Original Research Article

STUDY ON CHEMICAL COMPOSITION OF ESSENTIAL OIL, ETHANOL EXTRACT, AND ANTI-CANCER, ANTI- BACTERIAL ACTIVITY OF *ARTEMISIA RUTIFOLIA STEPH.ex SPRENG* GROWN IN MONGOLIA

ABSTRACT: Mongolia is rich in medicinal plants. In recent years, interest in plant-derived food additives has grown. This study was aimed to evaluate antioxidant, cytotoxic activities of aerial parts ethanol extracts from *Artemisia rutifolia Steph.ex Spreng* grown in Mongolia.

The antioxidant and cytotoxic activities of the essential oil and ethanol crude extracts were determined by using DPPH and MTT assays. The antioxidant activity of *Artemisia rutifolia Steph.ex Spreng* ethanol extract is 2.12 times higher than the antioxidant activity of the essential oil.

The essential oil of *Artemisia rutifolia Steph.ex Spreng* with a concentration of 150 mg/ml or 3µg/disk inhibits the growth of S.enterica 9.3 ± 0.76 mm, B.subtillus 10.3 ± 0.58 mm, S.aureus 9.6 ± 1.5 mm and has a moderate bacterial activity.

The results clearly showed that the essential oil presented satisfactory cytotoxic activity against two human tumor cell lines HepG2 (human liver cancer cell line); AGS (human stomach cancer cell line). Our work revealed that the ethanol extracts and essential oil of *Artemisia ritifolia* ~~*Steph.ex Spreng*~~ grown in Mongolia has potential as sources of ~~new~~ antioxidant, and cytotoxic compounds~~, respectively~~. The results of the study were certified by the utility model certificate of "Soap Fragrance Elixir" with the registration number 20-003068.

Keywords: *Artemisia rutifolia Steph.ex Spreng*, essential oil; antimicrobial; antioxidant, and cytotoxic activities

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Introduction

The Asteraceae family is endowed with essential oil-yielding plants, in particular, the genus Artemisia occupies a top position for its bio-prospection [1].

The genus consists of a small herbs and shrubs, found in northern temperate regions and comprises of about 500 species from South Asia, North America, and European countries[2]. In Mongolia, 103 Artemisia species have been found [3].

The Artemisia species have wide and various applications to the plant and human disease control, as well as in the pharmaceutical industry [5].

Artemisia rutifolia Steph.ex Spreng grows in Afghanistan, China, India, Kazakhstan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia and Tajikistan [7].

Steams and leaves of *Artemisia rutifolia Steph.ex Spreng* contain sugar, carbohydrates, nutrients, alkaloids, vitamin C, chlorophyll, sesquiterpene lactone-canine (Berekovskaya 1991), cirsilineol, tricin, chrosoeriol.[6] The aerial part of the *Artemisia rutifolia Steph.ex Spreng* contains high levels of phenolic compounds such as bile acids, caffeic acid, chlorogenic acid, cyanuric acid, p-coumaric acid, m-coumaric acid, ferulic acid, vanilla acid, myricetin and quercetin. [19].

The essential oil of the *Artemisia rutifolia Steph.ex Spreng* has a laxative effect on bacteria, fungi and helminths. Pharmacological studies have shown that canine is tolerant to cold and Chrysosplenetin has anti-cancer effects. [4]

Artemisia rutifolia Steph.ex Spreng has been used in traditional Pakistan medicine to treat fever, asthma, abdominal pain, inflammation, cancer and other diseases. [10]

The herbal teas have anti-asthma and anti-inflammatory, as well as diuretic properties. Fresh herbs are used to relieve toothache, sore throats, stomach and heart diseases. [9]

The *Artemisia rutifolia Steph.ex Spreng* essential oil with camphor and borneol has anti-inflammatory, anthelmintic and heart-stimulating properties [11].

In Russian traditional medicine, *Artemisia rutifolia Steph.ex Spreng* is used as a medicinal herb, while in veterinary practice it is used to bandage the wounds of pets. [11].

The aim of this study was to evaluate the antioxidant and cytotoxic effects of essential oil and ethanol extract from *Artemisia rutifolia Steph.ex Spreng* 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-diphenyl tetrazoliumbromide (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 *Artemisia rutifolia Steph.ex Spreng* 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 hepatocellular cell line (HepG2) and human gastric cancer cell lines (AGS) were 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 Kharkhiraa Mountain in September 2019 in Uvs aimag, Mongolia. Voucher specimens have been deposited in the herbarium of the *Khovd State University*, 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.[12] 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.

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Essential oil components were identified by comparison of their mass spectra and linear retention indices (relative to C_8–C_{24} alkanes) with those reported in database developed in our laboratory. [13,14]

Extraction and fractionation

The air-dried and powdered whole plant (150 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 (30g) 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.[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 by using Multi-detection Reader (Bio Tek Co.). Ethanol was used as negative control and -tocopherol as positive control. The scavenging capacity (SC) of the sample was calculated using the following formula:

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

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 *Artemisia rutifolia Steph.ex Spreng*, 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

HepG2 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 HepG2 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 IC_{50} 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. [16]

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 88.49% of the total oil. The terpenoides made up the largest component of the oil and had many representative volatiles. The oxygenated monoterpenes (7.44%), and oxygenated sesquiterpenes (12.08%), monoterpenes (50.82%), sesquiterpenes (18.15%). The main constituents were found to be santolina triene (22.38%), β-myrcene (21.84%),1,8cineol (4.63%), caryophyllene (7.19%) caryophyllene oxide (5.82%). These differences might have been derived from local, climatic and seasonal factors (Table 1).

Antioxidant activity

DPPH is free radical compound that has been widely used to determine free radical scavenging activity. [15]

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. [17]

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. [15] 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

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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 (table-2).

The antioxidant activity of *Artemisia rutifolia* S*teph.ex Spreng* ethanol extract is 2.12 times higher than the antioxidant activity of the essential oil.

The process of preperation of the soap fragrance using the essential oil of *Artemisia rutifolia* S*teph.ex Spreng*.

The acidity, pH, and antibacterial activity of soap fragrances were determined by the National Food Safety Reference Laboratory of the General Agency for Specialized Inspection of Mongolia.

The number of acids in soap fragrances was determined by titration method, the solution environment by standard method /MNS1474: 2000/, the acid number by standard method /MNS1131: 2018/, bactericidal ability by standard method /MNS6236: 2011/, alcohol content by standard method /MNF-2011/, the amount of Eschirichia coli bacteria by the standard method /MNS1595: 2017/, the amount of Staphylococcus aureus bacteria by the standard method /MNS1636: 2017/ and the amount of Pseudomonas aruginosa bacteria was determined by the standard method /MNS2173: 2017/.

Cytotoxic activity

To investigate the cytotoxic activity of ethanol extracts and essential oil from *Artemisia .rutifolia* S*teph.ex Spreng*, 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 *Artemisia .rutifolia.* S*teph.ex Spreng* 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 *Artemisia .rutifolia* S*teph.ex Spreng*, we evaluated its effect on four different bacteria, such as *S.enterica, B.subtillus, S.Aureus, E.coli* (See Table 2).

Conclusion

This study on essential oil chemical composition and biological activities of *Artemisia .rutifolia* S*teph.ex Spreng* grown in Mongolia were not well performed before.

Essential oils hydrodistilled from *Artemisia .rutifolia* S*teph.ex Spreng* were found to be rich in santolina triene, β-myrcene 1,8cineol, camphor, caryophyllene, caryophyllene oxide.

The essential oil of *Artemisia rutifolia* S*teph.ex Spreng* with a concentration of 150 mg/ml or 3µg/disk inhibits the growth of S.enterica 9.3 ± 0.76 mm, B.subtillus 10.3 ± 0.58 mm, S.aureus 9.6 ± 1.5 mm and has a moderate bacterial activity.

The antioxidant activity of the ethanol extracts were moderate than essential oil. 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 *Artemisia rutifolia* S*teph.ex Spreng* ethanol extracts as a new antioxidant and cytotoxic agents for human health.

The antibacterial activity of "Soap fragrance" preperation against Eschirichia coli, Staphylococcus aureus, and Pseudomonas aruginosa bacteria, meets the requirements of /MNS1595: 2017/, /MNS1636: 2017/, /MNS2173: 2017/ standards.

The results of the study were certified by the Utility model certificate of "Soap Fragrance preperation" with the registration number 20-003068.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Supplementary Material

Table 1. Main components (%) of the essential oil from *Artemisia .rutifolia Steph.ex Spreng* grown in Mongolia

peak #	R.T. min	corr. area	% of total	
1	6.583	84946209	22,38	santolina triene
2	7.356	1140134	0,30	a-pinene
3	7.810	780597	0,21	camphene
4	8.648	3112995	0,82	sabinene
5	9.247	82893338	21,84	b-myrcene
6	9.420	19989245	5,27	pseudo-limonene
7	10.554	17580383	4,63	1,8-cineol
8	14.575	8084964	2,13	camphor
9	15.839	2579144	0,68	terpinen-4-ol
10	23.029	3206867	0,84	b-elemene
11	23.672	820752	0,22	a-cedrene
12	23.888	27301871	7,19	caryophyllene
13	24.957	2220715	0,59	humulene
14	25.412	3429326	0,90	dehydro-sesquicineol
15	25.628	3681830	0,97	selina-4,11-diene
16	25.816	3744234	0,99	germacrene D
17	26.184	4561706	1,20	valencene
18	26.256	8225966	2,17	a-selinene
19	26.350	5094084	1,34	aciphyllene
20	26.567	3170156	0,84	a-bulnesene
21	26.819	1803784	0,48	g-cadinene
22	27.087	1596169	0,42	d-cadinene
23	28.725	7460070	1,97	spaphulenol
24	28.863	22107840	5,82	caryophyllene oxide
25	29.787	8628186	2,27	cedrol
26	30.205	2601482	0,69	eremoligenol
27	31.750	1585655	0,42	a-bisabolol
28	36.761	3439630	0,91	aciphilyc acid

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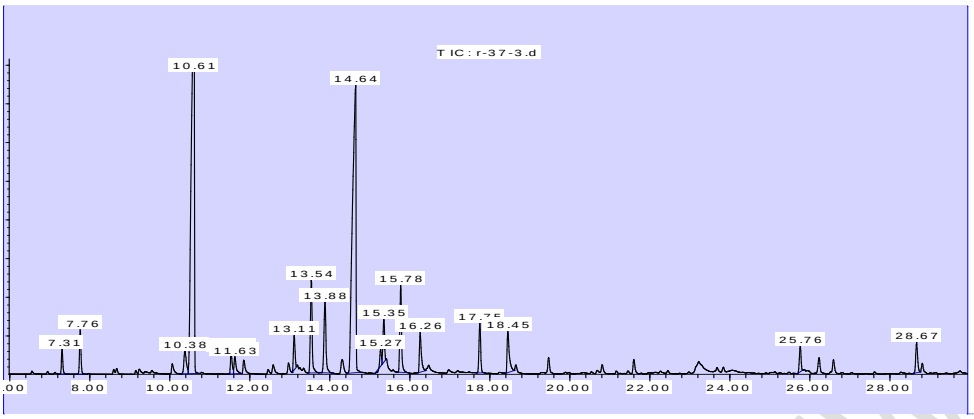

Figure 1. GC/MS analysis of essential oil from *Artemisia rutifolia* S*teph.ex Spreng* grown in Mongolia

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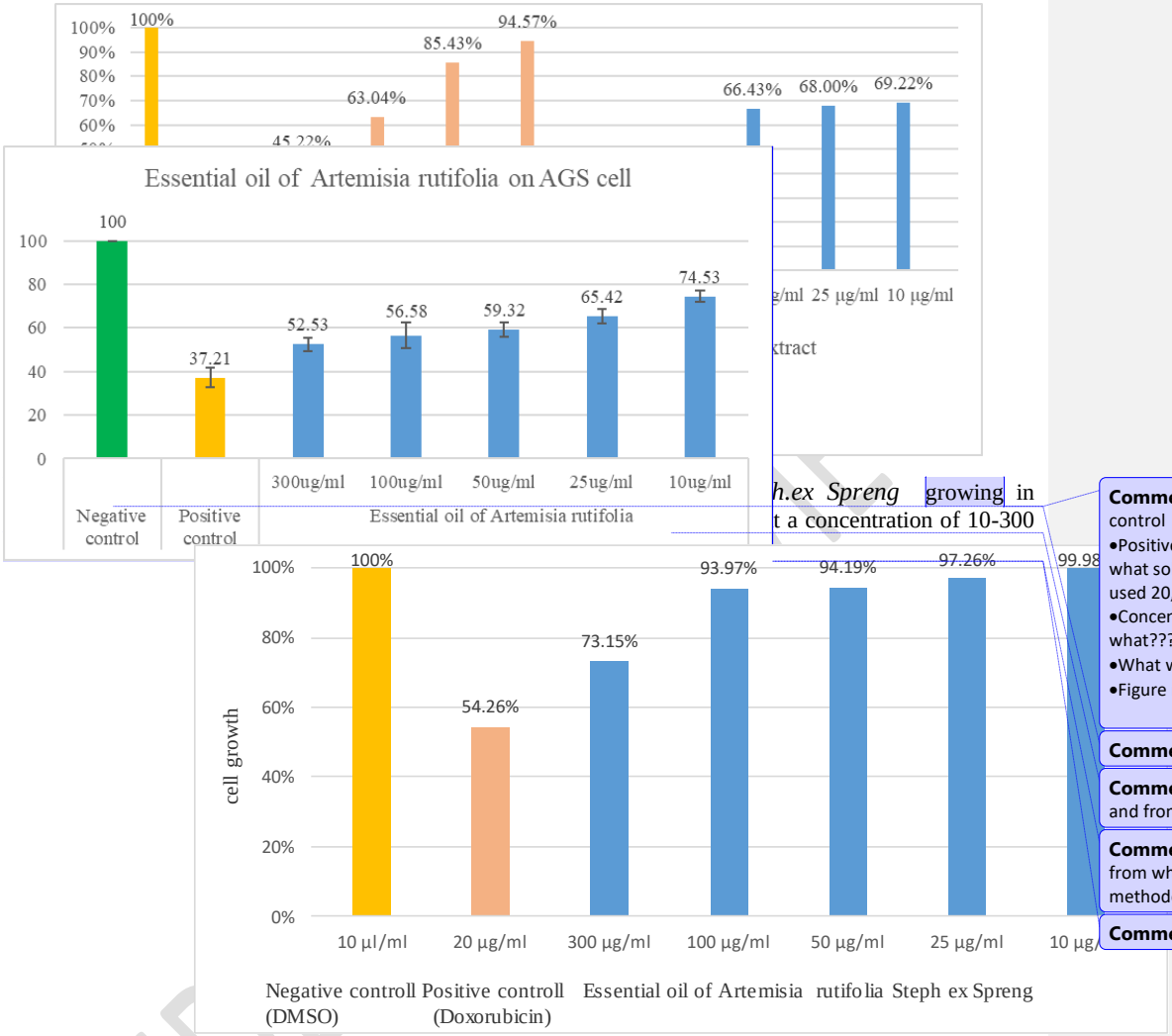

Figure 3. Cytotoxicity (%) of essential oil from *Artemisia rutifolia* S*teph.ex Spreng* growing in Mongolia against HepG2 cell line. Cell was treated with the fractions for 24 h at a concentration of 10-300 µl/ml.

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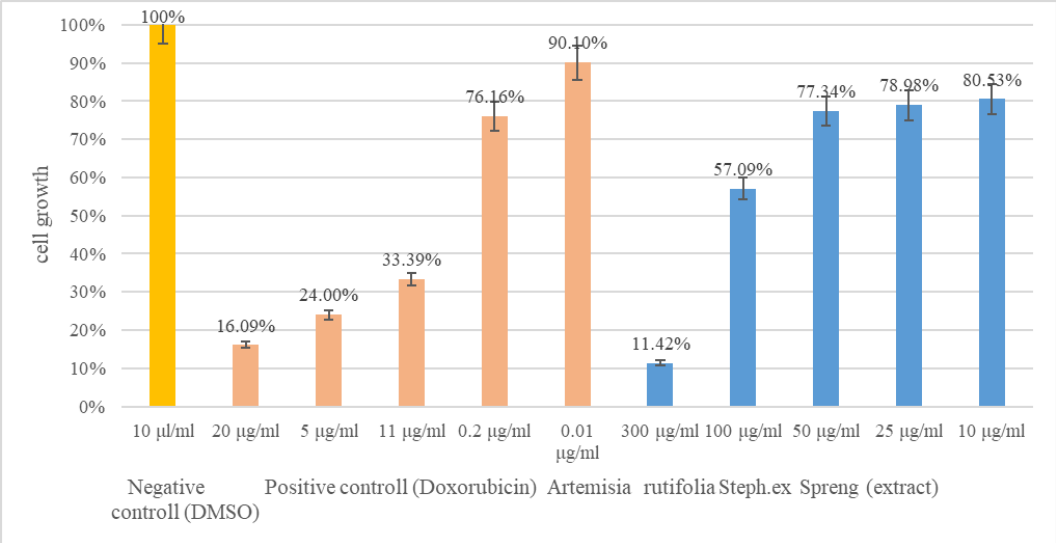

Figure 4. Growth inhibitory effect of ethanol extract *Artemisia rutifolia Steph.ex Spreng* on AGS cells after 24 hours treatment. The results are expressed as percentage of untreated control

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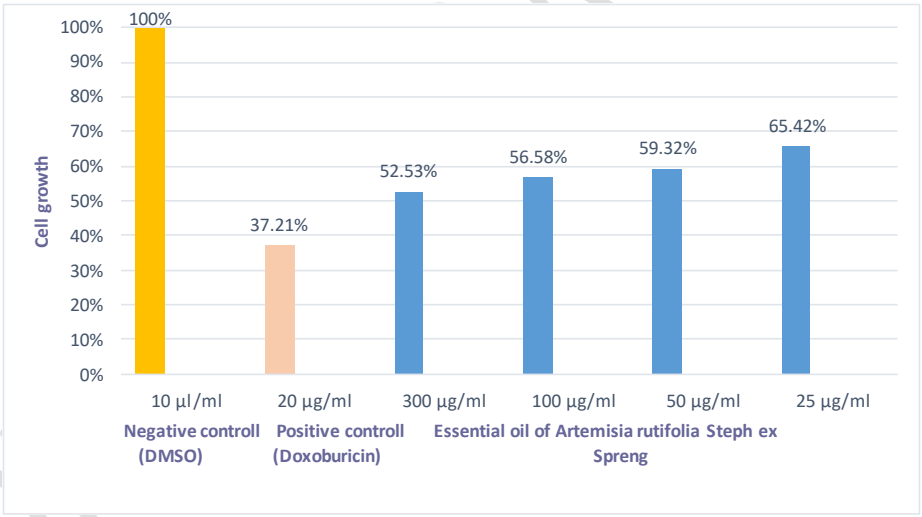

Figure 5. Growth inhibitory effect of essential oil *Artemisia rutifolia Steph.ex Spreng* on AGS cells after 24 hours treatment. The results are expressed as percentage of untreated control

Table 2. Growth inhibition effect of essential oil and ethanol extract from *Artemisia rutifolia Steph.ex Spreng* 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
Artemisia rutifolia Steph.ex Spreng (essential oil)	9.3	±0.7	6.6	±0.2	10.3	±0.5	6.5	±0.00
Artemisia rutifolia Steph.ex Spreng(ethanol extract)	7.8	±0.7	7.2	±0.5	7.0	±0.5	6.5	±0.5

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
Artemisia rutifolia Steph.ex Spreng (essential oil)	9.6	±0.5	6.0	±0.00	6.5	±0.0	6.5	±0.0
Artemisia rutifolia Steph.ex Spreng (ethanol extract)	6.8	±0.2	6.2	±0.2	6.0	±0.00	6.0	±0.00

Comment [U142]: Why the separate tables?

Comment [U143]: of

Comment [U144]: what product?

Comment [U145]: Why separate mean±SEM..... should be eg 9.3±0.7

Сүүлд ном зүй
5.Tan R.X., Zheng W.F., Tang H.Q. (1998) Biologically active substances from the genus Artemisia. J. Planta Med., 64, 294-302
1.Pandey A.K., Tripathi N.N. (2011) Aromatic plants of Gorakhpur forest division: Their antimycotic property and medicinal value. Int. J. Pharm. Sci. Rev. Res.,7, 142–147.

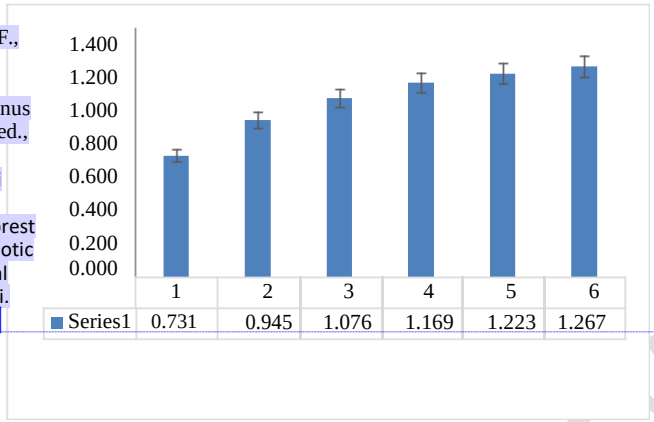

Figure 6. Radical scavenging activity of ethanol extract from *Nepeta sibirica* L. grown in Mongolia

Comment [U146]: Why here????

Comment [U147]: Is this your plant too????