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ESSENTIAL OIL COMPOSITION AND MOSQUITO LARVICIDAL ACTIVITY OF *ARTEMISIA NILAGIRICA* (C. B. CLARKE) PAMPAN. FROM SOUTH INDIA

L. LEEJA and JOHN E. THOPPIL*

Genetics and Plant Breeding Division, Department of Botany, University of Calicut, Kerala - 673 635, India.

** e-mail : leejalak4@rediffmail.com, jethoppi12004@rediffmail.com*

Artemisia nilagirica (C. B. Clarke) Pampan. (Asteraceae) collected from South India was hydro-distilled to produce an aromatic greenish oil (1.2%). Gas Liquid Chromatography reveals twenty-two compounds, among which β-caryophyllene (19.94%) and *p*-cymene (15.33%) were the major constituents. Larvicidal activity of the essential oil against *Aedes albopictus* Skuse, commonly seen in Calicut University Campus was also studied. LC_{50} value was found to be 5 μgml^{-1}. The essential oil of *A. nilagirica* can be used as an effective larvicide.

Keywords: *Aedes albopictus; Artemisia nilagirica;* Asteraceae; Essential oil; Gas liquid chromatography; Mosquito larvicidal activity.

Introduction

Artemisia (*Asteraceae*) is a rich source of sesquiterpenoids and is well known for its biological and chemical diversity[1]. *Artemisia nilagirica* (C. B. Clarke) Pampan., an aromatic weedy herb of Asteraceae is used as an emmenogogue, diuretic, aphrodisiac, appetizer, febrifuge, alexiteric[2], anthelmintic[2-4], expectorant and antiseptic[3]. Roots of *A. nilagirica* are used as tonic and antispasmodic[4]. Leaves and flowering tips are used for asthma[2, 5] and they are bitter, astringent, acrid, thermogenic, aromatic, anodyne, anti-inflammatory, digestive, haematinic[2] and are administered in nervous and spasmodic afflictions[5]. It can be used as a substitute for *Cinchona* leaves. *A. nilagirica* along with other plants have been used for the treatment of specific human ailments such as allergies, burns, cuts, wounds, inflammations, leucoderma, scabies, small pox and sexually transmitted diseases[6, 7]. The plant is reported to possess antimicrobial properties[4, 8-11]. The present study is an attempt to find out the chemical composition and mosquito larvicidal activity of the essential oil of *A. nilagirica* growing in South India.

Materials and Methods

Essential Oil Extraction : Artemisia nilagirica, a common weed in Wyanad district of Kerala, India, was collected and grown in the Calicut University Campus (Kerala, India) and authenticated at the Herbarium of Botany Department, University of Calicut, where voucher specimens (CALI 86002) were deposited. The shade dried aerial parts of the plants were hydro-distilled in a Clevanger apparatus at 100 °C for 4 hours. The aromatic greenish essential oil (1.2%) was collected and dried over anhydrous sodium sulphate. The pure oil was transferred to a small amber coloured bottle and stored at 4-6 °C.

GLC Analysis : A neat sample of the cooled essential oil was qualitatively analyzed on a PERKIN ELMER (USA) Autosystem Gas Chromatograph, equipped with FID and connected with a chromatograph data processor PE Nelson 1022. GLC conditions used were as follows: column character: SE-30 (Silicon E- 30), solid phase- CH W. HP (Chromosorb W - High Performance); mesh size: 100/ 100, column measurements: length - 183 cm, internal diameter - 2 mm, carrier gas: N_2; N_2 inlet pressure - 0.387 $kgcm^{-2}$, flow rate - 30 ml min^{-1}; temperature programme: from 80°C (initial temperature) to 220°C (final temperature) at a rate of 5°C min^{-1}; injector temperature 200°C, detector temperature 300°C.

Identification of Components : The percentage composition of the oil was computed from the GLC peak areas without using correction factors. The identity of the major components was assigned by comparing their GLC retention times with those of authentic standards, peak enrichment by co-injection with the standards and by comparison with literature data[12].

Mosquito larvicidal activity : The larvae of *Aedes albopictus* Skuse were reared according to the standard method[13]. The forth instar larvae were used to study the larvicidal activity. All bio-analysis were performed according to the method of WHO[14]. The oil obtained was used to prepare stock solution of 10 $mgml^{-1}$ in acetone. The stock solution was diluted to 10 ml with filtered tap water to obtain the test solutions of 10, 8, 6.6, 5.6, 5, 3.3, 2.5 and 2 μgml^{-1}. Two controls were maintained at a time, one consisted of acetone and the other tap water only. The fourth instar larvae (20 each) were tested at 8 different

Table 1. Major chemical components of the essential oil of *Artemisia nilagirica.*

Essential oil components	Concentration (%)	tμR* (min)
α-thujene	0.13	4.15
α-pinene	1.32	4.5
α-phellandrene	0.27	4.8
Sabinene	0.16	5.5
α-thujone	1.71	5.79
Myrcene	0.73	6.59
p-cymene	15.33	7.62
Limonene	3.75	7.77
1,8-cineole	0.98	8.37
Terpinen-4-ol	0.69	8.5
Camphene	3.24	9.04
Linalool	0.90	9.33
α-terpinene	0.78	9.5
γ-terpinene	0.56	9.7
Camphor	1.06	10.2
α-terpinolene	0.29	10.7
α-terpineol	4.22	12.21
α-cadinene	8.55	16.01
Eugenol	1.43	16.4
β-caryophyllene	19.94	17.56
α-humulene	1.34	18.33
γ-elemene	8.32	21.22

* Retention time

Table 2. Larvicidal activity of *Artemisia nilagirica* essential oil against *Aedes albopictus.*

Concentration of oil (μg ml^{-1})	Mortality** (%)
10	100 ± 0
8	80.16 ± 0.75
6.6	70.3 ± 0.52
5.7	60.16 ± 0.75
5	50 ± 1
3.3	29.3 ± 0.52
2.5	19.3 ± 0.52
2	9.5 ± 0.8

** Mean ± S. D.

LC_{50} Value = 5 μg ml^{-1}

test solutions as well as control in triplicate. The larval mortality was recorded after 24 hours. Toxic activity of oil was reported as LC_{50} that is concentration of oil that killed 50% larvae in 24 hours.

Results and Discussion

In the present investigation, the essential oil of *A. nilagirica* seems to be enriched with sesquiterpenes (39.58%) and monoterpenes (36.12%) together with small amount of phenolics (1.43%). Various components of the essential oil are listed in Table 1. The major components detected in the present investigation are β-caryophyllene (19.94%), a sesquiterpene hydrocarbon, followed by *p*-cymene (15.33%), a monoterpene.

Other volatile components detected includes α-cadinene (8.55%), α-humulene (1.34%), γ-elemene (8.32%), all being sesquiterpene hydrocarbons. In addition to that, substantial amounts of phenol such as eugenol (1.43%) and monoterpenes such as α-pinene (1.32%), α-thujone (1.71%), limonene (3.75%), camphene (3.24%), camphor (1.06%) and γ-terpineol (4.22%) were also detected. A few compounds were also detected in low amounts (Table 1). Previous reports[5, 15-16] confirms the occurrence of many of these compounds in *Artemisia.*

Among the different species of mosquitoes, *Aedes albopictus,* one of the vectors of yellow fever and dengue fever, which is common in Calicut University Campus. A silvery stripe on its mesotonum and whitish irregular patches on the lateral appendages of its thorax distinguish it[17]. The tree holes are the most widespread container habitats and *Aedes albopictus* are usually seen in the holes of trees such as *Tamarindus* sp., *Pongamia pinnata, Syzygium cumini* and *Caesalpinia* sp. etc.

Several essential oils and their isolates have been evaluated for use as indoor insect repellents due to their volatile nature, pleasant aroma and biodegradability[18-20]. In the present study, the essential oil of *A. nilagirica* was tested against *A. albopictus.* The plant oil showed extreme larvicidal activity against *A. albopictus.* The most effective treatment of *A. nilagirica* oil, which caused 100% mortality, was at a concentration of 10 μgml^{-1} (Table 2). On dilution, the activity was reduced slightly. LC_{50} value of the essential oil of *A. nilagirica* was 5 μgml^{-1}. No mortality was noticed when 2 controls - tap water and acetone were used. So the present study strongly indicates the efficacy of essential oil of *A. nilagirica* as larvicidal agents and their possible use in the biological control of *A. albopictus,* an important vector of several parasitic diseases.

The components detected in the present investigation possess reputed insecticidal and medicinal properties. β-caryophyllene together with limonene, camphene, α-thujone, eugenol, α-pinene, camphor, myrcene, linalool and α-phellandrene might be the probable reason for mosquito larvicidal activity of *A. nilagirica* essential oil. Such activity has already been reported on these volatile terpenoids. Various major components such as caryophyllene, limonene, camphene, camphor, eugenol, α-pinene, terpinen-4-ol, α-terpineol and myrcene were found to be in use as an insectifuge[21-23]. Moreover anticarcinogenic and antitumor activity of β-caryophyllene, β-pinene, camphor, limonene and eugenol has been reported[22, 23]. Probably due to the same reason, the essential oil of *A. nilagirica* could be effectively exploited for formulating potential and biodegradable therapeutic drugs for curing cancer as well as for the production of potential ecofriendly insecticides.

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