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Chemical Diversity and Biological Activity of the Volatiles of Five *Artemisia* Species from Far East Russia

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Abstract: *Artemisia argyi*, *A. feddei*, *A. gmelinii*, *A. manshurica*, and *A. olgensis* (Asteraceae) were collected in Far East Russia. Oils were hydrodistilled and simultaneously analyzed by GC-FID and GC/MS. Main constituents were found as follows in *Artemisia* oils: selin-11-en-4α-ol (18.0%), 1,8-cineole (14.2.0%), artemisia alcohol (12.9%), borneol (9.7%) in *A. argyi*; camphor (31.2%), 1,8-cineole (17.6%), α-thujone (5.7%) in *A. feddei*; longiverbenone (12.0%), isopinocamphone (8.9%), 1,8-cineole (6.7%), camphor (5.8%), *trans*-*p*-menth-2-en-1-ol (5.3%) in *A. gmelinii*; germacrene D (11.2%), rosifoliol (10.1%), caryophyllene oxide (6.8%), eudesma-4(15),7-dien-1β-ol (5.6%) in *A. manshurica*; eudesma-4(15),7-dien-1β-ol (6.9%), caryophyllene oxide (5.6%), guaia-6,10(14)-dien-4β-ol (5.1%) and hexadecanoic acid (5.0%) in *A. olgensis*. Oils were subsequently submitted for antifungal and antimosquito evaluations. *Artemisia* species oils showed biting deterrent effects in *Aedes aegypti* and *Artemisia gmelinii* oil with the most active biting deterrence index values of 0.82 ± 0.1 at 10 µg/mL. Larval bioassay of *A. gmelinii* and *A. olgensis* oils showed higher larvicidal activity against *Ae. aegypti* larvae with LD50 values of 83.8 (72.6 – 95.7) ppm and 91.0 (73.8 – 114.5) ppm, respectively. Antifungal activity was evaluated against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using direct overlay bioautography assay and all showed non-selective weak antifungal activity. Antioxidant evaluation of the oils was performed by using β-carotene bleaching, Trolox equivalent and DPPH tests. The tested *Artemisia* oils demonstrated moderate antioxidant activity.

Keywords: *Artemisia*; essential oil; antifungal; botanical insecticidal; mosquito control; antioxidant activity.

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14. ABSTRACT

Artemisia argyi, A. feddei, A. gmelinii, A. manshurica, and A. olgensis (Asteraceae) were collected in Far East Russia. Oils were hydrodistilled and simultaneously analyzed by GC-FID and GC/MS. Main constituents were found as follows in Artemisia oils: selin-11-en-4a-ol (18.0%), 1,8-cineole (14.2.0%), artemisia alcohol (12.9%), borneol (9.7%) in A. argyi; camphor (31.2%), 1,8-cineole (17.6%), a-thujone (5.7%) in A. feddei; longiverbenone (12.0%), isopinocamphone (8.9%), 1,8-cineole (6.7%), camphor (5.8%), trans-p-menth- 2-en-1-ol (5.3%) in A. gmelinii; germacrene D (11.2%), rosifoliol (10.1%), caryophyllene oxide (6.8%) eudesma-4(15),7-dien-1b-ol (5.6%) in A. manshurica; eudesma-4(15),7-dien-1b-ol (6.9%), caryophyllene oxide (5.6%), guaia-6,10(14)-dien-4b-ol (5.1%) and hexadecanoic acid (5.0%) in A. olgensis. Oils were subsequently submitted for antifungal and antimosquito evaluations. Artemisia species oils showed biting deterrent effects in Aedes aegypti and Artemisia gmelinii oil with the most active biting deterrence index values of 0.82 ?? 0.1 at 10 mg/mL. Larval bioassay of A. gmelinii and A. olgensis oils showed higher larvicidal activity against Ae. aegypti larvae with LD50 values of 83.8 (72.6 ??? 95.7) ppm and 91.0 (73.8 ??? 114.5) ppm, respectively. Antifungal activity was evaluated against the strawberry anthracnose-causing fungal plant pathogens Colletotrichum acutatum, C. fragariae and C. gloeosporioides using direct overlay bioautography assay and all showed nonselective weak antifungal activity. Antioxidant evaluation of the oils was performed by using b-carotene bleaching, Trolox equivalent and DPPH tests. The tested Artemisia oils demonstrated moderate antioxidant activity.

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1. Introduction

Artemisia L. is from the Asteraceae-Anthemideae-Artemisiinae and ranks among the most species-rich plant genera. The genus contains approximately 500 taxa at specific or subspecific levels almost exclusively found in the northern hemisphere. *Artemisia* is centered in and most likely originated from Central Asia [1, 2]. It was well documented in literature that *Artemisia* species have been used since ancient times for food and medicinal purposes [3]. The genus *Artemisia* has been the subject of numerous chemical and biological studies, yielding primarily sesquiterpene lactones [4,5], diterpenes, coumarins [1], polyacetylenes [6] and flavonoids [1,7,8] as the main metabolites. Biological activity of *Artemisia* species includes antitumor [9-16], antimalarial [17,18], antibacterial [19,20], antifungal [21,22], antimutagenic [22,23], repellent and antifeedant [24], larvicidal [25,26] and vasorelaxant [27] properties.

Previous chemical investigations of *A. argyi* demonstrated the presence of coumarins [9], flavones [8, 15, 23, 28-30], mono- and sesquiterpenes [31-36], lactones [11], ketones [27], sitosterols [37] and lipophilic constituents [38]. Pharmacological studies on *A. argyi* proved terpinen-4-ol and β-caryophyllene as the antiasthmatic principles of the oil [39-40]. Antitumor [9, 12, 15, 16, 28, 33, 41], cytotoxic [13], antimutagenic [23], anti-inflammatory, antioxidative [42], antibacterial [20], antifungal [21,22], antifeedant [24, 26] and vasorelaxant [27] activities of *A. argyi* were reported earlier. In Traditional Chinese Medicine (TCM), *A. argyi* is used as raw material and processed into moxa wool [43]. This plant is also identified as "Aeyup" and used as important medicinal material in traditional Korean medicine [44]. Huang and Liu (1999) [45] provided a basis for *A. argyi* and *A. indica* differentiation, application and utilization using their macroscopical and microscopical characteristics, TLC and UV spectra [45]. *A. feddei* was subjected earlier to investigation for coumarins [46], sesquiterpene lactones [47-50] and monoterpenes [51]. Scopoletin (coumarin) detected in the water extract of *Artemisia feddei* was reported as an inducible nitric oxide synthesis inhibitory active constituent. [46], while the oil demonstrated antibacterial activity [19]. *A. gmelinii* was also reported for coumarins with scopoletin as major constituent [52]. A literature search did not reveal any scientific reports on the composition of *A. manshurica* and *A. olgensis* volatiles.

As a continuation of our investigations on *Artemisia* oils, we studied the chemical composition and biological activity of essential oil from five *Artemisia* species: *Artemisia argyi* Lév. et Vaniot, *A. feddei* Lév. et Vaniot, *A. gmelinii* Web. ex Stechm., *A. manshurica* (Komarov) Komarov, *A. olgensis* (Vorobiev) Worosch. collected in Far East region of Russia. In a program aimed at discovering natural fungicides and insecticides as alternatives to conventional synthetic agrochemicals, unique *Artemisia* species from Russia were evaluated for biopesticide activity. The oils were tested for antifungal activity using direct bioautography assays against three *Colletotrichum* species and for deterrent and larvicidal activity against *Aedes aegypti* (L.). Also, in a second research effort to study natural sources for effective antioxidants, *Artemisia* essential oils were subjected to investigation by three antioxidant methods: (i) β-carotene bleaching assay, (ii) Trolox equivalent (TEAC) assay, (iii) 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. There are several reports on antioxidant activity of *Artemisia* oils [6, 53, 54] compared to α-tocopherol, butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). There has been a growing interest in research concerning natural antioxidant active compounds, including the plant extracts and essential oils that are less damaging to the mammalian health and environment. Antioxidants retard oxidation and are often added to numerous meat and poultry products to prevent or slow oxidative degradation of fats. Free radical scavenging, chelating of pro-oxidant metal ions or quenching singlet-oxygen formation mechanisms are involved in antioxidant action of natural antioxidants [7]. The present work is the first report on the chemical composition and biological activity of *Artemisia* oils from the Far East of Russia.

2. Materials and Methods

2.1. Plant materials

Plant materials, voucher specimens codes, plant parts studied and essential oil yields are given in Table 1. Voucher specimens were kept at the Herbarium of the Department of Botany of Far Eastern Federal University, Russia. Botanical identifications were carried out by R. Doudkin and P.

Gorovoy from Pacific Institute of Bioorganic Chemistry (Laboratory of Chemotaxonomy) of the Far Eastern Branch of the Russian Academy of Sciences.

Table 1. Collection data for the *Artemisia* species studied

***Artemisia* ssp.**	**Collection place**	**Voucher specimen No**	**Oil yield (%)**	**Plant part used for distillation**	**Oil color**
Artemisia argyi	Primorsky Krai, Nadejdinsky District, meadow around Terekhovka village	27891	0.36	Aerial	Turquoise
Artemisia feddei	Primorsky Krai, Nadejdinsky District, stream near to Terekhovka village	27883	0.20	Aerial	Green
Artemisia gmelinii	Primorsky Krai, Partizansky District, Nakhodkinsky crossing	27887	1.60	Aerial	Turquoise
Artemisia manshurica	Primorsky Krai, Partizansky District, Nakhodkinsky crossing	27876	0.16	Aerial	Yellowish
Artemisia olgensis	Primorsky Krai, Olginsky District, "Siniye Skali", west side of Olga village	27879	0.05	Aerial	Yellow-orange

2.2. Chemicals

All organic solvents and reagents used were of analytical or chromatographic grade. Anhydrous sodium sulfate (ACS-ISO, for analysis), *n*-hexane, acetone (ACS, for analysis) and dimethyl sulfoxide (DMSO) were purchased from Carlo Erba (Italy). Technical grade commercial fungicides benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc., West Chester, PA, USA) were used as fungicide standards at 2 mM in 2 μL of 95% ethanol. DPPH was supplied from Sigma-Aldrich Chemie (Steinheim, Germany). For the antifungal assay, potato-dextrose broth (Difco, Detroit, MI, USA), glass silica gel thin layer chromatography (TLC) plates with a fluorescent indicator (250 mm, Silica Gel GF Uniplate, Analtech, Inc., Newark, DE, USA), and a moisture chamber (398-C, Pioneer Plastics, Inc., Dixon, KY, USA) were used. Monobasic sodium phosphate (Fisher Scientific Chemical Co., Fairlawn, NJ) and adenine (Sigma-Aldrich, St. Louis, MO) were used for mosquito biting bioassay. DEET (99.1 % purity; N,N-diethyl-3-methylbenzamide, Sigma Aldrich, St. Louis, MO) was used as a positive control. Molecular biology grade ethanol was obtained from Fisher Scientific Chemical Co. (Fairlawn, NJ).

2.3. Isolation of essential oils

Air dried aerial parts of the collected *Artemisia* species were hydrodistilled separately for 3 h using a Clevenger-type apparatus according to the procedure published in European Pharmacopoeia. Percent yields (v/w) of the oils calculated on a moisture free basis are given in Table 1. The oils were dried over anhydrous sodium sulphate and stored in sealed vials in dark, at 4° C, until GC-FID and GC/MS analyses. The oils were dissolved in *n*-hexane to conduct chromatographic determination of their composition.

2.4. Gas Chromatography – Mass Spectrometry (GC/MS)

The oils were analyzed by capillary GC-FID and GC/MS techniques using an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). The same column and analytical conditions were used for both GC/MS and GC-FID. HP-Innowax FSC column (60m × 0.25mm, 0.25 μm film thickness, Agilent, Walt & Jennings Scientific, Wilmington, Delaware, USA) was used with helium as a carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted at 40:1. Flame ionization

detection and injector temperature were performed at 250°C. Mass spectrums were taken at 70 eV. Mass range was from *m/z* 35 to 450.

2.5. Gas Chromatography (GC-FID)

The GC-FID analysis was carried out using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). FID detector temperature was 300°C. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions.

2.6. Identification and quantification of compounds

Identification of the volatile constituents was achieved by parallel comparison of their retention indices and mass spectra with data published in the WILEY GC/MS Library (Wiley, New York, NY, USA), MASSFINDER *software 4.0* (Dr. Hochmuth Scientific Consulting, Hamburg, Germany) (Hochmuth, 2008), ADAMS Library and NIST Library (Adams 2007), and the in-house "Başer Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. A C9-C30 *n*-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples in the calculation of retention indices (RI). Quantification of volatiles components was performed on the basis of their GC-FID peak area using integration data.

2.7. Antifungal activity test

The oils were evaluated for antifungal activity against strawberry anthracnose-causing plant pathogens, *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using the direct overlay bioautography assay described by Wedge [55].

2.8. Insects

Ae. aegypti (L.) used in these studies were from a laboratory colony maintained since 1952 at the Mosquito and Fly Research Unit at Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida. This colony is maintained since 1952 using standard procedures [56]. The eggs were stored in laboratory to use as per need. Mosquitoes were reared to the adult stage by feeding the larvae on a diet of 2:1 alfalfa pellets (US Nutrition Inc. Bohemia, NY) and hog chow (Ware Milling 150 ALF Drive, Houston, MS 38851). The diet contents were ground in a grinder and passed through sieve no. 40, 425 micron (USA Standard Sieve, Humboldt MFG. Co. Norridge, IL 60706).

2.9. Mosquito biting bioassays

Experiments were conducted by using a six-celled in vitro Klun & Debboun (K & D) module bioassay system developed by Klun et al. 2005 [57] for quantitative evaluation of bite deterrent properties of candidate compounds for human use. This bioassay method determines specifically measured biting (feeding) deterrent properties of the chemicals. Briefly the assay system consists of a six well blood reservoir with each of the 3 cm × 4 cm wells containing 6 mL of blood. As reported earlier [58], female mosquitoes feed as well on the CPDA-1 (citrate-phosphate-dextrose-adenine) + ATP as they do on blood. Therefore, we used the CPDA-1 + ATP instead of human blood. CPDA-1 was prepared by dissolving 3.33 g sodium citrate, 0.376 g citric acid, 4.02 g dextrose, 0.28 g monobasic sodium phosphate and 0.346 g of adenine in 1026 mL of de-ionized water. ATP was added to CPDA-1 to yield 10^{-3} M ATP (AABB 2005). CPDA-1 and ATP preparations were freshly made on the day of the test. DEET was used as a positive control. Molecular biology grade ethanol was obtained from Fisher Scientific Chemical Co. (Fairlawn, NJ). Five *Artemisia* essential oils were tested in this study and used DEET at 25 nmol/cm^2 as a positive control. All the treatments were prepared in acetone. The stock solutions were kept in a refrigerator at 3-4°C. Treatments were prepared fresh at the time of bioassay.

The temperature of the solution in the reservoirs was maintained at 37°C by continuously passing the warm water through the reservoir using a circulatory bath. The reservoirs were covered with a layer of collagen membrane. This CPDA-1+ATP solution membrane unit simulated a human host for mosquito feeding. The test compounds were randomly applied to six 4 cm × 5 cm areas of organdy cloth and positioned over the membrane-covered CPDA-1+ATP solution with a separator placed between the treated cloth and the six-celled module. A six celled K & D module containing five females per cell was positioned over cloth treatments covering the six CPDA-1+ATP solution membrane wells, and trap doors were opened to expose the treatments to these females. The number of mosquitoes biting through cloth treatments in each cell was recorded after a 3 min exposure and mosquitoes were prodded back into the cells. These mosquitoes were then squashed to determine the number which has actually engorged the solution. A replicate consisted of six treatments: four test compounds, DEET (a standard mosquito repellent compound) and acetone treated cloth as solvent control. The 25 nmol DEET/cm^2 cloth dose was used as a standard, because it suppresses mosquito biting by 80% as compared to controls [57]. A set of replications was conducted on different days using new lots. Treatments were replicated 15 times in oil.

2.10. Larval bioassays

Bioassays were conducted by using the bioassay system described by Pridgeon et al. (2009) [59] to determine the larvicidal activity of five *Artemisia* species from Far East Russia against *Ae. aegypti*. In brief, the eggs were hatched under vacuum (1-h) by placing a piece of a paper towel with eggs in a cup filled with 100 mL of deionized water containing small quantity of larval diet. Larvae were removed from vacuum and held overnight in the cup in a temperature-controlled chamber maintained at a temperature of 27±2°C and 70 ± 5% RH at a photoperiod regimen of 12:12 (L:D) h. Five 1-d-old first instar *Ae. aegypti* were added to each well of 24-well plates placed on illuminated light box by using a disposable 22.5-cm Pasteur pipette with a droplet of water. Fifty µL of larval diet (2% slurry of 2:1 alfalfa pellets and hog chow) were added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). All chemicals to be tested were diluted in dimethyl sulfoxide (DMSO). Eleven microliters of the test chemical was added to the labeled wells, and in control treatments 11 µL of DMSO alone was added. Each well had a total volume of 1.1 mL. After the treatment, the plates were swirled in clockwise and counter clockwise motions and front and back and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24- and 48-h after treatment. Larvae that showed no movement in the well after manual disturbance of water by a pipette tip were recorded as dead. A series of dosages (4 - 5 concentrations) were used in each treatment to get a range of mortality. Treatments were replicated 15 times in oil. LD_{50} values for larvicidal data were calculated by using the Probit procedure of SAS (SAS Institute 2007). Control mortality was corrected by using Abbott's formula.

2.11. Statistical analyses

Since the K&D module bioassay system can handle only 4 treatments along with negative and positive controls, in order to make direct comparisons among more than four test compounds and to compensate for variation in overall response among replicates, repellency was quantified as Biting Deterrence Index (BDI). BDI's were calculated using the following formula:

$$[\mathrm{BDI}_{i,j,k}] = \left[\frac{\mathrm{PNB}_{i,j,k} - \mathrm{PNB}_{c,j,k}}{\mathrm{PNB}_{d,j,k} - \mathrm{PNB}_{c,j,k}}\right]$$

Where $PNB_{i,j,k}$ denotes the mean proportion of females not biting in test compound i for replication j and day k (i=1-4, j=1-5, k=1-2), $PNB_{c,j,k}$ denotes the mean proportion of females not biting in solvent control for replication j and day k (j=1-5, k=1-2) and $PNB_{d,j,k}$ denotes the mean proportion of females not biting in response to DEET (positive control) for replication j and day k (j=1-5, k=1-

2). This formula adjusts for variation in response among replication days and incorporates information from the solvent control as well as positive control.

A BDI value of 0 indicates an effect similar to acetone. A BDI value significantly greater than 0 indicates an anti-biting effect relative to ethanol. BDI values not significantly different from 1 are statistically similar to DEET. BDI values were analyzed by using the ANOVA procedure of SAS (SAS Institute 2007) and means were separated using the Ryan-Einot-Gabriel-Welsch Multiple Range method.

2.12. Antioxidant activity tests

Antioxidant activity was evaluated on three tests: TEAC [60], 1,1- Diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging test [61] and inhibition of β-carotene/linoleic acid co-oxidation [62, 63], which were carried out using procedures described previously.

3. Results and Discussion

Within scope of the present work, we carried out the gas-chromatography of the essential oils of *Artemisia argyi*, *A. feddei*, *A. gmelinii*, *A. manshurica*, *and A. olgensis*. Hydrodistillation of the dried aerial parts of five *Artemisia* species gave oils with specific odors and different colors (Table 1). The oil yield was found as highest in *A. gmelinii* (1.60%) and lowest in *A. olgensis* (0.05%). The list of detected compounds with their relative percentages, retention indices and percentages of compound is given in Table 2 in order of their elution time on the HP-Innowax FSC column. All the volatile constituents detected in the oils of the five *Artemisia* species were classified in order of abundance as monoterpene ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$) hydrocarbons, their oxygenated derivatives, and non-isoprenoid compound (Table 3). The oils of *A. argyi*, *A. feddei* and *A. gmelinii* were found to be rich with oxygenated monoterpenes, while the oils of *A. manshurica* and *A. olgensis* consisted primarily of oxygenated sesquiterpenes.

Table 2. Chemical composition of the volatiles of *Artemisia* species

Compound[a]	RRI[b]	RRI[c]	Ref.	Content (%)[d]				
				Aa[e]	Af[f]	Ag[g]	Am[h]	Ao[i]
Tricyclene	1014	1017	[64]	t[k]	0.1	-	-	-
α-Pinene	1032	1032	[65]	0.3	0.1	0.1	-	0.1
Santolina triene	1043	1031	[7]	t	-	-	2.6	-
Camphene	1076	1085	[64]	0.4	1.8	0.2	-	-
β-Pinene	1118	1118	[64]	0.2	0.1	1.1	-	0.5
Myrcene	1174	1156	[64]	-	-	-	t	0.5
α-Phellandrene	1180	1186	[66]	-	-	0.2	-	-
α-Terpinene	1188	1179	[64]	0.4	0.4	0.2	-	-
Dehydro-1,8-cineole	1195	1200	[67]	0.1	0.1	t	-	-
Limonene	1203	1205	[64]	t	-	0.1	-	t
1,8-Cineole	1210	1204	[68]	14.2	17.6	6.7	-	-
2-Pentyl furan	1244	1237	[69]	T	0.1	-	-	t
γ-Terpinene	1255	1256	[64]	0.9	0.9	0.3	-	t
(*E*)-β-Ocimene	1266	1252	[70]	-	-	-	t	0.3
p-Cymene	1280	1279	[64]	1.2	1.4	0.7	-	t
Terpinolene	1290	1283	[64]	0.2	0.3	0.1	-	-

Compound[a]	RRI[b]	RRI[c]	Ref.	Content (%)[d]				
				Aa[e]	Af[f]	Ag[g]	Am[h]	Ao[i]
1,2,3-Trimethyl benzene	1355			-	0.1	-	-	-
Artemisia ketone	1358	1358	[71]	0.1	-	0.7	-	
Nonanal	1390	1389	[72]	-	-	-	-	0.2
Yomogi alcohol	1403	1401	[73]	3.8	0.2	0.1	-	-
Santolina alcohol	1405	1413	[73]	0.1	-	-	-	-
Artemisyl acetate	1423			0.6	-	-	-	-
α-Thujone	1435	1428	[73]	0.1	5.7	1.2	-	-
β-Thujone	1445	1438	[74]	1.1	2.8	0.8	-	-
α-Cubebene	1467	1468	[75]	-	-	0.1	-	-
trans-Sabinene hydrate	1474	1474	[76]	3.2	1.0	0.3	-	-
α-Longipinene	1479	1469	[77]	-	t	0.2	-	-
α-Copaene	1497	1497	[70]	0.2	t	0.5	0.4	0.3
Decanal	1499			-	0.1	-	-	-
Artemisia alcohol	1510	1512	[71]	12.9	0.2	0.1	-	-
Chrysanthenone	1531	1540	[71]	T	1.3	0.1	-	-
Camphor	1533	1532	[64]	1.4	31.2	5.8	0.5	-
α-Gurjunene	1539	1529	[78]	-	t	0.1	-	-
Dihydroachillene	1547			-	0.3	t	-	-
β-Cubebene	1549	1547	[78]	-	-	0.4	0.1	0.2
cis-Sabinene hydrate	1556	1556	[79]	2.0	0.9	0.3	-	-
Isopinocamphone	1562			-	-	8.9	-	-
trans-*p*-Menth-2-en-1-ol	1571	1622	[80]	0.4	0.5	5.3	-	-
cis-Chrysanthenyl acetate	1582	1583	[71]	0.1	-	-	-	-
Pinocarvone	1584	1587	[78]	0.2	0.4	2.8	-	-
Bornyl acetate	1586	1582	[74]	0.3	0.3	0.4	0.8	0.5
Nopinone	1597	1597	[78]	-	t	0.4	-	-
β-Elemene	1600	1602	[81]	-	-	t	0.3	-
Thymol methyl ether	1604	1611		-	-	-	-	0.2
epi-Bicyclosesquiphellandrene	1605			-	-	-	0.2	-
β-Copaene	1609			-	-	0.1	-	-
Terpinen-4-ol	1611	1617	[66]	4.1	-	0.9	-	-
β-Caryophyllene	1612	1604	[70]	-	3.0	1.9	4.0	2.0
cis-*p*-Menth-2-en-1-ol	1640	1645	[66]	0.3	0.4	3.9	-	0.3
Thuj-3-en-10-al	1642			-	0.1	-	-	-
Myrtenal	1648	1648	[78]	0.2	0.3	0.9	-	-
Sabinaketone	1651	1651	[77]	-	0.1	0.1	-	-
Isobornyl propionate	1655	1676		0.1	-	-	-	-
Sabinyl acetate	1658			-	0.2		-	-
trans-Pinocarvyl acetate	1661			-	-	0.1	-	-

Compound[a]	RRI[b]	RRI[c]	Ref.	Content (%)[d]				
				Aa[e]	Af[f]	Ag[g]	Am[h]	Ao[i]
cis-Verbenol	1663	1663	[78]	0.1	0.1	-	-	-
(*Z*)-β-Farnesene	1668	1670	[82]	t	-	-	1.0	0.4
trans-Pinocarveol	1670	1646	[80]	0.3	0.5	1.0	-	-
epi-Zonarene	1675	1675	[78]	-	-	0.2	-	-
δ-Terpineol	1680	1680	[75]	0.3	0.2	0.1	-	-
trans-Verbenol	1683	1666	[83]	0.6	0.5	-	-	-
Isoborneol	1684	1669	[72]	-	-	0.2	-	-
cis-Piperitol	1687	1692	[66]	-	-	1.3	-	-
α-Humulene	1689	1689	[78]	0.1	0.1	0.5	1.2	0.3
Drima-7,9(11)-diene	1694			-	-	0.5	-	-
(*E*)-β-Farnesene	1695			0.2	-	-	-	-
Myrtenyl acetate	1704			-	-	0.1	-	-
γ-Muurolene	1704	1704	[78]	-	-	0.1	1.0	0.4
α-Terpineol	1706	1706	[78]	3.0	0.3	0.7	-	-
Borneol	1719	1719	[78]	9.7	3.6	3.3	-	-
Verbenone	1725	1725	[81]	-	0.3	-	-	-
Germacrene D	1726	1726	[78]	-	-	0.9	11.2	4.2
(*Z*,*E*)-α-Farnesene	1737			-	-	-	2.9	2.0
p-Mentha-1,5-dien-8-ol	1738	1714	[83]	-	t	0.2	-	-
α-Muurolene	1740	1742	[66]	-	-	-	0.6	0.7
β-Selinene	1742	1731	[66]	0.3	t	0.7	-	-
Piperitone	1746	1744	[66]	-	0.7	1.8	-	-
Carvone	1751	1750	[66]	0.1	0.3	-	-	-
trans-Piperitone oxide	1754			-	-	0.4	-	-
Bicyclogermacrene	1756	1756	[78]	-	-	0.3	0.5	0.2
cis-Piperitol	1757	1757	[71]	0.2	0.2	3.2	-	-
(*E*,*E*)-α-Farnesene	1758	1750	[84]	-	-	-	1.4	0.6
cis-Chrysanthenol	1764			4.4	0.1	0.2	-	-
δ-Cadinene	1773	1773	[77]	-	0.1	0.7	1.1	0.9
γ-Cadinene	1776	1779	[81]	-	-	0.1	0.4	0.2
cis-Carvyl acetate	1782			-	0.1	-	-	-
β-Sesquiphellandrene	1783	1783	[77]	-	-	-	0.3	-
ar-Curcumene	1786	1786	[77]	0.1	t	0.1	1.3	0.6
Cadina-1,4-diene (=*Cubenene*)	1799	1799	[78]	-	-	0.1	-	-
Benzene propanal	1800			-	0.3	-	-	-
Cumin aldehyde	1802			0.1	0.3	0.1	-	-
Myrtenol	1805	1807	[66]	0.2	0.3	1.1	-	-
α-Campholene alcohol	1806			0.1	-	0.3	-	-
Liguloxide	1811			-	-	0.1	-	-
Fragranol	1824			-	-	3.8	-	-

Compound[a]	RRI[b]	RRI[c]	Ref.	Content (%)[d]				
				Aa[e]	Af[f]	Ag[g]	Am[h]	Ao[i]
(*E,E*)-2,4-Decadienal	1827			t	0.1	-	-	0.2
trans-Carveol	1845	1845	[78]	0.2	0.1	0.3	-	-
cis-Calamenene	1853	1849	[85]	-	0.1	0.2	-	0.3
m-Cymen-8-ol	1856			-	-	0.2	-	-
Fragranyl isobutyrate	1866			-	-	0.2	-	-
p-Cymen-8-ol	1864	1865	[66]	0.1	0.2	-	-	-
(*E*)-Geranyl acetone	1868	1868	[77]	t	0.1	-	-	0.4
trans-Myrtanol	1872			-	-	0.3	-	-
Aplotaxene	1880			-	-	-	-	0.2
cis-Carveol	1882	1878	[78]	0.3	0.1	-	-	-
epi-Cubebol	1900			-	-	0.2	-	-
α-Calacorene	1931	1918	[84]	-	t	0.1	-	t
1,5-Epoxy-salvial(4)14-ene	1945			0.1	-	0.1	0.2	0.6
Palustrol	1949	1934	[75]	-	0.1		-	-
Cubebol	1957			-	0.1	0.2	-	-
Isocaryophyllene oxide	2001	2001	[77]	0.2	0.3	0.1	0.7	0.3
Caryophyllene oxide	2008	2008	[76]	2.2	4.4	1.6	6.8	5.6
Perilla alcohol	2029			0.1	t	0.4	-	
Salvial-4(14)-en-1-one	2037	2016	[86]	0.2	0.1	0.1	2.1	2.5
Pentadecanal	2041			-	0.1	-	-	-
Guaia-6,10(14)-dien-4-ol isomer*	2029			-	-	-	-	1.5
(*E*)-Nerolidol	2050	2049	[75]	t	t	0.2	1.0	0.5
Ledol	2057	2057	[78]	-	0.2	-	-	-
Humulene epoxide-II	2071	2071	[78]	0.2	0.3	0.4	1.5	0.8
Junenol	2073			-	0.1	-	0.9	1.1
Guaiyl acetate	2094			-	-	-	t	0.8
p-Mentha-1,4-dien-7-ol	2073			-	0.2	t	-	-
Caryophylla-2(12),6(13)-dien-5-one	2074			0.1	0.1	t	-	-
cis-Sesquisabinene hydrate	2085			0.1	-	-	0.4	-
Caryophyll-5-en-12-al	2098			0.1	-	-	-	-
Cubenol	2080	2080	[87]			0.2	-	-
Cumin alcohol	2100	2068	[88]	t	0.1	0.2	-	-
Salviadienol	2128			0.1	0.1	0.1	1.4	1.9
Hexahydrofarnesyl acetone	2131	2132	[89]	0.1	0.3	0.1	-	0.6
Rosifoliol	2144	2144	[90]	-	-	-	10.1	-
Spathulenol	2144	2150	[79]	0.3	0.4	1.5	0.4	4.9
Nor-Copaonone	2179			-	-	-	0.6	0.9
γ-Eudesmol	2185	2179	[81]	-	0.3	-	1.0	-
Eremoligenol	2183			-	-	-	1.1	-
Eugenol	2186	2186	[78]	0.2	-	-	-	-

Compound[a]	RRI[b]	RRI[c]	Ref.	Content (%)[d]				
				Aa[e]	Af[f]	Ag[g]	Am[h]	Ao[i]
T-Cadinol	2187	2189	[91]	-	-	-	-	1.6
Nonanoic acid	2192			-	0.3	-	-	0.4
T-Muurolol	2209	2209	[92]	-	-	-	0.7	0.6
Zingiberenol	2188			0.2	-	-	-	-
Clovenol	2205	2205	[90]	-	0.1	-	-	-
Torreyol	2207			-	-	-	-	0.6
ar-Turmerol	2214	2214	[77]	0.1	0.2	-	-	0.2
α-Bisabolol	2232	2233	[93]	-	-	-	-	0.2
Carvacrol	2239	2239	[65]	0.2	0.1	-	-	-
trans-α-Bergamotol	2247	2247	[77]	-	-	-	-	0.3
Torilenol	2248			0.1	0.1	-	3.3	3.8
α-Cadinol	2255	2231	[80]	-	-	-	1.5	1.4
β-Eudesmol	2257	2231	[80]	-	0.8	-	-	-
Longiverbenone (= *Vulgarone B*)	2265			-	0.4	12.0	-	1.2
Alismol (=*6,10(14)-Guaiadien-4β-ol*)	2272	2272	[77]	-	-	-	-	5.1
Selin-11-en-4α-ol	2273			18.0	0.6	-	-	0.5
Cyperenone	2276			0.1	-	-	-	-
Oxo-α-Ylangene	2289			-	0.5	-	-	-
Decanoic acid	2298	2288	[94]	-	-	-	-	1.0
Tricosane	2300	2300	[78]	-	0.1	-	-	-
(6S, 7R)-Bisabolone	2311			0.2	-	-	-	-
Caryophylla-2(12),6(13)-dien-5β-ol (=*Caryophylladienol I*)	2316	2316	[77]	0.3	0.3	-	-	-
Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	2324	2324	[77]	0.1	0.2	-	-	-
Eudesma-4(15),7-dien-ol isomer*	2326			0.4	-	0.6	-	2.5
Eudesm-4(15),7-dien-1β-ol	2362	2351	[95]	-	0.2	-	5.6	6.9
Manoyl oxide	2376	2347	[83]	-	-	-	-	2.2
Caryophylla-2(12),6-dien-5α-ol (=*Caryophyllenol I*)	2389	2389	[90]	0.1	0.2	-	-	0.6
Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	2392	2392	[77]	0.4	0.8	0.3	-	1.3
(Z)-Nuciferal	2393			-	0.1	-	-	-
Chamazulene	2420	2373	[64]	0.1	-	0.1	-	-
Pentacosane	2500	2500	[77]	-	0.1	-	-	-
Dodecanoic acid	2503	2504	[96]	-	-	t	-	0.8
14-Hydroxy-α-muurolene	2535			-	-	t	-	0.4
γ-Costol	2533			0.1	-	-	-	-
α-Costol	2604			-	0.1	-	-	-
β-Costol	2606			0.1	t	-	-	-
14-Hydroxy-δ-cadinene	2607			-	-	0.1	-	0.4

Compound[a]	RRI[b]	RRI[c]	Ref.	Content (%)[d]				
				Aa[e]	Af[f]	Ag[g]	Am[h]	Ao[i]
Phytol	2622	2620	[84]	0.1	0.1	0.1	-	t
Tetradecanoic acid	2670	2672	[97]	0.2	0.2	-	-	1.2
Heptacosane	2700	2700	[98]	-	t	-	-	0.5
Hexadecanoic acid	2931	2930	[85]	0.9	1.5	0.3	3.5	5.0
Total				**95.2**	**94.8**	**88.4**	**74.6**	**77.4**

[a] Compounds were identified by comparison of their RI (determined rel. to n-alkanes (C9–C30) and mass spectra with those of authentic compounds or with databases) (see Exper. part).
[b] Relative retention indices (RRI) experimentally determined against n-alkanes on HP-Innowax FSC column.
[c] Relative retention indices reported in literature.
[d] The contents (%) of the individual components were calculated based on the peak area (FID response).
[e] Aa: *Artemisia argyi*; [f] Af: *A. feddei*; [g] Ag: *A. gmelinii*; [h] Am: *A. manshurica*; [i] Ao: *A. olgensis*.
[k] t: Trace (< 0.1 %); * Correct isomer not identified.

Table 3. Distribution of main compound classes in the *Artemisia* oils studied

Compound class	%				
	Aa	Af	Ag	Am	Ao
Monoterpene hydrocarbons	3.6	5.2	3.0	2.6	1.4
Oxygenated monoterpenes	65.2	71.3	58.6	1.3	1.4
Sesquiterpene hydrocarbons	0.9	3.3	7.8	27.7	12.9
Oxygenated sesquiterpenes	24.1	11.5	18.1	39.5	49.6
Other	1.4	3.5	0.9	3.5	12.1

Aa : *A. argyi*; **Af**: *A. feddei*; **Ag**: *A. gmelinii*; **Am**: *A. manshurica*; **Ao**: *A. olgensis.*

Ninety six compounds representing 95.2 % of the oil were characterized in *A. argyi*. Monoterpenes (68.8%) consisted primarily of oxygenated forms (65.2%) were represented by 1,8-cineole (14.2%), artemisia alcohol (12.9%), borneol (9.7%), *cis*-chrysanthenol (4.4%), terpinen-4-ol (4.1%) and yomogi alcohol (3.8%) as major constituents. Sesquiterpenes (25.0%) were mostly comprised by oxygenated forms (24.1%) with selin-11-en-4α-ol (18.0%) and caryophyllene oxide (2.2%). General profile of *A. argyi* volatiles from Far East Russia was found to be quite different from that having been reported previously. Xu et al. 2007 [99] reported about 7- ethyl-1,4-dimethyl-azulene (17.3%), 1,8-cineole (10.3%) and β-limonenol (8.2%) as the main volatile components of the essential oil from A. *argyi*. The inflorescence oil of *A. argyi* collected in China was earlier reported to contain 1,8-cineole (4.5%), borneol (3.6%), terpineol (10.2%), spathulenol (10.0%), caryophyllene oxide (6.5%), juniper camphor (8.7%), chamazulene (2.0%), and camphor (3.5%) as major components [21]. The flowers of *A. argyi* were found to be rich in attractant substances for pollination: cylcofenchene, α-pinene, α-myrcene, D-limonene, caryophyllene, and germacrene D [100]. The composition of leaf oil was reported to be different from oil from flowers with α-cubebene, cadinene, bornyl acetate, germacrene D, borneol, D-limonene, α-myrcene and α-phellandrene as main constituents [101].

One hundred and nine compounds representing 94.8 % of the oil were characterized in *A. feddei*. Monoterpenes (76.5%) with dominance of oxygenated monoterpenes (71.3%) comprised the most abundance group in the oil with camphor (31.2%), 1,8-cineole (17.6%) and α-thujone (5.7%) as the major constituents. Sesquiterpenes (14.8%) were also comprised mostly by oxygenated forms with caryophyllene oxide (4.4%) as the main representative of this class. Comparison with literature data demonstrated diversity of the oil of *A. feddei* from Far East Russia. *A. feddei* oil from China was reported earlier had significant differences in composition with chamazulene (9.0%), α-terpineol (8.2%), α-phellandrene (5.8%), α-terpinyl acetate (5.1%), camphor (4.0%) and terpinen-4-ol (3.0%) [19].

Essential oil obtained from *A. gmelinii,* yielded 108 compounds representing 88.4% of the oil. More than half (61.6%) of the oil was comprised by monoterpenes with oxygenated terpenes predominating (58.6%). Isopinocamphone (8.9%), 1,8-cineole (6.7%), camphor (5.8%), *trans*-*p*-menth-2-en-1-ol (5.3%), *cis*-*p*-menth-2-en-1-ol (3.9%) and fragranol (3.8%) were found to be the main monoterpenes constituents. Sesquiterpenes (25.9%) were dominated by oxygenated forms which accounted for 18.1% of the oil with longiverbenone (12.0%) as major constituent. A literature search revealed compositional diversity of terpenoids in *A. gmelinii* from different localities. Artemisia ketone (28.2%) and 1,8-cineole (13.0%) were major constituents of the Himalayan species *A. gmelinii* (Mathela et al. 1994). *A. gmelinii* was recently reported to contain α-thujone (63.2%), germacrene D (2.2-36.5%), vulgarone B (18.9-38.5%), borneol (0.3-8.0%), β-caryophyllene (0.1-9.9%) and selin-11-en-4-α-ol (7.4%) as major volatiles [102]. Moldavian species, *A. gmelinii* oil was comprised of α-thujone [103]. *A. gmelinii* essential oil from Central Asia, yielded 1,8-cineol (21-40%), camphor (10-31%), borneol (4-17%) and terpinen-4-ol (4-8%) [104].

A. manshurica oil was characterized by 52 compounds constituting 83.6% of the oil. Sesquiterpenes (76.2%) were found in the highest abundance in the oil. Among sesquiterpene hydrocarbons (36.7%), germacrene D (11.2%) and β-caryophyllene (4.0%) were the major constituents, while rosifoliol (10.1%), caryophyllene oxide (6.8%) and eudesm-4(15),7-dien-1β- ol (5.6%) were the primary oxygenated forms (39.5%). Monoterpenes were present in scarce amounts (3.9%) in the oil.

Seventy eight compounds constituting 77.4% of the *A. olgensis* oil were detected. Sesquiterpenes (62.5%) were comprised of mostly oxygenated forms (49.6%) with eudesm-4(15),7-dien-1β-ol (6.9%), caryophyllene oxide (5.6%), guaiadien-4β-ol (5.1%), spathulenol (4.9%), and torilenol (3.8%) as the major constituents. Non-oxygenated sesquiterpene hydrocarbons (12.9%) were composed of primarily germacrene D (4.2%) and monoterpenes were detected in scarce amounts (2.8%).

Artemisia oils were evaluated for their antifungal activity against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using the direct overlay bioautography assay (Table 4). Anthracnose diseases of strawberry (*Fragaria* × *ananassa* Duch.) are caused by the fungal pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* [55]. *C. fragariae* is most often associated with anthracnose crown rot of strawberries grown in hot, humid areas such as the southeastern United States [55]. Evaluation of the five *Artemisia* essential oils in direct bioautography assay was evaluated using two concentrations at 80 μg and 160 μg applications against three *Colletotrichum* species. Three *Artemisia* oils (*A. argyi*, *A. feddei* and *A. manshurica*) demonstrated clear zones inhibition between 1.75 to 4.0 mm zone inhibitions which was less active than the commercial fungicide captan. *Artemisia olgensis* oil did not show activity whereas *A. gmelinii* oil demonstrated diffuse inhibitory zones where fewer fungal mycelia and spores grew on the bioautography plate. Therefore, no further antifungal studies were warranted on these five *Artemisia* species.

The five *Artemisia* essential oils were also evaluated for mosquito biting deterrency against *Ae. aegypti* by using the K & D module bioassay system. Based on biting deterrence index (BDI) values, *Artemisia gmelinii* showed the highest activity which was near to DEET, commercial standard (Figure 1). Many species of *Artemisia* are known to have repellent action against insects and are used in different parts of the world most often by burning plant parts [105, 106]. Hwang et al. (1985) [107] isolated compounds from essential oil of *A. vulgaris* and reported various levels of repellency in different compounds tested [107]. *A. argyi*, *A. feddei*, *A. manshurica* and *A. olgensis* oils showed some activity but not significant. Only *A. gmelinii* oil possess the monoterpenoid fragranol (3.8%) and sesquiterpenoid longiverbenone (12.0%). Therefore, these compounds may be the source of mosquito biting deterrent activity in *A. gmelinii*.

Table 4. Antifungal activity of five *Artemisia* essential oils using direct bioautography with three *Colletotrichum* test species.

Sample	Mean Fungal Growth Inhibition (mm) ± SEM[a]					
	C. acutatum		***C. fragariae***		***C. gloeosporoides***	
	80 *µ*g/spot	**160 *µ*g/spot**	**80 *µ*g/spot**	**160*µ*g/spot**	**80*µ*g/spot**	**160 *µ*g/spot**
A. argyi	2.5 ± 0.71	3.5 ± 0.71	2.5 ± 0.71	4.0 ± 0.00	3.0 ± 0.00	3.5 ± 0.71
A. feddei	2.0 ± 0.00	2.5 ± 0.71	3.0 ± 0.00	3.5 ± 0.71	3.0 ± 0.00	3.5 ± 0.71
A. gmelinii	diffuse zones	diffuse zones	diffuse zones	diffuse zones	diffuse zones	diffuse zones
A. manshurica	1.75 ± 0.35	2.0 ± 0.00	2.0 ± 0.00	2.5 ± 0.71	2.0 ± 0.00	2.5 ± 0.71
A. olgensis	not active	not active	not active	not active	not active	not active
Benomyl* at 1.16 µg	d		d		d	
Captan[b] at 1.2 µg	11.5 ± 0.71		9.5 ± 0.71		12.0 ± 0.82	
Cyprodinil[b] at 0.9 µg	d		31.75 ± 2.36		d	
Azoxystobin[b] at 1.61 µg	d		24.0 ± 0.82		d	

[a] Mean inhibitory zones and standard deviations (SD) were used to determine the level of antifungal activity against each fungal species.
[b] Technical grade agrochemical fungicides (without formulation) with different modes of action were used as internal standards.
[d] Diffuse zones on the bioautography plate where the fungal growth is visually in interspersed with few mycelia.

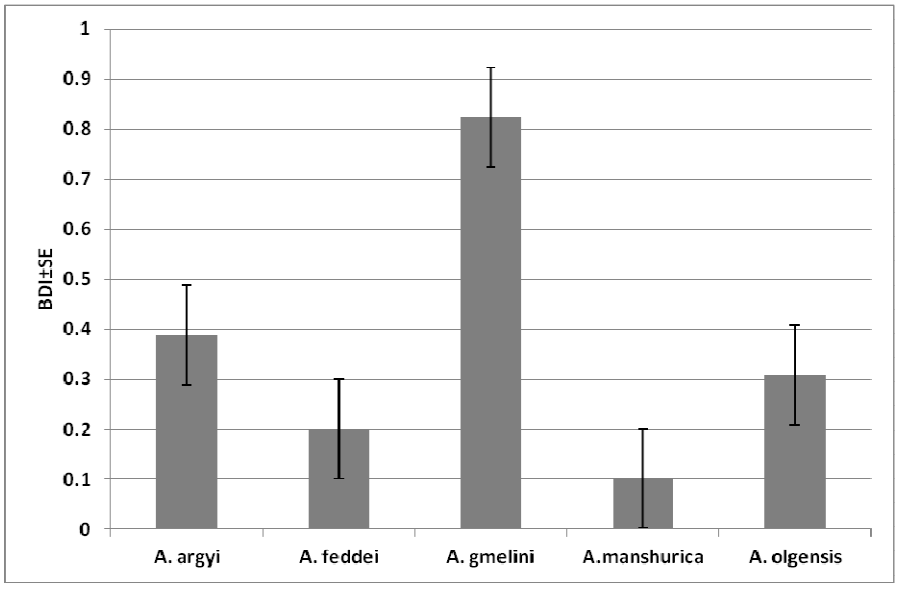

Figure 1. Mean values of Biting Deterrence Index (BDI ± standard error) of essential oils from aerial parts of *Artemisia* spp. tested against *Ae. aegypti* females. All oils were tested at the concentration of 10 µg/cm^2. Ethanol was solvent control and DEET at 25 nmol/cm^2 was used as positive control. Proportion not biting in DEET ranged between 0.87 to 0.90 whereas ethanol control showed values between 0.38 – 0.39.

Some *Artemisia* species have potent larvicidal properties and *A. vulgaris* oil and extract both were reported to be toxic to mosquito larvae [105, 108]. In the present study, *A. manshurica* and *A. olgensis* showed mosquito larvicidal activity against *Ae. aegypti* whereas *A. argyi*, *A. feddei* and *A. gmelinii* oils did not show any activity at the screening dose of 125 ppm. LD_{50} values in *A. manshurica* and *A. olgensis* oils were 82.4 (78.0-87.0) ppm and 89.4 (83.6-95.7) ppm and LD_{90}

values were 122.7 (113.7-162.7) ppm and 139. (125.6-162.7) ppm respectively at 24-h post treatment (Figure 2). There was no substantial increase in mortality at 48-h post treatment. *A. manshurica* and *A. olgensis* oils were rich in oxygenated sesquiterpenes which may be responsible for the larvicidal activity.

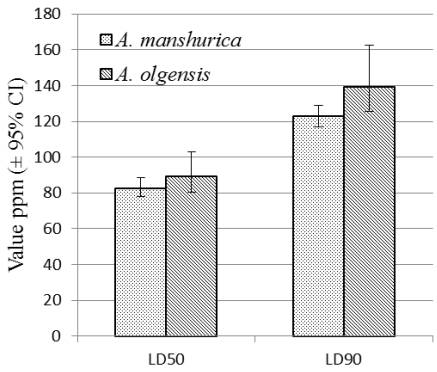

Figure 2. LD_{50} and LD_{90} values (± 95% CI) of *Artemisia* spp. against 1-d old *Ae. aegypti* at 24-h post treatment

The inhibitory effect of the *Artemisia* essential oils on lipid peroxidation was determined by the β-carotene/linoleic acid bleaching test. This test simulates the oxidation of the membrane lipid components in the presence of antioxidants inside the cells. Antioxidant activity of the oils was determined by measuring the ability of the volatile constituents to inhibit the conjugated diene hydroperoxide formation from linoleic acid and β-carotene coupled oxidation in an emulsified aqueous system loses its orange color when reacting with free radicals. The presence of the oil with antioxidant activity can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical formed in the system. In this test system, *A. feddei* and *A. argyi* oils demonstrated moderate antioxidant activity with 4.1-6.1% of inhibition (Figure 3).

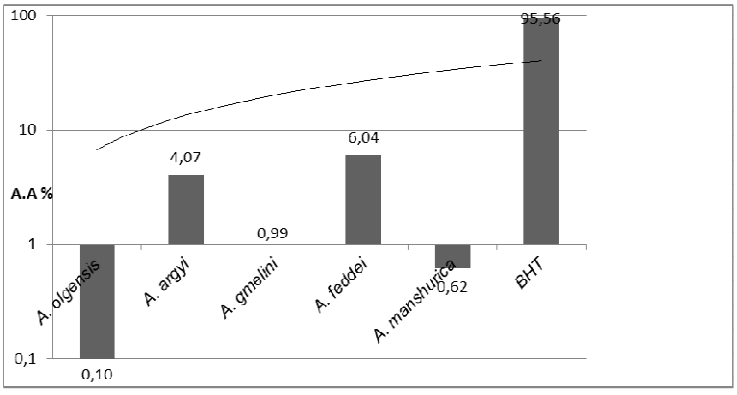

Figure 3. The inhibition percentage of peroxides formation in the presence of *Artemisia* oils as compared to BHT by β-carotene/linoleic acid bleaching test (logarithmic diagram).

We also measured the relative capacity of antioxidants to scavenge the $ABTS^{\circ+}$ radical compared to the antioxidant potency of Trolox (standard). *A. argyi* and *A. gmelinii* essential oils demonstrated moderate antioxidant activity. Highest TEAC values were estimated for *A. argyi* and *A. gmelinii* oils as 1.58 mM and 1.33 mM at 30 min, respectively. *A. manshurica* demonstrated lowest activity with TEAC as 0.5 mM. (Figure 4).

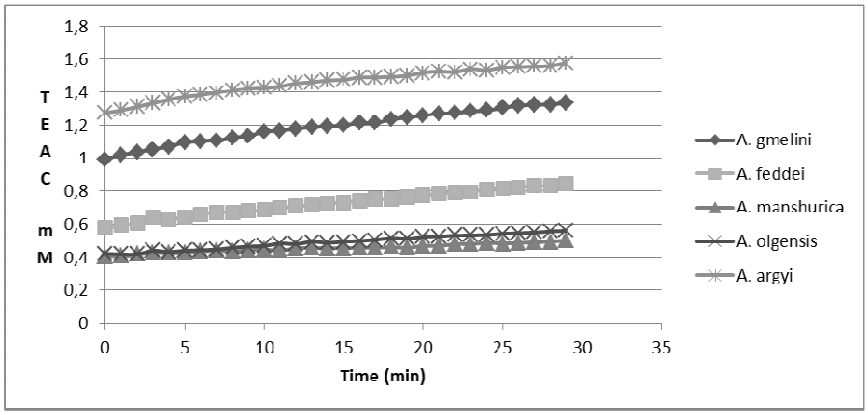

Figure 4. Determination of AOA of *Artemisia* oils by Trolox equivalent antioxidant capacity (TEAC) test

Each of the tested oils as well as BHT (standard) reduced DPPH to yellow colored product and the absorbance at 517 nm declined. Among the tested oils, *A. argyi* and *A. gmelinii* demonstrated noteworthy activity with an IC_{50} of 8 mg/mL and 13.7 mg/mL, respectively. The oils of *A. feddei*, *A. manshurica* and *A. olgensis* (40 mg/mL) did not possess such reducing effects and it was not possible to measure concentrations for a 50% inhibition (Table 5).

Table 5. Antioxidant activity of five *Artemisia* oils in DPPH test

Free radical scavenging activity, μg/mL	Aa	Af	Ag	Am	Ao	BHT*
DPPH IC_{50}	*8*	*>40*	*13.7*	*>40*	*>40*	10

* Standard; **Aa**: *A. argyi*; **Af**: *A. feddei*; **Ag**: *A. gmelinii*; **Am**: *A. manshurica*; **Ao**: *A. olgensis*.

In conclusion, the present work was a study into chemistry and biological activity of five *Artemisia* species from Far East Russia. *Artemisia* oils have unique chemical composition with their high contents of oxygenated mono- and sesquiterpenes. Five tested *Artemisia* oils demonstrated moderate antioxidant and antifungal activity, whereas three *Artemisia* species showed promising mosquito activity against the yellow fever mosquito *Aedes aegypti*. *A. gmelinii* oil offers potential for biting deterrent activity and further studies need to be focused on the active chemistry in the biting deterrent activity. *A. manshurica* and *A. olgensis* oils showed higher larvicidal activity and may be useful in the search for new natural mosquito larvicidal compounds. These results could be useful in the discovery for new natural mosquito repellent and larvicidal compounds. Bio-insecticides may be also effective, selective, bio-degradable and associated with little or no-resistance of the pest and less toxic to the environment.

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