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Phytochemical screening of fadogia ancylantha using gas chromatography-tandem mass spectrometry-electrospray ionization (gc-esi-qqq-ms/ms)

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Abstract

Ethnobotanical plants contain phytochemicals with antiviral, antibacterial, and antifungal properties. One of the ethnobotanical plants used by traditional healers in Tanzania to boost the immune system, tone muscles, and strengthen bones is Fadogia ancylantha, commonly known as "Makoni tea bush" in English. This study aimed to identify Fadogia ancylantha's phytochemicals in leaves, stems, and root barks. Phytochemicals were extracted using n-hexane, dichloromethane, and methanol solvents. Gas chromatography-tandem mass spectrometry (GC-MS/MS) and the National Institute of Standards and Technology Library (NIST) were used to determine phytochemicals' chemical composition and identity, respectively. Twenty-eight phytochemicals were identified from the leaves, 38 from the stem, and 30 from the roots. The order of number of compounds identified was found to be methanol solvent>DCM solvent >hexane solvent in all samples (leaves, stems, and roots). The screening of phytochemicals revealed the presence of flavonoids, phenolics, terpenoids, and plant sterols. These compounds were reported to have anticancer, antiviral, antibacterial, antifungal, and anti-inflammatory activities. The results show that Fadogia ancylantha is a rich source of phytochemicals with diverse biological activities.

Keywords: *Antiviral; Fadogia ancylantha; GC-MS/MS; Phytochemicals; Traditional medicine*

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Introduction

Traditional medicine, as defined by the World Health Organization (WHO, 2018), is the sum of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the prevention, diagnosis, improvement, or treatment of physical and mental illness. Traditional medicine has been used worldwide since ancient times. In 2002, WHO reported that more than 80% of the world population, mainly in developing countries, still relies on traditional medicine for primary healthcare (Sambo, 2010).

Most common traditional medicines in sub-Saharan Africa are reported to be used to treat various diseases, including opportunistic infectious diseases in people living with viral infections (Maroyi, 2014). Maroy, (2014) mentioned that among the 74 plant species from 37 families, only 50.6% were revealed to contain phytochemicals that exhibit anti-HIV activity. The researcher concluded that the high-performing families were Euphorbiaceae, Asteraceae, Fabaceae sensu lato, Lamiaceae, Combretaceae, and Myretaceae. Different parts of the plants had different performance, such as leaves (67.3 %), tree bark (43.2 %), roots (35.1%), root bark (31.1 %), shrubs (27 %), herbs (24.3 %) and climbers (5.4 %), bulbs and seeds (each 5.4 %), fruits and stems (each 2.4 %).

Studies on the antiviral activity of the hexane extracts from forty different Asian medicinal plant species revealed the crude extracts of both *Acorus calamus* L (Araceae) and *Artocarpus heterophyllus* Lam (Moraceae) to have superior inhibitory activity of 80% against the reverse transcriptase enzyme with IC_{50} of 32.96 and 34.69 g/mL, respectively. Other crude plant extracts with almost similar inhibitory activity were *Ocimum sanctum* L., *Quercus infectoria*, *Plumbage indica* L., *Allium sativum* L., *Sapium indica*, and *Cinnamomum loureiroi* (Silprasit *et al.,* 2011).

Plant species such as *Jatropha curcas* and *Hamalanthus nutan* (Euphorbiaceae), *Andrographic paniculata* (Acanthaceae), *Oldenlandia affinis*, and *Palicourea condensate* (Rubiaceae) have been shown to exhibit activities against viral infections, opportunistic infections, and cancer (Chinsembu and Hedimbi, 2010). However, further studies are recommended to identify several unknown active compounds and their mechanism of action against viral infections.

In Tanzania, most people depend on traditional medicine because of the high cost and insufficient supply of standard synthetic drugs due to population growth, but most importantly, because of easy availability, affordability, accessibility, and promising efficacy (Ekor, 2014). In addition to the above benefits, one plant might have multiple effects in treating more than one disease. Furthermore, it has been reported that over 60% of the population in Tanzania uses medicinal herbs to manage and reduce the effects of viral infections (Kisangau *et al.,* 2007).

Fadogia ancylantha, commonly known as "Makoni tea bush" in English, is a wild perennial shrub endemic to Eastern and Central Africa. It belongs to the Rubiaceae family and is widely distributed in different regions on the mainland of Tanzania. Apart from *F. ancylantha*, other species from the family Rubiaceae are commonly spread in Tanzania (Maregesi *et al.,* 2010).

The leaves of *F. ancylantha* are used to boost the immune system, tone muscles, and strengthen bones. The plant is also reported to have anti-poisonous, anti-ulcer, and aphrodisiac properties. Black tea, also known as Makoni tea, is made by fermentation and drying leaves and stems. Menchelin *et al.* (2010) reported that caffeine-free tea is rich in proteins, phenolic compounds, and zinc. Also, it exhibits antioxidant effects (Mencherini *et al.,* 2010). Furthermore, *F. ancylantha* has been reported to be used for the treatment of liver disease, which revealed hepatoprotective effects in alcohol-damaged liver (Tiya *et al.,* 2019).

Fadogia ancylantha has also been reported to be used in ethnomedicine to boost immunity for HIV/AIDS management by local people in Tanzania, whereby dry leaves of the plant are ground into powder form, mixed with hot water, and then given to sick people to drink while is hot (Tiya *et al.,* 2019).

A survey interview with thirty traditional medicine practitioners revealed about forty-one plant species belonging to different families used in treating various diseases (Kisangau *et al.,* 2007). The survey describes plant species of the family Euphorbiaceae (*Sapium communis*, *Jatrofa curcars*, *Phyllinthus reticulatus*, and *Antidesma venasum*) that are effective in the treatment of tuberculosis, cryptococcal meningitis, chronic cough, diarrhoea, and oral candidiasis. The roots of *Ximenia americana* (Olacaceae) are used for the treatment of skin rashes, whereas *Canthium zanzibarica*, *Tarenna graveolens*, and *Vangueria infausta* of the family Rubiaceae are used for the treatment of cryptococcal meningitis, and oral candidiasis., The plant (*X. americana*) has also exhibited antidiabetic, antioxidant, and antimicrobial activities (Nyirenda *et al., 2012*). The phytochemistry of *X. americana* has revealed the presence of tannin, triglycerides, antibacterial agents, and antidiabetic (Konservasi *et al.,* 2019). Generally, the species has been reported to be used as medicine, alcohol, lubrication, soap, food, and vegetables.

This study aimed to identify phytochemicals found in *F. ancylantha* using various solvents and their beneficial activities in combating diseases. Mufindi district is one of the locations in Tanzania where the *Fadogia ancylantha* plant grows abundantly. All samples used in this study were from this district to minimize variations caused by sample collection from multiple districts or regions.

Materials and Methods

Collection of plant material

The stem barks, leaves, roots, and root barks of *F. ancylatha* were collected from the Mufindi district in the Iringa region. The samples were air-dried under shade to avoid decomposition or evaporating volatile substances.

Identification of plant material

The collected plant materials were identified at the Department of Botany, University of Dar es Salaam, where the voucher specimen was deposited (COLL: No: FMM 4235).

Preparation of plant material

The collected plants were dried in the shade at room temperature, approximately 30^{o}C. The dried plant material (leaves, stems, and roots) was macerated into a powder using a macerator.

Chemicals and reagents

Chemicals and reagents used during phytochemical screening were 99.9 % methanol (MeOH) from Scharlau (Spain), dichloromethane (DCM), acetonitrile from Chem-lab (Belgium), n-hexane (n-Hex) from LiChrosolv® (Germany), ultra-pure water (Mill Q water), and nitrogen gas. All chemical solvents were HPLC grade.

Instrument

The GC-qqq-MS/MS from Agilent Company (7000D) with MassHunter software and NIST library.

Preparations of crude extracts of F. ancylantha (leaf, stem, and root)

200g of each plant material (leaves, stem, and root) were soaked in 1000mL of hexane, dichloromethane, and methanol for a consecutive week. The mixtures were filtered, and the obtained filtrates were evaporated under reduced pressure using a rotary evaporator to obtain three crude extracts.

Gas Chromatography Tandem Mass Spectrometry Analysis

An Agilent GC-MS Triple Quadrupole 7000D series with electron impact ionization was used for the phytochemical screening of the crude extracts. Gas chromatography DB-17MS capillary column of length 30 m x 0.25 mm thickness and helium (purity of 99.999%) as a carrier gas were used to analyze samples. 1 µL of the sample was injected into the Gas Chromatography at a constant flow rate of 1 mL/min. The temperature for the ion source was 230 ˚C, while 280 ˚C temperature was for the injector. The oven's initial temperature was set at 65 ˚C; after 2 min, the oven temperature was raised to 290 ˚C maintained for 1 min and stayed elevated for 15 ˚C/min for 30 min. The ionization energy was 70 eV at positive electron ionization. Scan range was from m/z 35 to m/z 500 fragments. Identification of the compounds was made based on retention time (Rt) in minutes (min) and spectral index from the National Institute of Standards Technology (NIST 14) in which molecular Mass (mol. Mass) of the specific compound was noted.

Results

The summary of the phytochemical screening from extracts from leaves, stems, and roots is presented in Figure 1 - 3 and Table. 1 – Table 4. Most compounds were found in methanol extract, followed by DCM and hexane extracts. Sterm contains most compounds, followed by roots and leaves (Figure 1, Figure2, and Figure3). This study identified 42 phytochemicals with different biological activities, as reported in the literature. The identified phytochemicals were classified into 15 classes. Some compounds were found in all samples (Table 1).

Table 1

GC-MS/MS results of *Fadogia ancylantha* indicating compounds recovered based on solvent.

ID	Name of compound	LEAVES			STEM			ROOT		
		MeOH	n-Hex	DCM	MeOH	n-Hex	DCM	MeOH	n-Hex	DCM
1	Benzeneacetaldehyde	X	-	-	X	-	-	X	-	-
2	Benzoic acid	X	-	X	X	-	X	X	-	X
3	Borneol	-	-	-	-	-	-	X	-	-
4	Benzaldehyde, 4-ethyl-	X	-	-	X	-	-	X	-	-
5	Caprolactam	-	-	X	-	-	X	-	-	X
6	Resorcinol	X	-	-	X	-	-	X	-	-
7	2-Hydroxy-iso-butyrophenone	-	-	X	-	-	X	-	-	X
8	Hexadecane	-	-	X	-	-	X	-	-	X
9	2,4-Di-*tert*-butyl-phenol	X	-	X	X	-	X	X	-	X
10	Ethanone, 1-(2,4-dihydroxy phenyl)-	X	-	-	X	-	-	X	-	-
11	3-Hydroxy-4-methoxybenzoic acid	-	-	-	X	-	-	-	-	-
12	Phenol, 3,4,5-trimethoxy-	-	-	-	-	-	-	X	-	-
13	6-Hydroxybenzofuran-3-one	-	-	-	X	-	-	-	-	-

ID	Name of compound	LEAVES			STEM			ROOT		
		MeOH	n-Hex	DCM	MeOH	n-Hex	DCM	MeOH	n-Hex	DCM
14	(*E*)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenyl	-	-	-	X	-	-	X	-	-
15	Myristic acid	-	-	-	-	X	X	-	-	-
16	Tetradecanoic acid	X	X	X	X	X	X	X	X	X
17	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	X	-	X	X	-	X	X	-	X
18	*p*-Coumaric acid	-	-	-	X	-	-	-	-	-
19	Tetradecanal	-	X	-	-	X	-	-	X	-
20	Pluchidiol	-	-	-	-	-	-	-	-	-
21	Neophytadiene	X	X	X	X	X	X	X	X	X
22	Pentadecanoic acid	-	-	-	X	-	-	-	-	-
23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	X	-	X	X	-	X	X	-	X
24	Hexadecanoic acid, methyl ester	X	-	-	X	-	-	X	-	-
25	Scopoletin	-	-	-	X	-	-	-	-	-
26	Tridecanal	-	X	-	-	X	-	-	X	-
27	Heptadecanoic acid	-	X	X	-	X	X	-	X	X
28	*n*-Hexadecanoic acid	X	X	X	X	X	X	X	X	X

ID	Name of compound	LEAVES			STEM			ROOT		
		MeOH	n-Hex	DCM	MeOH	n-Hex	DCM	MeOH	n-Hex	DCM
29	Octadecanal	-	-	-	-	-	X	-	-	-
30	9-Octadecen-1-ol, (*Z*)-	-	-	X	-	-	X	-	-	X
31	Ethyl (9*Z*,12*Z*)-9,12-octadecadienoate	X	-	-	X	-	-	X	-	-
32	Phytol	X	X	X	X	X	X	X	X	X
33	9,12-Octadecadienoic acid (*Z*,*Z*)-	-	-	-	X	-	-	-	-	-
34	9-Octadecenoic acid, (*E*)-	-	-	-	-	-	-	-	-	X
35	Oleic Acid	-	-	-	X	-	-	-	-	-
36	9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)-	X	X	X	X	X	X	X	X	X
37	Octadecanoic acid	X	X	X	X	X	X	X	X	X
38	α-Amyrin	-	X	-	-	X	-	-	X	-
39	Eicosanoic acid	X	X	-	X	X	-	X	X	-
40	Squalene	X	X	X	X	X	X	X	X	X
41	γ-Tocopherol	X	X	X	X	X	X	X	X	X
42	*dl*-α-Tocopherol	X	X	X	X	X	X	X	X	X

Table 2

Results of the GC-MS/MS analysis of the n-hexane, dichloromethane, and methanol extracts of Fadogia ancylantha leaves

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
1	Benzene acetaldehyde	-	-	-	-	119.9	8.62	C_8H_8O	Aldehyde
2	Benzoic acid	-	-	121.9	11.6	121.9	11.94	$C_7H_6O_2$	Aromatic carboxylic acid
4	Benzaldehyde, 4-ethyl-	-	-	-	-	132.8	12.79	$C_9H_{10}O$	Aldehyde
5	Caprolactam	-	-	155	13.63	-	-	$C_6H_{11}NO$	Polycarbonate
6	Resorcinol	-	-	-	-	109.9	14.07		Benzenediol
7	2-Hydroxy-*iso*-butyrophenone	-	-	120.9	14.27	-	-	$C_{10}H_{12}O_2$	Phenolic compound
8	*n*-Hexadecane	-	-	196.9	18.78	-	-	$C_{16}H_{34}$	Alkane
9	2,4-Di-*tert*-butylphenol	-	-	205.8	19.16	205.8	19.16	$C_{14}H_{22}O$	Phenolic compound
10	Ethanone, 1-(2,4-dihydroxyphenyl)-	-	-	-	-	151.9	19.76	$C_8H_8O_3$	Phenolic alcohol
16	Tetradecanoic acid	227.9	23.98	228	23.99	227.9	24.01	$C^{20}H_{40}O$	Fatty acid
17	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	-	-	211.8	24.13	193.8	24.15	$C_{17}H_{34}O_2$	Benzofurans
19	Tetradecanal	221.8	25.01	-	-	-	-	$C_{17}H_{34}O_2$	Fatty aldehyde

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
20	Pluchidiol	-	-	-	-	167.9	24.5	$C_{16}H_{32}O_2$	
21	Neophytadiene	278.3	25.35	278.1	25.36	278.1	25.37	$C_{18}H_{36}O$	Sesquiterpenoid
23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-	-	278	26.12	278	26.12	$C_{20}H_{40}O$	acyclic diterpene alcohol
24	Hexadecanoic acid, methyl ester	-	-	-	-	269.9	27	$C_{18}H_{30}O_2$	Palmitic Fatty acid
26	Tridecanal	250.2	28.73	-	-	-	-	$C_{30}H_{50}O$	Tridecyl fatty aldehyde
27	Heptadecanoic acid	270	29.23	269.9	29.22	-	-	$C_{20}H_{40}O_2$	Saturated Fatty acid
28	*n*-Hexadecanoic acid	256	27.95	256	27.91	256	27.95	$C_{14}H_{12}O_3$	Saturated Fatty acid
30	(*Z*)-9-Octadecen-1-ol	-	-	250.2	28.74	-	-	$C_{28}H_{48}O_2$	Unsaturated fatty alcohol
31	Ethyl (9Z,12*Z*)-9,12-octadecadienoate	-	-	-	-	263.5	29.63	$C_{29}H_{50}O_2$	Polyunsaturated omega-6 fatty acid
32	Phytol (3,7,11,15-Tetramethyl-2-hexadecane-1-ol)	196	29.75	280.1	25.46	196	29.75	$C_{20}H_{40}O$	acyclic diterpene alcohol
36	(*Z*, *Z*, *Z*)-9,12,15-Octadecatrienoic acid	264	30.11	263.9	30.08	263.9	30.09	$C_{18}H_{30}O_2$	Polyunsaturated fatty acid
37	Octadecanoic acid	284	30.28	284	30.27	284	30.28	$C_{18}H_{36}O_2$	Unsaturated fatty acid
38	α–Amyrin	425.8	31	-	-	-	-	$C_{30}H_{50}O$	pentacyclic triterpene
39	Eicosanoic acid	311.9	31.65	-	-	311.9	31.66	$C_{20}H_{40}O_2$	C_{20} Fatty acid

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
40	Squalene	366.8	35.12	366.8	35.11	366.8	35.11	$C_{30}H_{50}$	Triterpene
41	γ–Tocopherol	415.8	38.23	415.7	38.23	415.7	38.24	$C_{28}H_{48}O_2$	Plant sterols
42	*dl*-α-Tocopherol	429.7	39.68	429.7	39.68	429.7	39.69	$C_{29}H_{50}O_2$	Phenolics

Table 3

Results of the GC-MS/MS analysis of the n-hexane, dichloromethane, and methanol extracts from the stem of Fadogia ancylantha

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
1	Benzene acetaldehyde	-	-	-	-	-	8.62	C_8H_8O	Aldehyde
2	Benzoic acid	-	-	280.7	11.47	121.9	11.65	$C_7H_6O_2$	Carboxylic acid
4	Benzaldehyde, 4-ethyl-	-	-	-	-	280.6	12.77	$C_9H_{10}O$	Aldehyde
5	Caprolactam	-	-	139.8	13.63	-	-	$C_6H_{11}NO$	Polycarbonate
6	Resorcinol	-	-	-	-	109.9	14.07		Benzenediol
7	2-Hydroxy-*iso*-butyrophenone	205.9	19.16	205.8	19.16	205.8	19.16	$C_{14}H_{22}O$	Phenolic compound
8	*n*-Hexadecane	-	-	-	-	151.8	19.76	$C_8H_8O_3$	Alkane
9	2,4-Di-*tert*-butylphenol	-	-	-	-	167.8	20.13	$C_{14}H_{28}O_2$	Phenolic compound
10	Ethanone, 1-(2,4-dihydroxyphenyl)-	-	-	-	-	206.8	21.4	$C_{14}H_{28}O$	Phenolic alcohol

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
15	Myristic acid	300.552	23.97	300.552	23.96	-	-	$C_{17}H_{36}O_2Si$	Saturated Fatty acid
16	Tetradecanoic acid	-	-	-	-	179.9	23.51		Fatty acid
17	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	227.9	23.97	228	23.96	-	-	$C_{20}H_{38}$	Benzofurans
18	p-Coumaric acid	164.158	24.35	-	-	-	-	C9H8O3	Phenolics
19	Tetradecanal	-	-	-	-	228	24.01	$C^{20}H_{40}O$	Fatty aldehyde
20	Pluchidiol	-	-	-	-	163.8	24.35	$C_{13}H_{26}O$	Phenolics
21	Neophytadiene	-	-	-	-	278	25.37	$C_{18}H_{36}O$	Sesquiterpenoid
22	Pentadecanoic acid	-	-	-	-	241.8	25.73	$C_{15}H_{30}O_2$	Saturated Fatty acid
24	Hexadecanoic acid, methyl ester	-	-	-	-	241.8	25.73	$C_{20}H_{36}O_2$	Palmitic Fatty acid
26	Tridecanal	-	-	-	-	242.6	27.45	$C_{18}H_{36}O_2$	Tridecyl fatty aldehyde
27	Heptadecanoic acid	250.3	28.73	246	25.01	-	-	$C_{30}H_{50}O$	Saturated Fatty acid
28	*n*-Hexadecanoic acid	269.9	29.23		29.22	-	-	$C_{20}H_{40}O_2$	Saturated Fatty acid
30	(*Z*)-9-Octadecen-1-ol	256	27.83	256	27.91	256	27.95	$C_{18}H_{36}O$	Unsaturated fatty alcohol
31	Ethyl (9Z,12Z)-9,12-octadecadienoate	-	-	250.1	28.73	-	-	$C_{30}H_{50}$	Polyunsaturated omega-6 fatty acid
32	Phytol	206.7	29.75	206.6	29.75	196	29.74	$C_{20}H_{40}O$	acyclic diterpene alcohol

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
36	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	-	-	-	-	280	29.97	$C_{18}H_{32}O_2$	Polyunsaturated fatty acid
34	Octadecanoic acid	-	-	282.1	30.04	281.9	30.04	$C_{18}H_{34}O_2$	Unsaturated fatty acid
38	α–Amyrin	284	30.28	284	30.27	284	30.28	$C_{18}H_{36}O_2$	pentacyclic triterpene
39	Eicosanoic acid	425.9	31	-	-	-	-	$C_{30}H_{50}O$	C20 Fatty acid
40	Squalene	341	35.12	366.6	35.11	329.7	35.11	$C_{30}H_{50}$	Triterpene
41	γ–Tocopherol	415.8	38.23	-	-	415.6	38.24	$C_{28}H_{48}O_2$	Plant sterols
42	*dl*-α-Tocopherol	429.7	39.68	429.7	39.68	429.7	39.69	$C_{29}H_{50}O_2$	Phenolics

Table 4

Results of the GC-MS/MS analysis of the n-hexane, dichloromethane, and methanol extracts of the root of Fadogia ancylantha

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
2	Benzoic acid	-	-	-	-	280.7	11.94	$C_7H_6O_2$	Carboxylic acid
3	Borneol	-	-	-	-	138.9	11.78	$C_5H_9NO_2$	Bicyclic monoterpenoid
5	Caprolactam	113	13.59	113	13.63	113	12.79	$C_6H_{11}NO$	Polycarbonate
7	2-Hydroxy-*iso*-butyrophenone	-	-	120.9	14.27	-	-	$C_{10}H_{12}O_2$	Phenolic compound

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	**Rt (Min)**	**Mol. Mass**	**Rt (Min)**	**Mol. Mass**	**Rt (Min)**		
9	2,4-Di-*tert*-butyl-phenol	205.8	19.15	205.8	19.16	205.8	19.16	$C_{14}H_{22}O$	Phenolic compound
11	3-Hydroxy-4-methoxybenzoic acid	-	-	-	-	206.6	20.1	$C_{14}H_{28}O_2$	Phenolic acid
12	Phenol, 3,4,5-trimethoxy-	-	-	-	-	280.5	20.99	$C_{11}H_{16}O_3$	Phenolic compound
14	(*E*)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	-	-	-	-	179.8	23.5	$C_{10}H_{12}O_3$	Phenol (Phenylpropanoid)
19	Tetradecanal	221.8	25.01	-	-	-	-	$C_{17}H_{34}O_2$	Fatty aldehyde
24	Hexadecanoic acid, methyl ester	-	-	-	-	270	26.98	$C_{18}H_{30}O_2$	Palmitic Fatty acid
25	Scopoletin	-	-	191.8	27.45	191.8	27.45	$C_{10}H_8O_4$	hydroxycoumarin
26	Tridecanal	250.2	28.73	-	-	-	-	$C_{13}H_{26}O$	Tridecyl fatty aldehyde
28	*n*-Hexadecanoic acid	255.9	27.95	255.9	27.91	255.9	27.95	$C_{16}H_{32}O$	Saturated Fatty acid
29	Octadecanal	-	-	280.6	28.73	-	-	$C_{30}H_{50}$	Aldehyde
34	(*E*)-9-Octadecenoic acid	-	-	280.2	30	-	-	$C_{18}H_{34}O_2$	Unsaturated fatty acid
35	Oleic Acid	280	-	-	-	279.9	30	$C_{18}H_{34}O_2$	monounsaturated omega-9 fatty acid
37	Octadecanoic acid	-	30.28	283.9	30.27	283.9	30.28	$C_{18}H_{36}O_2$	Unsaturated fatty acid
38	α–Amyrin	-	31			425.9	30.99	$C_{30}H_{50}O$	pentacyclic triterpene
40	Squalene	340.8	35.09	340.7	35.11	366.8	35.11	$C_{30}H_{50}$	Triterpene

Peak ID	Name of compound	n-Hexane		Dichloromethane		Methanol		Molecular formula	Chemical Class
		Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)	Mol. Mass	Rt (Min)		
41	γ– Tocopherol	-	-	-	-	415.7	38.24	$C_{28}H_{48}O_2$	Plant sterols
42	*dl*-α-Tocopherol	429.7	39.64	-	-	429.7	39.69	$C_{29}H_{50}O_2$	Phenolics

Figure 1

MS chromatogram of leaves from F. ancylantha in (a) methanol, (b) DCM, and (c) n-hexane. A total of 28 phytochemicals were identified—20 compounds from methanol extract, 14 compounds from n-hexane extract, and 18 compounds from DCM extract.

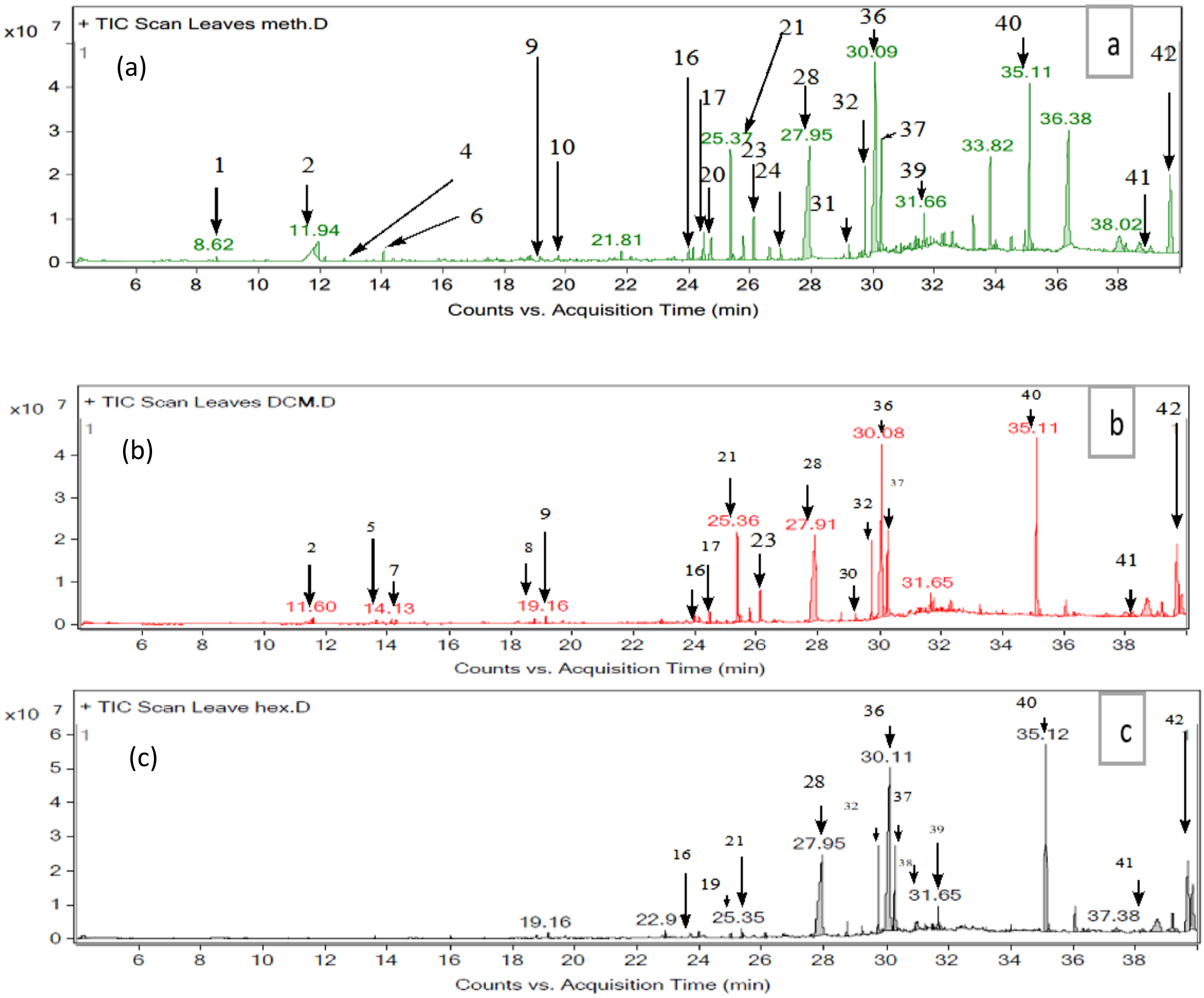


Figure 2

MS chromatogram of the stem from F. ancylantha from (a) methanol, (b) DCM, and (c) n-hexane. A total of 38 phytochemicals were identified in the stem: 28 compounds for methanol extract, 15 compounds for n-hexane extract, and 20 compounds for DCM extract

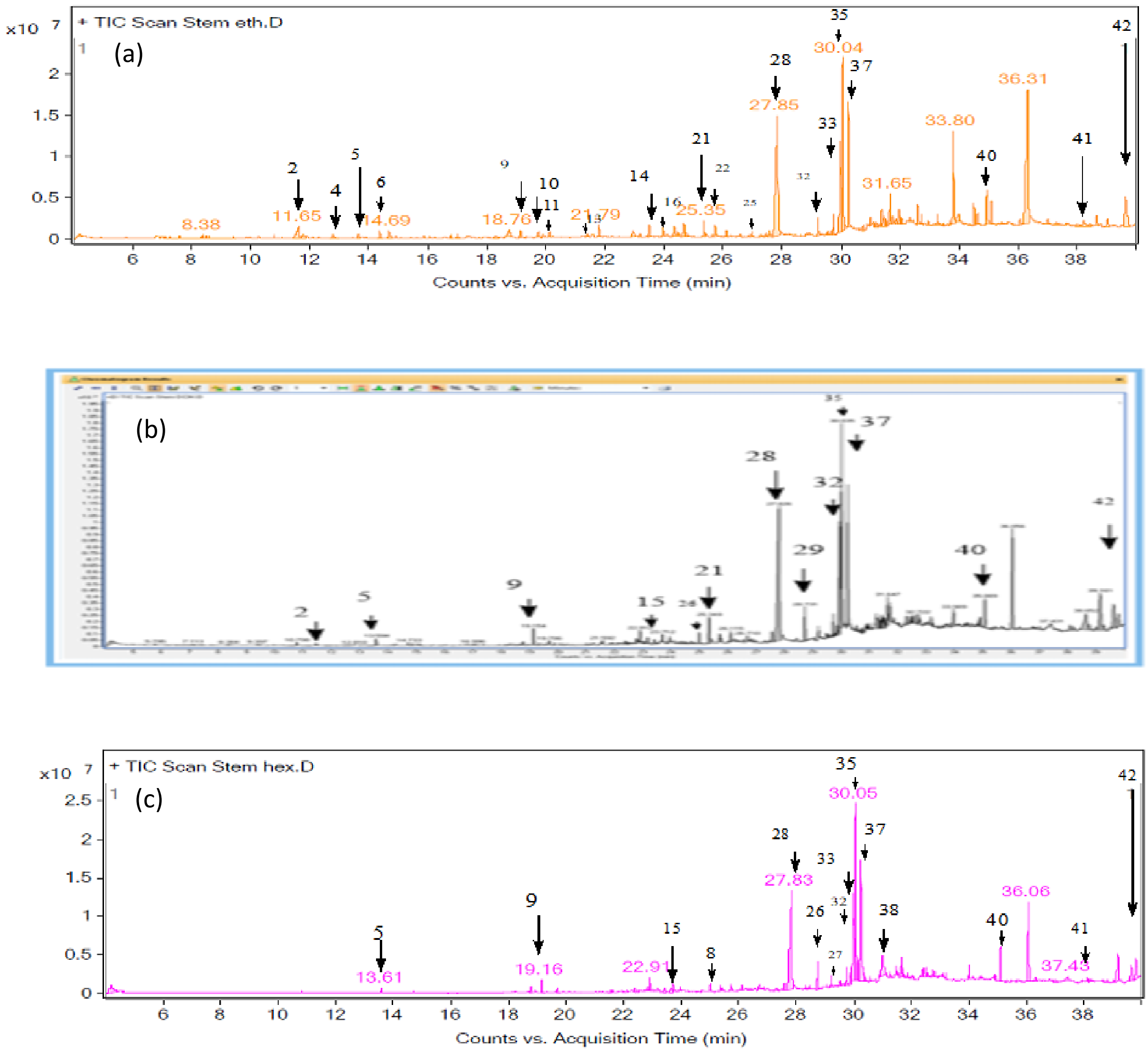


Figure 3

MS chromatogram of root from F. ancylantha from (a) methanol, (b) DCM, and (c) n-hexane. In stem extracts, compounds identified are 28 in methanol extract, 15 in n-hexane extract, and 20 in DCM extract.

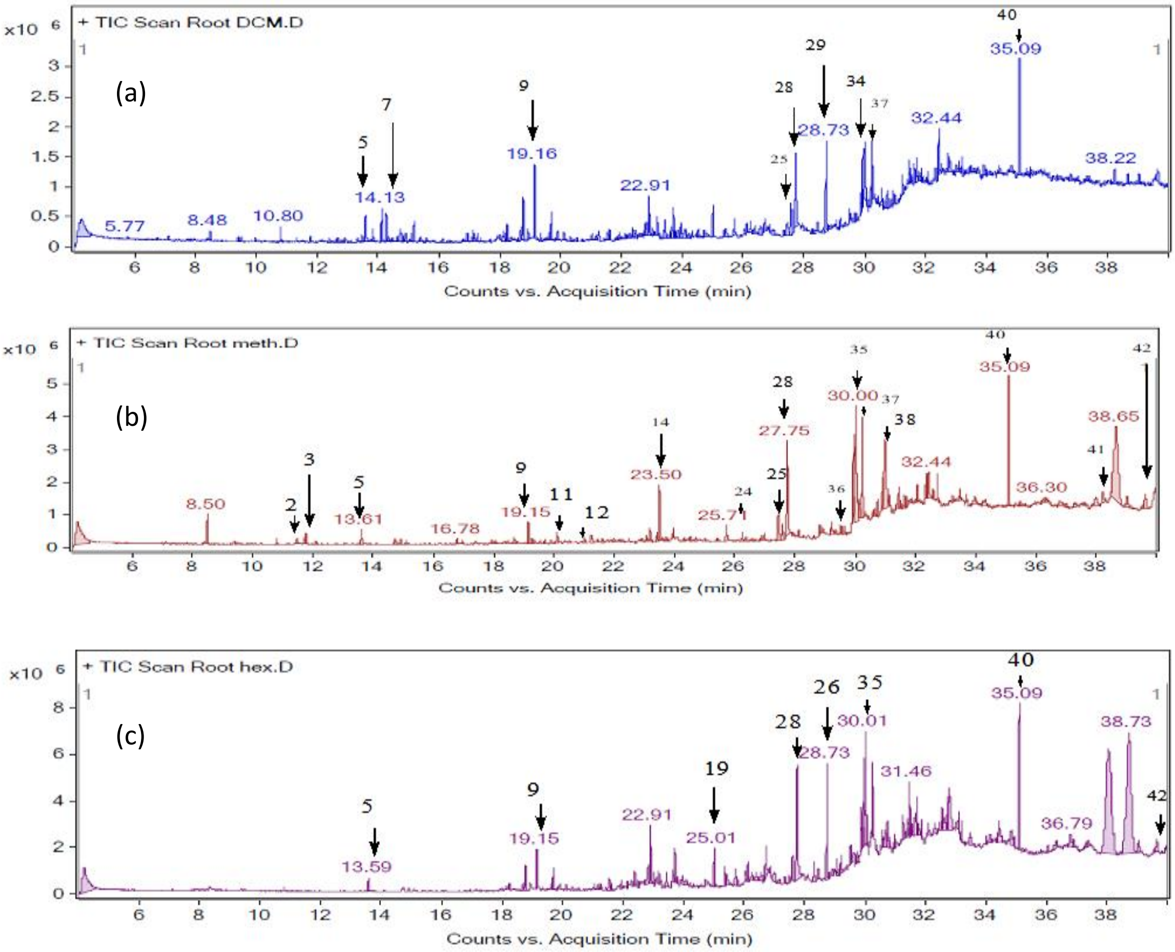
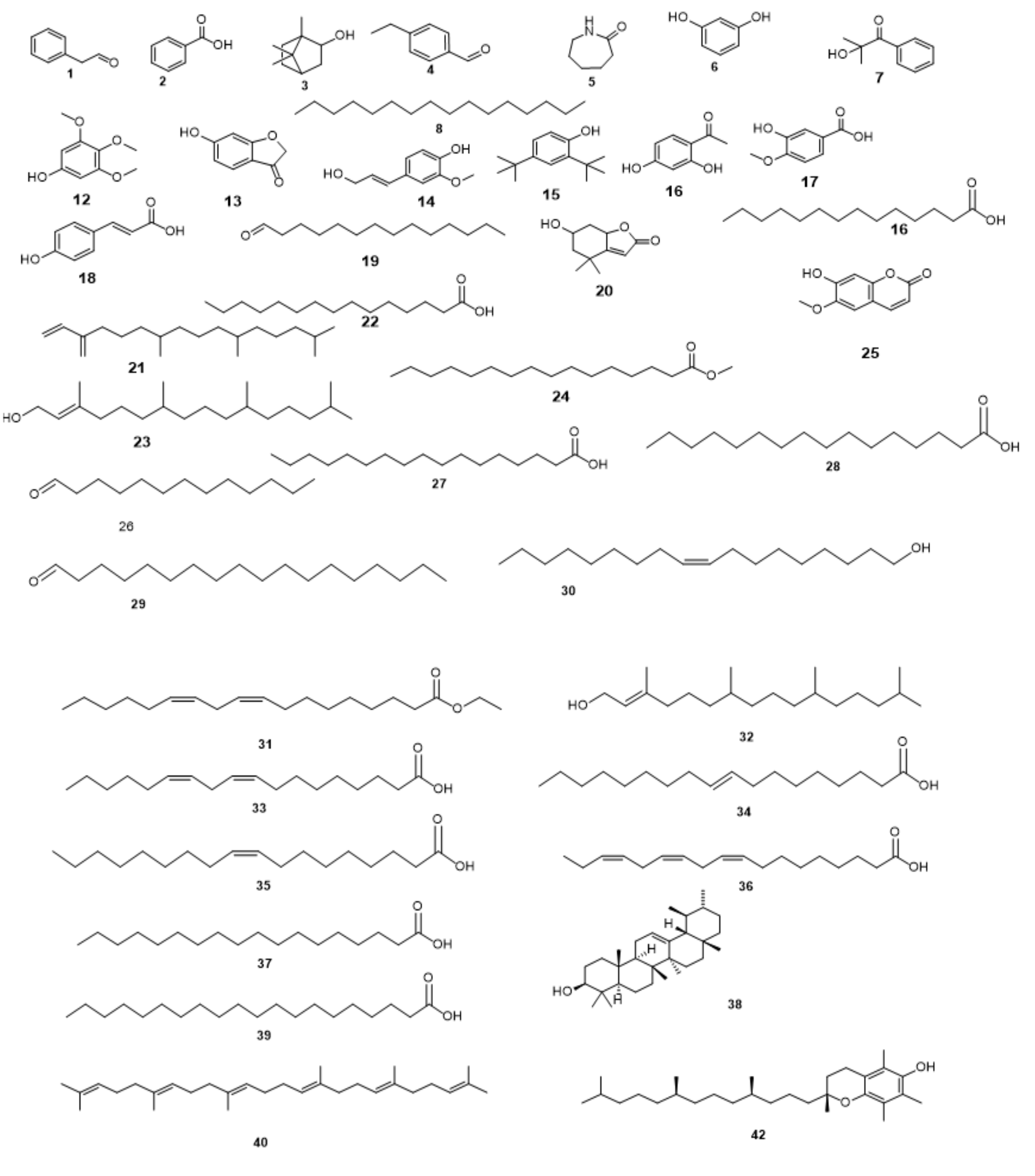


Figure 4

Structures of phytochemicals identified from leaves, roots, and stems of F. ancylantha using methanol, DCM and hexane solvents. Structures were determined using fragments obtained from GC-MS/MS and the National Institute of Standards and Technology Library (NIST).



Discussion

Methanol solvent was most effective at extracting compounds, followed by DCM and *n*-hexane solvents. Polar solvents like methanol and water can dissolve polar and non-polar compounds, while non-polar solvents like hexane dissolve only non-polar compounds. Polar compounds are preferred over non-polar solvents for extracting phytochemicals from plants used for traditional medicine (Nkwocha *et al.,* 2024). Water extraction is also a standard method that traditional healers use to extract phytochemicals from *F. ancylantha* and is most effective at recovering most of the compounds. Hot water is most effective at extraction but was found to reduce the activity of active metabolite, suggesting that cold water is better (Cheng *et al.,* 2023; Šola *et al.,* 2023; Zhang *et al.,* 2018). The target site of most of the phytochemicals and pharmaceutical drugs is inside the cell (Aqil *et al.,* 2013).

The methanol extracts of the root have more compounds (Figure 1-Figure3) compared to the stem and leaves, suggesting that the root has the most compounds. Similar findings were reported in the literature (Thouri *et al.,* 2017; Truong *et al.,* 2019). Peak pattern and elution time suggest that leaves, stems, and roots contain similar compounds, and thus, all these plant parts are potential sources of phytochemicals with medicinal properties.

The screening of compounds identified 42 compounds using the National Institute NIST database.

The analysis of the leaves, stems, and roots of *F. ancylantha* was successful, and various medicinal constituents with multiple pharmacological activities were identified.

Class of Phytochemicals

This study managed to identify 42 phytochemicals with different biological activities, as reported in the literature. The identified phytochemicals were classified into 13 classes, which lead by unsaturated fatty acids (18), followed by Phenolics (16%), terpenoids (13%), saturated fatty acids (13%), aldehyde (11%), alcohol (5%), plant sterols (5%), coumarins (3%), benzofurans (3%), palmitic fatty acids (3%), alkane (3%), benzenediol (3%), and polycarbonates (3%).

Bioactivities of Phytochemicals Identified

Benzeneacetaldehyde, also known as phenylacetaldehyde, is utilized in natural medicine for its antibiotic activity in maggot therapy (Arora *et al.,* 2011). This compound is biosynthetically derived from the amino acid phenylalanine and is found in various natural sources such as chocolate, buckwheat, flowers, and insect communication pheromones(Janeš *et al.,* 2009; Schnermann and Schieberle, 1997). It is also noted as a floral attractant for numerous species of Lepidoptera, particularly the cabbage looper moth (Heath *et al.,* 1992).

Benzoic acid has been studied for potential applications in improving gut functions and health in humans and animals, particularly in the context of feeds and food additives (Mao *et al.,* 2019). Adequation of benzoic acid improves gut functions, including digestion, absorption, and barrier. It can regulate enzyme activity, redox status, immunity, and microbiota, with potential applications as a gut health product in humans and animals (Diao *et al.,* 2015). It has also been suggested that benzoic acid may be used as an additive for improving food health, especially for convalescent patients. A concentration of 1000 mg/kg of benzoic acid improves intestinal morphology and enriches microbial composition, giving a positive modulated intestinal health function (Gong *et al.,* 2021; Mao *et al.,* 2019). Combining benzoic acid and essential oils reduces the inflammatory responses and modifies the cecal microbiome, improving finishing pigs' growth performance (Resende *et al.,* 2020).

Borneol is a bicyclic monoterpenoid. It is found in traditional medicine and has demonstrated that borneol has been associated with central nervous system effects, enhancing the blood-brain barrier's permeability and potentially improving drug delivery to the brain. In animal studies, borneol increased the amount of a marker, Evans blue, entering the brain and widened tight junctions in the blood-brain barrier (Zhang *et al.,* 2017). Borneol also has been found to have potential gastroprotective effects and vasorelactant properties (Zhang *et al.,* 2017). Borneol has been linked to skin-improving activity, including anti-wrinkle and whitening effects. It has been shown to inhibit ultraviolet irradiation-induced tyrosinase and matrix metalloproteinase activity while maintaining collagen type 1 synthesis (Kim *et al.,* 2023). Research has compared the safety and pharmacological activity of different stereo-chemical configurations of borneol, such as L-borneol, D-borneol, and synthetic borneol. L-borneol has been found to exhibit better potential in various aspects and may effectively replace expensive D-borneol in specific applications (Ma *et al.*, 2023). The antiviral effects of borneol and its derivatives have been extensively studied and have shown promising inhibitory activity against various viruses, including SARS-CoV-2, respiratory syncytial virus (RSV), influenza virus, and filoviruses (Sokolova *et al.,* 2022). Molecular modelling suggested potential binding sites of borneol derivatives on the glycoprotein S of SARS-CoV-2 viruses, indicating a mechanism of action related to the inhibitory effect on the virus's surface protein (Sokolova *et al.,* 2022). Borneol showed high activity against multiple SARS-CoV-2 strains, comparable to Remdesivir (Filimonov *et al.*, 2022). Antiviral Activity Against RSV - Studies have identified (-)-borneol derivatives as potent RSV entry inhibitors, with compounds 3b and 5a exhibiting more potency than the known antiviral agent Ribavirin (Sokolova *et al.,* 2022). Time-of-addition assay and temperature shift studies demonstrated that compounds 3b, 5a, and 6b inhibited RSV entry, potentially by interacting with the viral F protein that mediates membrane fusion (Sokolova *et al.,* 2022). Borneol derivatives have shown broad-spectrum antiviral activity, affecting various viruses' replication processes, including influenza A, orthopoxviruses, and coronaviruses (Sokolova *et al.,* 2022). It has been suggested that (-)-borneol-based esters affect filoviruses' glycoprotein-mediated membrane fusion process, showing inhibitory effects (Sokolova *et al.,* 2021). Molecular docking studies indicated that borneol derivatives can bind to different stem parts of the influenza A virus's hemagglutinin (HA) protein, leading to the blocking of viral and cell membrane fusion processes (Sokolova *et al.,* 2021).

In conclusion, borneol and its derivatives have demonstrated significant antiviral effects against SARS-CoV-2, RSV, influenza virus, and other viruses, making them potential candidates for developing broad-spectrum antiviral agents. Their mechanisms of action involve inhibiting viral replication processes and interfering with viral fusion proteins. Further research on borneol and its derivatives may contribute to developing novel antiviral treatments.

Caprolactam is primarily used to produce Nylon 6 and is widely employed in fibres, plastics, and other industrial applications. While caprolactam is not commonly associated with direct biological benefits, its hydrolysis product, aminocaproic acid, has medicinal applications. Caprolactam hydrolyses aminocaproic acid in water, which is utilized medicinally (Kantorowski and Kurth, 2000). Aminocaproic acid is used to control bleeding and in treating acute bleeding syndromes, such as in patients with haemophilia undergoing tooth extractions or in cases of excessive bleeding during surgery (Kantorowski and Kurth, 2000). The substance has been used in the synthesis of several pharmaceutical drugs, including pentylenetetrazol, meptazinol, and laurocapram.

Resorcinol is a phenol derivative reported by Hu *et al.* (2021) in the investigation of resorcinol derivatives with α-glucosidase, providing evidence to be a promising new antidiabetic alternative after a performed α-glucosidase inhibitory assay. A chemical analysis of *Syzygium samarangense* leaves methanol and ethanol extractions using Electrospray ionization (ESI) Triple Quadrupole (QQQ) mass spectrometry (Hu *et al.,* 2021).

2,4-Di-*tert*-butyl phenol is a standard natural product found in at least 169 species, including bacteria, fungi, plants, and animals. It exhibits potent toxicity against a wide range of organisms. The bioactivities and natural sources of butyl-phenol have been extensively investigated, and it is often found as a significant component of volatile or essential oils. The phenol (butyl phenol) has been studied for its potential antibacterial and anticancer agent (Seenivasan *et al.,* 2022; Zhao *et al.,* 2020).

(*E*)-2-(4-bromophenyl)-1-(2,4-dihydroxyphenyl) ethanone oxime (BDEO) has been reported to exhibit therapeutic effects on hyperuricemia through its dual inhibitory effects on xanthine oxidase (XOD) and renal urate transporter1 (URAT1). The compound was synthesized and found to possess potent anti-hyperuricemic activity, with the study aiming to investigate its inhibitory effects on XOD and URAT1 *in vitro*, as well as its anti-hyperuricemic activities in vivo (Hu *et al.,* 2017). The research indicated that BDEO may be a dual XOD and URAT1 inhibitor for treating hyperuricemia.

(*E*)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol, also known as coniferol, isolated from *Allium consanguineum*, has been reported to demonstrate potential biological benefits. In a study investigating the phytochemicals of *A. consanguineum*, coniferol displayed significant antidiabetic and antioxidant activities in *in vitro* assays (Mahnashi *et al.,* 2022). Coniferol exhibited notable potency against α-glucosidase and α-amylase, critical targets in diabetes management, as evidenced by its low IC_{50} values (Mahnashi *et al.,* 2022). Furthermore, coniferol demonstrated intense DPPH radical scavenging activity, indicating its antioxidant potential (Mahnashi *et al.,* 2022). Coniferol effectively lowered blood glucose levels in experimental animals, highlighting their potential to manage diabetes (Mahnashi *et al.,* 2022). Molecular docking studies suggested that Coniferol interacts with α-glucosidase and α-amylase enzymes, further supporting its antidiabetic properties (Mahnashi *et al.,* 2022). These findings indicate that coniferol has promising antidiabetic and antioxidant activities.

Myristic acid has been reported to exhibit potent cholesterol-upregulating action, which could lead to increased levels of total cholesterol and LDL cholesterol. It has been observed that myristic acid is associated with low plasma HDL cholesterol levels in specific populations, potentially leading to disturbances in lipid metabolism and has been associated with alterations in energy metabolism, insulin resistance, and hepatic steatosis in mice, indicating its diverse physiological effects (Saraswathi *et al.,* 2022). Myristic acid is also reported to be linked to a higher risk of ischemic heart disease (IHD) in some studies, indicating potential implications for cardiovascular health. Different dietary saturated fats, including myristic acid, have been shown to have varying effects on lipid profiles, with myristic and lauric acids increasing cholesterol fractions more than palmitic acid (Saraswathi *et al.,* 2022). In contrast to palmitic acid, stearic acid, another type of saturated fatty acid, does not appear to influence lipid metabolism significantly. Myristic acid has been found to play a role in balancing the stimulator of interferon genes (STING)-dependent autophagy and interferon responses, suggesting its potential as a target for the treatment of diseases caused by aberrant STING activation also inhibits the cyclic GMP-AMP synthase (cGAS)-dependent antiviral innate immunity (Jia *et al.,* 2023; Lu *et al.,* 2023). Chronic administration of myristic acid has been reported to improve hyperglycemia in a mouse model of congenital type 2 diabetes (Takato *et al.,* 2017).

6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (HTT) is a natural product found in Macaranga, *Hedlundia hybrid*, and other organisms. HTT has been the subject of scientific research for its potential applications in natural medicine. A study by Jayawardena *et al.,* (2019) evaluated the anti-inflammatory potential of the ethanol extract of *Sargassum horneri* from South Korea, focusing on its effect on LPS-stimulated RAW 264.7 macrophages. It also aimed to isolate and purify HTT using supportive data from NMR and other analytical sources. The study aimed to assess the defence of RAW 264.7 macrophages against LPS-induced damage through the down-regulation of NO production levels, oxidative stress proteins, and inflammation-associated gene expression (Jayawardena *et al.,* 2019).

***p*-Coumaric acid (*p*-CA)** exhibits various biological benefits. *p*-CA demonstrates radical scavenging ability and ferric ion-reducing antioxidant power, contributing to its potential as an antioxidant agent. Studies have revealed the anti-inflammatory properties of *p*-CA, indicating its potential in managing inflammatory reactions (Pragasam *et al.,* 2013). *p*-CA has also been shown to possess anticancer potential, highlighting its role in cancer management (Sakamula and Thong-asa, 2018). Furthermore, evidence suggests that *p*-CA may have therapeutic potential in managing diabetes-related conditions due to its ability to modulate glucose and lipid metabolism. Research indicates a neuroprotective effect of *p*-CA in mice with cerebral ischemia-reperfusion injuries. *p*-CA has been associated with protective effects against hyperlipidemia, indicating its potential for managing lipid-related disorders. Recent studies have revealed that *p*-CA can lessen the harmful effects of oxidative stress in the reproductive system and inhibit enzymes linked with erectile function. *p*-CA can potentially prevent necrosis and cholestasis induced by liver damage and exhibit anti-amoebic activities (Akdemir *et al.,* 2017). These diverse biological benefits of p-Coumaric acid make it a promising compound with potential therapeutic applications in various health conditions.

Pluchidiol. There is limited information available regarding the antiviral activities of Pluchidiol specifically. However, various polyhydroxylated alkaloids, to which Pluchidiol belongs, have been used for their antiviral properties (Kallassy, 2017). Additionally, traditional plant medicines have been known to have antiviral activities, and some examples of viruses that medicinal plants have inhibited are shown (Patra, 2012). Pluchidiol may also possess antiviral properties, but further research is required to determine its effectiveness against specific viruses.

Neophytadiene. According to Al-Rajhi *et al.,* (2022), neophyte diene has been shown to possess antimicrobial activity, such as against fungi (for example, C. albicans and M. cicinelloides), gram-negative bacteria (for example, *E.coli and P. aeruginosa*), and gram-positive bacteria (for example, *S. aureus* and *B. subt*le). Additionally, neophyte diene was identified to exhibit both antioxidant and antibacterial activities (El-fayoumy *et al.,* 2021).

Hexadecanoic acid methyl ester, also known as methyl palmitate, is a fatty acid ester in the root extract of the *Jatropha curcas* plant. It has been reported to have several potential therapeutic properties and applications in natural medicine. Research done by (Othman *et al.,* 2015) suggests that hexadecanoic acid methyl ester possesses anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, alpha-reductase inhibitor, and antiandrogenic properties. Othman *et al.* (2015) identified methyl palmitate using GC-Quadrupole-mass spectrometry and LC-Quadrupole-mass spectrometry tracer analysis of isotopes in water samples.

Scopoletin is a hydroxycoumarin naturally found in several plants, including *Scopolia carniolica*, *Artemisia capillaris*, and more (Gao *et al.,* 2024). Scopoletin has been reported to exhibit various potential health benefits, such as antioxidant properties, which can help protect cells from oxidative stress and damage caused by free radicals (Chao *et al.,* 2009). Further studies suggest that scopoletin exhibits anti-inflammatory effects (Muniandy *et al.,* 2018) and cardiovascular benefits, including vasorelaxant effects that could contribute to cardiovascular health and neuroprotective effects (Kim *et al.,* 2021). Scopoletin has also been investigated for its potential to address conditions such as diabetes, with some studies suggesting its role in modulating glucose metabolism (Kumar *et al.,* 2022). Furthermore, scopoletin has been found to have antiviral properties in *in-silico* studies against SARS-CoV-2 (Kalabegovic *et al., 2021*). The antiviral activity of scopoletin is attributed to the inhibition of SARS CoV-2 viral replication (Kalabegovic *et al.,* 2021). Studies have shown that coumarins have potential antiviral activity against the hepatitis C virus (Sharifi-Rad *et al.,* 2021). Das *et al.* (2020) confirmed the potent activity of scopoletin on *Candida*.

Ethyl (9*Z*,12*Z*)-9,12-octadecadienoate, also known as ethyl linoleate, is a long-chain fatty acid ethyl ester. It is a natural product in *Desmos cochinchinensis*, *Achillea millefolium*, and other organisms. This compound is an essential omega-6 polyunsaturated fatty acid and an isotopologue of linoleic acid (Zesiewicz *et al.,* 2018). Studies have indicated that the deuterated compound, resistant to lipid peroxidation, could have cell-protective properties, making it a subject of interest for potential health-related applications (Hill *et al.,* 2012). This compound also has a potential drug against neuronal, renal, and vascular degeneration and its role in inhibiting lipid autoxidation (Demidov, 2020). Further clinical trials may provide additional insights into its applications in natural medicine.

Phytol, also known as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, is a natural product in various organisms. The compound has been described as an acyclic diterpene alcohol with potential medicinal applications (Sawada *et al.,* 2009). Phytol is a constituent of chlorophyll and is commonly used as a precursor for manufacturing synthetic forms of vitamin E and vitamin K1 (Sawada *et al.,* 2009). It has been shown to modulate transcription in cells via transcription factors PPAR-alpha and retinoid X receptor (RXR) (Sawada *et al.,* 2009). Phytol has been studied for its antiviral activity against various viruses, including influenza (Swamy, 2020). Diterpenoid alkaloids have been reported to have antiviral activity. They are categorized as alkaloids with antiviral activity that inhibit the replication of IAV, coxsackieviruses, and respiratory syncytial virus in vitro (Guo *et al.,* 2022). While there is limited information on the antiviral effects of phytol specifically, algal-derived macromolecules, which include diterpenoids, have been found to have broad-spectrum antiviral effects and immunomodulatory properties (Liu *et al.,* 2021).

(*Z,Z,Z*)-9,12,15-Octadecatrienoic acid, an important polyunsaturated omega-3 fatty acid which exhibits antioxidant activities. Studies also suggested that linolenic acid may have anti-inflammatory and anticancer effects, particularly in *n*-hexadecanoic acid (palmitic acid), which is relatively high in certain extracts (Kim *et al.,* 2020).

Oleic acid, a monounsaturated omega-9 fatty acid, offers several biological benefits to overall health and well-being. It supports cardiovascular health by helping to reduce the risk of heart disease (Carter *et al.,* 1997), and it can assist in lowering low-density lipoprotein (LDL) cholesterol levels while maintaining or increasing high-density lipoprotein (HDL) cholesterol levels. Furthermore, it exhibits anti-inflammatory effects, which can help reduce inflammation (Bowen *et al.,* 2019; Alonso-Torre *et al.,* 2012; Santa-María *et al.,* 2023). Including sources of oleic acid in the diet may aid in weight management. It can increase satiety and reduce appetite, potentially assisting in weight loss and maintenance (Barbour *et al.,* 2015). Oleic acid is also thought to have a positive impact on brain health. It is an essential component of myelin, the protective sheath surrounding nerve fibres. Oleic acid potentially supports proper nerve functions and overall cognitive health. Some research suggests that oleic acid may improve insulin sensitivity, essential for regulating blood sugar levels. The compound benefits individuals with or at risk of developing type 2 diabetes. Oleic acid plays a role in enhancing the absorption of fat-soluble vitamins, such as vitamins A, D, E, and K. This can contribute to overall better nutrient uptake and utilization in the body. When used topically, oleic acid, often found in skincare products, can help moisturize and nourish the skin. It can aid in maintaining the skin's natural oil barrier and hydration, promoting healthy skin. Oleic acid has shown anticancer activities, and advised that more research is required to understand its effects fully (Banim *et al.,* 2018; Ben Fradj *et al.,* 2018). The antiviral activity of oleic acid has been investigated in various studies, demonstrating its potential to inhibit the replication and spread of viruses and antiviral activity against tobacco mosaic virus (TMV). A study reported in Pesticide Biochemistry and Physiology found that oleic acid, separated from cottonseed oil sludge, exhibited moderate antiviral activity against TMV, with effects comparable to those of the antiviral agent Ningnanmycin (Zhao *et al.,* 2017). Oleic acid was observed to increase resistance against TMV in tobacco leaves, potentially through the activation of defence-related genes and enzymes (Zhao *et al.,* 2017). Another study highlighted the antiviral activity of oleic acid against the enveloped bacteriophage φ6, demonstrating its potency in inactivating the virus and preventing its replication (Sands, 1977). Even at low concentrations, oleic acid significantly reduced the virus titer and disassembled the virion through its antiviral effects (Sands, 1977). The antiviral mechanism of oleic acid involves interference with viral fusion proteins, disassembly of the viral structure, and inhibition of the viral replication cycle (Sands, 1977). These findings collectively suggest that oleic acid possesses notable antiviral properties, making it a promising candidate for potential applications in antiviral agents and integrated control of plant viruses.

Octadecanoic acid, also known as stearic acid, is a versatile ingredient commonly used in cosmetics and skin care products due to its emollient and moisturizing properties. The compound in cosmetics functions as a moisture retention agent, reducing inflammation and treating skin conditions. It is commonly found in coconut oil (Varma *et al.,* 2019). In most animal and plant fats, it exists as glycerol ester (Zhen *et al.,* 2015).

Alpha-amyrin, a pentacyclic triterpene derived from the traditional medicinal plant Protium heptaphyllum resin, exhibits many biological benefits. Alpha-amyrin demonstrates anti-inflammatory effects, as evidenced by its ability to reduce proinflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Melo *et al.,* 2010). It has been found to ameliorate inflammatory conditions, including acute pancreatitis and acute periodontitis, through its anti-inflammatory actions (Melo *et al.,* 2010). Alpha-amyrin possesses antioxidant properties, as indicated by its ability to reduce myeloperoxidase (MPO) activity and thiobarbituric acid-reactive substances (TBARS) (Melo *et al.,* 2010). It has been associated with suppressing oxidative stress and lipid peroxidation, reflecting its antioxidant action (Melo *et al.,* 2010). Studies have demonstrated the antinociceptive properties of alpha-amyrin, suggesting its potential in alleviating pain sensations (Melo *et al.,* 2010). Alpha-amyrin exhibits gastroprotective and hepatoprotective effects, potentially protecting the gastrointestinal and hepatic systems (Melo *et al.,* 2010). Alpha-amyrin has been linked to antihyperglycemic and hypolipidemic effects, highlighting its potential in managing blood sugar levels and lipid profiles (Santos *et al.,* 2012). It has shown promise in preserving beta cell integrity and reducing plasma glucose levels, indicating its potential in diabetes management (Santos *et al.,* 2012). The observed biological benefits of alpha-amyrin suggest its potential as a lead compound for drug development effective in diabetes, atherosclerosis, and possibly other inflammatory conditions(Santos *et al.,* 2012). These properties collectively position alpha-amyrin as a valuable natural compound with diverse therapeutic potential, making it an intriguing subject for further research and potential pharmaceutical development. The α-amyrin and β-amyrin mixture derived from *Celastrus hindsii* has demonstrated notable biological activities, including antioxidant, anti-xanthine oxidase, and anti-tyrosinase properties. These findings align with the broader research on α-amyrin and β-amyrin, suggesting potential therapeutic applications in various health conditions. Notably, the mixture of α-amyrin and β-amyrin showed promising potential in combating gout and skin hyperpigmentation, reflecting their diverse pharmacological activities (Viet *et al.,* 2021). The structural and chemical characterization of the α-amyrin and β-amyrin mixture, as well as their isolation and purification from *C. hindsii*, have been thoroughly investigated using techniques such as gas chromatography-mass spectrometry (GC-MS), electrospray ionization-mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR). These analyses have confirmed the presence and quantity of α-amyrin and β-amyrin in the plant material (Viet *et al.,* 2021).

Eicosanoic acid, also known as arachidic acid, is a saturated fatty acid with 20 carbon atoms, which have well-established roles in regulating inflammation, immunity, and other physiological processes (Norman *et al.,* 2015). The eicosanoids group includes prostaglandins, thromboxanes, leukotrienes, and lipoxins, and their local, cell-specific actions make them essential in autocrine/paracrine hormone regulation. Certain drugs, like aspirin, ibuprofen, corticosteroids, and specific COX inhibitors, suppress eicosanoids from arachidonic acid (Norman *et al.,* 2015).

Squalene has been found to have antiviral activity against hepatitis C virus carriers and SARS-CoV-2 (Ebrahimi *et al.,* 2022). Terpenoids, a type of Squalene, have also been shown to have antiviral effects by inhibiting viral DNA synthesis and activating membrane-mediated mechanisms (Ebrahimi *et al.,* 2022). Additionally, a study suggests that Squalene may exert antiviral activity by fitting effectively into the binding site of the coat protein of GBNV (Sangeetha *et al.,* 2021).

Gamma-tocopherol, a form of vitamin E, consists of eight natural isoforms, including alpha, beta, gamma, and delta tocopherols and alpha, beta, gamma, and delta tocotrienols. Gamma-tocopherol lacks one of the electron-donating methyl groups on its chromanol ring, making it a somewhat less potent antioxidant than alpha-tocopherol (Kamal-Eldin and Appelqvist, 1996). However, it can trap nitrogen-based free radicals, which alpha-tocopherol cannot do. Gamma-tocopherol has gained attention for its potential health benefits, including anti-inflammatory, cardioprotective, anticancer, and overall anti-ageing properties. In unsupplemented humans, 50% of gamma-tocopherol is converted to a water-soluble metabolite called gamma-CEHC, excreted into urine (Jiang *et al.,* 2001). Preliminary evidence suggests that gamma-tocopherol may help protect against the onset of type 1 diabetes and reduce several potent inflammatory mediators, including leukotriene B4 and tumour necrosis factor-alpha (Helzlsouer *et al.,* 2000; Jiang and Ames, 2003). This metabolite has been shown to promote the excretion of excess sodium, exhibiting natriuretic activity. Vitamin E modulates differential gene expression by affecting the expression of an array of the gene encoding for proteins; the research conducted by Rota *et al.,* (2005) in the rat hippocampus due to its neuroprotective properties, the changes proved it is protective on Alzheimer's disease progression. Animal studies have shown that gamma-tocopherol supplementation led to a more potent decrease in platelet clumping and clot formation than supplementation with alpha-tocopherol, and it was a more effective inhibitor of ex-vivo lipid peroxidation and low-density lipoprotein (LDL) oxidation. Further research is needed; its potential role in disease prevention and treatment has garnered attention, and it is recommended for inclusion in dietary supplements for optimal nutrition.

(+)-α-Tocopherol. Recent studies have shown that water-soluble derivatives of (+)-α-tocopherol have potent antiviral activity (Precision *et al.,* 2021). Researchers in the United States have demonstrated that these derivatives (D-α-tocopherol polyethylene glycol succinate) exhibit antiviral solid activity and can even synergize with other antiviral drugs, such as remdesivir, which inhibits SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) (Precision *et al.,* 2021). A study on natural compounds evaluated the potential anti-DENV-2 activity of (+)-α-tocopherol and found it possesses significant antiviral activity (Paemanee *et al.,* 2018). Therefore, it can be concluded that (+)-α-tocopherol has shown promising antiviral activity in recent studies.

Conclusion

Based on the findings above, it has been confirmed that *F. ancylantha* has potential antiviral activity. The phytochemicals derived from the plant offer a rich source of antiviral compounds with diverse mechanisms of action. The medicinal plant has shown promising antiviral activity, providing a basis for developing new drugs. According to the literature, the chemical compounds found in these plants, including flavonoids, phenolic acids, terpenoids, and saponins, exhibit inhibitory effects on various stages of the virus life cycle.

Recommendation

The integration of indigenous knowledge with scientific research holds great potential for the discovery of novel antiviral therapeutics. Therefore, we recommend researching this plant to identify more phytochemicals and confirm its biological efficacy and safety.

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