STUDY ON ESSENTIAL OIL COMPOSITION AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL AND ETHANOL EXTRACT'S FROM ARTEMISIA SCOPARIA WALDST.ET KIT GROWN IN MONGOLIA

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Abstract:

Mongolia is rich in medicinal plants. Essential oils of aromatic and medicinal plants generally have a diverse range of activities because they possess many active constituents that work through a several modes of action. Artemisia, the largest genus of the family Asteraceae, has a number of effects against human and plant diseases [8].

This study was aimed to evaluate antioxidant, cytotoxic activities of aerial parts ethanol extracts from Artemisia scoparia Waldst.et Kit grown in Mongolia. The antioxidant and cytotoxic activities of the essential oil and ethanol extract was determined by using DPPH and MTT assays. The ethanol extracts showed higher antioxidant activity than essential oil. The essential oil of Artemisia scoparia Waldst.et Kit with a concentration of 150 mg/ml or 3µg/disk inhibits the growth of S.enterica 10.6±0.58 mm, B.subtillus 11.6±1.15 mm, and has a moderate bacterial activity.

The half-lethal dose (IC50) 56.542 µg/ml is of essential oil of Artemisia scoparia Waldst. et. Kit, so essential oil of Artemisia scoparia Waldst. et. Kit is active in inhibiting of liver cancer cell line (HepG2).

The half-lethal dose (IC50) 72.611 µg / ml is of essential oil Artemisia scoparia Waldst. et. Kit, which has the active in inhibiting the growth of human stomach cancer cell line (AGS).

The results clearly showed that the essential oil presented satisfactory cytotoxic activity against human stomach cancer cell line AGS. Our work revealed that the ethanol extracts and essential oil of Artemisia scoparia Waldst.et Kit grown in Mongolia has potential as sources of new antioxidant, and cytotoxic compounds, respectively.

Keywords: *Artemisia scoparia Waldst.et Kit; essential oil; antimicrobial; antioxidant, and cytotoxic activities*

INTRODUCTION

The genus *Artemisia* (*Asteraceae*) consists of about 500 species, distributing throughout the world, one of the most numerous plant groupings, which comprises about 1000 genera and over 20000 species [9,10]

Mongolia is rich in essential oil medicinal plants. Mongolian traditional medicine has long history of more than 2500 years.[1,5] There are about 60 clans, about 200 species[5], 300 kinds of essential oil plants and 600 kinds of herbal plants have been registered, among of them, 150-200 kinds are commonly used.[2] Many essential oil plants have not been studied yet. It is important to investigate their chemical compositions and biological activities by using traditional medicine.[3,4] The genus *Artemisia* is one of the largest and most widely distributed genera of the family *Astraceae* (Compositae). It is a heterogenous genus, consisting over 500 diverse species occurred predominantly in the temperate zones of Europe, Asia and North America [18]

Artemisia species are commonly utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses [12].

Various species of *Artemisia* seems to hold great potential for in-depth investigation for various biological activities, especially their effects on the central nervous and cardiovascular systems [9].

A.scoparia has medicinal properties like anti-chlosterolemic, antipyretic, antiseptic, antibacterial, diuretic, cholagogue, vasodilator [18].

The herb in Mongolian-Tibetan medicine is used for yellowing of the eyes, violations of the regulatory system mkkhris (the system responsible for food rhenium, absorption, energy processes, assis-milation) and Botkin's disease [19].

The major components of *Artemisia scoparia* oil were the following: *p*-cymene (0.6–15.2%), limonene (0.1–6.3%), α-pinene (0.2–10.1%), β-pinene (0.4–8.9%), *trans*-β-ocimene (0.3–5.4%),caryophyllene (4.6–13.8%), germacrene D (11.5–40.3%), spathulenol (4.0–11.7%), and caryophyllene oxide (4.3–15.6%)[14].

Twenty four compounds were identified in the oil of the *Atremisia scoparia* representing 93.6% of the oil. The main component of the oil were p-cymene-11.31%, limonene-10.27%, myrcene-9.61%, α-pinene-8.56%, γ-terpinene-7.81%, β-pinene+sabinene-5.55%, spathulenol-3.17%, 1,8 cineole-2.83%, caryophyllene-1.60%, Z-β-ocimene-1.64%,and E nerolidol-1.58% [15].

The major constituents of the *A. scoparia* oil were methyl eugenol (18.53%), caryophllene oxide (5.69%), spathulenol (4.37%), sabinene (1.24%).[16]

A.scoparia oil was dominated by the diacetylenes phenyl-2,4-pentadiyne (34.2%) and capillene (4.9%). Other major components were β-pinene (21.3%), methyl eugenol(5.5%), α-pinene (5.4%), myrcene (5.2%), limonene (5.0%), and (*E*)-β-ocimene (3.8%).%).[17]

In 1976, Shatar et al., examined the chemical composition of *Artemisia .scoparia* oil produced from plants grown in Mongolian Gobi.

The compounds identified in the GC were as follows: α-pinene (15.0%), camphene (12.0%), β-pinene (1.2%), sabinene(6.0%), 3-δ-carene (2.4%), myrcene (2.5%), terpinene (4.3%), α-phellandrene (3.5%), β-phellandrene (3.0%), p-cymene (5.0%), longicyclene (2.5%), longifolene (0.3%), β-bisabolene (0.6%), β-santalene (0.2%), α-himachalene (2.5%), γ-bisabolene (0.4%), δ-cadinene (1.8%) and curcumene (0.2%) [18]

The major constituents of the oil of *Artemisia scoparia* were β-pinene (16.10%), carvacrol (13.81%), limonene (8.82%), cis-ocimene (8.38%), methyl eugenol (7.62%), and transocimene (7.17%). [23]

The essential oil of *Artemisia .scoparia* extracted at the vegetative stage contain 20.3%, β-thujone, 55.4% α-thujone-5.9%, 1,8-cinelol and 9.4% camphor [24]

The essential oils of *Artemisia scoparia* show strong insecticidal activity against stored-product insects (Negahban et al., 2006) .[21]

Among these, the oils from mature and young leaf of *Artemisia scoparia* have IC50 of 65.9 lg/ml and 119.3 lg/ml, respectively; and it was significantly lesser than that of commercial antioxidant BHT (140.6 lg/ml).[22]

The aim of this study was to evaluate the antioxidant and cytotoxic effects of essential oil and ethanol extract from A*rtemisia scoparia* Waldst.et Kit grown in Mongolia.

The antioxidant activities of the essential oil and the ethanol extracts were tested by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT) colorimetric method was used for determining cytotoxic activity of samples.

To the best of our knowledge, there are no published reports on the biological activities of the essential oil and ethanol extracts of A*rtemisia scoparia* Waldst.et Kit grown in Mongolia.Therefore, it is important to develop a better understanding of their mode of biological action for new application in human health.

Methods Chemicals

Dimethyl sulfoxide (DMSO), and DPPH were purchased from Millipore-Sigma (Germany) and WST was purchased from DoGen (Korea). RPMI 1640 medium and fetal bovine serum were purchased from GIBCO (USA). Penicillin and streptomycin were purchased from Himedia (India). The human stomach cancer cell line (AGS) was purchased from ATCC (USA). All other chemicals were of analytical grade and purchased from Millipore-Sigma (Germany) and DUKSAN Co. (Korea).

Plant material

Samples were collected from Khovd aimag (Mongolia) on the July 2019. Voucher specimens have been deposited in the herbarium of the Branch Khovd of National University of Mongolia.

Isolation of the Essential oil

The aerial parts (1 kg) of the freshly collected plants were finely chopped and hydro-distilled for 3 h using a Clevenger-Adams type apparatus.[7]

The yield of the essential oil produced during the steam distillation was 0.96% (v/w). The oil was then stored at 4°C prior to analysis.

The GC-MS analysis of the essential oil sample was carried out using a Agilent 6890 gas chromatograph equipped with mass selective detector MSD 5973 (Agilent) on capillary column HP5 (5% diphenyl and 95% dimethylsiloxane, 30 m x 0.25 mm x 0.25 µm (film thickness)). The temperature of injector is 280°C. The column temperature was programmed as follows: 2 min at 50°C, temperature increase at a rate of 4 deg/min to 240°C and then at a rate of 20 deg/min to 280°C, isothermal period of 5 min. Helium was used as a carrier gas (1.0 ml/min). MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 30–650, data acquisition rate of 1.2 scan/s. 1.0 µl of sample (solution of the essential oil in hexane, 8.0 µl per 0.5 ml) was injected in a split mode with split ratio 100:1.

A mixture of normal hydrocarbons C_8–C_{24} was added to the sample as a standard for determining linear retention indices. Essential oil components were identified by comparison of their full mass spectra and linear retention indices (RI) with these parameters for authentic samples listed in the handbook [28].

Extraction and fractionation

The air-dried and powdered whole plant (170 g) was extracted with 99% 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 obtained ethanol crude extract was weighed (28 g) and stored in the refrigerator for the later analysis.

Determination of the Antioxidant activity

The assay was carried out according to the method of Brand-William et al.[29] 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 by using Multi-detection Reader (Bio Tek Co.). Ethanol was used as negative control and α-tocopherol as positive control.

The scavenging capacity (SC) of the sample was calculated using the following formula:

$$SC\ (\%) = [1-A_S/A_C]\ 100$$

Where, A_S=is the net absorbance of the sample, A_C=is the net absorbance of negative 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.

Determination of antimicrobial activity

To investigate the antimicrobial activity of essential oil and ethanol extract from A*rtemisia scoparia* Waldst.et Kit., we evaluated its effect on four different bacteria, such as *S.enterica, B.subtillus, S.aureus, E.coli* by Agar diffusion method.

Determination cytotoxic activity

AGS cell was cultured in RPMI-1640 medium supplemented with 0.2% sodium bicarbonate, 1% penicillin-streptomycin and 10% fetal bovine serum at 37°C in 5% CO_2 incubator. The four samples were prepared as 30 mg/ml stock solutions

in DMSO. The AGS cell was treated by samples with final concentration of 300 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and 10 µg/ml, and incubated for 24 hours. RPMI-1640 medium with 10% WST was added to the treated cells. After 1-hour incubation, the cultured cells were quantified by spectrophotometer, measuring the absorbance of the dye solution at 450 nm. Results of each extract were compared to that of DMSO only treated control cells, 1% v/v DMSO. The IC50 was calculated for each sample by IC50 Calculator by AAT Bioquest. Avoiding the possibility of metabolic activity alteration thus tetrazolium dye reduction without affecting cell viability, the results were then checked under microscope by examination of live condition[30]

Result and Discussion

Analysis of the Essential oil

The percentage contents of the essential oil component are summarized in Table 1. A total of 28 components were identified, representing 93.46% of the total oil. The terpenoides made up the largest component of the oil and had many representative volatiles.

The oxygenated monoterpenes (36.794%), and oxygenated sesquiterpenes (18.6%), monoterpenes (2.42%) sesquiterpenes (35.65%). The main constituents were found to be eugenol (10.86%), methyl eugenol (11.85%), ar curcumene(5.89%), capillene (20.75%), spaphulenol (11.79%), eugenol-2Me-butonate (7.66%), caryophyllene oxide (4.57%).

These differences might have been derived from local, climatic and seasonal factors (Table 1).

A comparison of the chemical composition of essential oil of A*rtemisia scoparia* Waldst.et Kit is shown. / Table-2 /. Essential oils of Mongolian A*rtemisia scoparia* Waldst.et Kit include α-pinene, β-pinene, p-cymol, limonene, 1.8 cineole, camphor, γ-terpinene, terpinene-4-ol, piperitone, eugenol, lavandulyl acetate, caryophyllene, α-zingiberene, humulene and β-farnesene.

Containing compounds such as E-nerolidol, caryophyllene oxide, spatulenol, this species is characterized by the hemotype characteristics of A*rtemisia scoparia* Waldst.et Kit essential oil.

For the first time, 9 terpent compounds were determined in essential oil from A*rtemisia scoparia* Waldst.et Kit, including α-copaene, γ-curcumene,

Ar curcumene, capillene, d-kadinene, dihydrocaryophyllene-5-one, eugenol isobutanoate, eugenol 2 methyl butanoate, and eugenol 3 methyl butanoate.

Antioxidant activity

DPPH is free radical compound that has been widely used to determine free radical scavenging activity.[29]
The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form DPPH (non radical) with the loss of this violet color. [31]

The DPPH assay is used to analyze antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. The method was applied according to Brand-Williams et al.[29]

Ethanol extract was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution (30 mg/mL) for antioxidant assays. The extract was prepared by two times dilution method in 96-well microtiter plate. Also, Gallic acid standard solutions were prepared in 96 well micro liter plate for building of standard curve, which is used for calculation of antioxidant activity of samples. The final results were expressed as ug/ml of Gallic acid equivalent (See Figure 6,7).
Antioxidant activity the essential oil of A*rtemisia scoparia* Waldst.et Kit is 29.608%

Cytotoxic activity

To investigate the cytotoxic activity of ethanol extracts and essential oil from A*rtemisia scoparia* Waldst.et Kit, we evaluated its effect on a selection of liver cancer cell line HepG2 and human stomach cancer cell line AGS by Rapid colorimetric assay.
These cell lines were submitted to growing concentrations of essential oil and ethanol extract A*rtemisia scoparia* Waldst.et Kit for 24 and 48 hours. As shown in Figure 2-5, the essential oil of plant significantly active against chosen human cancer cell lines tested the ethanol extract (See Figure 2-5).

Antimicrobial activity

To investigate the antimicrobial activity of essential oil and ethanol extract from A*rtemisia scoparia* Waldst.et Kit., we evaluated its effect on four different bacteria, such as *S.enterica, B.subtillus, S.Aureus, E.coli* (See Table 3).

Conclusion

In recent years, interest in plant-derived food additives has grown. Plant extracts might substitute synthetic food antioxidants, which may influence human health when consumed chronically. [13]

This study on essential oil chemical composition and biological activities of A*rtemisia scoparia* Waldst.et Kit grown in Mongolia were not well performed before.

Essential oils hydrodistilled from A*rtemisia scoparia* Waldst.et Kit were found to be rich in eugenol, methyl eugenol, ar-curcumene, capillene, spaphulenol, caryophyllene oxide.

The antioxidant activity of the ethanol extracts were moderate than essential oil.

Essential oil of *Artemisia scoparia* Waldst. et. Kit has a half-lethal dose (IC50) of 56.542 μg/ml, so essential oil of *Artemisia scoparia* Waldst. et. Kit is active in inhibiting liver cancer cells (HepG2).

The half-lethal dose (IC50) *Artemisia scoparia* Waldst. et. Kit of essential oil is 72.611 μg / ml, which has the effect of inhibiting the growth of gastric cancer cells (AGS).

The results clearly showed that the ethanol extracts presented satisfactory cytotoxic activity against 2 human cancer cell lines tested.

The results of this work also demonstrate the potential of A*rtemisia scoparia* Waldst.et Kit ethanol extracts as a new antioxidant and cytotoxic agents for human health.

Acknowledgments

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Supplementary Material

Table 1. Main components (%) of the essential oil from *Artemisia scoparia* Waldst.et Kit grown in Mongolia

peak #	R.T. min	corr. area	% of total	
1	7.356	744825	0,10	□-pinene
2	8.713	3080262	0,40	□-pinene
3	10.373	8911663	1,17	p-cymol
4	10.518	3391853	0,44	limonene
5	10.554	5191472	0,68	1,8-cineol
6	11.615	2353942	0,31	□-terpinene
7	14.597	2135571	0,28	camphor
8	15.860	2668290	0,35	terpinene-4-ol
9	18.575	3719805	0,49	piperitone
10	19.759	1050974	0,14	lavandulyl acetate
11	22.004	82828788	10,86	eugenol
12	22.502	2288874	0,30	□-copaene
13	23.513	90397869	11,85	methyl eugenol
14	23.910	37512803	4,92	caryophyllene
15	24.957	3374806	0,44	humulene
16	25.036	9663257	1,27	*E*-□-farnesene
17	25.751	6537486	0,86	□-curcumene
18	25.852	44921908	5,89	Ar-curcumene
19	26.264	4922013	0,65	□-zingiberene
20	26.408	158289426	20,75	capillene
21	27.087	4373031	0,57	□-cadinene
22	27.968	5621035	0,74	dihydrocaryophyllene-5-one
23	28.292	11463889	1,50	(*E*)-nerolidol
24	28.783	89970775	11,79	spaphulenol
25	28.899	34893880	4,57	caryophyllene oxide
26	31.086	17288562	2,27	eugenol isobutanoate
27	33.779	58474863	7,66	eugenol 2-Me-butanoate
28	33.909	16858117	2,21	eugenol 3-Me-butanoate

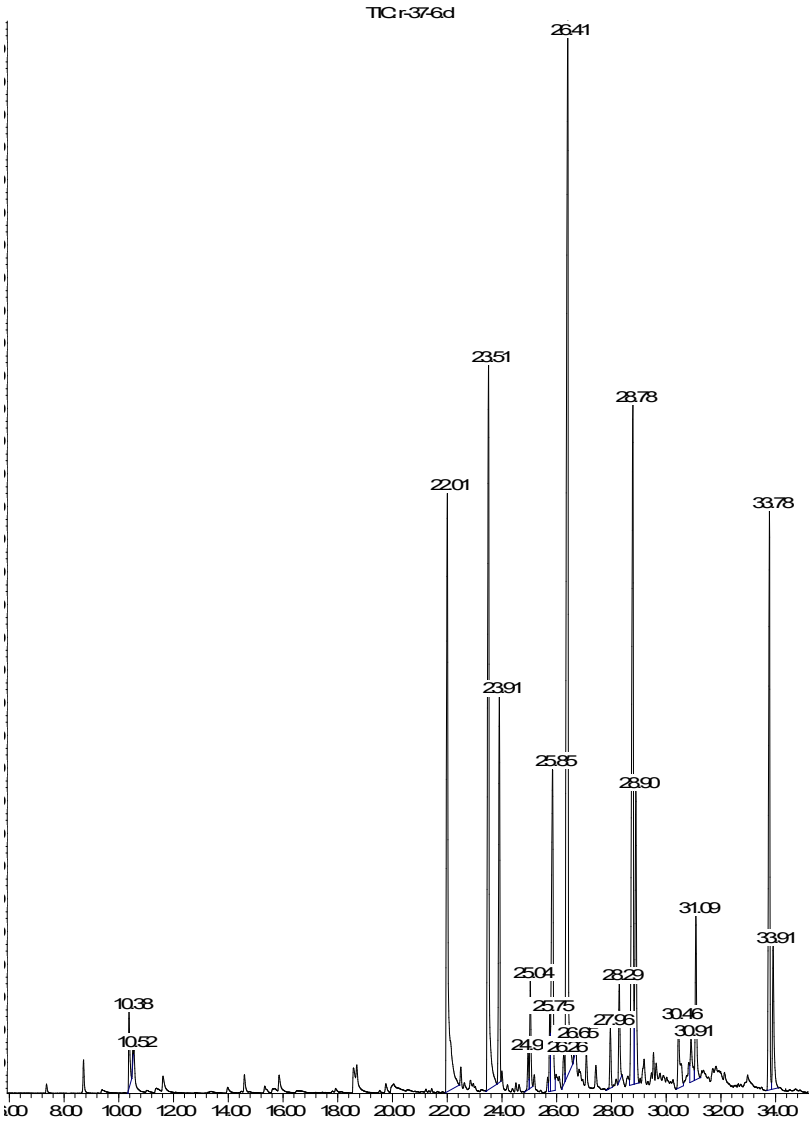

Figure 1. GC/MS analysis of essential oil from A*rtemisia scoparia* Waldst.et Kit grown in Mongolia

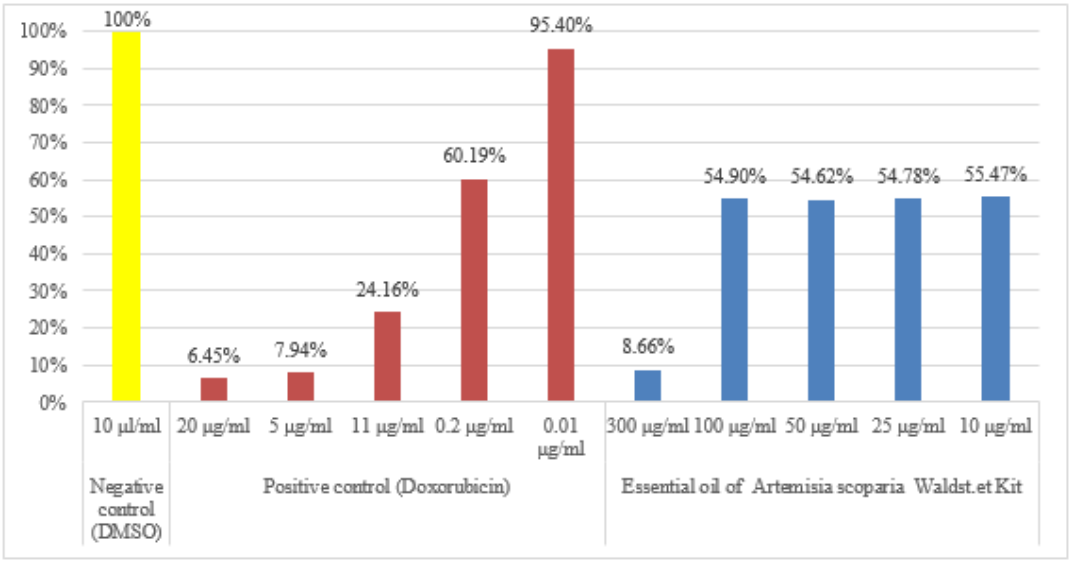

Figure 2. Cytotoxicity (%) of ethanol extract from A*rtemisia scoparia* Waldst.et Kit growing in Mongolia against AGS cell line. Cell was treated with the fractions for 24 h at a concentration of 10-300 µl/ml

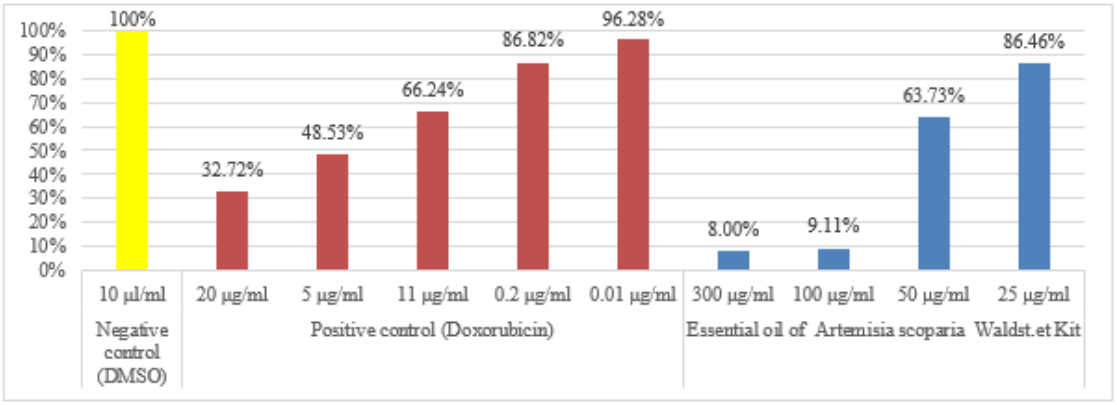

Figure 3. Cytotoxicity (%) of essential oil from A*rtemisia scoparia* Waldst.et Kit growing in Mongolia against HepG2 cell line. Cell was treated with the fractions for 24 h at a concentration of 10-300 µl/ml.

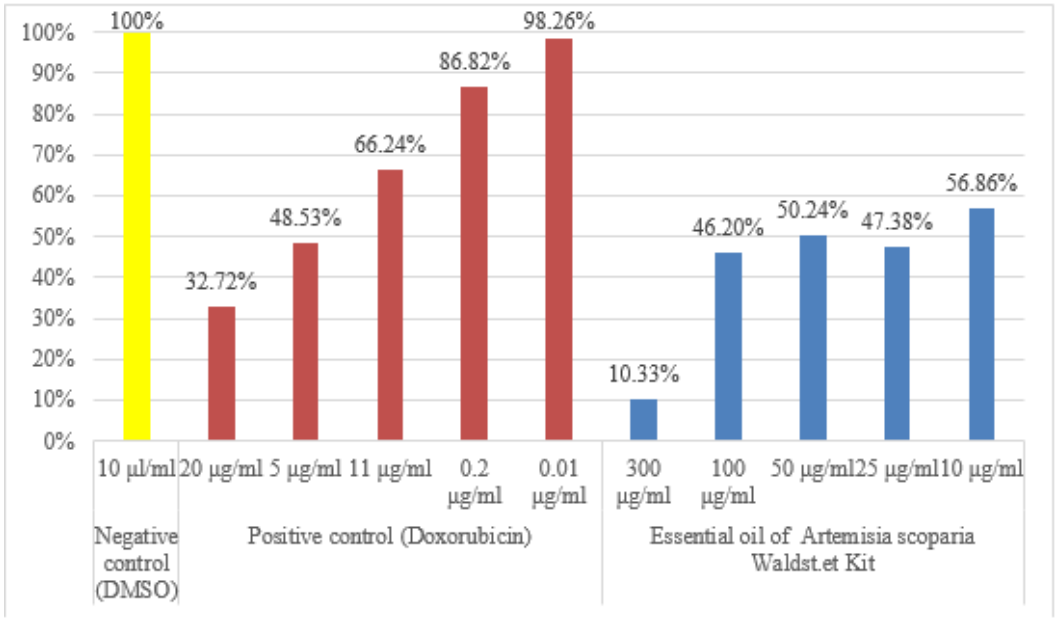

Figure 4. Growth inhibitory effect of ethanol extract A*rtemisia scoparia* Waldst.et Kit against HepG2 cells line. Cell was treated with the fractions for 24 h at a concentration of 10-300 µl/ml

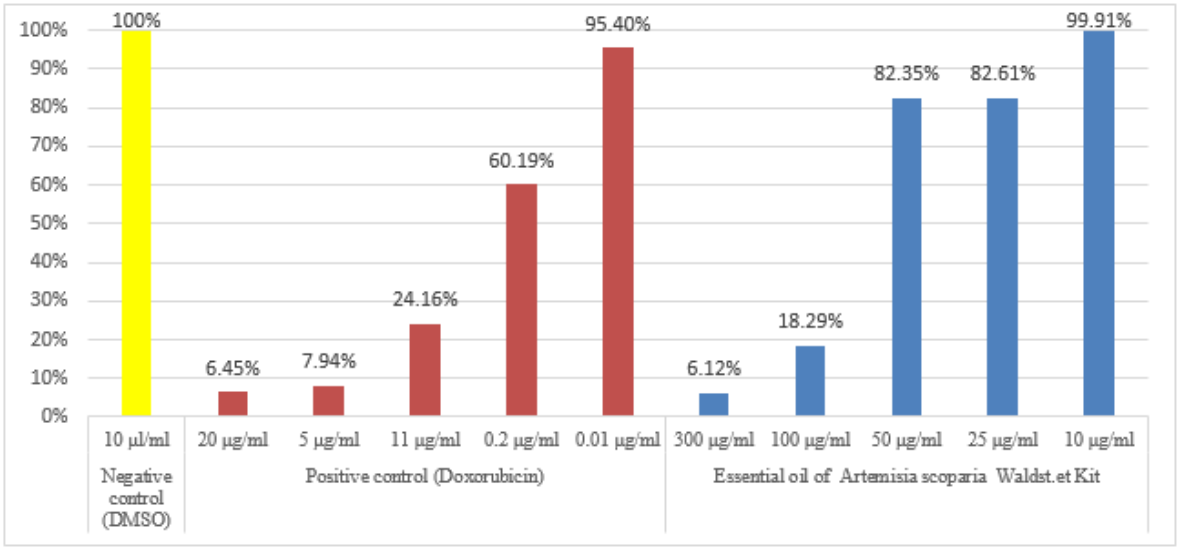

Figure 5. Growth inhibitory effect of essential oil A*rtemisia scoparia* Waldst.et Kit on AGS cells after 24 hours treatment. The results are expressed as percentage of untreated control

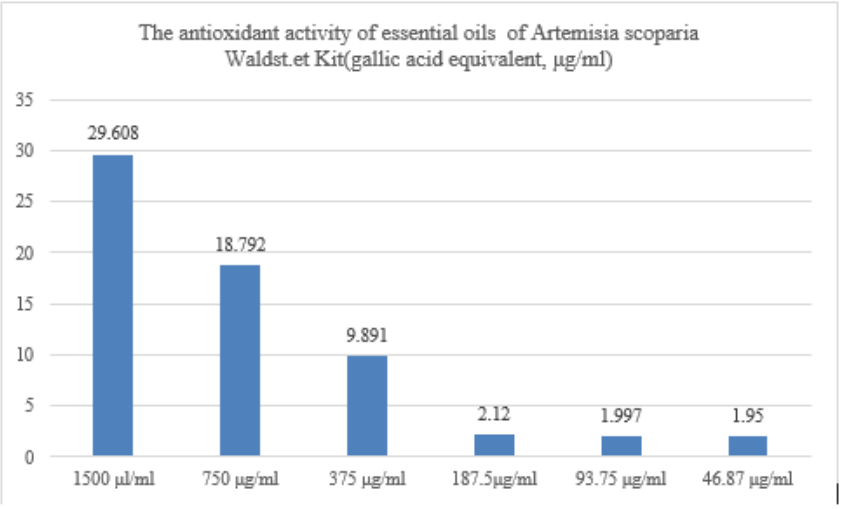

Figure 6. Radical scavenging activity of essential oil from A*rtemisia scoparia* Waldst.et Kit grown in Mongolia

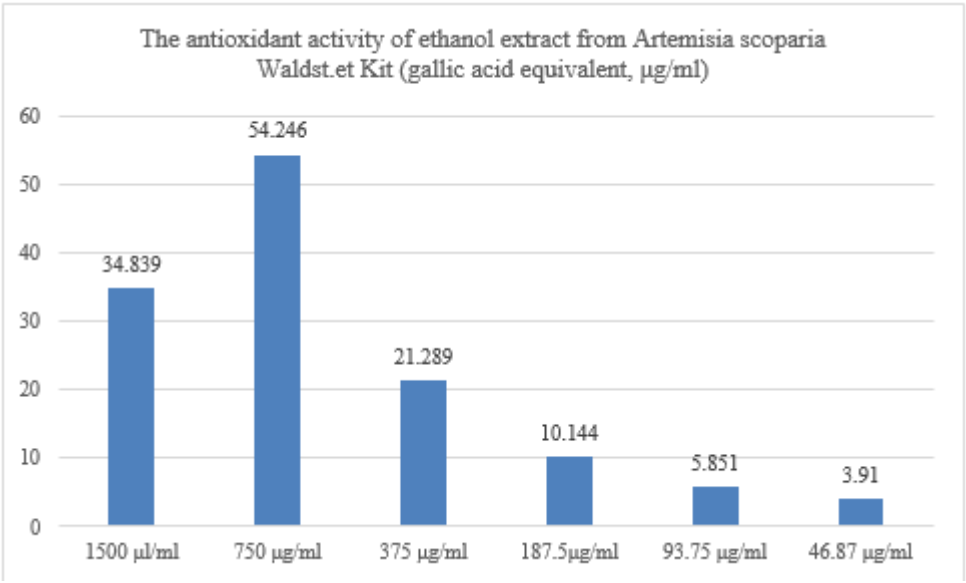

Figure 7. Radical scavenging activity of ethanol extract from A*rtemisia scoparia* Waldst.et Kit grown in Mongolia

Table 2. Comparison of chemical composition of *Artemisia scoparia* Waldst.et Kit essential oil (%)

№	Turpentine compounds	Content/ %/	Essential oil of Artemisia scoparia Waldst.et Kit [20]	Essential oil of Artemisia scoparia Waldst.et Kit [25]	Essential oil of Artemisia scoparia Waldst.et Kit [26]	Essential oil of Artemisia scoparia Waldst.et Kit [27]
1	α-pinene	0.10	15.186	15.21	15.0	15.18
2	β-pinene	0.40	5.552	5.25		5.55
3	p-cymol	1.17				0.46
4	limonene	0.44	10.268	10.23		10.26
5	1.8 cineole	0.68	2.597	2.83	+	2.59
6	γ-terpinene	0.31	4.797	7.80		4.79
7	camphor	0.28		+	7.9	-
8	terpinene-4-ol	0.35	0.189	+	+	0.18
9	piperitone	0.49			2.7	
10	lavandulyl acetate	0.14	0.120			
11	eugenol	10.86			15.5	
12	α -copaene	0.30				
13	methyl eugenol	11.85		+		
14	β-caryophyllene	4.92	1.601	+		1.60
15	humulene	0.44	0.115	+		0.11
16	β-farnesene	1.27	0.447	+		
17	γ-curcumene	0.86				
18	ar- curcumene	5.89				
19	α-zingiberene	0.65				2.26
20	capillene	20.75				
21	d-kadinene	0.57				
22	dihydrocaryophyllene-5-one	0.74				
23	E-nerolidol	1.50	1.571	1.29		1.57
24	spatulenol	11.79		3.17		0.10
25	caryophyllene oxide	4.57	0.130	+		0.13
26	eugenol isobutanoate	2.27				
27	eugenol 2 methyl butanoate	7.66				
28	eugenol 3 methyl butanoate	2.21				

Table 3. Growth inhibition effect of essential oil and ethanol extract from *Artemisia scoparia* Waldst.et Kit on gram positive and negative bacteria

Sample	Zones of growth inhibition in mm							
	S.enterica				***B.subtillus***			
	Product 3µg/disc		0.6 µg/disc		Product 3µg/disc		0.6 µg/disc	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
A*rtemisia scoparia* Waldst.et Kit ***(essential oil)***	10.6	±0.58	8.5	±0.5	11.6	±1.15	9.3	±0.58
A*rtemisia scoparia* Waldst.et Kit ***(ethanol extract)***	10.6	±0.58	8.5	±0.00	11.3	±0.58	8.3	±0.58

continued

Sample	Zones of growth inhibition in mm							
	S.aureus				***E.coli***			
	Product 3µg/disc		0.6 µg/disc		Product 3µg/disc		0.6 µg/disc	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
A*rtemisia scoparia* Waldst.et Kit ***(essential oil)***	8.3	±0.58	6.5	±0.00	6.0	±0.00	6.0	±0.00
A*rtemisia scoparia* Waldst.et Kit ***(ethanol extract)***	8.0	±0.00	6.6	±0.29	6.5	±0.00	6.5	±0.00