

杨梅叶精油成分分析及其对肺癌 A549 细胞增殖的抑制作用

朱丽云^{1,2}, 张春苗¹, 高永生^{1,2*}, 宋林珍¹, 陈 玮³, 李素芳¹

(¹中国计量大学浙江省海洋食品品质及危害物控制技术重点实验室, 杭州 310018;
²安徽汉芳生物科技有限公司, 安徽淮北 235000; ³浙江理工大学生命科学学院, 杭州 310018)

摘要:【目的】比较分析‘丁香梅’‘东魁’‘晚稻杨梅’和‘荸荠种’4个品种杨梅叶精油的成分与抗癌活性差异。【方法】以水蒸气蒸馏法提取4种杨梅叶精油, 采用GC-MS技术分析其组成成分, 并以3-(4,5-二甲基噻唑-2)-2,5-二苯基四氮唑溴盐比色法(MTT法)评价了4种杨梅叶精油对肺癌A549细胞增殖的抑制活性。【结果】经GC-MS分析, ‘丁香梅’‘东魁’‘晚稻杨梅’和‘荸荠种’4种杨梅叶精油分别鉴定出34、33、41和33种成分, 包括萜烯、萜醇和萜醚3大类主要成分和脂肪醛、烷酮和酚等微量成分。‘丁香梅’‘东魁’‘晚稻杨梅’和‘荸荠种’4种杨梅叶精油中共有的成分为11种, 特有成分分别为7、6、9和5种。 β -石竹烯(12.85%~23.87%)、 α -葎草烯(19.27%~24.255%)、橙花叔醇(1.15%~23.47%)、氧化香橙烯(3.82%~7.51%)、氧化石竹烯(2.93%~5.26%)和d-杜松烯(0.8%~5.2%)是杨梅叶精油中的主要成分, 但含量存在显著差异。根据MTT结果, ‘丁香梅’‘东魁’‘晚稻杨梅’和‘荸荠种’4种杨梅叶精油对肺癌A549细胞增殖抑制作用均存在时间和剂量依赖性, 作用24 h后A549细胞半数致死量IC₅₀分别为127.24、127.14、118.03和123.62 $\mu\text{g}\cdot\text{mL}^{-1}$, ‘晚稻杨梅’叶精油的抗增殖活性略优于其他3种杨梅叶精油。【结论】‘丁香梅’‘东魁’‘晚稻杨梅’和‘荸荠种’4种杨梅叶精油成分差异显著, 对肺癌A546细胞增殖抑制活性较强, 不同品种间无显著差异($p > 0.05$)。

关键词: 杨梅叶精油; 成分分析; 3-(4,5-二甲基噻唑-2)-2,5-二苯基四氮唑溴盐 MTT; 肺癌细胞; 增殖抑制

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Identification of essential oils in *Myrica rubra* leaves and their inhibitory effect on the proliferation of lung cancer A549 cell

ZHU Liyun^{1,2}, ZHANG Chunmiao¹, GAO Yongsheng^{1,2*}, SONG Linzhen¹, CHEN Wei³, LI Sufang¹

(Key Laboratory of Marine Food Quality and Hazard Controlling Technology of Zhejiang Province, China Jiliang University, Hangzhou 310018, Zhejiang, China; ²Anhui Hanfang Biotechnology Co., Ltd., Huabei 235000, Anhui, China; ³College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou 310018, Zhejiang, China)

Abstract:【Objective】The secondary metabolites of essential oils in *Myrica rubra* leaves (MEO) have antioxidant, antibacterial, anticancer and other biological activities. In this paper, the chemical compositions and anti-cancer activity of MEO extracted from the leaves of four bayberry cultivars, ‘Ding’ao’ ‘Dongkui’ ‘Wandao’ and ‘Biqi’, were analyzed. This study could provide a theoretical basis for the development of natural anti-cancer drugs.【Methods】The essential oils were extracted with steam distillation from the leaves of the four cultivars, ‘Ding’ao’ (grown in Wenzhou, Zhejiang), ‘Dongkui’ (grown in Xianju, Zhejiang), ‘Wandao’ (grown in Zhoushan, Zhejiang) and ‘Biqi’ (grown in Yuyao, Zhejiang). GC-MS was used to analyze the components and contents of essential oils. The chromatographic analysis column was a HP-5 type (30 m×0.25 mm×0.25 μm). The initial was 50 °C, which was kept for 1 min, rose to 250 degrees at 8 min and then maintained for 5 min. Carrier gas was high purity helium with a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. The temperature of the vaporizing chamber was 220 °C. The in-

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作者简介: 朱丽云, 女, 副教授, 博士, 从事芳香精油品质控制与功效研究。Tel: 0571-86835703, E-mail: zly@cjlu.edu.cn

*通信作者 Author for correspondence. Tel: 0571-86835772, E-mail: gaoys@cjlu.edu.cn

jection volume was 1 μL . The split ratio was 20:1. Mass spectrometry conditions included an EI source with an ion source temperature of 200 $^{\circ}\text{C}$, a solvent delay time of 2 min, and a mass range of 50-500 u. Components were identified based on the NIST' 08 standard mass spectrometry database and related literatures. The relative GC contents of volatile oils were calculated by area normalization method. And then the compositions and contents of essential oils were comparatively analyzed. Human lung cancer A549 cells were used as the material to examine the inhibitory activities of four MEOs on the proliferation of the lung cancer A549 cells using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (MTT assay). The concentrations of MEOs used were 50, 75, 100, 125, 150, 175 and 200 $\mu\text{g} \cdot \text{mL}^{-1}$. The action durations were 24, 48, 72, 96 and 120 h, and the proliferation inhibition effect on human lung cancer A549 cells was measured at these treatment time points. 【Results】 After analysis with GC-MS, 34, 33, 41 and 33 components of MEOs were identified from 'Ding' ao' 'Dongkui' 'Wandao' and 'Biqi', respectively, and the contents of the identified components accounted for 99.35%, 91.23%, 98.34% and 97% of total essential oils, respectively. MEOs included the three major components, such as terpenes, terpenols and terpene ethers, and trace components, such as fatty acids, pyrrolidone, and phenols, and so on. The contents of terpenes accounted for 63.58%, 74.13%, 72.12% and 52.42% of total essential oils in 'Ding' ao' 'Dongkui' 'Wandao' and 'Biqi', respectively. The terpene compounds of the essential oils in 'Dongkui' and 'Wandao' were significantly higher than those in 'Dingao' and 'Biqi'. There were 11 components of the essential oils found in the four varieties of *Myrica rubra*. Furthermore, 7, 6, 9 and 5 endemic components were found in the four kinds of MEOs from *Myrica rubra*, 'Ding' ao' 'Dongkui' 'Wandao' and 'Biqi', respectively. The main components in the MEOs included 12.85%-23.87% of beta caryophyllene, 19.27%-24.26% of alpha olefin, 1.15%-23.47% of humulus nerolidol, 3.82%-7.51% of aromadendrene oxide, 2.93%-5.26% of caryophyllene oxide and 0.8%-5.2% of d-cadinene. However, there were significant differences in the contents and components of MEOs from different *Myrica rubra* varieties. The results displayed that the inhibitory effects of MEOs on lung cancer A549 cells were dependent on both the action time and dose. After the action of 24 h, the lethal dose IC₅₀ of MEOs from 'Ding' ao' 'Dongkui' 'Wandao' and 'Biqi' leaves were 127.24, 127.14, 118.03 and 123.62 $\mu\text{g} \cdot \text{mL}^{-1}$, respectively. The antiproliferative activity of MEO from 'Wandao' was slightly better than those from the other three varieties. 【Conclusion】 There were significant differences in the essential oil compositions among the four cultivars. And all the MEOs had a remarkable inhibitory activity on lung cancer A546 cells. However, the inhibitory effect of essential oils on the proliferation of A546 cells did not differ significantly ($p > 0.05$) among different *Myrica rubra* varieties.

Key words: *Myrica rubra* essential oil (MEO); Component analysis; 3-(4,5-Dimethylthiazol -2-yl)-2,5-diphenyltetrazolium bromide (MTT); Lung cancer; Proliferation inhibition

杨梅(*Myrica rubra* Sieb. et Zucc.)是杨梅科多年生常绿乔木,主产于浙江省,'东魁''荸荠种''丁岙梅'和'晚稻杨梅'是浙江省4大良种,在全省乃至全国均有广泛种植^[1-2]。杨梅叶中存在多种活性成分,如原花青素、单宁、槲皮素、杨梅素、香豆素衍生物、三萜化合物等,具有抗抑郁^[3]、调节脂质与糖代谢^[4-5]、抗氧化^[6-9]、消炎^[10-11]、止痛^[12]、抑瘤抗癌^[13-14]等作用。杨梅叶精油,是一类通过水蒸气蒸馏、超临界萃取、同时蒸馏萃取等方法提取获得的水不溶性的挥

发性液体,成分复杂多样,以萜烯类成分为主。杨梅叶精油对肠腺癌CaCo-2细胞的抗增殖作用显著,能够提高癌细胞内多柔比星(癌症治疗中最重要的细胞抑制剂)的浓度、增加癌细胞核中活性氧产生量和多柔比星的积累,并增强其在癌细胞中的抗增殖和促氧化作用^[14]。Lenka等^[15]采用水蒸气蒸馏法提取了杨梅叶精油,发现其能以浓度依赖性方式抑制人结肠癌、回肠腺癌细胞系癌细胞的增殖。

近年来,全球肺癌发病率不断增加,化学治疗药

物存在耐药性和肿瘤复发性^[16],易产生骨髓抑制、贫血、胃肠病、心脏毒性、脱发、记忆丧失和抑郁症等复杂的副作用,收效甚微^[17]。寻求自然和身体良性互补的植物抗癌药物已受到普遍关注^[18]。笔者在前期实验中发现杨梅叶精油对肠系癌细胞^[15]、人肺癌A549细胞具有较强的抗增殖作用。基于浙江省内杨梅种植优势和杨梅叶次级代谢物潜在的抗癌药物开发前景,笔者开展了对浙江省四大良种杨梅叶精油成分差异和对人肺癌A549细胞增殖抑制活性的研究,为开发替代化学药物的植物源抗癌制剂研究提供理论基础,对提高杨梅产业附加值具有重要的意义。

1 材料和方法

1.1 材料

‘丁岙梅’叶(浙江温州茶山)、‘东魁’叶(浙江仙居)、‘晚稻杨梅’叶(浙江舟山)、‘荸荠种’叶(浙江余姚),杨梅叶片均为2 a(年)生以上枝条的老叶。

细胞系:人非小细胞肺癌细胞株A549细胞,购于上海生化与细胞所细胞库。

仪器设备:气相色谱-质谱联用仪 Agilent 6890/5973(美国安捷伦)、相差倒置显微镜(Nikon公司)、二氧化碳培养箱(Thermo公司)、多功能酶标仪(Thermo公司)。

1.2 方法

1.2.1 杨梅叶精油的提取 新鲜杨梅叶粉碎,以1:6(*V*:*V*)料水比装入蒸馏瓶,180 °C回流提取4 h,收集挥发油,加入无水亚硫酸钠脱水干燥,过膜备用。4个品种杨梅叶精油的提取相同,均提取10次合并杨梅叶精油。

1.2.2 杨梅叶精油的GC-MS成分分析 色谱条件:色谱柱为HP-5,30 m × 0.25 mm × 0.25 μm;升温程序:初始温度50 °C,保持1 min,以8 °C ·min⁻¹升至250 °C,保持5 min;载气为高纯氦气,流速为1 mL ·min⁻¹;汽化室温度为220 °C;进样量1 μL,分流比20:1。

质谱条件:离子源为EI源,离子源温度为200 °C;溶剂延迟2 min,质量范围为50~500 u。采用美国NIST'08标准质谱库及相关文献进行定性;以面积归一化法计算挥发油成分相对GC含量。

1.2.3 杨梅叶精油的抗癌细胞增殖活性分析 (1)肺癌细胞A549培养与铺板。将冻存的肺癌细胞A549复苏,于37 °C、5% (ϕ)二氧化碳及饱和湿度环

境下培养。收集对数生长期的A549肿瘤细胞,用血细胞计数板计算细胞数目,并将细胞浓度调整为每mL 3 000个,接种于96孔板中,每孔体积为90 μL,试验组分别加50、75、100、125、150、175和200 μg ·mL⁻¹杨梅叶精油10 μL,对照组加培养液10 μL。

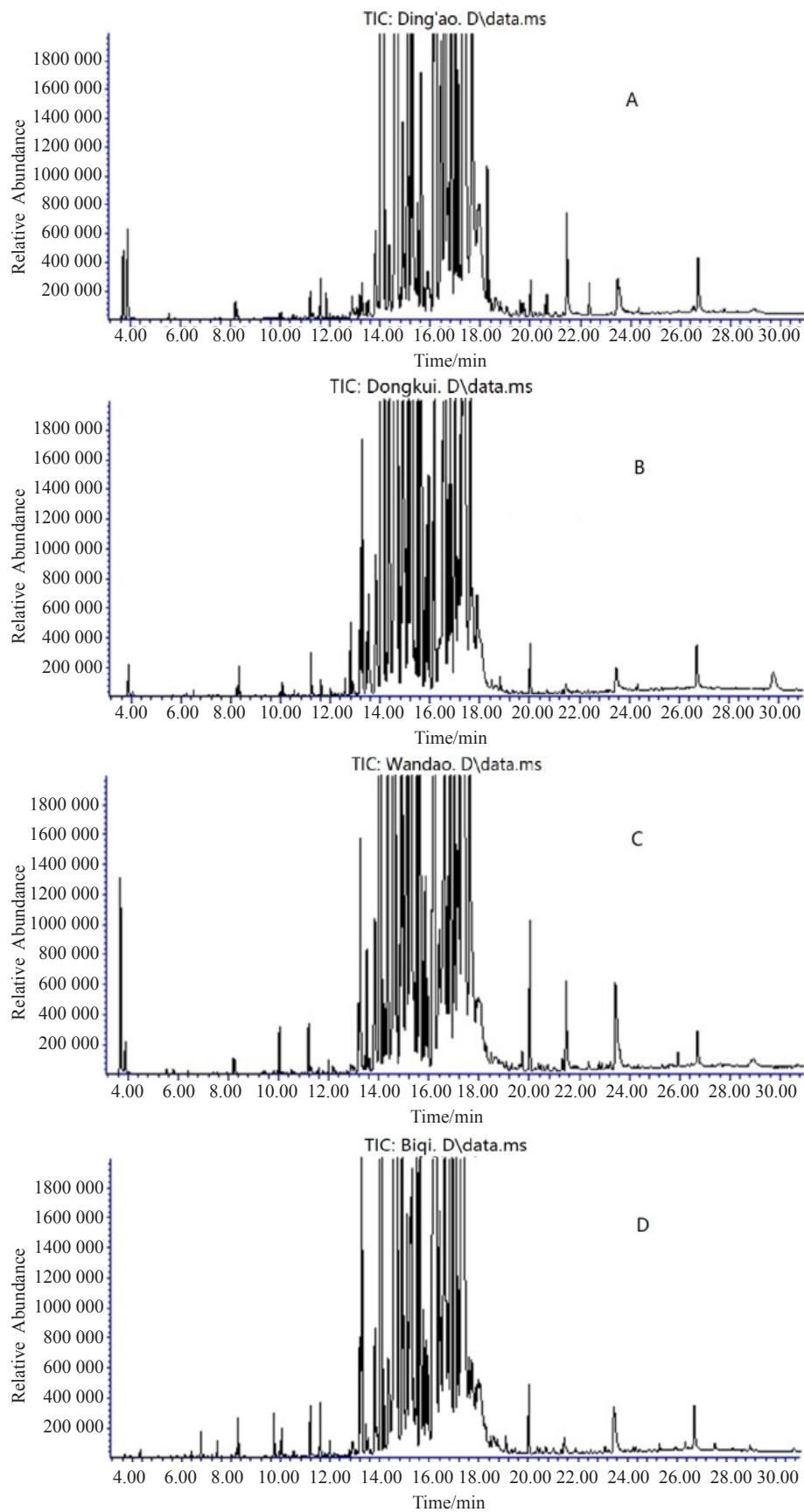
(2)MTT实验。在24、48、72、96、120 h 5个时间点培养结束前4 h,向每孔加入10 μL(5 mg ·mL⁻¹)MTT,培养4 h后吸掉上层液体,向每孔加入150 μL DMSO溶解紫色结晶,用摇床振荡10 min使结晶物充分溶解,用酶标仪检测各孔的吸光度(OD₅₇₀)值。以抑制率%=(加药组OD值-对照组OD值)/对照组OD值×100,计算杨梅叶精油对肺癌细胞A549的抑制率。

2 结果与分析

2.1 不同品种杨梅叶精油的成分差异性分析

采用水蒸气蒸馏法提取获得杨梅叶精油,呈淡黄色澄清液体,具有浓郁的植物芳香气味,从香气特征上不易分辨杨梅叶精油的品种来源。经GC-MS技术分析,‘丁岙梅’‘东魁’‘晚稻杨梅’和‘荸荠种’4种杨梅叶精油分别鉴定出34、33、41和33种成分,被鉴定成分的含量分别占各自挥发油总量的99.35%、91.23%、98.34%和97%,其色谱-质谱总离子流图见图1,由图1可知,4种杨梅叶精油峰形比较一致,主要成分均集中在14~18 min出峰,但不同保留时间的峰高存在差异性,意味着4种杨梅叶精油在成分、含量上存在一定的差异,如‘丁岙梅’和‘晚稻杨梅’品种在4 min前出现的峰较高,‘东魁’较低,而‘荸荠种’几乎没有。4种杨梅叶精油成分的相对百分含量按峰面积归一化法计算得到,结果见表1。

由表1可知,‘丁岙梅’‘东魁’‘晚稻杨梅’‘荸荠种’4大品种杨梅叶精油的主要成分为萜烯类、萜醇类及醚类氧化物等,萜烯类在‘丁岙梅’‘东魁’‘晚稻杨梅’‘荸荠种’4个品种杨梅叶精油中各占63.58%、74.13%、72.12%和52.42%,均以β-石竹烯和α-葎草烯为主,其中4个品种精油中共有的萜烯类化合物有5种,分别为β-石竹烯、α-葎草烯、1-异丙基-7-甲基-4-亚甲基-1,2,3,4,4a,5,6,8a-八氢萘、(+)-g-古芸烯、d-杜松烯,在‘东魁’和‘晚稻杨梅’叶精油中萜烯类化合物含量显著高于‘丁岙梅’和‘荸荠种’原因为‘晚稻杨梅’中存在特有的佛手烯和β-广藿香烯含量占8.97%,而α-芹子烯、d-杜松烯和α-杜松烯在‘东



A. 丁岙梅; B. 东魁; C. 晚稻杨梅; D. 莴荞种。
A. Dingao; B. Dongkui; C. Wandao; D. Biqi.

图1 杨梅叶精油色谱-质谱总离子流

Fig. 1 Total ion chromatogram of chromatographic and mass spectrometry of essential oils from *Myrica rubra* leaves

表 1 4个品种杨梅叶精油组分分析

Table 1 Analysis of essential oil components in the leaves of the four varieties of *Myrica rubra*

序号 No.	化合物 Components	分子式 Molecular formula	类型 Type	相对含量 The relative content/%			
				丁香梅 Dingao	东魁 Dongkui	晚稻杨梅 Wandao	荸荠种 Biqi
1	2-环己烯-1-醇 2-Cyclohexen-1-ol	C ₆ H ₁₀ O	烯醇 Enol	-	0.10	-	-
2	叶醇 Leaf alcohol	C ₆ H ₁₂ O	烯醇 Enol	0.17	-	0.48	-
3	正己醇 Hexyl alcohol	C ₆ H ₁₄ O	饱和醇 Alkanol	0.36	-	0.11	-
4	4-萜烯醇 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	C ₁₀ H ₁₈ O	萜醇 Terpene alcohol	-	-	-	0.11
5	水杨酸甲酯 Methyl salicylate	C ₈ H ₈ O ₃	苯基酯 Phenyl chromone	-	-	0.12	0.07
6	2-环己烯-1-醇 2-Cyclohexen-1-ol	C ₆ H ₁₀ O	烯醇 Enol	-	-	0.11	0.12
7	α-毕澄茄油烯 α-Cubebene	C ₁₅ H ₂₄	萜烯 Terpene	0.13	0.18	-	2.42
8	α-衣兰烯 α-ylangene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.43	0.21	0.30
9	α-蒎烯 α-Pinene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.72	0.71	-
10	β-榄香烯 β-elemene	C ₁₅ H ₂₄	萜烯 Terpene	0.93	0.44	0.36	0.57
11	(-)异石竹烯 (-)-Caryophyllene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.85	0.02	0.27
12	α-石竹烯 α-Caryophyllene	C ₁₅ H ₂₄	萜烯 Terpene	0.40	-	-	0.41
13	β-紫罗酮 β-Lonone	C ₁₃ H ₂₀ O	萜酮 Terpene ketone	-	-	0.19	0.09
14	α-古芸烯 α-Gurjunene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	0.70	-
15	β-石竹烯 β-Caryophyllene	C ₁₅ H ₂₄	萜烯 Terpene	23.87	21.55	12.85	10.19
16	雪松烯 Cedrene	C ₁₅ H ₂₄	萜烯 Terpene	0.21	-	-	0.26
17	1-异丙基-7-甲基-4-亚甲基-1,2,3,4,4a,5,6,8a-八氢萘 1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6, 8a-octahydronaphthalene	C ₁₅ H ₂₄	萜烯 Terpene	0.65	0.16	0.23	1.57
18	去氢白菖烯 Calamenene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	0.22	-
19	(+)-香橙烯 Aromandendrene	C ₁₅ H ₂₄	萜烯 Terpene	0.25	1.84	1.60	-
20	α-葎草烯 α-Humulene	C ₁₅ H ₂₄	萜烯 Terpene	20.60	20.59	19.27	24.25
21	(+)-g-古芸烯 (+)-g-curjunene	C ₁₅ H ₂₄	萜烯 Terpene	0.48	0.18	0.30	0.59
22	香树烯 (-)-alloaromadendrene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.45	0.48	0.38
23	β-芹子烯 β-Selinene	C ₁₅ H ₂₄	萜烯 Terpene	1.50	6.04	-	1.13
24	α-姜黄烯 α-Curcumene	C ₁₅ H ₂₄	萜烯 Terpene	0.19	-	0.67	-
25	d-长叶烯 d-Longifolene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.27	-	-
26	3,7,11-三甲基-1,3,6,10-十二碳-四烯 1,3,6,10-Dodecatetraene,3,7,11-trimethyl-,(3E,6E)-	C ₁₅ H ₂₄	萜烯 Terpene	3.68	-	2.61	0.71
27	(-)-g-杜松烯 (-)-g-Cadinene	C ₁₅ H ₂₄	萜烯 Terpene	0.42	-	-	-
28	d-杜松烯 d-Cadinene	C ₁₅ H ₂₄	萜烯 Terpene	0.80	5.20	4.43	2.67
29	α-衣兰油烯 α-Murolene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.38	0.54	0.89
30	β-广藿香烯 β-patchoulene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	2.58	-
31	佛术烯 Eremophilene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	6.39	-
32	α-芹子烯 α-Selinene	C ₁₅ H ₂₄	萜烯 Terpene	1.44	7.09	7.11	-
33	α-杜松烯 α-Cadinene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.50	2.52	1.04
34	1,2,3,4,4a,7-六氢-1,6-二甲基-4-(1-甲基乙基)-萘 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1- methylethyl)-Naphthalene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.21	0.29	0.39
35	α-柏木烯 α-Cedrene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	0.27	-
36	1,2,3,6-四甲基双环[2.2.2]辛-2-烯 1,2,3,6-Tetramethylbicyclo[2.2.2]oct-2-ene	C ₁₂ H ₂₀	烷烃 Alkanes	1.16	0.45	-	-
37	橙花叔醇 Nerolidol	C ₁₅ H ₂₆ O	萜醇 Terpene alcohol	14.23	1.15	12.21	23.47
38	喇叭烯氧化物(-) Ledene oxide-(II)	C ₅ H ₁₀ O ₂	萜醚 Terpene ether	1.97	-	0.87	-
39	氧化石竹烯 Caryophyllene oxide	C ₁₅ H ₂₄ O	萜醚 Terpene ether	4.32	5.26	2.93	3.02
40	4-甲氧基-1-甲基吲哚 1H-Indole,4-methoxy-1-methyl-	C ₁₀ H ₁₁ NO	吲哚 Indole	0.62	-	-	-
41	莰烯 Camphene	C ₁₀ H ₁₆	萜烯 Terpene	3.16	-	-	-
42	(+)-匙叶桉油烯醇 (+)-spathulenol	C ₁₅ H ₂₄ O	萜醇 Terpene alcohol	-	-	0.52	-
43	香橙烯氧化物 Aromadendrene oxide	C ₁₅ H ₂₄ O	萜醚 Terpene ether	3.82	4.47	4.11	7.51
44	α-布藜烯 α-loulnesene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	0.66	-
45	α-二去氢菖蒲烯 α-Calacorene	C ₁₅ H ₂₀	萜烯 Terpene	-	-	-	0.31
46	未鉴定 Not identified	C ₁₃ H ₁₈ O ₂	-	-	-	0.54	-
47	十氢萘 Decahydronaphthalene	C ₁₀ H ₁₈	萜烯 Terpene	-	-	-	0.58
48	3,4-二甲基-3-环己烯-1-甲醛 3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	C ₉ H ₁₄ O	脂肪醛 Aldehydes	3.63	1.43	2.60	7.48
49	1,5-二乙烯基-2,3-二甲基-(1a,2a,3a,5β)-环己烷 Cyclohexane,1,5-diethenyl-2,3-dimethyl-(1a,2a,3a,5β)-	C ₁₂ H ₂₀	烷烃 Alkanes	-	0.81	-	-
50	檀紫三烯 1,4-Hexadiene,3-ethenyl-2,5-dimethyl-	C ₁₀ H ₁₆	萜烯 Terpene	-	1.50	-	3.84

表1(续) Table 1(continued)

序号 No.	化合物 Components	分子式 Molecular formula	类型 Type	相对含量 The relative content/%			
				丁香梅 Dingao	东魁 Dongkui	晚稻杨梅 Wandao	荸荠种 Biqi
51	10,10-二甲基-2,6-二亚甲基双环[7.2.0]十一烷-5-醇 10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5β-ol	C ₁₅ H ₂₄ O	萜醇 Terpene alcohol	3.67	-	-	-
52	异戊二烯环氧化物 1,3b,6,6-Tetramethyldecahydro-1H-cyclopropa[7,8]azuleno[4,5-b]oxirene	C ₁₅ H ₂₄ O	萜烯 Terpene	3.05	-	-	-
53	[1S-(1a,4a,5a)]-4-甲基-1-(1-甲基乙基)二环[3.1.0]己烷-3-酮 4-methyl-1-(1-methylethyl)-[1S-(1a,4a,5a)]-Bicyclo[3.1.0]hexan-3-one	C ₁₀ H ₁₆ O	萜酮 Terpene ketone	-	0.69	-	-
54	香橙烯环氧化物 Alloaromadendrene oxide	C ₁₅ H ₂₄ O	萜醚 Terpene ether	-	0.85	-	-
55	α-毕澄茄醇 1-Naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-(1R,4S,4aR,8aR)-	C ₁₅ H ₂₆ O	萜醇 Terpene alcohol	-	1.65	-	0.39
56	2-异丙基-5-甲基-9-亚甲基[4.4.0]癸-1-烯 Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1-methyl-6-methylene-4-(1-methylethyl)-	C ₁₅ H ₂₄	萜烯 Terpene	-	3.88	4.14	-
57	β-葎草烯 1,5-Cycloundecadiene,1,4,4-trimethyl-8-methylene-,(1E,5E)-	C ₁₅ H ₂₄	萜烯 Terpene	1.82	-	-	-
58	β-人参烯 β-Panasiensene	C ₁₅ H ₂₄	萜烯 Terpene	-	-	2.88	-
59	1-乙烯基-1-甲基-4-亚甲基-2-(2-甲基丙-1-烯基)环庚烷 1-ethenyl-1-methyl-4-methylidene-2-(2-methylprop-1-enyl)cycloheptane	C ₁₅ H ₂₄	萜烯 Terpene	-	-	-	0.64
60	(-)反式-松香芹醇 Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-, (1S,3R,5S)-	C ₁₀ H ₁₆ O	萜酮 Terpene ketone	-	-	-	0.42
61	(+)-喇叭烯 (+)-Ledene	C ₁₅ H ₂₄	萜烯 Terpene	-	0.55	0.03	-
62	4-异丙-1,6-二甲萘 Cadalin	C ₁₅ H ₁₈	萜烯 Terpene	-	1.12	0.05	-
63	香叶芳樟醇 Geranyl linalool	C ₂₀ H ₃₄ O	萜醇 Terpene alcohol	0.11	-	-	-
64	6,10,14-三甲基-2-十五烷酮 2-Pentadecanone,6,10,14-trimethyl-	C ₁₈ H ₃₆ O	烷酮 Alkanone	0.13	0.14	0.39	0.19
65	棕榈醛 Hexadecanal	C ₁₆ H ₃₂ O	脂肪醛 Fatty aldehydes	0.43	-	-	-
66	棕榈酸 Palmitic acid	C ₁₆ H ₃₂ O	脂肪酸 Fatty acid	0.47	-	0.35	0.08
67	植醇 Phytol	C ₂₀ H ₄₀ O	脂肪醇 Fatty alcohol	0.38	-	0.53	0.40
68	2,2'-亚甲基双-(4-甲基-6-叔丁基苯酚) 2,2'-Methylenebis(6-tert-butyl-4-methylphenol)	C ₂₃ H ₃₂ O ₂	酚 Phenol	0.30	0.20	0.16	0.24

魁’和‘晚稻杨梅’品种中含量显著高于‘丁香梅’和‘荸荠种’,具体原因尚不可知。萜烯类化合物在不同品种杨梅叶精油中差异显著,可能与不同品种生长环境不同会引起单萜烯之间发生互变异构有关。萜醇类化合物是含量上仅次于萜烯类化合物的一类化合物,除在‘东魁’品种中含量仅2.8%以外,其他3个品种中含量均高于12.21%,在‘荸荠种’品种含量为23.47%,4个品种中主要成分均为橙花叔醇。萜醚类化合物主要由石竹烯氧化物和香橙烯氧化物组成,含量不高,为7.04%~10.53%,醚类氧化物一般由萜烯类化合物氧化而成,可能是萜烯类化合物接触空气、受热等原因引起氧化所致。杨梅叶精油中除萜烯类、萜醇类和醚类这3类主要化合物,还有微量的脂肪醛、萜酮、烷烃、脂肪醇等成分存在,这些化合物种类很少、含量很低,在杨梅叶精油特征香气的形成上有一定贡献。‘丁香梅’‘东魁’‘晚稻杨梅’‘荸荠种’4种杨梅叶精油均存在特有化合物,分别有7种

(11.46%)、6种(4.22%)、9种(14.76%)和5种(2.06%),杨梅叶精油间成分的差异性与萜烯类容易氧化、变构,以及与杨梅叶品种、栽培地区的地理环境、气候和收获季节等因素有关^[19]。

2.2 不同品种杨梅叶精油对肺癌A549细胞的增殖抑制作用分析

将‘丁香梅’‘东魁’‘晚稻杨梅’和‘荸荠种’杨梅叶精油分别按照梯度稀释的试验浓度,分别加入到各个试验孔中,在24、48、72、96、120 h 5个时间点时测定肺癌A549细胞存活率,结果见图2。

从图2中可以看出,肺癌A549细胞活性均随杨梅叶精油质量浓度的增大而减小,呈现剂量依赖性。杨梅叶精油在25 μg·mL⁻¹和50 μg·mL⁻¹的低质量浓度下,随着作用时间增加细胞存活率变化不显著($p > 0.05$),其他浓度下,随着作用时间的增加,细胞存活率均呈现明显下降趋势,说明杨梅叶精油对癌细胞的药效具有持续性,且存在加强的现象。

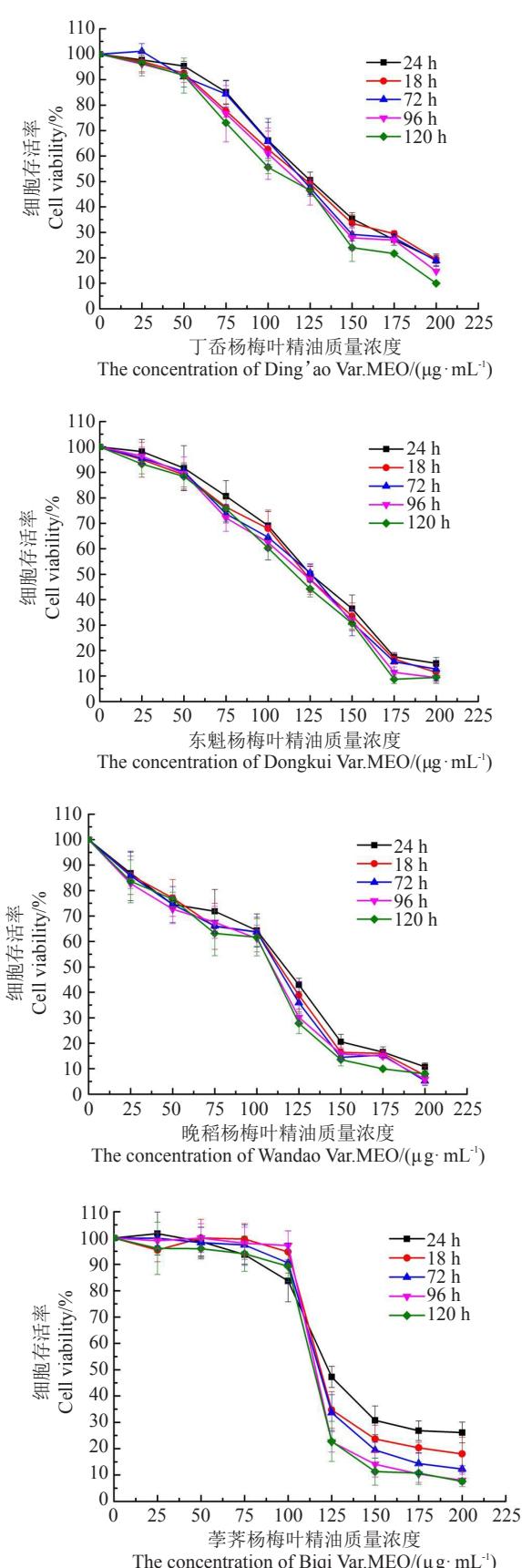


图 2 杨梅叶精油对肺癌 A549 细胞增殖抑制作用分析
 Fig. 2 The proliferation inhibitory effect on the lung cancer A549 cell of MEO from different varieties

以作用 24 h 为例,计算‘丁岙梅’‘东魁’‘晚稻杨梅’和‘荸荠种’杨梅叶精油对肺癌 A549 细胞的半数致死量 IC_{50} 分别为 127.24、127.14、118.03 和 123.62 $\mu\text{g}\cdot\text{mL}^{-1}$,而作用 48 h 的半数致死剂量 IC_{50} 分别为 124.47、126.87、113.61 和 118.40 $\mu\text{g}\cdot\text{mL}^{-1}$,作用 72、96 和 120 h 的半数致死剂量 IC_{50} 测定结果与作用 24 h 和 48 h 的结果类似,作用不同时间‘晚稻杨梅’杨梅叶精油对肺癌 A549 细胞的增殖抑制作用均略强于其他 3 种杨梅叶精油,但不存在显著差异。

3 讨 论

3.1 杨梅叶精油抗癌活性发挥的主要成分剖析

根据 GC-MS 分析结果,‘丁岙梅’‘东魁’‘晚稻杨梅’‘荸荠种’4 个品种杨梅叶精油中包含萜烯类(52.42%~74.13%)、萜醇类(2.8%~23.47%)、萜醚氧化物(7.04%~10.53%)等主要成分和脂肪醛(1.43%~7.48%)、萜酮(0~0.51%)、烷烃(0~1.26%)、脂肪醇(0.1%~1.12%)等微量成分,在抗癌活性发挥上,脂肪醛、萜酮、烷烃、脂肪醇等微量成分因含量低无法有效发挥作用,肺癌 A549 细胞增殖抑制活性应以萜烯、萜醇和萜醚氧化物为主。杨梅叶精油中含量最高的 2 种萜烯类化合物为同分异构体的 β -石竹烯和 α -葎草烯,在 4 种杨梅叶精油中占 32.12%~44.47%。富含 α -葎草烯和 β -石竹烯的植物精油抑制癌细胞生长的报道很多^[20~22]。Amiel 等^[23]从细胞角度和分子水平等研究了 β -石竹烯对鼠淋巴瘤细胞(BS-24-1)和 EB 病毒转化人 B 淋巴细胞(MoFir)的抗癌作用,结果发现 9.6 $\mu\text{mol}\cdot\text{L}^{-1}$ β -石竹烯引起肿瘤细胞 DNA 碎片化,且激活 caspase-3 活性引起肿瘤细胞系凋亡发挥抗癌作用,此结果与 Ghelardini 等^[24]和 Kubo 等^[25]的研究结果相似,说明 β -石竹烯抗肿瘤的效果已得到广大研究者的认可。 β -石竹烯作为植物天然产物,具有被用于预防肿瘤发展的膳食补充剂的潜力^[26]。 α -葎草烯衍生物对小鼠 P-338 淋巴瘤、人肺 A-549 细胞癌、HT-29 结肠癌和 MEL-28 黑色素瘤均有防治作用^[27]。橙花叔醇,是除 β -石竹烯和 α -葎草烯以外的第三大组分,在抑制癌细胞生长方面尚未见报道。值得关注的还有含量较高的 β -广藿香烯和石竹烯氧化物,Park 等^[28]报道了石竹烯氧化物对人类前列腺癌和乳腺癌细胞具显著的细胞增殖抑制和诱导凋亡的作用。 β -广藿香烯常用于中药炎症性疾病治疗^[29~30],Liu 等^[31]报道了 β -广藿香烯对人肝细胞癌细胞系

HepPG-2、乳腺癌细胞系4T1、结肠直肠癌细胞系HCT116、结肠癌细胞系CT-26、急性骨髓性白血病细胞系MV4-11和人肺癌细胞系A549和NCI-H1975的抗增殖活性。‘晚稻杨梅’杨梅叶精油对人肺癌A549细胞的增殖抑制作用优于‘丁香梅’‘东魁’和‘荸荠种’3种精油,可能与‘晚稻杨梅’精油中存在特有的 β -广藿香烯(含量占2.58%)有关。

3.2 杨梅叶精油生物活性分析与应用潜力

根据MTT实验结果,发现‘丁香梅’‘东魁’‘晚稻杨梅’‘荸荠种’4个品种杨梅叶精油在50~200 $\mu\text{g}\cdot\text{mL}^{-1}$ 质量浓度下对肺癌A549细胞具有较强的增殖抑制作用,且存在时间和剂量依赖性,与前期杨梅叶精油对人结肠和回盲肠腺癌细胞系的作用效果相似,不同细胞系之间存在差异,对肠系癌细胞的增殖抑制效果略优于对肺癌细胞,其抗癌效果可能与半胱氨酸蛋白酶家族显著增加诱导细胞凋亡有关。杨梅叶精油除抗癌作用以外,另有抗氧化^[32]、抗菌^[33]、消炎^[34]等报道,因为杨梅为中国特色经济作物,国外研究报道较少。作为主要成分的 β -石竹烯、 α -葎草烯、橙花叔醇和 β -广藿香烯等除抗癌活性外,有诸多抗焦虑、抗菌、消炎和抗肿瘤作用等生物活性的报道^[35-36]。杨梅叶精油是杨梅植株上常绿叶片中的次级代谢产物,具有潜在的抗癌、抑菌、消炎等药物开发前景,因其组成成分的复杂性其作用机制的研究较少,有待于进一步深入。

4 结 论

‘丁香梅’‘东魁’‘晚稻杨梅’‘荸荠种’4种杨梅叶精油经GC-MS分析,共鉴定出68种成分,主要由萜烯、萜醇和醚类组成, β -石竹烯和 α -葎草烯是主要成分,占总成分含量的32.12%~44.47%。杨梅叶精油组成成分的差异引起对肺癌A549细胞增殖抑制活性的差异,相较而言,‘晚稻杨梅’杨梅叶精油优于其他3种。

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