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Essential Oil and Its Antimicrobial Activity from Ethiopian *Acokanthera schimperia*

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Abstract

Background: *Acokanthera schimperia* is a medicinal plant, which has been used by traditional healers as a curing agent in Ethiopia. **Objective:** The constituents of the essential oil, which was extracted from the leaves of *A. schimperia*, were investigated, and its antibacterial and antifungal activities were studied. **Materials and Methods:** The essential oil was extracted by an ordinary steam distillation process, and its chemical constituents were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Antibacterial and antifungal activities of the oil were studied by micro-dilution method against *Escherichia coli*, *Bacillus subtilis* (for bacteria), and *Candida albicans* (for fungus) respectively. **Result:** From the essential oil, 21 compounds were recognized, and making up 56.06 %. However, the essential oil doesn't show any antimicrobial activities. **Conclusion:** This is the first research on *A. schimperia* concerning its essential oil and antimicrobial activities.

Keywords: *Acokanthera schimperia*, Essential oil, GC-MS, Antimicrobial activity.

Introduction

Plants have been used by Traditional healers for centuries as a source of curative agent, and it has been spread-out globally and is gaining fame [1]. In Ethiopia, about 80% of the people, especially those who are living outside the cities are still dependent on traditional medicines [2,3]. Among different Ethiopia plants, *Acokanthera schimperi* (A. DC.), which belongs to Apocynaceae family, is a well-known African arrow poison plant and dispersed in Eritrea, Ethiopia, Tanzania, West Uganda, Rwanda and Eastern DR Congo. Outside Africa, It is also found in southern Yemen [4]. In Ethiopia, it is a tree of dry woodland, thickets, and grasslands in Dry and Moist agro-climatic zones in nearly all regions [5].

A. schimperi is a plant locally called "መረንዝ", which means "Toxic" used for the preparation of arrow poison in East Africa. It is either used on its own or mixed with other plants or animal parts. The bark, wood, and roots are the usual ingredients for arrow poison, and they are also used for suicide and homicide. The poison from this plant is also used for hunting wild animals and stray dogs from fields and homes [6]. The leaves and bark are used to treat different disease and shows antiviral activity in the cases of skin disorders caused by viruses, and antimalarial activity [7]. In addition, Ethiopian traditional healers used this indigenous plant for the treatment of epilepsy, amnesia, eye disease, syphilis, rheumatic pain, elephantiasis, scabies, leprosy, wound, eczema and warts [8].

Essential oils are combinations of volatile constituents that present at little amount in the plant, and used as flavor and fragrances agent in food, pharmaceutical and perfumery industries [9]. In this paper, we will focus on the essential oil from the leaves of *A. schimperi*, including its chemical constituents and bioactivities.

Materials and Methods

Plant material

In September 2015, the leaves of *A. schimperi* were collected from a well-known monastery called Debre Libanos which is 90km far from Addis Ababa, the capital city of Ethiopia and the species of the plant was identified by Amare Seifu Assefa, a botanist from the Ethiopian Biodiversity Institute.

Extraction of essential oil

200 g coarsely grinded leaves of *A. schimperia* were socked in 350 mL tap water for 12 hrs and transferred to 3L volume Clevenger's apparatus (flask). Then 1.5 L distilled water was added to maintain the level of water above the sample. After that the sample was subjected to steam distillation process for eight hrs. The distillates were saturated with NaCl and extracted by diethyl ether. The organic phase was dried

by anhydrous Na_2SO_4 as water absorbent agent. Diethyl ether was removed from the oil by putting it at open air until the essential oil remains. Finally, the essential oil was stored at 4 °C in refrigerator for further use.

Analysis of essential oil

The investigation of the essential oil were done by an Agilent 6890 gas chromatograph interfaced with an Agilent 5973N mass spectrometer engaged with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The condition for Gas Chromatography (GC): the temperature programmed at 70 ℃ for the first 2 minutes, increased sequentially with a rate of 5 ℃/minute until it reaches 300 ℃. After that, the temperature was set to remain constant for the next 2 minutes; The injector temperature and volume were set at 250 ℃ and 1µL respectively; the carrier gas was Helium with flow rate 1 mL/min under split ratio of 1:20; Mass Spectrometry (MS) condition: EI ionization mode, 70eV, scan range 30-500 Amu, ion spring temperature was 230℃. Each constituent's mass spectra were compared with the spectrometer database (NIST 11). For quantification purposes, relative area percentages were used without the use of correction factors.

Antimicrobial activities

Escherichia coli (ATCC 25922) and *Bacillus subtilis* (ATCC 6633) were used for evaluation of antibacterial activities and *Candida albicans* (ATCC 60193) was used for antifungal evaluation. The bioassays tests were made in 96-well decontaminated micro-plates using a micro-dilution method [10, 11]. *E. coli* and *B. subtilis* were cultured for 18-hrs and added to Lysogeny Broth (LB) medium (1 L water, 10 g tryptone, 5 g yeast, and 10 g NaCl) to attain 1 × 105 CFU/ml, and *C. albicans* was grown for 4-days and added to Potato Dextrose Broth (PDB) medium (potato 20%, glucose 2%) to get 1 × 103 spores/mL. The test samples were dissolved by Dimethyl Sulfoxide (DMSO) to attain from 0.5 to 512 µg/mL concentration ranged, which were made by 2-fold sequential dilution method. The wells holding test strains and diluted samples were incubated for 24 hrs in isothermal condition (37 ℃) for antibacterial bioassay. With the same condition for antifungal bioassay, the sample was incubated for 4 days at 28 ℃. The wells which contain a culture suspension and DMSO were set as negative controls. Kanamycin and Nystatin were set as positive controls for bacteria and fungi respectively. All tests were performed twice. The Minimal Inhibitory Concentration (MIC) was well-thought-out as the lowest antibiotic concentration that indicates a total production inhibition for the tested microorganisms.

Results and Discussion

The essential oil extracted from the leaves of *A. schimperia* gave pale yellow oil (yield 0.007 % w/w). The constituents of the essential oils with their retention time and relative percentages were given in **Figure 1** and **Table 1**. A total of twenty-one compounds were identified representing 75.52 % of the total essential oil of *A. schimperi*, and damascenone (14.83 %), dihydroactinidiolide (10.87 %), kaur-16-ene (5.74 %) and (E)-2-tridecenal(5.51 %) were the main components. Sesquiterpenoids, including six compounds, were found as major in the oil and represented 32.98 % of the total identified components. The essential oil extracted from the leaves of *A. schimperi* was tested for its antibacterial activity against *E. coli* (Gram-negative) and *B. subtilis* (Gram-positive), and its antifungal activities against *C. albicans* (fungi). The results as shown in **Table 2** indicated that the essential oil from *A. schimperia* did not show any antimicrobial activities.

Conclusion

This report shows that the essential oil of *A. schimperia* is rich in terpenoids. However, the oil was not found to show any significant antimicrobial activities against *E. coli*, *B. subtilis* and *C. albicans*. Nevertheless, this is the first report on the essential oil of *A. schimperia*. According to literatures, *A. schimperia* can be found an attractive medicinal plant. Although, several research works have been done on some plants of this genus till to date, but a large number of this plant is still chemically or pharmacologically unknown. Consequently, a broad future research remains possible in which the isolation of new active principles from *A. schimperia* would be a great scientific worth.

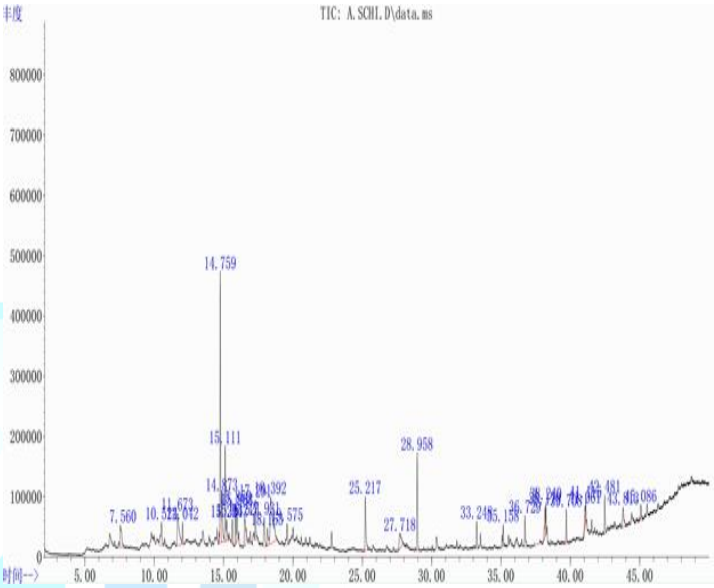

Figure 1: GC-MS profile of essential oil from *A. schimperia*.

Components	Retention time (min.)	Area
Nonanal	7.56	3.06
beta-Cyclocitral	10.52	2.84
(E)-2-tridecenal	11.67	5.51
Damascenone	14.76	14.83
Caryophyllene	15.63	1.37
alpha-Ionone	15.85	2.17
Nerylacetone	16.52	2.3
beta-Ionone	17.29	2.77
(+)-delta-Cadinene	18.16	1.47
Dihydroactinidiolide	18.39	10.87
1-Methyl-6-methylenebicyclo[3.2.0]heptane	19.57	1.83
Trimethylpentadecan-2-one	25.226,10,14-	4.64
3-Deoxyestradiol	27.72	2.77
Kaur-16-ene	28.96	5.74
Tetracosane	35.15	1.3
Pentacosane	36.73	2.26
1-Heptacosanol	38.18	2.12
Eicosane	38.24	1.44
6,6-Diethylhoctadecane	39.71	1.92
Cyclooctacosane	41.06	0.72
Octadecane	42.48	3.59

Table 1: Chemical components (%) of the essential oil from *A. schimperia*.

	E. coli	*B.subtilis*	*C.albicans*
***A. schimperia* essential oil**	> 128	> 128	> 128
Kanamycin	4	4	-
Nystatin	-	-	4

Table 2: Antimicrobial activities of the essential oil (MIC: µg/ml).

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