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CHEMICAL COMPOSITION AND CYTOTOXIC ACTIVITY OF THE ESSENTIAL OILS OF *CYMBOPOGON CITRATUS* L. GROWN IN PHU THO PROVINCE

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SUMMARY

Culms and leaves of *Cymbopogon citratus* L. were collected from two regions of Phu Tho province (Thanh Son and Phu Ninh) and used as materials for essential oil extraction. Oils obtained were steam-distilled, analyzed for chemical composition and evaluated for cytotoxic activity against three different cancer cell lines. The GC/MS analysis showed that citral is the major content of the steam-distilled essential oils which was found in the range of 64.15-76.22%. Camphene was found only in culm oils of both regions but it was not detected in the leaf oils. Interestingly, the isomer forms of ocimene present at higher content in the culm oils than in the leaf oils whereas myrcene content in the leaf oils is higher than that in the culm oils. In a cytotoxicity test, four essential oils of culms and leaves of *C. citratus* from Thanh Son and Phu Ninh showed potent activity against A549 (human lung carcinoma) cell line with the IC_{50} values ranging from 4.01±0.39 to 6.3±0.54 µg/ml. The essential oils (culms and leaves) from Phu Ninh exhibited moderate effects on the Hela (human cervical adenocarcinoma) cells with the IC_{50} values of 19.43±1.16 and 42±2.41 µg/ml, respectively. However, they were inactive against the human hepatocellular carcinoma Hep3B cell line. The essential oils from Thanh Son exhibited potent cytotoxic activity against Hela and Hep3B cell lines with the IC_{50} values ranging from 1.18±0.26 to 8.91±0.32 µg/ml. The results indicated that the essential oils of *C. citratus* from Thanh Son, Phu Tho could be considered as a promising candidate for the natural sources of anticancer agents.

Keywords: *Cymbopogon citratus* L.*, essential oil, chemical composition, cytotoxic activity*

INTRODUCTION

Aromatic grasses have been used in traditional medicine for the treatment of many diseases since ancient times. Lemongrass, *Cymbopogon citratus* L., belonging to the genus *Cymbopogon* (Gramineae) is known to be one of the main sources for essential oil extraction. This species has been used in the treatment of gastrointestinal disturbance and in the perfume industry (Loi, 2006). It has been reported to exert various pharmacological activities such as antiplasmodial, anti-inflammation and anti-cancer (Akhila, 2010). The activity of the plant can be referred to the present of essential oils such as citral, myrcene, and geraniol. Citral, a natural mixture of two geometrical isomeric acrylic monoterpenes geranial (*trans*-citral) and neral (*cis*-citral), is the major content of essential oils of *C. citratus*, other major terpenes vary to some extent. Myrcene is also reported as a major constituent of the *C. citratus* oils. It presents at high content in the oils of of *C. citratus* Stapf grown in Zambia (18%) and Nigeria (25.3%). Lemongrass oil has also been reported to have antimicrobial (Chalchat *et al*., 1997; Handique, Singh, 1990), insecticidal (Sukari, 1992), insect repellant activity (Ansari and Razdan, 1995), and found to have cytotoxic properties on some cancer cell lines such as human epidemic (HaCat) (Koba *et al*., 2009), Chinese Hamster Ovary (CHO) (Kpoviessi *et al*., 2014), and P388 leukemia cells (Dubey *et al*., 1997). A study of Bidinotto demonstrated that the essential oil from lemon grass presented a protective role against early

MNU-induced mammary gland alterations in BALB/c mice (Bidinotto *et al*., 2011). In Vietnam, there have been few papers describing the chemical composition and biological activity of lemongrass essential oils (Dieu, 2010).

In this study, the chemical composition and the cytotoxic activity of hydro-distilled essential oils of *C. citratus* collected in Thanh Son and Phu Ninh, Phu Tho were investigated. Essential oils from the two different parts of *C. citratus* culms and leaves were prepared, analysed by GC/MS, and evaluated for the cytotoxic activity against A549, Hela, and Hep3B cell lines.

MATERIALS AND METHODS

Materials and essential oil isolation

The lemongrasses (*C. citratus*) were freshly collected in two regions Thanh Son and Phu Ninh, Phu Tho province in Spring, 2016. The leaves and culms of each sample (500 g each) were hydro-distilled in a Clevenger-type apparatus for 4 h, after which the essential oils were separated, dried with anhydrous Na_2SO_4. The obtained oils were stored at -5°C until used.

GC/MS analysis of essential oils

Essential oil analysis: GC/MS analysis was performed using a Agilent GC7890A apparatus coupled to a Mass Selective Detector (Agilent 5976C). A HP-5MS fused silica capillary column (60 m × 0.25 mm id. × 0.25 µm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 240 ^oC and the oven temperature program was as follows: 60 ^oC to 220 ^oC at 4 ^oC/min, and then at 20 ^oC/min to 240 ^oC with an inter-phase temperature of 280 ^oC. The split injection mode was 1:142, the detector temperature 270 ^oC, and the injection volume 0.1 µL. The MS interface temperature was 270 ^oC, MSs mode, E.I. detector voltage 1300 V, and mass range 40-400 Da at 1.0 scan/s. Identification of components was achieved based on their retention indices, and by comparison of their mass spectral fragmentation patterns with those stored on the MS library (NIST08, Wiley09). Component relative contents were calculated based on total ion current without standardization. Data processing was MassFinder4.0.

Cell lines and cell culture

The cancer cell lines A549 (human lung carcinoma), Hela (human cervical adenocarcinoma), and Hep3B (human hepatocellular carcinoma) were kindly provided by Prof. Jeong-Hyung Lee, Department of Biochemistry, College of Natural Sciences, Kangwon National University, Korea. The cells were cultured at 37 °C in either RMPI1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin in a 5% CO_2 incubator. Cells between 5 and 20 passages were used for the assays.

Cell Proliferation Assay

An MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide] assay was used to measure the viability of cells after extract treatment. Cells were seeded in 96-well plates at a density of 2.0×10^5 cells/ml. Cells were treated with various concentrations of essential oils (1, 3, 10, 30 and 100 µg/ml) and then incubated at 37°C for 48 h. Cells were subsequently incubated at 37°C with MTT (0.5 mg/ml) for 4 h. After removal of supernatant, formazan crystals were dissolved in isopropanol and the optical density was measured at 570 nm (Binh *et al*., 2015). Etoposide was used as a positive control.

Statistics

Results were given as the mean standard error for the mean (SEM). IC_{50} values and statistical analyses were performed with GraphPad Prism Software.

RESULTS AND DISCUSSION

Essential oils of *C. citratus* including TSC (culms collected in Thanh Son), TSL (leaves collected in Thanh Son), PNC (culms collected in Phu Ninh), and PNL (leaves collected in Phu Ninh) were obtained in yields of 0.62%, 0.71%, 0.81%, and 0.65%, respectively (Table 1). Chemical analysis by GC/MS showed that monoterpenes and sesquiterpenes are the major chemical groups of four essential oils. However, the contents among the four hydrodistilled oils vary greatly. The number of compounds found in PNL (37 compounds) is doubled in comparison with those found in TSC (17 compounds). Interestingly, camphene was found only in the culm oils but not in the leaf oils. Mycene appears to have higher content in the leaf oils than those in the culm oils. In contrast, citronellol and the isomers of ocimene, present at significantly higher content in the culms than in the leaves. Citral (neral and geranial) was identified as the most abundance component in all essential oils which

accounts from 64.15 to 76.22% (Table 1). This composition accounts in the same range with those of the previous reports (Blanco *et al*., 2009; Nonviho *et al*., 2010).

Table 1. Chemical composition and yield of *C. citratus* essential oils collected in Phu Tho province. [a] Yield calculated based on the fresh materials; RI: retention index; TSC: culm oil collected in Thanh Son; TSL: leaf oil collected in Thanh Son, PNC: culm oil collected in Phu Ninh; PNL: leaf oil collected in Phu Ninh.

Components	**(RI)**	**TSC (%)**	**TSL (%)**	**PNC (%)**	**PNL (%)**
α-Pinene	936	-	-	0.16	-
Camphene	953	0.18	-	0.33	-
6-Methylhept-5-en-2-one	984	0.86	1.47	1.00	1.37
Myrcene	989	6.15	10.70	3.59	7.59
dehydro-1,8-Cineole	993	0.27	0.28	0.14	-
Limonene	1031	-	-	0.11	-
(*Z*)-β-Ocimene	1035	2.18	0.85	2.65	0.73
(*E*)-β-Ocimene	1046	1.04	0.49	1.09	0.38
Linalool	1100	1.33	1.30	1.37	0.89
Lavandulol	1144	-	0.25	0.24	0.34
Citronellal	1152	0.28	0.17	0.51	-
Chrysanthemol trans	1154	-	0.15	-	-
Isoneral	1164	1.13	0.90	0.90	0.95
Mentha-1,5-dien-8-ol	1172	-	0.24	-	-
Isogeranial	1181	1.93	1.65	1.53	1.59
α-Terpineol	1197	-	-	0.12	-
Citronellol	1227	1.25	0.70	2.33	1.01
Nerol	1230	0.47	0.24	1.45	0.58
Neral	1245	33.62	32.42	28.92	34.25
Geraniol	1254	3.33	3.72	5.17	3.66
Geranial	1274	42.53	40.09	35.23	41.97
Methyl geranate	1323	-	-	0.42	-
Geranic acid	1353	-	-	0.43	-
Geranyl acetate	1380	0.76	1.14	0.23	0.60
(*E*)-Caryophylene	1433	-	0.24	0.57	0.35
α-*trans*-Bergamotene	1441	-	0.13	0.33	-
cis-Dihydroagarofuran	1522	-	-	0.20	-
D-Cadinene	1531	-	-	0.54	-
Caryophyllene oxide	1598	-	-	0.41	0.42
5-epi-7-epi-α-Eudesmol	1617	-	-	0.77	0.29
Selin-11-en-4-a-ol	1633	-	-	3.24	1.39
Eudesmol	1635	-	-	0.63	0.30
Valerianol	1647	-	-	0.47	-
epi-α-Cadinol	1651	-	-	0.32	-
epi-α-Muurolol	1652	0.27	0.32	0.32	-
Hinesol	1656	-	-	0.29	-
α-Cadinol	1665	-	-	1.37	0.59
(*E,E*)-Farnesol	1717	-	-	0.13	-
(*E,Z*)-Farnesol	1738	-	-	0.15	-
Total identified (%)		98.99	98.82	98.26	99.25
Yield[a] (%)		0.62 ± 0.04	0.71 ± 0.03	0.81 ± 0.04	0.65 ± 0.02

Table 2. Cytotoxic activity of *C. citratus* essential oils collected in Phu Tho province. [a] Experiments were carried out in triplicated; [b] positive control.

Samples	IC_{50}[a](µg/ml)		
	A549	**Hela**	**Hep3B**
TSC	5.07 ± 0.52	8.91 ± 0.32	5.2 4± 0.29
TSL	6.3 ± 0.54	1.18 ± .26	5.37 ± 0.31
PNC	4.01 ± 0.39	19.43 ± 1.16	>100
PNL	4.2 ± 0.21	42 ± 2.41	>100
Etoposide[b] (µM)	2.68 ± 0.89	3.29 ± 0.05	17.53 ± 1.12

All the essential oils were evaluated for the cytotoxic activity against three different cell lines including A549 (human lung carcinoma), Hela (human cervical adenocarcinoma), and Hep3B (human hepatocellular carcinoma). Remarkably, all the *C. citratus* oils had the cytotoxic effects on at least two tested cell lines. The tested oils showed potent cytotoxic activity against A549 cells with the IC_{50} ranging from 4.01±0.39 to 6.3±0.54 µg/ml (Table 2). The essential oils (culms and leaves) from Phu Ninh also exhibited moderate effects on the Hela cells with the IC_{50} values of 19.43±1.16 and 42±2.41 µg/ml, respectively. However, they were inactive against the hepatocellular carcinoma Hep3B cell line. The essential oils from Thanh Son displayed potent cytotoxic effect on all tested cell lines with the IC_{50} values ranging from 1.18±0.26 to 8.91±0.32 µg/ml. The difference on IC_{50} value may be due to the difference in the origin of sample and the composition of the essential oils.

Recent studies demonstrated that the main component, citral, played an essential role in determining the cytotoxic activity of lemongrass oils. Citral was reported to induce the apoptosis in tumor cell lines by activating the procaspase-3 in several cancer cell lines (Dudai *et al*., 2005). Citral dose and time-dependently induced the total and pi-class-specific activities of glutathione S-transferase (GST) in mouse skin. GST played a role the cellular detoxification of oxidative damaging, genotoxic, and carcinogenic chemicals. The results suggested for the use of citral for skin cancer prevention (Nakamura *et al*., 2003). In an in vivo study, oral treatment with lemongrass (*C. citratus* Stapf) essential oil in female Balb/C mice administered with N-methyl-N-nitrosurea (MNU) lead to the prevention effect of this oil on the leukocyte DNA damage and carcinogenesis (Bidinotto *et al*., 2011). A further study on the mechanism of actions of the *C. citratus* essential oils on tested cancer cell lines should be performed.

CONCLUSIONS

The chemical composition analysis of the culm and leaf of *Cymbopogon citratus* essential oils from two regions Thanh Son and Phu Ninh showed the similarity on the major content. However, some compositions such as myrcene, isomers of ocimene, and camphene can be the indicators to differentiate the culm and leaf part. For the first time the cytotoxic effect of these essential oils on A549, Hela, and Hep3B cell lines were reported. The results indicated that the oils from Thanh Son region exhibited potent anticancer effects on these three cell linesand could be an important material for further anticancer study.

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH DIỆT TẾ BÀO UNG THƯ CỦA TINH DẦU SẢ *CYMBOPOGON CITRATUS* THU HÁI TẠI TỈNH PHÚ THỌ

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TÓM TẮT

Tinh dầu từ bộ phận củ và lá của cây sả chanh (*Cymbopogon citratus* L.) thu hái tại Thanh Sơn và Phù Ninh, Phú Thọ đã được phân tích thành phần hóa học và đánh giá hoạt tính diệt tế bào ung thư trên 3 dòng thử nghiệm. Kết quả phân tích thành phần bằng phương pháp sắc ký khí kết nối khối phổ cho thấy hợp chất citral là thành phần chính của các mẫu tinh dầu nghiên cứu chiếm từ 64,15% đến 76,22% tổng lượng các tinh dầu. Phân tích về tỷ lệ thành phần giữa các mẫu sả củ và sả lá cho thấy hai đồng phân của ocimene có hàm lượng cao hơn ở tinh dầu sả củ so với sả lá. Camphene chỉ phát hiện ở hai mẫu sả củ mà không phát hiện được ở các mẫu sả lá thử nghiệm. Ngược lại, myrcene có hàm lượng cao hơn ở tinh dầu sả lá. Các mẫu tinh dầu được thử nghiệm hoạt tính diệt 3 dòng tế bào ung thư gồm: ung thư phổi (A539), ung thư cổ tử cung (Hela), ung thư gan (Hep3B). Các tinh dầu sả Phù Ninh và Thanh Sơn đều cho hiệu quả diệt tế bào ung thư phổi A549 rất mạnh (IC_{50} khoảng từ 4,01±0,39 đến 6,3±0,54 µg/ml). Đáng chú ý, mẫu tinh dầu sả củ và sả lá Thanh Sơn còn cho hiệu quả diệt hai dòng tế bào ung thư còn lại Hela và Hep3B rất mạnh với IC_{50} trong khoảng 1,18±0,26 to 8,91±0,32 µg/ml. Kết quả này gợi ý cho khả năng ứng dụng tinh dầu sả Thanh Sơn, Phú Thọ trong phát triển các phương thuốc điều trị ung thư trong tương lai.

Từ khóa: *Cymbopogon citratus* L.*, tinh dầu, thành phần hóa học, tác dụng diệt tế bào ung thư*