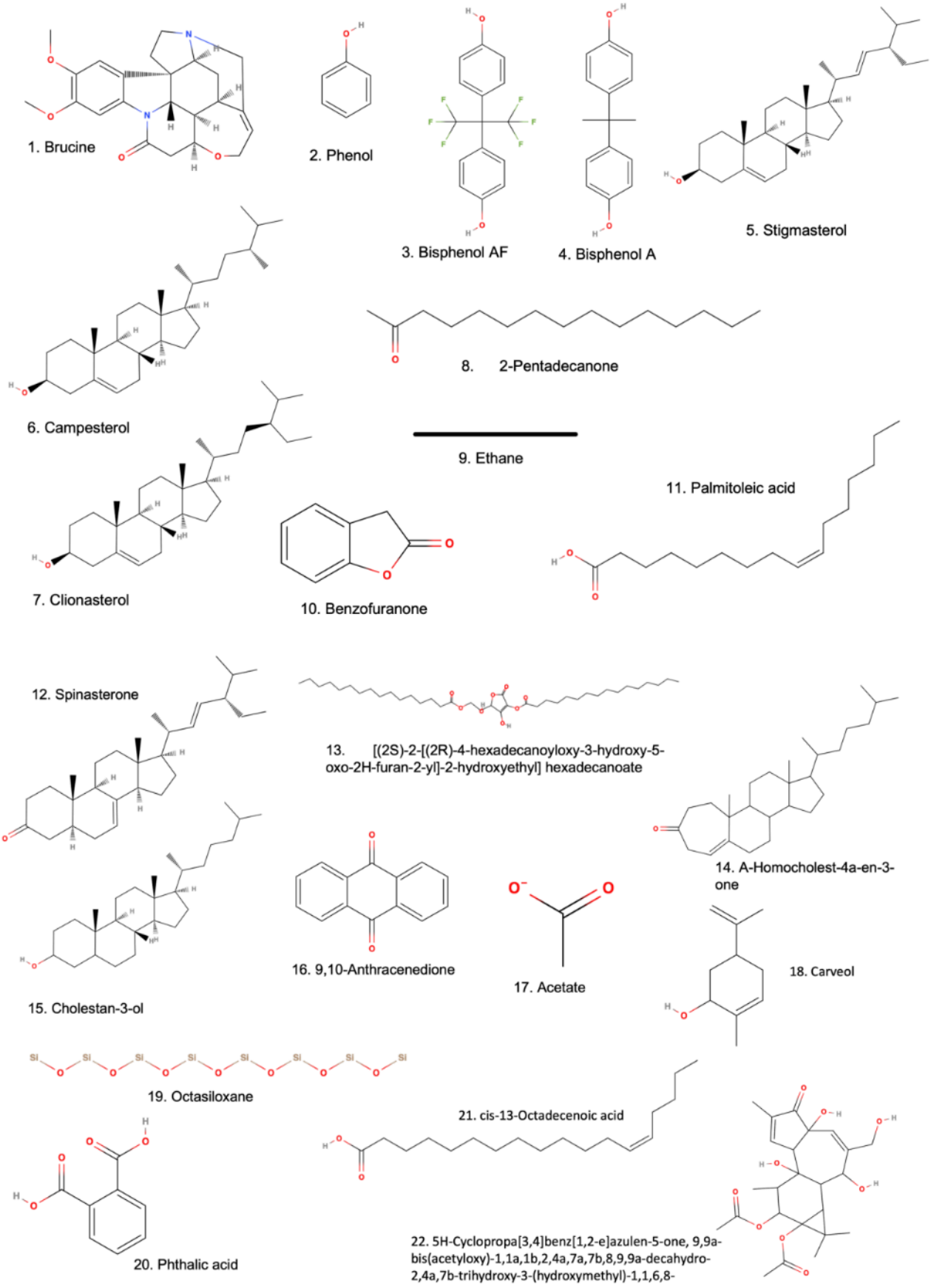

Figure 5: Chemical structure of compounds detected in *H. formicarium* tuber.

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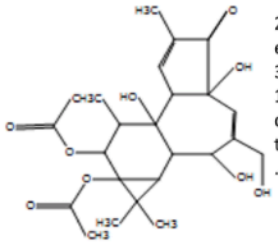
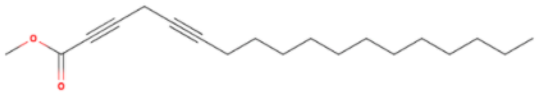
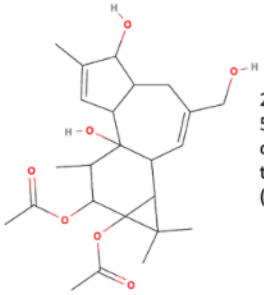
Figure 5 *(continued)*



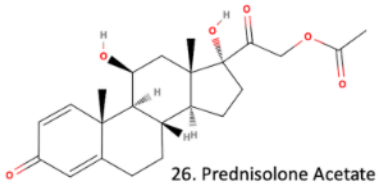
23. 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-,[1aR-(1a.α.,1b.β,2..α,3.β.,4a.β,7a.α,7b .α.,8.α



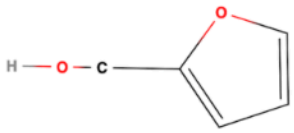
24. 2,5-Octadecadiynoic acid, methyl ester



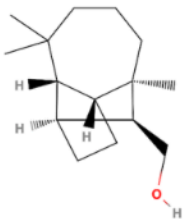
25. 1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1a.α,1b.β,4a.β.,5.β,7a.α,7b.α,8.α,9.β,9a.α.)]-



26. Prednisolone Acetate



27. 2 Furanmethanol



28. 1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1.α.,3a.β,4.α,8a.β,9R*)]-

4. CONCLUSION

By using GC-MS analysis, 28 different volatile compounds were identified in the hexane, ethyl acetate, and methanolic extracts of *H. formicarum* tubers. Therefore, *in vitro* studies need to be explored in future to evaluate the bioactivities of compounds extracted from *H. formicarum* tubers.

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