SHORT REPORT

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Chemical Composition, Antibacterial and Cytotoxic Activities of the Essential Oil from *Ficus tikoua* Bur.

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Abstract: The chemical composition and biological activities of the essential oil from *Ficus tikoua* Bur. were reported for the first time. Fifty-three compounds, accounting for 99.60% of the total essential oil composition, were identified and the main components were palmitic acid (51.13%) and linoleic acid (47.54%). The essential oil revealed significant antibacterial activity with the inhibition zones (7.89–10.59 mm), MIC (0.20–6.25 mg/mL) and MBC (0.20–12.50 mg/mL) against *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The essential oil exhibited significant cytotoxicity against A549, NCI-H1299, PC-3 and K562 tumor cells with the IC_{50} values of 131.08, 50.32, 120.58 and 31.68 μg/mL, respectively. The essential oil exhibited selective cytotoxic activity to human tumor cell lines, with a significantly lower cytotoxicity to human normal cell line (MRC-5, IC_{50} = 161.75 μg/mL) than to tumor cells. Additionally, palmitic acid, as the major compound, aslo revealed significant antibacterial and cytotoxic activities.

Keywords: *Ficus tikoua* Bur.; essential oil; GC-MS; palmitic acid; antibacterial activity; cytotoxic activity.

1. Plant Source

F. tikoua was collected in August 2018, from Guizhou Province of China. Dry whole plant was obtained by air-dried in the dark at room temperature. The plant material was identified by Prof. Yuanxing Xiong of Guizhou University. Voucher specimens (NO.1997) were deposited at Guizhou Engineering Center for Innovative Traditional Chinese Medicine and Ethnic Medicine, Guizhou University.

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2. Previous Studies

Ficus tikoua Bur. is a prostrate woody plant of *Ficus* genus, mainly distributed in South China, Northeastern India, Laos and Vietnam [1]. *F. tikoua* is widely used in traditional folk medicine to treat oedema, diarrhea, rheumatism, dysentery, impetigo, chronic bronchitis, jaundice, amenorrhoea and bruise [2,3]. In previous studies, the extracts from *F. tikoua* possess a wide spectrum of pharmacological properties such as antimicrobial [3-5], antioxidant [6,7], antidiabetes [7] and cytotoxic activities [2,8]. Previous phytochemical studies on *F. tikoua* resulted in the isolation of a number of phenolic glycosides [8], phenolic acid [9], flavonoids [2-4,10], benzofuran glucosides [6]. A literature review shows that there are no reports on the chemical constituents and pharmacological properties of the essential oil from *F. tikoua*. Therefore, the purpose of this investigation was to study the chemical constituents of *F. tikoua* essential oil and evaluate its antibacterial and cytotoxic activities. Furthermore, the antibacterial and cytotoxic activities of palmitic acid, a major constituent of *F. tikoua* essential oil, were evaluated.

3. Present Study

The dry whole plant of *F. tikoua* was cut into pieces and placed in a Clevenger-type apparatus and submitted to hydrodistillation (4 h). The essential oil was dried over anhydrous Na_2SO_4 and filtered, then stored at 4°C.

The hydrodistillation of the dried whole plant of *F. tikoua* yielded the essential oil at 0.11% (w/w). Fifty-three chemical compounds, accounting for 99.60% of the total essential oil composition, were identified by GC-FID/MS and presented in Table 1. The main components of the essential oil were palmitic acid (51.13%) and linoleic acid (47.54%).

The antibacterial ability of the *F. tikoua* essential oil and palmitic acid was qualitatively determined by the disc agar diffusion method, and quantitatively assessed by the broth microdilution method. The results were expressed as mean ± SD (Table S1 and Table 2). The essential oil showed broad spectrum antibacterial capacity with the inhibition zones ranging from 7.89–10.59 mm and displayed significant activity against *S. aureus* (MIC = 0.20 mg/mL, MBC = 0.20 mg/mL), *B. subtilis* (MIC = 0.39 mg/mL, MBC = 0.39 mg/mL), *E. faecalis* (MIC = 3.13 mg/mL, MBC = 3.13 mg/mL), *P. aeruginosa* (MIC = 6.25 mg/mL, MBC = 12.50 mg/mL), *E. coli* (MIC = 6.25 mg/mL, MBC = 12.50 mg/mL) and *P. vulgaris* (MIC = 6.25 mg/mL, MBC = 12.50 mg/mL). The *F. tikoua* essential oil showed stronger antibacterial capacity to Gram-positive bacteria than Gram-negative bacteria. Palmitic acid, as a major compound of *F. tikoua* essential oil, aslo revealed significant antibacterial activity against all tested strains with the diameter of inhibition zones (6.75–8.88 mm), MIC (0.63–1.25 mg/mL) and MBC (1.25–2.50 mg/mL). In previous studies, palmitic acid exhibited good antibacterial capacity against *Rhodobacteraceae bacterium R11 A* with the EC_{50} values of 44 μg/mL and exhibited broad spectrum antibacterial activity [16]. The linoleic acid has been well-known for its remarkable antibacterial activity [17]. The antibacterial capacity of *F. tikoua* essential oil against Gram-positive species was more effective than Gram-negative species, which might be responsible for the linoleic acid possessing higher antibacterial capacity against Gram-positive bacteria than Gram-negative bacteria [18]. Therefore, the antibacterial activity of *F. tikoua* essential oil could be attributed to the predominant compounds, palmitic acid and linoleic acid.

The cytotoxic activity of *F. tikoua* essential oil and palmitic acid was investigated against human non-small cell lung cancer (NCI-H1299), lung adenocarcinoma (A549), leukemic (K562), prostatic carcinoma (PC-3) cell lines and normal human fetal lung fibroblasts cell line (MRC-5) by MTT method. The results were expressed as mean ± SD (Table 3). Essential oil and palmitic acid inhibited the growth of all tested cell lines in a time- and concentration-dependent manner. The cytotoxic activity of *F. tikoua* essential oil was determined against the MRC-5 normal cell line (IC_{50} = 161.75 ± 7.89 μg/mL), and against the A549, NCI-H1299, PC-3 and K562 tumor cell lines with IC_{50} values of 131.08 ± 6.39**,** 50.32 ± 3.77, 120.58 ± 9.20 and 31.68 ± 4.31 μg/mL for 72 h incubation, respectively.

Table 1. Chemical composition of *F. tikoua* essential oil

Compounds[a]	RI[b]	RI[c]	Area %	Identification[d]
Octane	800	800[a,b]	0.01	MS, RI
2,4-Dimethylheptane	818	807-821[a,b]	0.02	MS, RI
4-Methyloctane	858	840-863[a,b]	0.01	MS, RI
Styrene	896	880-893[a,c]	t[e]	MS, RI
Nonane	900	896-900[a,b,d,e]	0.01	MS, RI
α-Pinene	937	933-937[a,c,e]	0.01	MS, RI
Camphene	953	952-954[a,c]	0.01	MS, RI
Benzaldehyde	963	960-963[a,b,d,e,f]	t[e]	MS, RI
Hexanoic acid	965	968-990[a,d,e]	t[e]	MS, RI
β-Pinene	982	979-983[a,c,e]	0.01	MS, RI
p-Cymene	1027	1025-1027[a,c,e]	t[e]	MS, RI
L-Limonene	1031	1026-1032[a,c,d,e]	t[e]	MS, RI
1,8-Cineole	1035	1023-1035[a,c,e]	0.01	MS, RI
Benzeneacetaldehyde	1046	1040-1046[a,d,e]	t[e]	MS, RI
cis-Linalool oxide	1075	1066-1075[a,e]	t[e]	MS, RI
trans-Linalool oxide	1091	1086-1094[a,e]	t[e]	MS, RI
Guaiacol	1091	1090[a,c]	t[e]	MS, RI
Linalool	1101	1094-1099[a,c,d,f]	0.01	MS, RI
Nonanal	1105	1100-1105[a,b,c,d,f]	t[e]	MS, RI
Camphor	1150	1143-1150[a,d,e,f]	t[e]	MS, RI
Octanoic acid	1166	1171-1180[a,e]	t[e]	MS, RI
Terpinen-4-ol	1182	1177-1182[a,c,e.f]	t[e]	MS, RI
α-Terpineol	1195	1183-1196[a,c,e,f]	0.01	MS, RI
2,6,11-Trimethyldodecane	1280	1275-1443[a,b]	0.02	MS, RI
l-Bornyl acetate	1290	1284-1285[a,c]	0.05	MS, RI
Thymol	1292	1280-1291[a,d,f]	0.01	MS, RI
β-Damascenone	1389	1380-1386[a,c]	0.01	MS, RI
β-Caryophyllene	1428	1418-1419[a,c,f]	0.01	MS, RI
β-Selinene	1495	1485-1486[a,f]	0.01	MS, RI
δ-Cadinene	1530	1518-1524[a,f]	0.02	MS, RI
Dodecanoic acid	1561	1563-1568[a,e]	0.01	MS, RI
d-Nerolidol	1566	1560-1564[a,d]	0.01	MS, RI
Spathulenol	1586	1572-1619[a,d]	0.01	MS, RI
Caryophyllene oxide	1593	1581-1592[a,c,e,f]	0.01	MS, RI
α-Cedrol	1612	1596-1598[a,f]	0.02	MS, RI
α-Cadinol	1663	1653[a,f]	0.01	MS, RI
2-Pentadecanone	1699	1696-1700[a,d,e]	0.02	MS, RI
Pentadecanal	1720	1715-1718[a,e]	0.03	MS, RI
Methyl myristate	1731	1723-1725[a,e]	0.01	MS, RI
Tetradecanoic acid	1768	1752-1777[a,d,e,f]	0.03	MS, RI
Neophytadiene	1844	1780-1852[a,d,e,f]	0.01	MS, RI
Hexahydrofarnesyl acetone	1850	1844-1848[a,e]	0.07	MS, RI
Pentadecanoic acid	1867	1865-1880[a,c,e]	0.04	MS, RI
2-Heptadecanone	1903	1890-1905[a,b,e]	0.04	MS, RI
Farnesyl acetone	1925	1910-1919[a,d]	0.02	MS, RI
Methyl palmitate	1929	1915-1926[a,d]	0.16	MS, RI
Palmitic acid	1996	1959-1977[a,c,d,e,f]	51.13	MS, RI
Linoleic acid	2173	2130-2133[a,d]	47.54	MS, RI
Totarol	2362	2302-2303[a,f]	0.02	MS, RI
4,8,12,16-Tetramethylheptadecan-4-olide	2374	2364-2366[a,e]	0.05	MS, RI
Pentacosane	2499	2500-2504[a,b,d,e,f]	0.01	MS, RI
Octacosane	2800	2800[a,d]	0.02	MS, RI
Squalene	2845	2832-2847[a,d]	0.10	MS, RI
		Total	**99.60**	

[a]Compounds are listed in order of their elution from a HP-5MS column.
[b]Retention index on HP-5MS column, calculated using homologous series of C_8–C_{30} alkanes.
[c]Retention index of literature, a) NIST 14 and Wiley 275 databases, b) [11], c) [12], d) [13], e) [14], f) [15].
[d]Identification: MS, based on computer matching with NIST 2014 and Wiley 275 MS databases; RI, based on comparison of calculated RI with that of the published values in the literature, NIST 2014 and Wiley 275 databases.
[e]t: trace (trace < 0.01%).

Table 2. The MIC and MBC values of *F. tikoua* essential oil and palmitic acid using microdilution assay

Microorganism	MIC and MBC[a]					
	Essential oil (mg/mL)		Palmitic acid (mg/mL)		Streptomycin (μg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive						
Enterococcus faecalis ATCC 19433	3.13	3.13	1.25	1.25	12.50	25.00
Staphylococcus aureus ATCC 6538P	0.20	0.20	1.25	2.50	0.39	0.78
Bacillus subtilis ATCC 6633	0.39	0.39	1.25	2.50	0.39	0.78
Gram negative						
Pseudomonas aeruginosa ATCC 9027	6.25	12.50	0.63	1.25	1.56	6.25
Escherichia coli CICC 10389	6.25	12.50	0.63	1.25	0.20	1.56
Proteus vulgaris ACCC 11002	6.25	12.50	1.25	1.25	0.39	1.56

[a]MIC: Minimal inhibitory concentration; MBC: Minimal bactericidal concentration; Streptomycin as positive control.

Table 3. Cytotoxic activity of *F. tikoua* essential oil and palmitic acid using MTT assay

Cell line[b]	Time (h)	IC_{50} (μg/mL) and SI[a]					
		Essential oil		Palmitic acid		Cisplatine	
		IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
MRC-5	24	290.23 ± 11.82	-	63.25 ± 2.65	-	12.73 ± 0.37	-
	48	242.38 ± 9.75	-	41.73 ± 0.93	-	4.875 ± 0.52	-
	72	161.75 ± 7.89	-	32.13 ± 1.77	-	2.81 ± 0.81	-
A549	24	166.61 ± 9.26[c]	1.74	89.13 ± 9.49	-	30.86 ± 1.70	-
	48	149.87 ± 12.13[c]	1.62	57.19 ± 5.30	-	14.40 ± 3.29	-
	72	131.08 ± 6.39[c]	1.23	43.66 ± 3.58	-	8.862 ± 1.8	-
PC-3	24	202.01 ± 10.34[c]	1.44	64.15 ± 5.93	-	40.59 ± 2.69	-
	48	140.70 ± 5.76[c]	1.72	60.29 ± 8.23	-	23.76 ± 3.17	-
	72	120.58 ± 9.20[c]	1.34	49.13 ± 2.97	-	10.88 ± 2.09	-
K562	24	79.50 ± 5.67[c]	3.65	56.08 ± 5.53	1.13	20.85 ± 1.75	-
	48	58.48 ± 8.34[c]	4.14	32.57 ± 2.36	1.28	10.31 ± 0.81	-
	72	31.68 ± 4.31[c]	5.11	13.82 ± 1.92[c]	2.32	5.76 ± 0.28	-
NCI-H1299	24	124.84 ± 6.48[c]	2.32	103.59 ± 7.95	-	43.89 ± 0.61	-
	48	96.12 ± 5.91[c]	2.52	61.52 ± 6.93	-	9.41 ± 0.59	-
	72	50.32 ± 3.77[c]	3.21	36.47 ± 4.28	-	7.69 ± 0.288	-

[a]IC_{50}: The sample concentration reduced cells growth by 50% (after 24, 48 and 72 hours incubation), expressed as the mean ± SD of triplicate experiments. SI (Selectivity index): The ratio of IC_{50} values (MRC-5 cell line) to IC_{50} values (tumor cell lines). Cisplatin: positive control.

[b]Cell line: MRC-5 (human fetal lung fibroblasts cells), A549 (human lung adenocarcinoma cell line), PC-3 (human prostatic carcinoma cell line), K562 (human leukemic cell line), NCI-H1299 (human non-small cell lung cancer cell line).

[c]Significantly different from the normal cell line (MRC-5) ($p < 0.05$).

The IC_{50} values of the essential oil against A549, NCI-H1299, PC-3 and K562 tumor cells were significantly lower compared to that against the normal cell line ($p < 0.05$), with selectivity indexes of 1.23, 3.21, 1.34 and 5.11 for 72 h incubation, respectively. The palmitic acid exhibited significant cytotoxicity against the MRC-5 normal cell line (IC_{50} = 32.13 ± 1.77 μg/mL), and against A549 (IC_{50} = 43.66 ± 3.58 μg/mL), NCI-H1299 (IC_{50} = 36.47 ± 4.28 μg/mL), PC-3 (IC_{50} = 49.13 ± 2.97 μg/mL) and K562 (IC_{50} = 13.82 ± 1.92 μg/mL) tumor cell lines for 72 h incubation. In previous studies, palmitic acid showed cytotoxic activity against tumor and normal cell lines, such as human leukemic cells [19], neuroblastoma cells [20], murine and human melanoma cells [21], granulotic cells [22] and pancreatic islets [23]. In previous studies, linoleic acid exhibited *in vitro* cytotoxicity against distinct human tumor cell types such as melanoma cells [21], MOLT-4 leukemia cells [24] and gastric adenocarcinoma cells [25]. Although, palmitic acid showed significant cytotoxicity against all tested cell lines, the observed cytotoxicity of the essential oil could be attributed to these specific chemical components and/or the synergistic effect between various constituents.

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Supporting Information

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