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CHEMICAL COMPOSITION AND PLANT GROWTH INHIBITORY EFFECT OF ESSENTIAL OIL FROM *ARTEMISIA NILAGIRICA* L. COLLECTED FROM NORTHEAST INDIA

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ABSTRACT
Essential oil extracted from aerial part of the *Artemisia nilagirica* by hydro-distillation was subjected to Gas Chromatographic analysis and a total of 36 major compounds were identified, comprising 86.5% of total value by weight. The major compounds of this essential oil were found Sabinene (3.5%), p-cymene (14.1%), 1,8, cineole (18.2%), artemesia ketone (5.7%), Artemesia alcohol (3.2%), camphor (9.1%), β-eudesmol (5.8%) and δ-cadinene (3.1%). The plant growth inhibitory effect of the essential oil was tested against *Vigna radiate*, *Lens culinaris*, *Vigna mungo*, *Vigna unguiculata* and *Vigna aconitifolia*. A significant reduction on seed germination was observed on tested seeds of those plants in response to the different concentrations of the oil. Even at low concentration (200ppm), the radical growth was significantly reduced. At higher concentrations (500ppm and 600ppm), the inhibitory effect was higher, as no radical developed. Amongst all the tested seeds, *Vigna mungo* was the least sensitive and *Vigna unguiculata* was found to be the most sensitive for seed germination.

KEY WORDS: *Artemisia nilagirica*, Essential oil, Growth inhibition, Hydro-distillation.

INTRODUCTION

The genus Artemisia belonging to the family Asteraceae is represented by more than 800 species, being one of the largest genus of the family (Davis, 1982). It is a wild, annual, aromatic, hardy plant, with a habit ranging from herbs to shrubs. This plant forms monospecific strands along water canals, agricultural fields, roadsides and wasteland (Anonymus, 1993). Most of the species are found growing abundantly all over temperate and cold temperate zones of the world. Their volatile oils have been in use since antiquity in flavour and fragrances, medicines, as antimicrobial and insecticidal agents and as insect repellents (Bakkali *et al.*, 2008; Batish *et al.*, 2008). The essential oil obtained by hydro distillation, is pale greenish yellow in colour and is used in the perfumery industry and in aromatherapy for balancing, stimulating, energizing and toning. It also finds use in the cure of sore throats, bronchitis, coughs, colds, fever, colic, loss of appetite, headache, earache, intestinal worms and malaria. The roots, stems and leaves of the plant are also used in a number of ways as enemas, poultices, infusions, body washes, lotions, for smoking, as snuff and also drunk as tea. The odour emanated by the plant is powerful, fresh camphoraceous, somewhat bittersweet, with a cedar- leaf-like top note and sage rosemary-like body note. The flavour of the oil is warm, almost pungent and bittersweet, with a slight cooling effect in higher dilution; traditionally it is used as an emollient, soothing agent and muscle relaxant.

MATERIALS & METHODS

Plant material

The *Artemesia nilagirica* plants were collected in the vegetative stage from the North Eastern Hill University campus, Shillong (location: 25°36′36″N and 91°54′5″E). The aerial part of the plant is hydro-distilled using Clevenger's apparatus for extracting the essential oil. The oil is stored in opaque plastic vials at ambient temperature (4^{o}C -8^{o}C) for chemical characterization.

Extraction of the essential oil

The plant samples (100 g) were subjected to hydro distillation in a Clevenger's Apparatus for 3hrs. For each batch of distillation, three replicates of 20g each of fresh plant material were dried at 60^{o}C for 48 hrs to determine moisture content. The extracted oil was dried over anhydrous sodium sulphate and stored at 4^{o}C -8^{o}C for the subsequent experiments.

GC-MS analysis of the oil

The oil obtained by hydro-distillation was marked as fresh oil and shade-dried oil as dry oil and subjected to GC-MS analysis for identifying its chemical composition and its constituents. The volatile constituents were separated in a glass column of 2mm i.d. (internal diameter) × 2m length, filled with 15% SE 52 on gas chrom Q 80/100 mesh. The column temperature was programmed from 100˚ to 200˚C at the rate of 3˚C /minute after initial loading time of ten minutes. A Varion model 3700 gas chromatograph with flame ionization detector (FID) was used during the course of analysis. The major identified compounds and their percentage under different seasons are presented in Table: 2.

Bioassay of Artemesia oil

Seeds from the test plants viz. Green gram (*Vigna radiate*), Lentil (*Lens culinaris*), Black gram (*Vigna mungo*), Lobia (*Vigna unguiculata*) and Moth (*Vigna aconitifolia*) were imbibed in water for 6hrs and these pre-imbibed seeds were placed for germination in petri-plates (90 mm Diameter) on thin cotton pad covered with filter paper with 5ml water-methanol-oil solution. Petri-dishes containing 25 seeds were incubated in the dark at 27± 2^{o}C. To test the inhibitory effect of the oil, 100µl of the oil was mixed with 9.9 ml methanol. Then concentrations of 200, 300, 400, 500 and 600 ppm of the oil were evaluated for phyto-toxicity in dose-response manner under laboratory conditions. The treated petri-dishes were sealed with parafilm and placed at 27 ± 2^{o}C. A set of petri-dishes with required quantity of methanol and water served as the control. Each concentration was tested in five replicates. After incubation, the numbers of germinated seeds were counted and the length of the radicle was measured.

RESULTS & DISCUSSIONS

Artemesia oil yield

The oil yield varied with the moisture content of the plant sample (Table 1). The oil yield was lower in the fresh sample as compared to the shade-dried sample. The oil yield from the fresh leaf was 0.44% (v/w) on an average. Shade-dried leaves yielded 1.4% oil (v/w). However, in the case of the whole plant (leaf + stem) average oil yield was 1.02% and only stem yielded only 0.29% oil. Oil yield obtaining by hydro-distillation of different Artemisia species were quite low in fresh aerial parts. However, shade-dried sample yielded around 1% (v/w). Nezhhadali *et al.* (2008) reported 1.19% (v/w) from *A. herba*, Ramezani *et al* (2004) reported 1.25% (v/w) from *A. khorassanica*, Ghorbani-Ghouzhdi *et al.* (2008) reported 1.4% (v/w) from *A. sieberi*, Dob *et al.* (2005) reported 0.1% (w/w) from *A. campestris*, Pal Singh *et al.* (2009) reported 0.17% (v/w) from *A. scoparia*, Judzentiene and Buzelyte (2006) reported about 0.3% (v/w) oil yield from *A. vulgaris*. *A. annua* yielded about 1.2% (v/w) essential oil, reported by Verdian-rizi *et al.* (2008). Basher *et al.* (1997) reported that *A. balchanorum*, *A. leucodes*, *A. rhodantha* and *A. scoparia* yield 1.2, 0.28, 0.75 and 0.72% essential oil on hydro-distillation respectively. In the present study, *Artemisia nilagirica* yield 1.03% (v/w) essential oil.

TABLE 1: Oil Yield and recovery pattern of different parts of *Artemesia nilagirica*

Source	Oil Recovery	Oil Recovery Pattern (Time in minutes), %				
		30	60	90	120	150
Leaf	1.471 ± 0.08	63.31±0.10	16.21±0.44	8.92±0.19	6.15±0.37	5.73±0.06
Stem	0.297 ± 0.11	35.7± 0.28	21.43±0.08	21.44±0.08	21.39±0.12	0.0±0.0
Leaf + Stem	1.045± 0.04	64.4±0.17	13.82±0.15	10.31±0.11	8.06±0.23	3.33±0.06

GC-MS amalysis of the oil

Gas Chromatography and Mass Spectrometry analysis of the oil from fresh and shade-dried leaves showed the following chemical composition and constituents of the oil.

TABLE 2: Composition of essential oil obtain from *Artemisia nilagirica*

Sl. No.	Compound	Value (% weight)
1	Santolinatriene	0.3
2	α- thuzene	1.0
3	α-pinene	0.5
4	Camphene	2.0
5	Sabinene	3.5
6	β-pinene	0.2
7	Myrcene	0.2
8	α- Phellendrene	0.1
9	2,5,5- trimethyl 3, 6- heptadiene-ol	1.3
10	α- terpinene	2.4
11	p-cymene	14.1
12	1,8, cineole	18.2
13	artemesia ketone	5.7
14	Artemesia alcohol	3.2
15	Terpinolene	0.1
16	α- Thujone	0.9
17	β-Thujone	0.4
18	p- menthe-2-en-1-ol	0.1
19	Camphor	9.1
20	Hydrocinnamaldehyde	0.8
21	Borneol	2.0
22	Terpinene-4-ol	1.2

23	p-menth-1en-8-ol	1.0
24	Cis-carveol	0.2
25	Piperitone	1.0
26	Copaene	0.5
27	δ-cubenene	0.5
28	α-gurjunene	0.4
29	Caryophyllene	1.9
30	γ- humulene	0.4
31	Germacrene-D	1.2
32	δ-cadinene	3.1
33	δ-calacorene	0.9
34	β-eudesmol	5.8
35	Valeranone	1.4
36	α-bisabolol	0.9

In the present investigation, using gas chromatographic technique, 36 major compounds were identified, comprising 86.5% of total value by weight. Out of these, Sabinene (3.5%), p-cymene (14.1%), 1,8, cineole (18.2%), artemisia ketone (5.7%), artemisia alcohol (3.2%), camphor (9.1%), β-eudesmol (5.8%) and δ-cadinene (3.1%). Ramezani *et al.* (2004) reported *A. khorassanica* essential oil contained 1, 8-cineol (17.7%) as major constituent. Kordali *et al.* (2005) reported that *A. spicigera* produced camphor 34.9%. *A. annua* essential oil contains 48% camphor, reported by Verdian-rizi *et al.* (2008). *A. vulgaris* essential oil contains trans-thujone as major compound (20.2%), reported from north Lithuania (Judzentiene and Buzelyte, 2006). β- myrcene was found to be major compound (29.27%) in *A. scoparia* essential oil, reported by Pal Singh *et al.*, 2009.

Bioassay of Artemesia oil

The effect of the oil was tested on the germination and seedling growth of the seeds of five plant species namely green gram (*Vigna radiata*), lentil (*Lens culinaris*), black gram (*Vigna mungo*), lobia (*Vigna unguiculata*) and moth (*Vigna aconitifolia*). A significant reduction on seed germination was observed in the tested seeds, in response to the different concentrations of the oil (Table- 3).

TABLE-3: Germination % of different seeds after 72 hours treatment

Concentration, ppm	No. of seeds observed	Germination %, after 72 hours				
		Vigna radiata	*Lens culinaris*	*Vigna unguiculata*	*Vigna mungo*	*Vigna aconitifolia*
0	25	99.97±0.50	84.28±0.25	88.38±0.48	96.01±0.14	100.05±0.17
200		92.04±0.17	72.23±0.27	16.01±0.09	88.13±0.35	92.19±0.18
300		76.12±0.12	64.26±0.33	0.00±0.00	80.17±0.18	83.98±0.19
400		60.12±0.54	52.26±0.34	0.00±0.00	71.99±0.17	76.06±0.27
500		39.98±0.27	16.00±0.05	0.00±0.00	63.86±0.58	67.88±0.52
600		0.00±0.00	0.00±0.00	0.00±0.00	56.00±0.03	59.96±0.48

It was also observed that *Vigna unguiculata* seeds are more sensitive on exposure to essential oil even at low concentration (200 ppm) and germination is completely inhibited after 200 ppm. *Vigna mungo* and *Vigna aconitifolia* seeds are less effective on exposure to the oil. However, except *Vigna unguiculata*, the entire tested seeds exhibits dose dependent response on germination.

TABLE 4: Radicle length of germinated seeds after 72 hours (cm)

Concentration	No. of seeds observed	Radicle length(cm), after 72 hours				
		Vigna radiata	*Lens culinaris*	*Vigna unguiculata*	*Vigna mungo*	*Vigna aconitifolia*
0	25	3.89±0.05	4.15±0.12	4.08±0.03	3.73±0.12	3.94±0.07
200		1.23±0.10	2.92±0.06	0.42±0.05	3.11±0.13	2.94±0.07
300		0.73±0.07	1.29±0.09	0.00±0.00	2.56±0.06	2.46±0.06
400		0.42±0.06	0.87±0.11	0.00±0.00	2.10±0.07	1.70±0.05
500		0.33±0.07	0.30±0.04	0.00±0.00	1.47±0.05	1.61±0.08
600		0.00±0.00	0.00±0.00	0.00±0.00	1.07±0.05	1.00±0.05

The radical growth was significantly reduced even at low concentration (200ppm) to all the tested seeds. *Vigna unguiculata* seeds exhibits most sensitivity and developed only 0.43 cm radical length in 200 ppm concentration. At higher concentrations of essential oil, the inhibitory effect was higher, as no radical or a little radical length developed. Amongst all the tested seeds, *Vigna mungo* was the least sensitive and *Vigna unguiculata* was found to be most highly sensitive for seed germination (Table-4).

TABLE 5: Shoot length of different seeds after 72 hours (cm)

Concentration	No. of seeds observed	Seedling length(cm), after 72 hours				
		Vigna radiata	*Lens culinaris*	*Vigna unguiculata*	*Vigna mungo*	*Vigna aconitifolia*
0	25	1.10±0.05	1.23±0.03	0.59±0.04	0.41±0.03	0.51±0.04
200		0.46±0.07	0.81±0.02	0.00±0.00	0.24±0.03	0.40±0.03
300		0.36±0.02	0.59±0.01	0.00±0.00	0.16±0.01	0.34±0.02
400		0.21±0.02	0.50±0.03	0.00±0.00	0.12±0.02	0.27±0.01
500		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.26±.01
600		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Development of shoot were adversely effected and significantly reduced on increasing the essential oil concentration (Table-5). No shoot was developed in *Vigna unguiculata* even in low concentration also. At higher concentration all of the experimental seeds developed no shoots except *Vigna aconitifolia*.

TABLE 6: Secondary root length of different seeds after 72 hours (cm)

Concentration	No. of seeds observed	Secondary root length, after 72 hours				
		Vigna radiata	*Lens culinaris*	*Vigna unguiculata*	*Vigna mungo*	*Vigna aconitifolia*
0	25	0.22±0.02	0.25±0.01	0.37±0.06	0.30±0.01	0.37±0.01
200		0.15±0.005	0.11±0.04	0.00±0.00	0.25±0.02	0.25±0.01
300		0.11±0.01	0.00±0.00	0.00±0.00	0.15±0.005	0.21±0.01
400		0.00±0.00	0.00±0.00	0.00±0.00	0.11±0.01	0.10±0.002
500		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
600		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Out of the 25 seeds of each concentration, only a few of them produced secondary roots. The lengths were reduced significantly as the concentration of the oil increased. In higher concentrations no secondary root development was observed among all the species (Table-6). The allelopathic potentials of *Artemisia* essential oils in terms of inhibition of seed germination as well as inhibition of seedling growth against *Lactuca sativa* and *Lolium perenne* reported by Satyal *et al* (2012), where both root (radicle) and shoot (plumule) growth of *L. sativa* and *L. perenne* were notably inhibited by the essential oils obtained from *Artemisia dubia*, *A. indica*, and *A. vulgaris*. Barney *et al* (2005) reported that *Artemisia annua* essential oil has growth inhibitory effect on several broadleaf weeds and crops. In the present investigation, *Artemisia nilagirica* oils exhibited notable allelopathic activity in terms of seed germination, radical length, shoot length and secondary root development as well.

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