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ESSENTIAL OIL COMPOSITION AND BIOLOGICAL ACTIVITY OF EXTRACTS *SCHIZONEPETA MULTIFIDA* GROWN IN MONGOLIA

This study was designed to determine essential oil chemical composition and evaluate antioxidant, cytotoxic activities, hepatoprotective effects of aerial parts serial fractions from *Schizonepeta multifida* grown in Mongolia. The composition of the essential oil of *S. multifida* grown in Mongolia was analyzed by Gas chromatography-Mass spectrometry (GC-MS). Among the 33 components (Z)-β-ocimene (38,3 %), 1,8-cineole (28,11 %) terpinolene (10,52 %), α-terpineol (3,63 %) were found to be the major ones.

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The ethanol crude extract of *S. multifida* was suspended in water and consequently fractionated with hexane, chloroform, ethyl acetate and butyl alcohol. All the fractions were examined for their antioxidant and cytotoxic activities, hepaprotective effects by using DPPH and MTT assays, respectively. Ethyl acetate fraction showed the highest DPPH radical scavenging activity at concentration of 50 μg/mL by 93,69 % with the IC50 value of 10.56 μg/mL. The essential oil exhibited lower antioxidant activity (9,57; 3,47 %) than fractions. MTT results showed that ethyl acetate fraction demonstrated significant cytotoxicity against A549 cell line, with values of 64.10 and 84,60 %. The water fraction notably protected HepG2 cells against the cytotoxicity of *t*-BHP than EGCG.

Key words: *Schizonepeta multifida, essential oil, antioxidant, cytoxicity, hepatoprotection activities.*

Many natural compounds extracted from plants exhibit important biological activities. Among these diverse natural compounds, essential oils extracted from aromatic plants are attracting special attention [1] by the pharmaceutical industry due to their multiple functions [2,3].

In Mongolia, the medicinal and food plants have a long history [4; 5]. The Schizonepeta genus have been used in Mongolian traditional medicine as antiseptic agents [6; 7]. The aerial parts of *S. multifida* have been commonly marketed for the treatment of common cold and fever along with headache, sore throat, allergic dermatitis, pruritus and eczema in traditional medicine [8; 9] and essential oil has been shown to possess antiviral (influenza virus H1N1), antibacterial and antifungal activities as well as anti-human lung cancer activity [10]. However the antioxidant activities, cytotoxicity, hepatoprotectice effects of organic fractions have not been investigated so far.

The essential oil components are listed in Table 1. Thirty three compounds, constituting about 98,60 % of the total oil, were identified. The major components of essential oil were (Z)-β-ocimene (38,3 %), 1,8-cineole (28,11 %) terpinolene (10,52 %), α-terpineol (3,63 %) and 3-octanone (2,94 %)].

Table 1

Essential oil components of *S. multifida* grown in Mongolia

939	α-Pinene	0.54
976	Sabinene	2.55
978	1-Octene-3-ol	0.68
980	β-Pinene	1.25
986	3-Octanone	2.94
991	Myrcene	0.16
1005	α-Phellandrene	0.10
1018	α-Terpinene	0.03
1026	p-Cymene	1.77
1031	Limonene	28.11
1034	1,8-Cineole	38.30
1040	(Z)-β-Ocymene	3.59
1050	(E)-β-Ocymene	10.52
1088	Terpinolene	0.16
1091	2-Nonanone	0.03
1097	(E)- Sabinene hydrate	0.37
1098	Linalool	0.04
1111	Dehydro-p-cymene	0.03
1173	Mentol	0.06
1177	Terpinene-4-ol	0.74
1189	α-Terpineol	3.63
1193	Myrtenol	0.05
1237	Pulegone	0.32

Ending of table 1

1252	Piperitone	0.03
1255	Geraniol	0.03
1289	Lavandulyl acetate	0.21
1290	Thymol	t
1298	Carvacrol	t
1355	Thymol acetate	t
1440	α-Humulene	0.05
1480	Germacrene-D	1.27
1527	δ-Cadinene	0.26
	Total identified	***98.60***
	Monoterpene hydrocarbons	61.91
	Oxygenated monoterpenes	35.11
	Sesquiterpene hydrocarbons	15.80
	Oxygenated sesquiterpenes	-

t, trace amount

The DPPH scavenging abilities of essential oil were 9,57 % and 3,47 % (50; 10 μg/mL in ethanol). Among all fractions the ethyl acetate fraction showed strong free radical scavenging activity in DPPH assay system with IC50 value of 8.54±3.5% (Figure 1).

MTT results showed that ethyl acetate fraction demonstrated significant cytotoxicity against A549 cell line (Figure 2). The IC50 value for ethanol extract was 35,8 μg/mL.

The t-BHP results suggested that the *S. multifida* extracts, specially water, ethanol, buthanol fractions contained a variety of hepatoprotective compounds distributed in all of the solvent fractions (Figure 3).

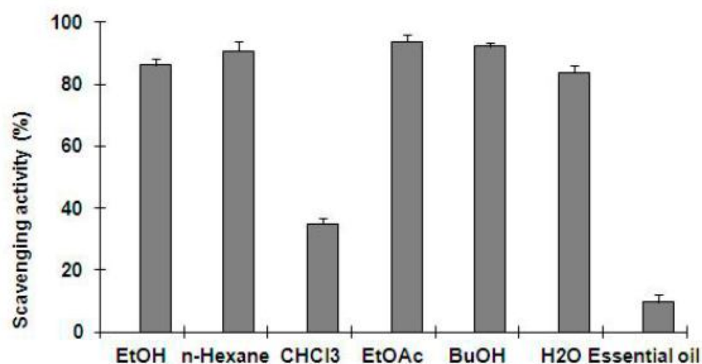

Figure 1. Free radical scavenging activity (%) by the DPPH assay of serial fractions and essential oil *S. multifida* at a concentration of 50 μg/mL.

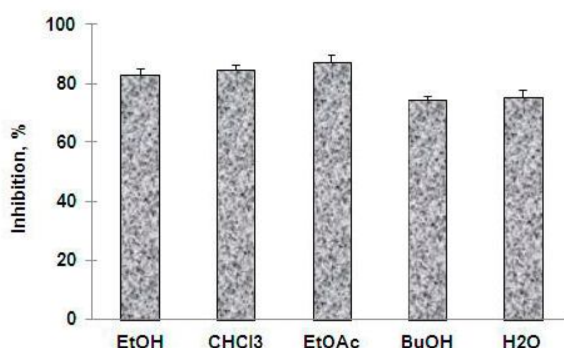

Figure 2. Cell inhibition (%) of organic fractions from *S. multifida* against A549 cell line. Cells were treated with the fractions for 48 h at a concentration of 50μg/mL

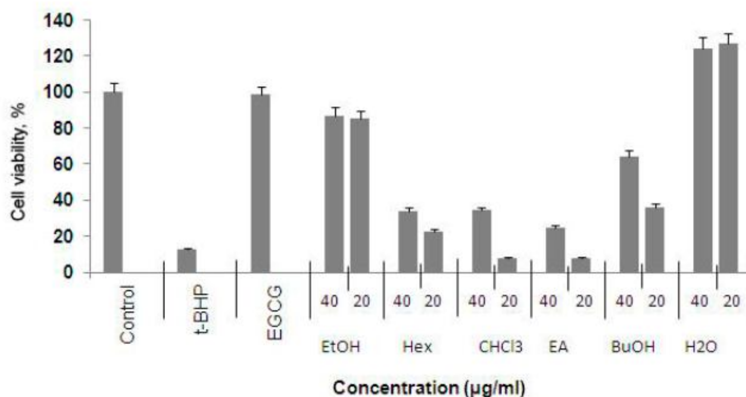

Figure 3. The cytoprotective effects of the serial solvent fractions from the aerial parts of *S. multifida* on prevention of injury of HepG2 cells by tert-butyl hyperoxide (*t*-BHP)

EXPERIMENTAL

Plant material: Samples were collected from Yolyn Am (Lammergeier Valley), Gurvan Saikhan Mountains of southern Mongolia in July 2009. Voucher specimens have been deposited at the herbarium of the Institute of Botany of the Mongolian Academy of Sciences, Ulaanbaatar, Mongolia.

Isolation of the Essential oil: The aerial parts (1,1 kg) of the freshly collected plants were finely chopped and hydro-distilled for 3 h using a Clevenger-Adams type apparatus [11]. The yield of the essential oil produced during the steam distillation was 98,6 %. The oil was then stored at 4°C prior to analysis.

Extraction and fractionation: The air-dried and powdered whole plant (170 g) was extracted with 70 % ethanol (2 L × 3) using sonicator under room temperature. The resultant extracts were combined and evaporated in a rotary vacuum evaporator (Buchi R-205, Switzerland) at 40°C to afford crude extracts. The ethanol crude extract (28 g) were suspended in water and then fractionated successively with n-hexane, chloroform, ethyl acetate and butyl alcohol by using the separation funnel, respectively.

Analysis of the essential oil: GC-MS analyses were carried out using an HP-5890 gas chromatograph coupled with a Finnigan MAT 800 ion Trap detector a J&W DB-5 column (30×0.25 film thickness 0,25 mm) was used with He as carrier gas (linear velocity 30 cm/s). The temperature programming was performed as follows: 50°C isothermal for 10 min then increased from 50°C to 220°C at 4°C/min, and finally isothermal at 220°C for 5 min. The injector temperatures were 250°C, the volume injected 1.0 μl of 1 % solution (diluted in hexane). The EI/MS spectra were recorded at 70eV (ion source temperature: 220°C). The mass range was 35 to 425 m/z.

The essential oil components were identified on the basis of their mass data and the comparison of their relative retention index (RRI) [12]. Their EI-mass spectra were either compared with the NIST/NBS and Wiley library spectra found in the literature [13], or were confirmed by comparison with data published in a reference book [14].

Antioxidant activity measurement: DPPH assay was carried out according to the method of Brand-William et al. [15] to investigate the free radical scavenging activity of samples. Briefly, the samples were dissolved in ethanol at the concentration of 100 mg/ml and then serially diluted by ethanol. On each well of a 96-well plate, 100 μl of samples of different concentration were mixed together with 100 μl of 60 μM DPPH prepared in ethanol. After incubation of 20-30 minutes for reaction, the absorbance of supernatants was measured at 517 nm [16; 17]. Ethanol was used as negative control and α-tocopherol as positive control. The IC_{50} value of a sample is the concentration of sample at which 50 % activity of DPPH (absorbance) is inhibited. It was calculated by linear regression.

Cell lines and culture: The human alveolar basal epithelial cell line (A549) was purchased from Korean cell line bank. The cells were grown in RPMI 1640 supplemented with 10 % fetal bovine serum (FBS), 1 % (w/v) glutamine, sodium pyruvate 5 %, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were cultured in a humidified atmosphere at 37°C in 5 % CO_2.

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) colorimetric method was used for determining cytotoxicity of samples as describing by Mosman [18]. In brief, each sample was dissolved in DMSO at concentration of 100 mg/ml and serially diluted into different concentration of 1-100 µg/ml with culture media. On a 96-well plate, 200 µL of cell suspension at density of $2x10^4$ (in ml) were seeded. After 24 h incubation at 37°C, 5 % CO_2 to allow cell attachment, the culture media were removed and replaced by 200 µl of cultured media containing different concentration of samples and incubated for 24 and 48 h at the same condition. In control wells, the media were added without any samples. Finally, after 4 h of MTT reagent addition (final concentration of 0,5 mg/ml), the formazan crystals formed was resolved in DMSO (200 µl/well) and absorbance were measured at 570 and 630 nm.

In vitro hepatoprotective effect against tert-butyl hydroperoxide (t-BHP): To evaluate hepatoprotective effects of the fractions *S. multifida*, the protective activity against *t*-BHP toxicity was measured in human hepatocellular carcinoma HepG2 cells with the MTT cell viability assay. HepG2 cells were obtained from the American Type Culture Collection and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin and 100 µg/mL streptomycin.

HepG2 cells ($1×10^4$ cells per well) were plated onto 96-well plates, incubated in DMEM at 37°C for another 24 h, then preincubated with the fractions (20; 40 µg/mL) in DMEM without fetal bovine serum at 37°C for 24 h. Cell viability was measured with the EZ–cyTox Cell Viability Assay Kit, as described previously [19].

An epigallocatechin-3-gallate (EGCG) pretreatment, used as a positive hepatoprotection control, for 24 h at a concentration of 10 µM maintained the viability of HepG2 cells at 98,37 % of the uninjured control level following administration of *t*-BHP.

The total extract and each of its fractions were administered to HepG2 cells at concentrations of 20 and 40 µg/mL. Pretreatment of HepG2 cultures for 24 h with any of these samples substantially prevented *t*-BHP induced cell damage. The H_2O fraction protected against *t*-BHP injury to the uninjured control level at 20 and 40 µg/mL concentrations, it was more effective than EGCG. The protective effects of EtOH fraction against *t*-BHP injury were almost similar to that of EGCG. The cells treated with n-Hex and $CHCl_3$, EA fractions showed low cell viabilities to uninjured control cells at all tested concentrations.

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СОСТАВ ЭФИРНОГО МАСЛА И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ ЭКСТРАКТОВ *SCHIZONEPETA MULTIFIDA*, ВЫРАЩЕННОГО В МОНГОЛИИ

Данное исследование было проведено для определения химического состава эфирного масла и оценки антиоксидантной, цитотоксической активности, гепатопротекторного действия серийных фракций надземных частей *Schizonepeta multifida*, выращенного в Монголии. Состав эфирного масла *S. multifida*, выращенного в Монголии, был проанализирован методом газовой хроматографии-масс-спектрометрии (ГХ-МС). Среди 33 компонентов (Z) -β-оксимен (38,3 %), 1,8-цинеол (28,11 %), терпинолен (10,52 %), α-терпинеол (3,63 %) оказались основными.

Этанольный неочищенный экстракт *S. multifida* суспендировали в воде и, следовательно, фракционировали с помощью гексана, хлороформа, этилацетата и бутилового спирта. Все фракции были исследованы на их антиоксидантную и цитотоксическую активность, гепапротекторные эффекты с использованием анализов DPPH и MTT соответственно. Фракция этилацетата показала самую высокую активность по удалению радикалов DPPH при концентрации 50 мкг/мл на 93,69 % при значении IC50 10,56 мкг/мл. Эфирное масло проявляло более низкую антиоксидантную активность (9,57; 3,47 %), чем фракции. Результаты MTT показали, что фракция этилацетата продемонстрировала значительную цитотоксичность в отношении клеточной линии A549 со значениями 64,10 и 84,60 %. Водная фракция в большей степени защищала клетки HepG2 от цитотоксичности *t*-BHP, чем EGCG.

Ключевые слова: *Schizonepeta multifida, эфирное масло, антиоксидант, цитоксичность, гепатопротекторная активность.*