2016

Essential oil of solanum spirale fruits and its biological activities

Sukanya Keawsa-Ard
Chiang Mai University

Boonsom Liawruangrath
Chiang Mai University, liawruangrath@gmail.com

Saisunee Liawruangrath
Chiang Mai University, liawruangrath@gmail.com

Aphiwat Teerawutgulrag
Chiang Mai University

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

Publication Details

Keawsa-Ard, S., Liawruangrath, B., Liawruangrath, S., Teerawutgulrag, A. & Pyne, S. G. (2016). Essential oil of solanum spirale fruits and its biological activities. Chiang Mai Journal of Science, 43 (3), 546-554.

Essential oil of solanum spirale fruits and its biological activities

Abstract
The essential oil of Solanum spirale Roxb. unripe fruits was analyzed for the first time using GC-MS. Twenty-nine constituents were identified, constituting 82.11% of the total chromatographical oil components. The major components were n-hexadecanoic acid (56.01%), linolelic acid (9.71%), octadecanoic acid (4.41%), methyl plamitate (1.69%), tetradecanoic acid (1.55%), (E)-phytol (1.18%), n-hexanal (0.91%), methyl salicylate (0.83%), 4-hydroxy-4- methylpentan-2-one (0.81%), pentadecanoic acid (0.71%) and β-selinene (0.56%). The oil exhibited anticancer activities against MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) with the IC50 values of 23.17 and 49.07 μg/mL, respectively. It exhibited antituberculosis activity against Mycobacterium tuberculosis H37Ra with the MIC value of 50.0 μg/mL and it also showed moderately antibacterial activity against Escherichia coli and Staphylococcus aureus with the MIC values of 118 μg/mL.

Disciplines
Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details
Keawsa-Ard, S., Liawruangrath, B., Liawruangrath, S., Teerawutgulrag, A. & Pyne, S. G. (2016). Essential oil of solanum spirale fruits and its biological activities. Chiang Mai Journal of Science, 43 (3), 546-554.

Chiang Mai J. Sci. 2016; 43(3) : 547-555
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Essential Oil of *Solanum spirale* Fruits and Its Biological Activities

Sukanya Keawsa-ard [a], Boonsom Liawruangrath* [b], Saisunee Liawruangrath [c], Aphiwat Teerawutgulrag [c] Stephen G. Pyne [d]

[a] Department of Basic Science, Faculty of Science, Payap University, Chiang Mai 50000, Thailand.
[b] Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
[c] Department of Chemistry and Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
[d] School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia.
*Author for correspondence; e-mail: liawruangrath@gmail.com

Received: 6 August 2014
Accepted: 28 October 2014

ABSTRACT

The essential oil of *Solanum spirale* Roxb. unripe fruits was analyzed for the first time using GC-MS. Twenty-nine constituents were identified, constituting 82.11% of the total chromatographical oil components. The major components were *n*-hexadecanoic acid (56.01%), linolelic acid (9.71%), octadecanoic acid (4.41%), methyl plamitate (1.69%), tetradecanoic acid (1.55%), (*E*)-phytol (1.18%), *n*-hexanal (0.91%), methyl salicylate (0.83%), 4-hydroxy-4-methylpentan-2-one (0.81%), pentadecanoic acid (0.71%) and β-selinene (0.56%). The oil exhibited anticancer activities against MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) with the IC_{50} values of 23.17 and 49.07 μg/mL, respectively. It exhibited antituberculosis activity against *Mycobacterium tuberculosis* H37Ra with the MIC value of 50.0 μg/mL and it also showed moderately antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with the MIC values of 118 μg/mL.

Keywords: *Solanum spirale*, unripe fruits, essential oil, anticancer activity, cytotoxic activity, antituberculosis activity, antibacterial activity

1. INTRODUCTION

Many plants are getting more popular for their commercial use in the cosmetic, pharmaceutical and food industries. Thai medicinal plants are still widely and legally used in traditional Thai medicine. These have the possibility of containing bioactive substances for the search of new innovative drugs, especially to treat many diseases. Pharmaceutical properties of medicinal plants are mainly related to the presence of essential oils and their terpenic and non-terpenic constituents [1]. The essential oils are very interesting natural plant products and among other qualities they possess various biological properties such as anticancer, antiviral, antiphlogistic, antibacterial,

antifungal and antioxidant activities [2].

Solanum spirale Roxb. belongs to the family Solanaceae. It is an ethnomedicinal plant native to South Asia and distributed mainly in China, India, Bengal, Myanmar, Thailand, Laos, Vietnam, Indonesia and Australia [3]. This plant, commonly known as "pak dit" in the north of Thailand, has been used as a vegetable. It is used in folk medicine in many countries for narcotic, diuretic and anaesthetic [4] purposes. In Thailand, the leaves are used in the treatment of fevers and colds [5]. The leaves and the leaf oil of *S. spirale* showed anticancer and antibacterial activities [6,7]. The chemical compositions and biological activities of the essential oil of *S. spirale* unripe fruits have not been studied previously. The aim of this study was to investigate the chemical constituents and evaluate the biological activities of this essential oil. We describe here the chemical constituents, anticancer, cytotoxic, antituberculosis and antibacterial activities of the unripe fruits oil from this medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant Material

The unripe fruits of *S. spirale* Roxb. were collected from Phayao Province, Thailand in July 2008. The specimen was identified by J.F. Maxwell, a botanist at the Herbarium of Biology Department, Faculty of Science, Chiang Mai University where a voucher specimen has been deposited under the code number S. Keawsa-ard 01.

2.2 Essential Oil Hydrodistillation

The unripe fruits (5 kg) of *S. spirale* were washed with distilled water and subjected to hydrodistillation in a Clevenger-type apparatus for 8 h. At the end of distillation, the essential oil was collected, dried with anhydrous sodium sulfate and kept at a temperature of 4 °C for further analysis.

2.3 Analysis of the Essential Oil

Analysis of essential oil was performed on a Hewlett-Packard GC6850 gas chromatograph (FID) equipped with a HP-5 (Hewlett-Packard 19091J-433E) fused silica capillary column, 30 m × 0.25 mm, 0.25 μm film thickness composed of 5% phenylmethylsiloxane. The column temperature was programmed from 40 °C to 275 °C at 6 °C/min and held for 12 min. The carrier gas was Helium at a flow of 20.0 mL/min. The injector and detector temperature were 260 °C and 280 °C, respectively. Sample was injected by splitting and the split ratio was 1:20. The GC/MS analysis was performed on the Hewlett-Packard GC6850 coupled with a HP 5973N mass selective detector under the same capillary column and conditions as for GC. Significant quadrupole MS operating parameters:interface temperature 240 °C; electron impact ionization at 70 eV with scan mass range of 29-550 m/z at a sampling rate of 1.0 scan/s. The identification of the compounds in the essential oil was based on their GC retention index (RI) relative to n-alkanes (C_7-C_{30}) indices on HP-5 column and computer matching of spectral MS data with the Wiley and NIST libraries data of the GC/MS, as well as by comparison of the fragmentation pattern of the mass spectra with the data published in the literature data [8-12]. The percentage area of each composition was calculated using the following equation:

$$\%\ \text{area of composition} = \frac{\text{area of composition}}{\text{total area}} \times 100$$

2.4 Biological Activities

2.4.1 Anticancer activity

The anticancer activity of the essential oil of *S. spirale* unripe fruits was determined by Resazurin Microplate Assay (REMA) using three cancer cell lines: KB (Oral Cavity

cancer, ATCC CCL-17), MCF-7 (Human breast adenocarcinoma, ATCC HTB-22) and NCI-H187 (Human small cell lung carcinoma, ATCC CRL-5804). This assay was performed using the method described by Brien *et al.* [13]. Ellipticine and doxorubicin were used as positive controls and 0.5% DMSO was used as negative control. The fluorescence signal was measured using a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA). The IC_{50} values were derived from dose-response curves by the SOFTMax Pro software. Triplicate determinations were performed.

2.4.2 Cytotoxic activity

The cytotoxicity assay against *Vero* cells line (African green monkey kidney) was performed using a Green Fluorescent Protein (GFP)-based assay [14]. The GFP-expressing *Vero cell* line was generated in-house by stably transfecting the African green monkey kidney cell line (*Vero*, ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). Ellipticine and 0.5% DMSO were used as a positive and negative controls, respectively. The IC_{50} values were also calculated. Triplicate determinations were performed.

2.4.3 Antituberculosis activity

The antituberculosis activity of the essential oil was determined by green fluorescent protein microplate assay (GFPMA) [15]. Green fluorescent protein (GFP) expressing *M. tuberculosis* strain $H_{37}Ra$ was established by Changsen *et al.* [16]. Rifampicin, streptomycin, isoniazid and ofloxacin were used as positive controls and 0.5% DMSO was used as a negative control. Triplicate determinations were performed. The lowest drug concentration that inhibits cell growth by 90% was reported as the minimum inhibitory concentration (MIC).

2.4.3 Antibacterial activity

The minimum inhibitory concentration (MIC) of the essential oil was determined against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) using the microtiter broth method [17] with some modifications. The tested bacteria were cultured in Mueller Hinton Broth (MHB; Difco™; Becton Dickinson, Sparks, MD). They were then adjusted a turbidity of the cultured as equal to McFarland Standard No. 0.5. Essential oil was resuspended in 95% ethanol initially adjusted to 236 mg/mL and then serially two-fold diluted with MHB in microtiter plate. A positive control (without tested sample) and a negative control (without tested bacteria) were applied in each well. After incubation for 24 h at 37 °C, bacterial growth was determined by measuring the absorbance at 600 nm using the labsystems multiskan EX type 335 microplate reader (Helsinki, Finland). The MIC was the concentration at which microorganism did not demonstrate visible growth. The same protocol was used to determine the MIC of Amoxicillin for the inhibition of all tested pathogenic bacteria. Triplicate determinations were performed.

3. RESULTS AND DISCUSSION

3.1 Analysis of the Essential Oil

The percentage yield of the essential oil of *S. spirale* unripe fruits was obtained in 0.004 % w/w, based on the fresh weight of the sample as pale yellow liquid. The essential oil was analysed by means of GC and GC-MS. The components of the oil were identified by their retention indices (RI) relative to *n*-alkanes indices on a HP-5 column and by a comparison of mass spectra from Wiely and NIST libraries, as well as by comparison of the fragmentation patterns of the mass spectra with the data reported in the literature. Retention indices

were determined using retention times of n-alkanes (C_7-C_{30}) that have been injected to the same instrument and under the same chromatographic conditions. The components identified from the essential oil with their retention time (RT), percentage composition (%) and retention index (RI) are summarized in Table 1. Twenty-nine constituents were identified, constituting 82.11% of the total chromatographical oil components. The major components of this essential oil were *n*-hexadecanoic acid (56.01%), linolelic acid (9.71%), octadecanoic acid (4.41%), methyl plamitate (1.69%), tetradecanoic acid (1.55%), (*E*) phytol (1.18%), *n*-hexanal (0.91%), methyl salicylate (0.83%), 4-hydroxy-4-methylpentan-2-one (0.81%), pentadecanoic acid (0.71%) and β-selinene (0.56%). The oil was found to contain 7 carboxylic acids (72.89%), 4 esters (2.79%), 4 ketones (1.83%), 5 hydrocarbons (1.76%), 2 alcohols (1.46%), 5 aldehydes (1.15%) and 2 ethers (0.23%). The major components of *S. spirale* essential oil which possessed anticancer, antituberculosis and antibacterial activities were *n*-hexadecanoic acid and (*E*)-phytol. Linolelic acid was a cancer preventive. It also inhibited antituberculosis and antimicrobial activities.

Table 1. Chemical compositions of the essential oil from the unripe fruits of *S. spirale*.

Compound	RT[a] (min)	Area (%)	RI[b] (Exp)	RI[c] (lit)	ID[d]	Reference
n-Hexanal	3.45	0.91	806	802	RI,MS	[8] Adams, 2001
Furfural	4.10	0.05	848	836	RI,MS	[8]
4-Hydroxy-4-methylpentan-2-one	4.14	0.81	850	849	RI,MS	[9] Leffingwell and Alford, 2011
Benzaldehyde	6.62	0.06	974	960	RI,MS	[8]
2-Pentylfuran	7.13	0.06	995	997	RI,MS	[9]
Hexanoic acid	7.26	0.16	999	1022	RI,MS	[10] Boussaada et al., 2009
5-Ethenyldihydro-5-methyl-2(3H)-furanone	8.38	0.19	1050	1043	RI,MS	[9]
(*E*) 2-Octenal	8.79	0.05	1067	1059	RI,MS	[11] Miyazawa et al., 2008
1,3-Dimethoxybenzene	10.90	0.17	1156	1169	RI,MS	[8]
Octanoic acid	11.74	0.34	1190	1189	RI,MS	[12] Liolios et al., 2007
Methyl salicylate	12.02	0.83	1201	1192	RI,MS	[8]
(*E,E*) 2,4-Decadienal	14.3	0.08	1300	1317	RI,MS	[8]
Methyl anisate	15.30	0.08	1348	1373	RI,MS	[8]
Dihydro-β-ionone	17.30	0.11	1342	1444	RI,MS	[9]
β-Selinene	18.39	0.56	1494	1490	RI,MS	[8]
3-Keto-β-ionone	21.83	0.27	1674		MS	
Tetradecanoic acid	23.61	1.55	1774	1768	RI,MS	[9]
6,10,14-Trimethyl-2-pentadecanone	24.82	0.64	1846	1849	RI,MS	[9]

Table 1. Continued.

Compound	RT[a] (min)	Area (%)	RI[b] (Exp)	RI[c] (lit)	ID[d]	Reference
Pentadecanoic acid	25.28	0.71	1873	1870	RI,MS	[11]
Methyl hexadecanoate	26.20	1.69	1929	1922	RI,MS	[8]
Isophytol	26.52	0.28	1949	1948	RI,MS	[8]
n-Hexadecanoic acid	27.22	56.01	1992	1989	RI,MS	[11]
(*E*)-Phytol	29.09	1.18	2115	2109	RI,MS	[11]
Linoleic acid	29.69	9.71	2156	2143	RI,MS	[9]
Octadecanoic acid	30.02	4.41	2178	2167	RI,MS	[9]
Tricosane	31.72	0.24	2298	2300	RI,MS	[8]
Pentacosane	34.35	0.16	2498	2500	RI,MS	[8]
Heptacosane	36.79	0.39	2698	2700	RI,MS	[8]
Nonacosane	39.07	0.41	2897	2900	RI,MS	[8]
Carboxylic acids		**72.89**				
Esters		**2.79**				
Ketones		**1.83**				
Hydrocarbons		**1.76**				
Alcohols		**1.46**				
Aldehydes		**1.15**				
Ethers		**0.23**				
Total		**82.11**				

[a] RT Retention time; [c] RI values from literature data;

[b] RI retention indices calculated from retention times relative to n-alkanes C_7-C_{30} on the HP-5 column.

[d] Methods of identification: MS, comparison of the mass spectrum with MS libraries; RI of literature.

3.2 Biological Activities

3.2.1 Anticancer and cytotoxic activities

The anticancer activity of the essential oil from *S. spirale* unripe fruits was determined by Resazurin Microplate assay (REMA) using KB-Oral, MCF-7 breast and NCI-H187 lung cancer cell lines. Results are presented in Table 2.

The essential oil showed significant activities against MCF-7 and NCI-H187 cell lines with the IC_{50} values of 23.17 and 49.07 μg/mL, respectively, but it did not show activity against KB cell line. The cytotoxicity of this essential oil was tested against the primate cell line using the green fluorescent protein (GFP) detection. The oil showed non-cytotoxicity against *Vero* cells (Table 2).

The essential oil showed significant anticancer activities against the human cell lines, NCI-H187 (small cell lung cancer) and MCF7 (breast cancer). The bioactivity of some compounds present in this essential oil have been reported to exhibit anticancer activity; in particular, *n*-hexadecanoic acid, a major compound in *S. spirale* oil, showed cytotoxicity to human leukemic cells MOLT-4 and it also showed *in vivo* antitumor activity in mice [18,19], linoleic acid has been reported to possess cytotoxicity against human leukemic cells K562 [20] while

conjugated linoleic acid (CLA) differs from the normal form of linoleic acid only in the position of two of the bonds that join its atoms and has been reported to exhibit anticancer activity [21]. (*E*)-Phytol is one part of chlorophyll which is important for plant biosynthesis. It possessed anticancer activity against HT-29 human colon cancer cells, MG-63 osteosarcoma cells and AZ-521 gastric cancer cells [22]. Methyl hexadecanoate has been reported to possess cytotoxicity in human gastric cancer cells [23]. Methyl salicylate and tetradecanoic acid were the dominant compounds in this oil, they have been used in folk medicine as cancer-preventive [24]. Consistently, It is reported that the anticancer activity of *S. spirale* could be attributed to the presence of a mixture of organic compounds.

Table 2. Anticancer and cytotoxic activities of the oil from *S. spirale* unripe fruits.

Sample	IC_{50} (µg/mL)			
	NCI-H187	KB-oral	MCF-7	*Vero* cell
Essential oil	49.07	Inactive	23.17	non-cytotoxic
Ellipticine[a]	0.875	0.619	-	1.335
Doxorubicin[a]	0.050	0.162	0.858	-

[a]Drugs used as positive controls

3.2.2 Antituberculosis activity

The antituberculosis activity of the essential oil was determined by green fluorescent protein microplate assay. The oil showed antituberculosis activity against *M. tuberculosis* H_{37}Ra with the MIC value of 50.00 µg/mL. Results are shown in Table 3.

Mycobacteria have recently increased their virulence and tuberculosis (TB) is the most lethal infection worldwide. Thirty million people are expected to catch tuberculosis in the near future by World Health Organization (WHO); one million children per year die from this disease [25].

The essential oil of *S. spirale* unripe fruits consisted of some active compounds that have been reported to exhibit antituberculosis activity. Fatty acids, *n*-hexadecanoic acid, oleic acid and linoleic acid possessed antituberculosis activity against *Mycobacterium aurum*, *Mycobacterium smegatis*, *Mycobacterium fortuitum* and *Mycobacterium phiei*, while linoleic acid exhibited activity against *M. aurum* [26]. (*E*)-phytol, a dominant compound in this oil, exhibited antituberculosis activity against *Mycobacterium tuberculosis* [27-28].

Table 3. Antituberculosis activity of the oil from *S. spirale* unripe fruits.

Sample	MIC (µg/mL)
Essential oil	50.00
Rifampicin[b]	0.003
Streptomycin[b]	0.156
Isoniazid[b]	0.023
Ofloxacin[b]	0.391

[b] Drugs used as positive controls

3.2.3 Antibacterial activity

The MIC of the essential oil from *S. spirale* unripe fruits was determined against *E. coli* and *S. aureus* using the microtiter broth method. The results are presented in Table 4. The oil exhibited antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive bacteria (*S. aureus*) with the MIC values of 118 µg/mL.

Table 4. Antibacterial activity of the oil from *S. spirale* unripe fruits.

Sample	MIC (μg/mL)	
	E. coli	S. aureus
Essential oil	118	118
Amoxicillin[c]	2.93	2.93

[c] Antibiotic used as a positive control

Some fatty acids present in this essential oil have been reported to possess antibacterial activity against *S. aureus* such as hexanoic acid, octanoic acid, tetradecanoic acid, pentadecanoic acid, *n*-hexadecanoic acid, octadecanoic acid and linoleic acid [29]. Some aldehydes in this oil such as *n*-hexanal, (*E*) 2-octenal and (*E,E*) 2,4-decadienal have been demonstrated to exhibit antibacterial activity against Gram-positive and Gram-negative bacterial strains [30-31]. Benzaldehyde exhibited antibacterial activity against *Listeria monocytogenes*, *Salmonella enteritidis* and *Lactobacillus plantarum* [32-34]. (*E*)-Phytol is an acyclic terpenoid which possessed antibacterial activity against *S. aureus* [35]. Results indicated that this essential oil may be used for treatment of the infectious diseases caused by Gram-positive and Gram-negative bacteria such as *S. aureus* and *E. coli*.

4. CONCLUSIONS

In conclusion, the chemical compositions of the essential oil from *S. spirale* unripe fruits have been analysed by GC and GC/MS. The major components of this essential oil were *n*-hexadecanoic acid (56.01%), linolelic acid (9.71%), octadecanoic acid (4.41%), methyl plamitate (1.69%), tetradecanoic acid (1.55%) and (*E*)-phytol (1.18%). The essential oil exhibited anticancer activity against MCF-7 and NCI-H187 cell lines, antituberculosis activity against *M. tuberculosis* H37Ra and antibacterial activity against *E. coli* and *S. aureus*. This study is the first report which describes the chemical constituents and biological activities of the essential oil from *S. spirale*. This essential oil may play an important role in new drug development, especially anticancer, antituberculosis and antimicrobial drugs.

ACKNOWLEDGEMENT

We would like to express our sincere thank to Department of Pharmaceutical Science, Faculty of Pharmacy and the Graduate School, Chiang Mai University, Chiang Mai, Thailand for their partial support. Additionally, we also would like to thank Materials Science Research Center, Faculty of Science, Chiang Mai University, Thailand Research Fund (TRF) and Commission on Higher Education for their partial support.

REFERENCES

[1] Petrovic S., Pavlovic M. and Popovic V., *J. Essent. Oil Res.* 2009; **21**: 467-470.

[2] Buchbauer G., *Handbook of Essential Oils: Science, Technology and Applications*, CRP Press, London, New York. 2010.

[3] Teng X.F., Zhang Y.J. and Yang C.R.. *Acta Botanica Yunnanica,* 2008; **30**: 239-242. DOI 10.3724/SP.J.1143.2008.07175.

[4] Joy P.P., Thomson J., Mathew S. and Skaria B.P., *Medical Plants*, Kerala Agricultural University, Aromatic and Medicinal Plants Research Station, Ernakulam District, Kerala, India. 2001.

[5] Inta A., Shengji P., Balslev H., Wangpakapattanawong P. and Trisonthi C., *J. Ethanopharmacol.*, 2008; **116**: 508-517. DOI 10.1016/j.jep.2007. 12.015.

[6] Keawsa-ard S. and Kongtaweelert S., *Chiang Mai J. Sci.*, 2012; **39(3)**: 455-463.

[7] Keawsa-ard S., Natakankitkul S., Liawruangrath S., Teerawutgulrag A.,

Trisuwan K., Charoenying P., Pyne S.G. and Liawruangrath B., *Chiang Mai J. Sci.*, 2012; **39(3)**: 445-454.

[8] Adams R.P., *Identification of Essential Oils Components by Gas Chromatography/ Quadrupole Mass Spectroscopy*, 3rd Edn., Allured publishing Co., Carol Stream, Illinois, USA, 2001.

[9] Leffingwell J.C. and Alford E.D., *J. Environ. Agric. Food Chem.,* 2005; **4**: 1-16.

[10] Boussaada O., Ammar S., Mahjoub M.A., Saidana D., Chriaa J., Chraif I., Daami M., Helal A.N. and Mighri Z., *J. Essent. Oil Res.,* 2009; **21**: 179-184. DOI 10.1080/10412905.2009.9700142.

[11] Miyazawa M., Nagai S. and Oshima T., *J. Oleo Sci.,* 2008; **57**: 139-143. DOI 10. 5650/jos.57.139.

[12] Liolios C., Laouer H., Boulaacheb N., Gortzi O. and Chinou I., Chemical composition and antimicrobial activity of the essential oil of Algerian *Phlomis bovei* De Noe subsp. *Bovei*, Molecules. 2007; **12**: 772-781. DOI 10.3390/12040772.

[13] Brien J.O., Wilson I., Orton T. and Pognan F., *Eur. J. Biochem.*, 2000; **267**: 5421-5426. DOI 10.1046/j.1432-1327. 2000.01606.x.

[14] Hunt L., Jordan M., Jesus M.D. and Wurm F.M., *Biotechol. Bioeng.*, 1999; **65**: 201-205. DOI 10.1002/(SICI)1097-0290(19991020)65:2<201::AID-BIT10> 3.0.CO;2-H.

[15] Collins L.A., Torrero M.N. and Franzblau S.G., *Antimicrob. Agants Chemoter.*, 1998; **42**: 344-347.

[16] Changsen C., Franzblau S.G. and Palittapongarnpim P., *Antimicrob. Agents Chemother.,* 2003; **47**: 3682-3687. DOI 10.1128/AAC.47.12.3682-3687.2003.

[17] Amsterdam D., Susceptibility Testing of Antimicrobials in Liquid Media; in Lorian V., ed., *Antibiotics in Laboratory Medicine*, 4th Edn., Williams & Wilkins, Baltimore, MD, USA., 1996; 52-111.

[18] Semary N.A.E., Ghazy S.M. and Naby M.M.A.E., *Aust. J. Basic Appl. Sci.*, 2009; **3**: 1540-1551.

[19] Harada H., Yamashita U., Kurihara H., Fukushi E., Kawabata J. and Kamei Y., *Anticancer Res.*, 2002; **22**: 2587-2590.

[20] Aburai N., Koshino H., Nishizawa N. and Kimura K., *Biosci. Biotechnol. Biochem.,* 2007; **71**: 2061-2064. DOI 10.1271/bbb. 70068.

[21] Whigham L.D., Cook M.E. and Atkinson R.L., *Pharmacological Res.*, 2000; **42**: 503-510. DOI 10.1006/phrs.2000. 0735.

[22] Yuenyongsawad S. and Tewtrakul S., *Songklanakarin J. Sci. Technol.*, 2005; **27**: 497-501.

[23] Lee K., Sook-Hee R. and Kum-Young P., Anticancer activity of phytol and eicosatrienoic acid identified from Perilla leaves. Han'guk Sikp'um Yongyang Kwahak Hoechi. (J. written in Korean), Abst Sci Finder, 1999; **28**: 1107-1112.

[24] Stitt PA. *Why George Should Eat Broccoli.* Dougherty Co, Milwaukee, WI, 1990; 399.

[25] Baser K.H.C., Kurkcuoglu M., Askun T. and Tumen G., *J. Essent. Oil Res.*, 2009; **21**: 572-575. DOI 10.1080/10412905. 2009.9700248.

[26] Seidel V. and Taylor P.W., *Int. J. Antimicrob. Agents*, 2004; **23**: 613-619. DOI 10.1016/ j.ijantimicag.2003.11.008.

[27] Chen J.J., Lin W.J., Shieh P.C., Chen I.S., Peng C.F. and Sung P.J., *Chem. Biodivers.*, 2010; **7**: 717-721. DOI 10.1002/cbdv. 200900198.

[28] Rajab M.S., Cantrell C.L., Franzblau S.G. and Fischer N.H., *Planta Med.*, 1998; **64**: 2-4. DOI 10.1055/s-2006-957354.

[29] Cañas-Rodriguez A. and Smith H.W., *Biochem. J*., 1966; 79-82.

[30] Patrignani F., Iucci L., Belletti N., Gardini F., Guerzoni M.E. and Lanciotti R., *Int. J. Food Microbiol*., 2008; **123**: 1-8. DOI 10.1016/j. ijfoodmicro. 2007.09.009.

[31] Trombetta D., Saija A., Bisignano G., Arena S., Caruso S., Mazzanti G., Uccella N. and Castelli F., *Lett. Appl. Microbiol*., 2002; **35**: 285-290. DOI 10.1046/j.1472-765X.2002.01190.x.

[32] Ramos-Nino M.E., Clifford M.N. and Adam M.R., *J. Appl. Bacteriol*., 1996; **80**: 303-310. DOI 10.1111/j. 1365-2672.1996.tb03224.x.

[33] Ramos-Nino M.E., Ramirez-Rodriguez C.A., Clifford M.N. and Adam M.R., *J. Appl. Microbiol*., 1998; **84**: 207-212.

[34] Bisignano G., Laganá M.G., Trombetta D., Arena S., Nostro A., Uccella N., Mazzanti G., *FEMS Microbiol. Lett*., 2001; **198**: 9-13. DOI 10.1111/j.1574-6968. 2001.tb10611.x.

[35] Inoue Y., Hada T., Shiraishi A., Hirose K., Hamashima H. and Kobayashi S., *Antimicrob. Agents Chemother*., 2005; **49**: 1770-1774. DOI 10.1128/AAC.49. 5.1770-1774.2005.