NPC Natural Product Communications

2015
Vol. 10
No. 7
1297 - 1300

Chemical Compositions and Biological Activities of Essential Oils of *Beilschmiedia glabra*

Wan Mohd Nuzul Hakimi Wan Salleh[a], Farediah Ahmad[a*], Khong Heng Yen[b] and Razauden Mohamed Zulkifli[c]

[a]*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), Skudai, 81310 Johor Bahru, Johor, Malaysia*

[b]*School of Chemistry and Environment Studies, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Sarawak, JalanMeranek, 94300 Kota Samarahan, Sarawak, Malaysia*

[c]*Department of Bioscience and Health Sciences, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, Malaysia*

farediah@kimia.fs.utm.my

Received: March 28th, 2015; Accepted: April 13th, 2015

This study was designed to examine the chemical compositions of essential oils from *Beilschmiedia glabra* and their antioxidant, antimicrobial, antityrosinase, acetylcholinesterase and anti-inflammatory activities. In total, 47 components were identified in the essential oils, which made up 86.8% and 89.7% of the leaf and bark oils, respectively. The leaf oil is composed mainly of β-eudesmol (15.4%), β-selinene (12.2%), caryophyllene oxide (8.1%) and γ-gurjunene (5.2%), while the bark oil contains high percentages of β-eudesmol (19.3%), β-selinene (16.9%), δ-cadinene (15.8%), germacrene D (9.8%) and β-caryophyllene (5.5%). Antioxidant activity showed that the leaf oil has the highest phenolic content at 233.4 mg GA/g, while the bark oil showed potent activity in the β-carotene/linoleic acid bleaching assay. However, both oils showed weak activity in the DPPH and ABTS assays. For antimicrobial activity, the leaf and bark oils displayed strong activity against *Candida glabrata* and *Saccharomyces cerevisiae* with MIC values of 31.3 and 62.5 µg/mL, respectively. Percentage inhibitions against tyrosinase (leaf 73.7%; bark 76.0%) and acetylcholinesterase (leaf 48.1%; bark 45.2%) were tested at a concentration of 1 mg/mL, while anti-inflammatory activity (leaf 59.7%; bark 48.9%) was evaluated at a concentration of 100 µM. Evaluation of these assays indicated moderate levels of activity.

Keywords: Essential oil, *Beilschmiedia*, Antioxidant, Antimicrobial, Antityrosinase, Acetylcholinesterase, Anti-inflammatory.

The genus *Beilschmiedia* comprises about 200 plant species widely distributed in the world's inter-tropical region and is known as *medang*. Most of the species grow in tropical climates, but a few of them are native to temperate regions. They are widespread in tropical Asia, Africa, Australia, Central America and South America [1-2]. The chemical compositions of essential oils from several species of *Beilschmiedia* were previously described [3-8]. As a continuation of our systematic studies of the pharmacologically active volatiles of Malaysian plants we describe in this paper studies of the chemical composition of the essential oils from the leaf and bark oils of *B. glabra* together with their antioxidant, antimicrobial, acetylcholinesterase, anti-tyrosinase and anti-inflammatory activities. As far as our literature survey could ascertain, this study is the first report on the biological activities of this species.

Hydrodistillation of the fresh samples of leaf and bark of *B. glabra* yielded 0.38 g (0.13%) and 0.12 g (0.04%) of essential oils, respectively. The list of chemical components identified in the oils is shown in Table 1. The GC and GC-MS analysis of the essential oils revealed the presence of 47 components, of which 45 were identified in the leaf oil (86.8%) and 16 in the bark oil (89.7%). Both oils were characterized by the presence of high concentrations of sesquiterpene hydrocarbons (53.1-66.4%), followed by oxygenated sesquiterpenes (23.3-29.8%). The most abundant components of the leaf oil were β-eudesmol (15.4%), β-selinene (12.2%) and caryophyllene oxide (8.1%). In addition, the bark oil was characterized by its richness in β-eudesmol (19.3%), β-selinene (16.9%) and δ-cadinene (15.8%). The other components present in appreciable amounts were β-caryophyllene (5.5%), valencene (4.1%), germacrene B (3.8%) and α-copaene (3.8%). β-Eudesmol, found in *Atractylodes lancea* rhizome, has a desensitizing channel blocking action to nicotinic acetylcholine receptors, anti-angiogenic action in vascular endothelium and neuronal differentiation actions. These multiple pharmacological actions are favorable for treating angiogenic diseases, including the complications of diabetes, namely retinopathy, nephropathy and cancer [9].

The essential oils were screened for their possible antioxidant activity by using β-carotene/linoleic acid, DPPH radical scavenging, ABTS radical scavenging and total phenolic contents. The results of the antioxidant activity are shown in Table 2. DPPH radical scavenging activity of the essential oils was expressed as IC_{50} values, which are the concentrations of analytes required for the conversion of half of the DPPH radicals to their more stable molecular counterparts, 2,2-diphenyl-1-picrylhydrazines. Both oils exhibited weak activity for DPPH and ABTS radical scavenging activity compared with the standard antioxidant, BHT. The highest results were given by the leaf oil which displayed IC_{50}values of 104.9 and 228.6 µg/mL in DPPH and ABTS, respectively. The scavenging of the ABTS radical by the oils was found to be much higher than that of the DPPH radical. Factors including radical stereoselectivity or sample solubility in different testing systems have been reported to affect the capacity of essential oils to react and quench different radicals [10]. In the β-carotene/linoleic acid assay, the effectiveness of the leaf (56.2%) and bark (77.6%) oils was compared with that of BHT (125.5%), but the inhibition values observed were significantly lower than that of BHT. The β-carotene/linoleic acid test is usually used for the estimation of the antioxidant potential ability to delay lipid peroxidation by reacting with the chain propagating peroxyl radicals faster than the reaction of these radicals with proteins or fatty acid side chains [11].

Table 1: Chemical components identified from *B. glabra* oils.

Components	KI[a]	KI[b]	Percentage (%)	
			Leaf	Bark
Camphene	945	946	0.5	-
δ-3-Carene	1005	1008	0.1	-
α-Terpinene	1016	1014	0.2	-
p-Cymene	1018	1020	0.1	-
1,8-Cineole	1026	1026	2.4	-
Terpinen-4-ol	1174	1174	0.2	-
α-Terpineol	1190	1186	0.1	-
Bornyl acetate	1283	1287	0.3	-
α-Cubebene	1345	1345	0.4	0.9
Cyclosativene	1370	1369	0.1	-
α-Ylangene	1370	1373	3.8	-
α-Copaene	1372	1374	1.7	3.8
β-Patchoulene	1377	1379	0.4	-
β-Bourbonene	1387	1387	0.2	-
β-Cubebene	1388	1387	0.2	-
β-Elemene	1390	1389	0.4	1.9
iso-Longifolene	1390	1389	0.3	1.1
α-Gurjunene	1405	1409	0.1	-
α-Cedrene	1412	1410	0.8	-
β-Caryophyllene	1415	1417	0.5	**5.5**
β-Gurjunene	1430	1431	1.0	-
α-Guaiene	1437	1437	1.1	-
Aromadendrene	1439	1439	2.3	-
α-Humulene	1450	1452	0.3	1.6
Alloaromadendrene	1459	1458	0.4	-
Dehyroaromadendrene	1460	1460	1.0	-
γ-Gurjunene	1470	1475	**5.2**	-
α-Amorphene	1482	1483	2.1	-
Germacrene D	1484	1484	-	9.5
β-Selinene	1489	1489	**12.2**	**16.9**
Cadina-1,4-diene	1495	1495	3.2	-
Valencene	1495	1496	4.0	4.1
α-Selinene	1498	1498	0.6	0.9
α-Muurolene	1501	1500	0.7	-
α-Bisabolene	1505	1506	1.7	-
γ-Cadinene	1518	1513	1.1	-
δ-Cadinene	1520	1522	2.7	**15.8**
cis-Calamenene	1525	1528	0.7	-
α-Calacorene	1545	1544	2.3	-
Germacrene B	1460	1559	1.2	3.8
Caryophyllene oxide	1585	1582	**8.1**	-
Globulol	1588	1590	-	1.7
Caryophylla-4(12), 8(13)-dien-5β-ol	1640	1639	2.5	-
Alloaromadendrene epoxide	1640	1639	1.6	-
β-Eudesmol	1645	1649	**15.4**	**19.3**
α-Cadinol	1650	1652	2.2	2.3
Guaiazulene	1775	1779	0.4	0.6
Group components				
Monoterpene hydrocarbons			0.9	-
Oxygenated monoterpenes			3.0	-
Sesquiterpene hydrocarbons			53.1	66.4
Oxygenated sesquiterpenes			29.8	23.3
Identified components (%)			**86.8**	**89.7**

[a]Kovat indices (KI) experimental; [b]KI from the literature [19].

For total phenolic content, the highest value of 233.4 mg GA/g of the leaf oil could be attributed to monoterpenoids and sesquiterpenoids. The antioxidant activity observed for the essential oils could be explained partially by the major components recorded in these oils. Both minor and major compounds could have a significant contribution to the oils' activity [12].

Table 2: Antioxidant activity of the essential oils of *B. glabra*.

Samples	β-carotene (I%)[a]	DPPH IC_{50} (μg/mL)[b]	ABTS IC_{50} (μg/mL)[b]	TPC (mg GA/g)[c]
Leaf oil	56.2 ± 0.2	104.9	228.6	233.4 ± 0.6
Bark oil	77.6 ± 0.2	107.5	231.7	191.0 ± 0.3
BHT	125.5 ± 0.3	18.5	52.2	-

[a]Percentage inhibition at a concentration of 2 g/L; [b]IC_{50} value at concentrations of 200-25 μg/mL; [c]TPC at a concentration of 1 mg/mL

The *in vitro* antimicrobial activity of the essential oil of *B. glabra* against six bacterial and three fungal species was evaluated by disc diffusion and minimum inhibitory concentration (MIC) using the broth dilution method. Table 3 shows the antimicrobial activity of the essential oil. The leaf oil showed strong activities against the fungal strains, but moderate activity against the bacterial strains. The inhibition zones and MIC values forthe microbial strains that are sensitive to the essential oils were in the range 7.5-12.6 mm and 31.3-500 μg/mL, respectively. The leaf oil showed a strong effect towards *Candida glabrata* (MIC value of 31.3 μg/mL) and *Saccharomyces cerevisiae*, while the bark oil exhibited MIC values of 62.5 μg/mL against the same strains. In addition, both oils also displayed moderate activity against all Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis*) with MIC values of 125 μg/mL. The major components of the essential oils such as β-eudesmol, δ-cadinene, caryophyllene oxide and β-caryophyllene have been reported to have antibacterial and antifungal activities and therefore, they could be responsible for the observed results from these essential oils [13-14]. The results showed that the Gram-positive strains were more sensitive. This was in accordance with several other reports where essential oils were shown to be more active towards Gram-positive than Gram-negative bacteria [15].

Table 3: Antimicrobial activity of the essential oils of *B. glabra*.

Test microbes		Leaf oil	Bark oil	SS
Gram-positive				
Bacillus subtilis	DD	10.5 ± 0.1	10.5 ± 0.1	18.5 ± 0.2
	MIC	125	125	7.8
Staphylococcus aureus	DD	8.2 ± 0.1	9.8 ± 0.2	19.2 ± 0.2
	MIC	125	125	7.8
Enterococcus faecalis	DD	11.0 ± 0.1	10.2 ± 0.1	18.6 ± 0.1
	MIC	125	125	15.6
Gram-negative				
Pseudomonas aeruginosa	DD	9.0 ± 0.2	7.4 ± 0.3	18.8 ± 0.2
	MIC	250	250	7.8
Escherichia coli	DD	7.8 ± 0.3	7.5 ± 0.3	19.5 ± 0.1
	MIC	500	500	7.8
Klebsiella pneumoniae	DD	7.5 ± 0.1	8.0 ± 0.2	19.2 ± 0.2
	MIC	500	500	15.6
Fungal /yeast				**NYS**
Aspergillus niger	DD	11.5 ± 0.1	10.0 ± 0.1	19.5 ± 0.2
	MIC	62.5	125	15.6
Candida glabrata	DD	12.6 ± 0.1	11.2 ± 0.1	20.4 ± 0.1
	MIC	31.3	62.5	7.8
Saccharomyces cerevisiae	DD	12.2 ± 0.2	10.8 ± 0.2	20.2 ± 0.2
	MIC	31.3	62.5	7.8

DD – disc diffusion (mm); MIC – Minimum inhibitory concentration (μg/mL); SS - Streptomycin sulfate; NYS - Nystatin

The tyrosinase and acetylcholinesterase inhibitory activities and anti-inflammatory activities of the essential oils were assessed by measurement of the mushroom tyrosinase enzyme, AChE enzymes and lipoxygenase assay, respectively. The results are shown in Table 4. The oxidation of L-dopa catalysed by mushroom tyrosinase was inhibited by the essential oils in a dose-dependent manner. The enzyme activity was not suppressed, but rather rapidly decreased. Tyrosinase inhibition activity of the leaf and bark oils was significant, producing 73.7% and 76.0% inhibition, respectively, yet the values were lower than the positive control, kojic acid (94.8%). AChE plays an important role in the central nervous system. It is one of the fastest known enzymes and catalyses the cleavage of acetylcholine in the synaptic cleft after depolarisation. Inhibitors of AChE, such as galanthamine, are used frequently in the pharmacotherapy of Alzheimer disease [16]. In the acetylcholinesterase inhibition assay, the leaf and bark oils exhibited moderate activity against *Electrophorus electricus* AChE enzyme with percentage inhibition values of 48.1% and 45.2%, respectively at the concentration of 1 mg/mL. In previous studies, evaluation of many common essentialoil components (γ-terpinene, carvone, *trans*-anethole, camphene, borneol, farnesol, nerol, 1,8-cineole, linalool, α-pinene, β-pinene, eugenol) had shown

Table 4: Tyrosinase and acetylcholinesterase inhibitory activities and anti-inflammatory activities of the essential oils of *B. glabra.*

Samples	Tyrosinase (I%)[a]	AChE (I%)[a]	Anti-inflammatory (I%)[b]
Leaf oil	73.7 ± 0.2	48.1 ± 0.3	59.7 ± 0.3
Bark oil	76.0 ± 0.3	45.2 ± 0.3	48.9 ± 0.2
Kojic acid	94.8 ± 0.1	-	-
Galantamine	-	95.9 ± 0.2	-
Quercetin	-	-	81.9 ± 0.2

[a]Percentage inhibition at a concentration of 1000 µg/mL; [b]Percentage inhibition at a concentration of 100 µM.

significant cholinesterase inhibitory activity. Nevertheless, it should be suggested that cholinesterase inhibitory activity of the essential oils may not be correlated only to the β-eudesmol amount, but synergistic effects should be considered [17].

Anti-inflammatory activity was evaluated by using the lipoxygenase assay according to the standard protocol. The leaf and bark oils showed moderate activtiy with inhibtions of 59.7% and 48.9%, respectivelyat the concentration of 100 µM. This inhibition activity may be attributed to the presence of β-caryophyllene, limonene and caryophyllene oxide, which are known as 5-LOX inhibitors. Generally, inflammation involves the formation of both prostaglandins and leukotrienes as mediators followed by the liberation of neutrophils and the production of the reactive oxygen species [18].

Experimental

Plant materials: A sample of *B. glabra* was collected from Kluang, Johor in October 2014. The species was identified by Shamsul Khamis from the Institute of Bioscience (IBS), UPM and the voucher specimen (SK2570/14) deposited at the Herbarium of IBS.

Solvents and chemicals - Antioxidants: β-Carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (Germany). Folin-Ciocalteu's reagent, anhydrous sodium sulfate, sodium carbonate, polyoxyethylenesorbitanmonopalmitate (Tween-40), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and potassium persulfate were purchased from Merck (Germany). All other chemicals and solvents were analytical grade. ***Antimicrobials:*** Nutrient agar, nutrient broth, sabouraud dextrose agar and sabouraud dextrose broth, streptomycin sulphate and nystatin were purchased from Oxoid (Italy). All tested microorganisms were purchased from Mutiara Scientific (Malaysia). ***Antityrosinases:*** Mushroom tyrosinase enzyme (EC1.14.18.1), kojic acid and L-dopa were purchased from Sigma-Aldrich (Germany). ***Acetylcholinesterase (AChE):*** *Electrophorus electricus* AChE (EC3.1.1.7, Type VI-S), acetylthiocholine iodide, 5,5'-dithio-bis(2-nitrobenzoic)acid (DTNB) and galantamine were purchased from Sigma-Aldrich (Germany). ***Anti-inflammatory:*** Lipoxygenase inhibitor screening assay kit (Item No. 760700) was purchased from Cayman Chemical Company (USA).

Extraction of essential oils: The fresh leaves and bark were each subjected to hydrodistillation in an all glass Dean-Stark apparatus for 8 h. The oils obtained were dried over anhydrous magnesium sulfate and stored at 4-6°C. The oil yields (w/w) were calculated based on fresh weight-basis.

Gas chromatography (GC): GC analysis was performed on a Hewlett Packard 6890 series II A gas chromatograph equipped with an Ultra-1 column (100% polymethylsiloxanes) (25 m long, 0.33 µm thickness and 0.20 mm inner diameter). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperature were set at 250°C and 280°C, respectively. Oven temperature was kept at 50°C, then gradually raised to 280°C at 5°C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated 3 times and the peak area percentages were reported as means ±SD of triplicates. Calculation of peak area percentage was carried out by using the GC HP Chemstation Software (Agilent Technologies).

Gas chromatography-mass spectrometry (GC-MS): GC-MS chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with anUltra-1 column (25 m long, 0.33 µm thickness and 0.20 mm inner diameter). Helium was used as a carrier gas at a flow rate of 1 mL/min. Injector temperature was 250°C. Oven temperature was programmed from 50°C (5 min hold) at 10°C/min to 250°C and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu.

Identification of components: The chemical components of the essential oils were identified by comparing their MS with the reference spectra in the computer library (Wiley) and also by comparing their retention and Kovats indices with the literature data [19]. The quantitative data were obtained electronically from FID area percentage without the use of a correction factor.

Antioxidant activity - β-Carotene/linoleic acid bleaching: The β-carotene-linoleic acid bleaching assay was carried out as given previously with minor modifications [20].

DPPH radical scavenging: The free radical scavenging was measured using the DPPH method with minor modifications [20].

Total phenolic content (TPC): TPC of essential oil was determined using a gallic acid equivalent with minor modifications [20].

ABTS radical scavenging: The antioxidant capacity was estimated in terms of the ABTS radical scavenging activity that follows the procedure as described previously [21].

Antimicrobial activity - Microbial strains: Nine microorganisms; three Gram-positive bacteria; *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC29737), *Enterococcus faecalis* (ATCC19433), three Gram-negative bacteria; *Pseudomonas aeruginosa* (ATCC9027), *Escherichia coli* (ATCC10536), *Klebsiella pneumonia* (ATCC13883) and three fungal/yeast *Aspergillus niger* (ATCC16888), *Candida glabrata* (ATCC2001) and *Saccharomyces cerevisiae* (ATCC7754) were used. The strains were grown on nutrient agar for the bacteria and sabouraud dextrose agar for the fungal/yeast. For the activity tests, nutrient broth for bacteria and sabouraud dextrose broth for fungal/yeast strains wereused.

Disk diffusion: Antimicrobial tests were carried out using the disc diffusion method with slight modifications, as given previously [22].

Minimum inhibitory concentration (MIC): The MIC was determined by a broth micro dilution method using 96-well microplates with modifications [22].

Antityrosinase activity: Tyrosinase inhibition assay was performed according to the previous method, with slight modifications [22].

Acetylcholinesterase activity (AChE): AChE inhibitory activities were measured by slightly modifying the spectrophotometric method developed previously [23,24].

Anti-inflammatory activity: The reagents were prepared according to the standard protocol lipoxygenase inhibitor screening assay [25].

Statistical analysis*:* Data obtained from essential oil analysis and bioactivities was expressed as mean values. The statistical analyses were carried out by employing one way ANOVA (p>0.05) by using statistical package (SPSS version 11.0).

Acknowledgments - The authors thank the MOSTI, Malaysia for financial support under research vote QJ130000.2526.03H93 and the Department of Chemistry, Faculty of Science, UTM for research facilities.

References

[1] Kochummen KM. (**1989**) In *Tree flora of Malaya: A manual for foresters*, Longman, Selangor, Malaysia.
[2] Corner EJH. (**1997**) *Wayside trees of Malaya*, 4th Ed., United Selangor Press, Kuala Lumpur, Malaysia.
[3] Kumamoto J, Scora RW. (**1970**) Structure of sarisan, an isomer of myristicin, isolated from the leaf oil of *Beilschmiedia miersii*. *Journal of Agricultural and Food Chemistry*, ***18***, 544–545.
[4] Scora RW, Scora PE. (**2001**) Essential leaf oil of *Persea* subgenus, *Eriodaphne* and closely related *Perseoid* genera. *Journal of Essential Oil Research*, ***13***, 37–42.
[5] Setzer WN, Haber WA. (**2007**) Leaf essential oil composition of five species of *Beilschmiedia* from Monteverde, Costa Rica. *Natural Product Communications*, ***2***, 79–83.
[6] Chaverri C, Ciccio JF. (**2010**) Essential oils from *Beilschmiedia pendula* (Sw.) Hemsl.(Lauraceae) from Costa Rica. *Journal of Essential Oil Research*, ***22***, 259–262.
[7] Su YC, Ho CL. (**2013**) Composition and *in vitro* cytotoxic activities of the leaf essential oil of *Beilschmiedia erythrophloia* from Taiwan. *Natural Product Communications*, ***8***, 143–144.
[8] Salleh WMNHW, Ahmad F, Khong HY. (**2015**) Chemical compositions and biological activities of the essential oils of *Beilschmiedia madang* Blume (Lauraceae). *Archives of Pharmacal Research*, ***38***, 485–493.
[9] Kimura I. (**2006**) Medical benefits of using natural compounds and their derivatives having multiple pharmacological actions. *Yakugaku Zasshi*, ***126***, 133–143.
[10] Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. (**2002**) Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, ***50***, 1619–1624.
[11] Ebrahimabadi AH, Mazoochi A, Kashi FJ, Djafari-Bidgoli Z, Batooli H. (**2010**) Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss from Iran. *Food Chemical Toxicology*, ***48***, 1371–1376.
[12] Wang W, Wu N, Zu YG, Fu YJ. (**2008**) Antioxidative activity of *Rosmarinus officinalis* L. Essential oil compared to its main components. *Food Chemistry*, ***108***, 1019–1022.
[13] Mann CM, Cox SD, Markham JL. (**2000**) The outer membrane of *Pseudomonas aeruginosa* NCTC6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Letters in Applied Microbiology*, ***30***, 294–297.
[14] Ho CL, Liao PC, Wang EI, Su YC. (**2011**) Composition and antifungal activities of the leaf essential oil of *Neolitsea parvigemma* from Taiwan. *Natural Product Communications*, ***6***, 1357–1360.
[15] Gutierrez J, Rodriguez G, Barry-Ryan C, Bourke P. (**2008**) Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables: Antimicrobial and sensory screening. *Journal of Food Protect*, ***71***, 1846–1854.
[16] Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, de Cindio B, Houghton PJ, Menichini F, Frega NG. (**2009**) Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. *Food Chemistry*, ***117***, 174–180.
[17] Orhan I, Aslan S, Kartal M, Sener B, Baser KHC. (**2008**) Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. *Food Chemistry*, ***108***, 663–668.
[18] Schmitz ML, Bacher S. (**2005**) Novel molecular targets in the search for anti-inflammatory agents. *Phytochemistry Reviews*, ***4***, 19–25.
[19] Adams RP. (**2001**) *Identification of essential oil by gas chromatography-quadrupole/mass spectroscopy*, 4th Ed. Allured Publishing Corporation, Carol Stream IL, USA.
[20] Salleh WMNHW, Ahmad F, Khong HY, Sirat HM. (**2012**) Chemical compositions, antioxidant and antimicrobial activity of the essential oils of *Piper officinarum* (Piperaceae). *Natural Product Communications*, *7*, 1659–1662.
[21] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. (**1999**) Antioxidant activity applying an improved ABTS radical cationdecolorization assay. *Free Radical Biology and Medicine*, ***26***, 1231–1237.
[22] Salleh WMNHW, Ahmad F, Khong HY. (**2014**) Chemical composition of *Piper stylosum* Miq. and *Piper ribesioides* Wall. essential oils and their antioxidant, antimicrobial and tyrosinase inhibition activities. *Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants*, ***13***, 488-497.
[23] Ellman GL, Courtney KD, Andres V, Feather-Stone RM. (**1961**) A new and rapid colorimetric determination of acetylcholinesterase activity.*Biochemical Pharmacology*, ***7***, 88–95.
[24] Salleh WMNHW, Hashim NA, Ahmad F, Khong HY. (**2014**) Anticholinesterase and antityrosinase activities of ten *Piper* species from Malaysia. *Advanced Pharmaceutical Bulletin*, ***4***, 527-531.
[25] Kemal C, Louis-Flamberg P, Krupinsky-Olsen R, Shorter AL. (**1987**) Reductive inactivation of soybean lipoxygenase I by catechols: A possible mechanism for regulation of lipoxygenase activity. *Biochemistry*, ***26***, 7064-7072.