佳木斯地区引种薰衣草抗菌活性研究

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摘要 采用气相色谱 - 质谱(GC - MS)联用仪,检测分析由索氏提取法获取的引种在佳木斯地区的薰衣草的茎叶精油,并结合生长速率法评价其体外抗菌活性。薰衣草的茎叶精油中最常见的成分有34种,芳樟醇(16.66%)、乙酸芳樟酯(12.01%)、龙脑(8.67%)、石竹烯氧化物(7.72%)和T - 杜松醇(5.61%)是其中的主要成分,5 - 甲基 - 2 - (1 - 甲基乙烯基) - 4 - 己烯 - 1 - 醇乙酸酯(5.77%)、5 - 甲基 - 2 - (1 - 甲基乙烯基) - 4 - 己烯 - 1 - 醇(4.45%)、α - 檀香醇(3.80%)和 α - 松油醇(3.65%)的含量次之。抗菌活性试验结果表明,薰衣草的茎叶提取物与百菌清和丁香酚对6种病原真菌均有显著的抗菌效果,可进一步研究薰衣草的茎叶提取物的抗菌作用机理,以替代化学农药用于防治真菌等病害来促进果蔬植物的生长和延长果蔬的储存时间。

关键词 薰衣草;GC - MS;抗菌活性;佳木斯地区
中图分类号 S - 3　**文献标识码** A　**文章编号** 0517 - 6611(2018)08 - 0012 - 03

Study on the Antibacterial Activities of *Lavandula angustifolia* in Jiamusi

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Abstract The essential oils from dried stem leaf of *Lavandula angustifolia* introduced in Jiamusi, obtained by soxhlet extraction was analyzed by GC-MS and evaluated by the growth rate method for *in vitro* antimicrobial activity. There were 34 common components usually found in *Lavandula angustifolia* stem leaf essential oils. The major constituents were linalool (16.66%), linalyl acetate (12.01%), endo-Borneol (8.67%), caryophyllene oxide (7.72%) and tau-Cadinol (5.61%). Other constituents present in fairly good amounts were 5-methyl-2-(1-methylethenyl) -4-hexene-1-alcohol acetate (5.77%), 5-methyl-2-(1-methylethenyl)-4-hexene -1-alcohol (4.45%), alpha-santalol (3.80%) and alpha-terpineol (3.65%). The antibacterial activity test results showed that the extraction of *Lavandula angustifolia* stem leaf and chlorothalonil and eugenol had remarkable antibacterial effect on the six pathogenic fungi, however ,further research was required to evaluate the antibacterial mechanism of *Lavandula angustifolia* stem leaf extraction to replace chemical pesticides used in the prevention and treatment of diseases to promote the growth of fruit and vegetable plants and lengthen the storage time of fruits and vegetables.

Key words *Lavandula angustifolia*; GC-MS; Antibacterial activities; Jiamusi area

近年来薰衣草提取的精油因其显著的抗菌性能和广泛的抗菌范围引起研究者的热议[1]。薰衣草精油是一种无毒、高效的天然抗菌剂[2,3],不仅能够很好地弥补传统的化学抗菌剂毒性大、污染环境和药物残留的缺点,而且还有可能成为一种潜在的抗菌剂应用于各种果蔬产品。尽管薰衣草精油已经被许多专家探究多年,但是主要是有关薰衣草精油提取的工艺优化、化学成分的鉴定和抗细菌活性的研究[4-5],然而关于薰衣草精油的抗真菌活性研究很少。因此,探究薰衣草精油在抗真菌方面的性能是非常有意义的工作:一方面能够发挥其无毒、无害和无残留的优良特性;另一方面实现其作为新型天然抗真菌剂的应用潜能,这将为以后实现工业化和实际应用提供理论指导和技术支持[3]。因传统获取薰衣草精油的方式(如超临界二氧化碳萃取法等)存在收率低和成本高的缺点,而且得到的精油易挥发和不宜保存[6],故拟采用超声波辅助乙醇溶液提取佳木斯地区引种的薰衣草的茎叶成分的方法,同时联合气相色谱 - 质谱(GC - MS)技术鉴定和分析其提取的精油的化学结构成分[7],通过抗菌试验证实薰衣草的茎叶提取物可作为一种植物源抗菌剂以控制真菌等病害,也为相关的无毒、无污染和无残留的抗菌剂的研制开发奠定理论基础[8]。

1 材料与方法

1.1 试验材料 薰衣草种子在2016年春种植于佳木斯地区,于第2年采摘新鲜的薰衣草茎叶,阴干,粉碎,过50目筛。供试菌种由吉林农业大学农学院植物病理研究所馈赠。

1.2 试验方法

1.2.1 薰衣草茎叶精油的提取方法。索氏提取器中用乙醇提取干燥的薰衣草的茎叶粉末(5.00 g)8 h,提取精油期间控制温度为40 ~ 60 ℃,获得金黄色、具有特殊香味的精油,经真空干燥后密封储存于4 ~ 6 ℃冰箱。

1.2.2 精油成分分析。气相色谱条件:HP - 5毛细管柱(60 m × 250 μm × 0.25 μm);载气为He;流量为1 mL/min。程序升温条件:初始温度30 ℃保持10 min,以5 ℃/min升温至320 ℃,保持10 min。进样量1.0 μL。进样口温度280 ℃,分流比为25∶1,溶剂延迟时间3 min。

质谱条件:电离方式为EI;电子能量70 eV;离子源温度230 ℃;四级杆温度150 ℃;扫描范围40 ~ 450 m/z,其扫描结果的数据采用NIST数据库进行比对检索。

1.2.3 薰衣草茎叶提取物的制备。称取不同份数干燥的薰衣草的茎叶粉末(总量1 000 g)于干净的烧杯中,同时分批添加总体积15 L质量分数为70%乙醇溶液,静置浸泡24 h后,分别进行超声振荡(温度60 ℃、提取40 min)后过滤。重复3次上述操作。获取的全部浸提液用旋转蒸发仪减压浓缩。所得产品进行干燥、称重,并计算产率。

1.2.4 抗菌试验。分别称取适量、干燥的薰衣草的茎叶提

基金项目 黑龙江省自然科学基金项目(H2016087);黑龙江省大学生科技创新项目(201710222059);佳木斯大学国家级培育项目(JMSUJCGP2016 - 002)。
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收稿日期 2017 - 12 - 31

取物、百菌清和丁香酚,依次加入 400 mL 配制的马铃薯培养基中制成带药的培养基,获得不同浓度薰衣草的茎叶提取物培养基(100.00、50.00、25.00、12.50 和 6.25 mg/mL)、百菌清培养基(20.00、10.00、5.00、2.50 和 1.25 mg/mL)和丁香酚培养基(0.250 00%、0.125 00%、0.062 50%、0.031 25% 和 0.015 63%)依次分装入锥形瓶中,包扎后灭菌。在无菌的环境下,将含有药物的培养基倒入培养皿中,制作成平板,用打孔器制取 6 种培养状态良好的供试真菌菌饼(直径 6 mm)放入各个平板中央,于生化培养箱中恒温 29 ℃培养 96 h。每种菌平行重复 3 皿,百菌清和丁香酚培养基为阳性对照组。观察并记录试验组与对照组菌落直径,十字交叉测菌落直径,结果取 3 次重复试验的平均值。通过生长速率法的原理观察真菌生长状态,并记录其抑菌率(%)和最小抑菌浓度(MIC)。

2 结果与分析

2.1 精油成分分析 薰衣草的茎叶精油中各化学成分的含量根据峰面积归一化法确定,采用标准的 NIST 数据库检索总离子流图中各峰的质谱图,并筛选出质谱匹配度较高的成分,依据 NIST 标准库检索其中鉴定出 34 个化学成分,占总含量的 94.24%。检测结果见图 1 和表 1。

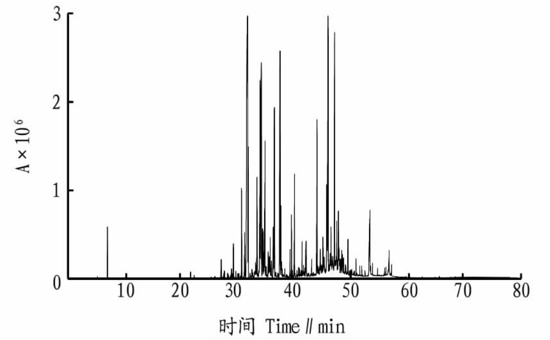

图 1 薰衣草茎叶精油 GC-MS 的总离子图

Fig. 1 GC-MS total ion chromatogram for essential oil of *Lavandula angustifolia* stem leaf

表 1 薰衣草茎叶精油的主要成分及含量

Table 1 Main chemical constituents and content of essential oil of *Lavandula angustifolia* stem leaf

序号 No.	化学成分 Components	分子式 Molecular formula	相对含量 Relative contents∥%
1	2-甲氧基-2-甲基丙烷(2-methoxy-2-methyl- Propane)	$C_5H_{12}O$	0.66
2	蘑菇醇(1-Octen-3-ol)	$C_8H_{16}O$	0.31
3	辛烷(Eucalyptol)	$C_{10}H_{18}O$	0.51
4	芳樟醇(3,7-dimethyl- 1,6-Octadien-3-ol)	$C_{10}H_{18}O$	16.66
5	异丁酸叶醇酯(cis-3-Hexenyl iso-butyrate)	$C_{10}H_{18}O_2$	1.92
6	异松香芹醇(Isopinocarveol)	$C_{10}H_{16}O$	0.33
7	樟脑[(+)-2-Bornanone]	$C_{10}H_{16}O$	1.76
8	5-甲基-2-(1-甲基乙烯基)-4-己烯-1-醇[5-methyl-2-(1-methylethenyl)-4-Hexen-1-ol]	$C_{10}H_{18}O$	4.45
9	龙脑(endo-Borneol)	$C_{10}H_{18}O$	8.67
10	2-乙烯基-1,3,3-三甲基环己烯(2-ethenyl-1,3,3-trimethyl- Cyclohexene)	$C_{11}H_{18}$	1.77
11	α-4-三甲基苯甲醇(alpha.,4-trimethyl- Benzenemethanol)	$C_{10}H_{14}O$	0.54
12	α-松油醇(alpha-Terpineol)	$C_{10}H_{18}O$	3.65
13	(-)-桃金娘烯醇((-)-Myrtenol)	$C_{10}H_{16}O$	0.36
14	顺式-马鞭草烯醇(cis-Verbenol)	$C_{10}H_{16}O$	0.47
15	乙酸龙脑酯(Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester)	$C_{12}H_{20}O_2$	0.38
16	2-甲基-3-苯基丙醛(2-methyl-3-phenyl- Propanal)	$C_{10}H_{12}O$	0.78
17	乙酸芳樟酯(Linalyl acetate)	$C_{12}H_{20}O_2$	12.01
18	5-甲基-2-(1-甲基乙烯基)-4-己烯-1-醇乙酸酯[5-methyl-2-(1-methylethenyl)-4-Hexen-1-ol, acetate]	$C_{12}H_{20}O_2$	5.77
19	对异丙基苯甲醇(p-Cymen-7-ol)	$C_{10}H_{14}O$	0.20
20	橙花乙酸酯((Z)-3,7-dimethyl-2,6-Octadien-1-ol, acetate)	$C_{12}H_{20}O_2$	2.41
21	α-依兰烯(alpha-ylangene)	$C_{15}H_{24}$	0.22
22	香豆素(Coumarin)	$C_9H_6O_2$	0.83
23	紫罗兰酮[4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- 3-Buten-2-one]	$C13H_{20}O$	0.32
24	α-檀香醇(alpha-Santalol)	$C_{15}H_{24}O$	3.80
25	β-檀香醇(beta-Santalol)	$C_{15}H_{24}O$	2.93
26	人参炔醇(Falcarinol)	$C_{17}H_{24}O$	0.20
27	石竹烯氧化物(Caryophyllene oxide)	$C_{15}H_{24}O$	7.72
28	二十二碳六烯酸(Doconexent)	$C_{22}H_{32}O_2$	1.78
29	二十碳五烯酸(cis-5,8,11,14,17-Eicosapentaenoic acid)	$C_{20}H_{30}O_2$	0.85
30	T-杜松醇(tau-Cadinol)	$C_{15}H_{26}O$	5.61
31	α-红没药烯(cis-Z-.alpha.-Bisabolene epoxide)	$C_{15}H_{24}O$	2.59
32	6,10,14-三甲基-2-十五烷酮(6,10,14-trimethyl-2-Pentadecanone)	$C_{18}H_{36}O$	0.23
33	十六烷酸(n-Hexadecanoic acid)	$C_{16}H_{32}O_2$	2.40
34	9,12,15-亚麻酸((Z,Z,Z)-9,12,15-Octadecatrienoic acid)	$C_{18}H_{30}O_2$	1.15

由表 1 可知,佳木斯地区引种的薰衣草的茎叶精油中组分含量较多的是醇类、酯类和萜类。鉴定的成分中含量最高的是芳樟醇,占 16.66%。其他依次为 12.01% 的乙酸芳樟酯、8.67% 的龙脑、7.72% 的石竹烯氧化物和 5.61% 的 T-杜松醇。由于芳樟醇、乙酸芳樟酯、龙脑、α-松油醇和香豆素等均有相似的抑菌效果,故薰衣草的茎叶可作为上述抗菌成分的提取来源。

2.2 抗菌试验结果 抗菌试验结果表明,薰衣草的茎叶提

取物(MIC=50 mg/mL)抑菌效果最显著的菌种分别是辣椒疫病菌、黄瓜枯萎病菌和茄子褐纹病菌;而百菌清(MIC = 2.5 mg/mL)对辣椒炭疽病菌和番茄灰霉病菌有明显抑制作用;丁香酚(MIC = 0.312 5‰)对辣椒疫病菌和番茄灰霉病菌有显著抗菌作用。根据抑菌率可看出,薰衣草的茎叶提取物、百菌清和丁香酚对 6 种病原真菌均有显著的抑制作用。百菌清是一种广谱、保护性杀菌剂;丁香酚主要是从丁香花、辛夷花等香草类植物中提取的成分,因其杀菌力强且具有防腐作用,常用作杀菌剂和防腐剂。因此,与两者相比,薰衣草的茎叶提取物可考虑作为一种杀菌剂用以防治真菌等病害。结果见表 2 ~ 4。

表 2 不同浓度的薰衣草茎叶提取物对病原真菌的生长抑制作用及最小抑菌浓度

Table 2 Growth inhibition effect of plant pathogens and minimum inhibitory concentration of *Lavandula angustifolia* stem leaf extraction

菌种 Strain	100.00 mg/mL		50.00 mg/mL		25.00 mg/mL		12.50 mg/mL		6.25 mg/mL		0 mg/mL 时生长直径 Growth diameter mm	MIC mg/mL
	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %		
辣椒炭疽病菌 *Colletotrichum capsici*	0	100	1.97	93.47	10.58	64.91	9.02	21.13	13.46	16.69	30.15	100
辣椒疫病菌 *Phytophthora capsici*	0	100	0	100	0.73	95.73	3.08	81.99	6.07	64.50	17.10	50
黄瓜枯萎病菌 *Fusarium oxysporiumf*. sp. *cucumeris*	0	100	0	100	5.79	80.24	8.05	72.53	19.42	33.72	29.30	50
茄子褐纹病菌 *Phomopsis vexans*	0	100	0	100	6.33	79.83	13.07	58.35	18.82	40.03	31.38	50
番茄灰霉病菌 *Botrytis cinerea*	0	100	1.95	93.46	8.00	73.18	18.90	36.64	28.37	4.89	29.83	100
番茄晚疫病菌 *Phytophthora infestans*	0	100	2.29	76.69	6.76	64.13	16.61	36.44	22.27	5.61	23.52	100

表 3 不同浓度的百菌清对病原真菌的生长抑制作用及最小抑菌浓度

Table 3 Growth inhibition effect of plant pathogens and minimum inhibitory concentration of chlorothalonil

菌种 Strain	20.00 mg/mL		10.00 mg/mL		5.00 mg/mL		2.50 mg/mL		1.25 mg/mL		0 mg/mL 时生长直径 Growth diameter mm	MIC mg/mL
	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %		
辣椒炭疽病菌 *Colletotrichum capsici*	0	100	0	100	0	100	0	100	13.16	56.35	30.15	2.5
辣椒疫病菌 *Phytophthora capsici*	0	100	2.64	84.56	4.89	71.40	9.82	42.57	11.52	32.63	17.10	20.0
黄瓜枯萎病菌 *Fusarium oxysporiumf*. sp. *cucumeris*	0	100	0	100	2.83	90.34	3.70	87.37	12.47	57.44	29.30	10.0
茄子褐纹病菌 *Phomopsis vexans*	0	100	0	100	3.21	89.77	5.24	83.33	8.15	74.03	31.38	10.0
番茄灰霉病菌 *Botrytis cinerea*	0	100	0	100	0	100	0	100	1.69	94.33	29.83	2.5
番茄晚疫病菌 *Phytophthora infestans*	0	100	0	100	8.07	65.69	9.46	59.78	11.39	51.57	23.52	10.0

表 4 不同浓度的丁香酚对病原真菌的生长抑制作用及最小抑菌浓度

Table 4 Growth inhibition effect of plant pathogens and minimum inhibitory concentration of eugenol

菌种 Strain	0.250 00 mg/mL		0.125 00 mg/mL		0.062 50 mg/mL		0.031 25 mg/mL		0.015 63 mg/mL		0 mg/mL 时生长直径 Growth diameter mm	MIC ‰
	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %	生长直径 Growth diameter mm	抑制率 Inhibition rate %		
辣椒炭疽病菌 *Colletotrichum capsici*	0	100	0	100	0	100	3.90	89.21	6.61	81.72	30.15	0.062 50
辣椒疫病菌 *Phytophthora capsici*	0	100	0	100	0	100	0	100	8.19	64.55	17.10	0.031 25
黄瓜枯萎病菌 *Fusarium oxysporiumf*. sp. *cucumeris*	0	100	0	100	0	100	8.99	74.53	28.60	13.31	29.30	0.062 50
茄子褐纹病菌 *Phomopsis vexans*	0	100	0	100	0	100	10.83	71.03	21.94	38.63	31.38	0.062 50
番茄灰霉病菌 *Botrytis cinerea*	0	100	0	100	0	100	0	100	8.76	75.55	29.83	0.031 25
番茄晚疫病菌 *Phytophthora infestans*	0	100	5.25	77.68	6.32	73.13	6.58	72.02	22.33	5.06	23.52	0.250 00

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3　结论

以佳木斯地区引种的薰衣草的茎叶为试验材料，探究其含有的各成分的化学结构、含量以及抗菌活性。GC – MS 检测数据分析显示，薰衣草的茎叶提取的精油中大多数有效成分具有抑菌作用。试验表明，薰衣草的茎叶提取物对植物的 6 种病原真菌存在显著的抗菌效果。虽然薰衣草的茎叶提取物与百菌清和丁香酚的抑菌效果存在差异，但可以解释为薰衣草茎叶的提取物仅有一部分活性成分发挥作用，因此，其最小抑菌浓度比其他 2 种药物更低。故薰衣草的茎叶提取物中的抗菌活性成分被分离和纯化后将展现出更强的抗菌能力，并为筛选高效的植物病原菌抗菌剂替代高毒、易残留的农药奠定基础和合理利用薰衣草资源提供科学的理论依据。

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科技论文写作规范——引言

扼要地概述研究工作的目的、范围、相关领域的前人工作和知识空白、理论基础和分析、研究设想、研究方法和实验设计、预期结果和意义等。一般文字不宜太长，不需做详尽的文献综述。在最后引出文章的目的及试验设计等。“引言”两字省略。