ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF FRUITS OF *Iryanthera ulei* W. FROM COLOMBIA

LUIS E CUCA*, FREDDY A BERNAL, CARLOS A COY, ERICSSON D COY

Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Química, Laboratorio de Investigación en Productos Naturales Vegetales, Cra 30 45-03, A.A. 14490, Bogotá, Colombia

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ABSTRACT

The essential oil was obtained by steam distillation from fruits of *Iryanthera ulei* Warb. growing in Colombia. The oil was analyzed by GC/FID and GC/MS. The main components were α -muurolol (13.2%), spathulenol (12.1%), α -cadinol (7.8%), guaiol (4.0%), oplopanone (3.7%), 1,10-diepi-cubenol (3.4%) and limonene (3.3%) constituting a sesquiterpene-type enriched mixture. In addition, oil was tested for its antimicrobial activity against some Gram-positive and Gram-negative bacteria by means of a modified agar-well diffusion method. *S. aureus* strain was found to be the most sensitive microorganism.

Keywords *Iryanthera ulei*, Myristicaceae, essential oil composition, α -muurolol, spathulenol, antimicrobial activity.

INTRODUCTION

Iryanthera ulei Warb. is a tree which grows up to 30 m in height. The fruit has a perianth carnosous or thin carnosous, often glabrescent, cupuliform, around 3 mm long¹ and it grows in the wild in Amazonian Brazil, Venezuela, Colombia and Peru. *I. ulei* has been used by some Colombian natives, like Barasana tribe that uses it to clean infected wounds and Puinave tribe as baiting fish.² In addition, this plant is traditionally used to treat diarrhea, oral infections, malaria and anemia,³ as well as, other *Iryanthera* species has been described as useful for treating infected wounds⁴ and fever.⁵

Previous studies of *I. ulei* revealed the presence of diarylpropanes, flavonoids, butanolides and neolignans together with one flavonolignoid from extracts of both trunk wood and bark.⁶⁻⁸ However, to the best of our knowledge there are no previous both chemical and biological activity investigations on the volatile components of *I. ulei*.

The most of the essential oil from plants exhibited significant biological activities, such as antimicrobial,⁹ antioxidant¹⁰ among others.¹¹ Therefore, the aim of this paper is to determine the essential oil composition of fruit of this Myristicaceae species growing in Colombia and its antimicrobial activity against Gram-positive and Gram-negative bacteria by means of a modified agar-well diffusion assay.

EXPERIMENTAL

Plant Material

Fruits of *I. ulei* were collected in Vereda Bajo Brasil, Florencia municipality (Caquetá, Colombia) in February 2006. A voucher specimen, numbered COL519611, has been deposited at the Herbario Nacional Colombiano of the Instituto de Ciencias Naturales – Universidad Nacional de Colombia.

Essential oil extraction

The fruits (1234 g) of the freshly collected plant were finely chopped and steam distilled for 3 h to obtain 0,596 g essential oil. It was dried over anhydrous sodium sulfate.

Analysis of essential oil

Oil was analyzed by capillary GC and GC/MS. GC analysis was carried out on a HP 5890 series II gas chromatograph equipped with a FID and operated in split mode (1:15, injected volume, 1 μ L), using a fused silica capillary column HP-5, 30 m x 0.25 mm, 0.5 μ m coating thickness. The operational conditions used were as follows: temperature program from 50°C (4 min) to 300°C (20 min) at 4°C/min, split/splitless injector (300°C), carrier gas was 1.0 mL of He/min; and makeup gas was nitrogen at a 30 mL/min flow rate. Quantitation was by using the Class 5000 software. Percentages were calculated by electronic integration of FID, peak areas without the use of response factor correction. Retention indices (RI) were calculated to help identification by using linear hydrocarbons (C₈-C₂₄).

GC/MS analyses were carried out on a Shimadzu GC-17A Gas Chromatograph coupled to a Shimadzu GCMS-QP5050A mass spectrometer (70 eV) using a fused silica capillary column HP-5ms, 30 m x 0.25 mm, 0.5 μ m coating thickness, with similar temperature programmed as in GC. Interface temperature 300°C. Detector voltage: 1.20 kV. Acquisition mass range: 42-800u. Acquisition mode: full scan; scan interval: 0.35 s. Solvent delay: 3 min.

The components of the oil were identified by comparison of their mass spectra with those of a computer library search and confirmed by comparison of their retention indices.¹²⁻¹⁴ The compounds identified on the oil are listed in Table 1.

Antibacterial assay

Bacterial strains. Gram-positive: *Staphylococcus aureus* ATCC 65380, *Enterococcus faecalis* ATCC 29212. Gram-negative: *Escherichia coli* ATCC 25922 and two *Salmonella typhimurium* strains: ATCC 14028s (mouse virulent smooth strain) and MS7953 (PhoP gene modified macrophage survival mutants strain). All strains were a gift from Professor E. A. Groisman of the Department of Molecular Microbiology at the University of Washington, St. Louis, MO, USA).

The antibacterial activity of the oil was tested by means of agar-well diffusion method modified from Lehrer *et al.* reported methodology.¹⁵ Briefly, the mid-logarithmic phase of bacteria was mixed uniformly in Trypticase soy agar plates at the concentration of 4×10^7 CFU/mL. Small wells in 2-mm in diameter were punched, followed by addition of essential oil. Following diffusion of oil into agar for 18 h, the plates were overlaid with nutrient agar (Difco) and incubated overnight at 37°C. Optical density (OD) of an aliquot was measured at 620 nm and, based on the relationship $OD_{620} 0.20 = 5 \times 10^7$ CFU/mL, a volume containing 1 to 4×10^6 CFU was added to 10 mL previously autoclaved, warm 10 mL buffer containing 3 mg powdered TSB medium, 1% w/v low-electroendosmosis-type agarose (Sigma Chemical Co, Saint Louis, MO, USA) and a final concentration of 0.02% v/v Tween-20 (Sigma). The diameters of the bacterial clearance zones were recorded by a caliper. The results were reported as 0.1 mm of inhibition zone is equivalent to 1 Activity Unit (AU). These radial diffusion positive samples were tested for antibacterial activity in a quantitative assay. Bacterial inhibition was calculated in quantitative assays as being bacterial growth inhibition % = $100 \times (OD_{620} \text{ bacteria in control} - OD_{620} \text{ medium}) - (OD_{620} \text{ sample in test} - OD_{620} \text{ blank}) / (OD_{620} \text{ bacteria in control} - OD_{620} \text{ blank})$.

RESULTS AND DISCUSSION

The gas chromatogram of the *I. ulei* fruit volatiles showed 50 compounds, from which 39 were identified representing 80.5% (listed in table 1). The main compounds of the essential oil were α -muurolol (13.2%), spathulenol (12.1%) and α -cadinol (7.8%). Furthermore, it was found the presence of eleven unknown compounds (17.8%) including three components (3.7%) possess a mass spectra profile that could be attributable to 1,2-dihydroxy-1-methylalkanes ($[M-C_2H_5O]^+ m/z 45(100)$), and five components (9.8%) which exhibit in the mass spectrum the characteristic peaks for oxygenated sesquiterpenes ($[M]^+ m/z 220$).

A review of the literature showed that no oil from any *Iryanthera* species has been studied yet. However, the oil composition of four Myristicaceae species has only been reported: *Virola surinamensis*¹⁶⁻¹⁷, *Myristica fragrans*¹⁸⁻²⁰, *Myristica malabarica*²¹, and *Gymnacranthera canarica*²¹. Monoterpenes were the main constituents of two first American and Asian species (terpinen-4-ol and α -pinene for *M. fragrans*, and limonene, β -cimene and α -pinene for *V. surinamensis*). Nevertheless, the two last have sesquiterpene hydrocarbon-type compounds as major constituents (β -caryophyllene, α -humulene, and α -copaene for *M. malabarica*, and β -caryophyllene, linalool and α -humulene for *G. canarica*). In this case, the essential oil from fruits of *I. ulei* was found to contain oxygenated sesquiterpene-type compounds as main constituents. It's known that, due to geographical position and seasonal variations (intrinsic and external factors), the content and composition of the oil is affected (the former had been demonstrated to the American Myristicaceae species *V. surinamensis*¹⁷).

Table I. Percentage Composition of the fruit oil of *Iryanthera ulei* Warb.

RI ^b	Compounds ^a	%	RI ^b	Compounds ^a	%
813	unknown 1 ^c	2.0	1654	α -muurolol	13.2
833	unknown 2 ^c	0.3	1660	α -cadinol	7.8
838	unknown 3 ^c	0.5	1668	unknown 7 ^c	7.1
844	unknown 4 ^c	1.2	1682	guaia-3,10(14)-dien-11-ol	1.2
856	unknown 5 ^c	1.9	1691	khusinol	0.4
942	α -pinene	0.9	1697	eudesma-4(15),7-dien-1 β -ol	1.8
984	β -pinene	0.3	1719	cedroxide	0.5
1039	limonene	3.3	1734	oplopanone	3.7
1388	α -copaene	0.3	1753	cedrenol acetate	1.0
1404	β -elemene	1.5	1779	14-hydroxy- α -muurolene	0.8
1436	β -caryophyllene	2.7	1818	epi- α -bisabolol acetate	0.2
1469	α -humulene	0.8	1829	cyclopentadecanolide*	0.6
1495	germacrene D	1.5	1840	nuciferol acetate	1.3
1507	γ -amorphene	0.9	1848	unknown 8 ^c	0.1
1512	α -muurolene	0.9	1871	lanceol acetate	0.8
1527	γ -cadinene	0.9	1875	β -santalol acetate	1.2
1529	δ -cadinene	0.9	1879	unknown 9 ^c	0.5
1537	zonarene	2.7	1908	unknown 10 ^c	0.7
1550	α -cadinene	0.2	1960	unknown 11 ^c	1.1
1565	unknown 6 ^c	0.2	1969	unknown 12 ^c	0.4
1573	(<i>E</i>)-nerolidol	1.6	2097	unknown 13 ^c	0.8
1585	dendrolasin	0.5	-	unknown 14 ^c	1.5
1596	spathulenol	12.1	-	unknown 15 ^c	0.3
1599	globulol	2.5			
1606	viridiflorol	0.3	Monoterpenes hydrocarbons(%)		3(4,6%)
1619	guaiol	4.0	Sesquiterpenes hydrocarbons(%)		11(13,3%)
1624	1,10-di- <i>epi</i> -cubenol	3.4	Oxygenated sesquiterpenes(%)		18(54,0%)
1627	1- <i>epi</i> -cubenol	0.9	Sesquiterpenones(%)		1(3,7%)
1633	eremoligenol	0.4	Sesquiterpenlactones(%)		1(0,6%)
1640	<i>allo</i> -aromadendrene epoxide	2.5	Acetylated sesquiterpenes(%)		5(4,3%)
1645	<i>epi</i> - α -cadinol	0.2	Unknown(%)		15(18,5%)

^acompounds are listed in order of their elution time from a HP5 column; ^b RI = retention indices as determined on HP5 using the homologous series of n-alkanes C8-C24; ^c unknown (1): 115(M, 5), 103(18), 73(62), 45(100); unknown (2): 96(M, 52), 91(100), 79(5), 65(12), 51(7); unknown (3): 75(M, 3), 57(11), 55(5), 47(7), 46(4), 45(100), 44(7), 43(12); unknown (4): 90(M, 1), 75(2), 57(11), 55(3), 47(7), 45(100), 44(6), 43 (15); unknown (5): 91(M, 6), 73(38), 61(32), 45(100), 43(53); unknown (6): 203(M, 30), 177(63), 159(55), 145(53), 131(71), 123(100), 109(68), 91(94); unknown (7): 220(M,3), 204(33), 161(65), 121(67), 105(55), 95(86), 81(56), 43(100); unknown (8): 187(M, 334), 177(46), 163(38), 147(90), 134(51), 119(73), 91(51), 77(60), 59(100); unknown (9): 204(M, 49), 177(47), 161(93), 140(48), 127(62), 123(67), 79(100), 55(66); unknown (10): 220(M, 9), 159(24), 147(21), 131(24), 117(15), 105(21), 91(23), 43(100); unknown (11): 220(M, 21), 177(30), 159(49), 133(14), 105(19), 91(25), 55(32), 43(100); unknown (12): 220(M, 20), 193(29), 177(40), 147(45), 121(55), 107(75), 91(96), 43(100); unknown (13): 278(M, 6), 137(10), 123(35), 109(24), 95(56), 81(64), 71(100), 43 (96); unknown (14): 280(M, 2), 167(34), 149(100), 83(9), 113(9), 71(32), 57(49), 43(33); unknown (15): 207 (M, 7), 145(6), 127(7), 99(14), 85(30), 71(59), 57(100), 43(57); *correct isomeric form not identified.

The results for antibacterial activity of essential oil are exposed in figure 1. *S. aureus* was found to be the most sensitive microorganism (about 53% growth inhibition) which is comparable to the standard antibiotic kanamycin (about 60% growth inhibition), whilst the % growth inhibition against *E. faecalis* was 35%. The oil was found to be totally ineffective against *E. coli*, however the standard antibiotic ampicillin showed a 40% growth inhibition toward this strain. Although the *S. typhimurium* MS7953 strain is known to be more sensitive,²² the oil showed similar growth inhibition value on comparing with the activity against the *S. typhimurium* 14028S virulent strain.

Figure 1. Antibacterial activity against some Gram-positive and negative bacteria of *I. ulei* essential oil. EO: essential oil; A: ampicillin; K: kanamycin; T: tetracycline; 1: *E. faecalis* S29212; 2: *E. coli* 25922; 3: *S. aureus* 6538; 4: *S. typhimurium* 14028S; 5: *S. typhimurium* MS7953

In conclusion, the essential oil from fruits of *I. ulei* can be taken as an excellent source of oxygenated sesquiterpene-type compounds which were characterized by means of GC-MS, containing α -muurolol (13.2%), spathulenol (12.1%), α -cadinol (7.8%), guaial (4.0%), oplopanone (3.7%), 1,10-diepicubenol (3.4%) and limonene (3.3%) as main components. In addition, the oil was found to have potential to inhibit the growth of *S. aureus* along other bacteria tested, but it was ineffective against *E. coli*, and non-selective between virulent and mutant strains for *S. typhimurium*. These results are in accordance with the colombian traditional medicine uses for this plant.

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