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Research Article

The Cytotoxicity Study of *Lantana camara* Linn Essential Oil on HeLa Cancer Cells Line

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ABSTRACT

Lantana camara Linn (Verbenaceae) is a natural plant that thrives in tropical climates and is relatively easy to cultivate. In Indonesia, this plant is still often considered as a weed. When held, the unpleasant smell and sticky hand make people dislike this plant even though the flowers are diverse. The essential oil was extracted from the leaves of *L. camara* by hydrodistillation. This study aimed to see how cytotoxic *L. camara* essential oil was against HeLa carcinoma cells. This research aimed to discover if *L. camara* essential oil was cytotoxic to HeLa cancer cells. The GC-MS investigation of an essential oil recognized ten compounds; two main constituents of the oil were Caryophyllene (27.65%) and Germacrene D (23.01%). The essential oil showed cytotoxicity on HeLa cervical cancer cell lines. The cytotoxic effect of oil was determined using MTT, IC_{50} values were 44.86 µg/mL ± 0.07.

Key word: Cytotoxicity, HeLa, Hydrodistillation, *Lantana camara*, Cervical cancer.

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INTRODUCTION

Lantana camara Linn. (Verbenaceae) is a tropical American native that has been used as an ornamental and hedge plant in many other nations. As an invasive noxious weed, this plant has spread swiftly and been naturalized in more than 60 countries. Because native plant species' regeneration processes are interrupted, *L. camara* usually creates dense monospecies stands.[1] The plant *L. camara* possesses various medicinal values and has different ethnomedicinal uses. Antioxidants, antiprotozoal, antimicrobials, and insecticides have been discovered in *L. camara* leaves and flower extracts.[2,3] The plant essential oil shows effectiveness towards various microbial strains indicating its antimicrobial properties.[4,5]

Essential oils extracted from plants play an essential role in medicine, in addition to their tremendous value in the cosmetics and nutraceutical industries.[6] The use of essential oils of aromatic plants for food and medical purposes has been a focus of research in health sciences due to their varied biological features such as antibacterial, analgesic, anti-inflammatory, antiparasitic, antioxidant, and antitumoral activity.[7] Many aromatic therapeutic plant species have been the subject of studies examining the impact of time on the chemical makeup of essential oils. This occurs because the time it takes to collect the materials is an important factor to consider when dealing with essential oil production.[8]

Cervical cancer is a primary cancer of the cervix whose mechanism begins with epithelial metaplasia in the transformation zone, namely the meeting of the vaginal mucosa with the cervical canal. This cancer, which affects women between the ages of 35 and 55, is the world's second most common type of cancer in women.[9] The main cause of cervical precancer and cancer is persistent infection with one or more of the high-risk oncogenic types of human papillomavirus (HPV). HPV causes various modifications in the epithelial cells of the cervix's transformation zone, disrupting their normal functioning.[10] Cervical cancer prevention and the impact of screening programs on cervical cancer-related mortality have garnered major attention in rich countries, in contrast to the modest effort found in most low- and middle-income countries.[11] *Garcinia cowa*, for example, has been studied for its anticancer activity on the HeLa cell line.[12] This species show cytotoxicity activities on several cancer cell lines.[13,14-18]

The purpose of this study was to learn more about the chemical makeup of *L. camara* essential oils obtained from Padang using GC–MS and to assess theirs in vitro cytotoxicity activities on HeLa cancer cells.

MATERIALS AND METHODS

Extraction of oils

L. Camara leaves were taken near the Universitas Andalas campus in Limau Manis, Padang. Dr. Nurainas identified the voucher specimens in the Herbarium Universitas Andalas, Indonesia by Number 472/K-ID/ANDA/XII/2017. The leaves of the plants that have been collected are then cleaned and cut into pieces. These pieces are then dried until dry leaves are obtained. The essential oil is isolated by distillation of water. A sample of ± 1 kg of leaves is put into a distillation flask as a container for the sample in a series of water distillation devices. Then the appliance is heated for 4 hours, starting from the first drop. The essential oil obtained after distillation is separated from the water layer using a separating funnel and collected into a brown glass bottle covered with aluminum foil. The essential oil that has been separated from water is drained from the water, which is still present in the essential oil using anhydrous sodium sulfate. The purpose of this study was to learn more about the chemical makeup of *L. camara* essential oils obtained from Padang using GC–MS and to assess theirs in vitro cytotoxicity activities on HeLa cancer cells.

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Determination of the chemical contents of essential oil

Chemical compounds in the essential oil of leaves sample were analyzed using a GC-MS tool with the following conditions: Instrument: GC Agilent® 7890A, Detector: MS Agilent® 5975C, Column: HP-5ms (Agilent®), diameter 0.25 mm, thick 0.25 µm, length 30 m. Flow rate of gas: 1 ml / min. Temperature in the column: 50-300 ^{0}C (temperature constant 50^{0}C for 2 minutes, the temperature is increased to 80^{o}C in 2^{o}C/ minute increments, then increased to 150^{o}C in 5^{o}C/min increments, then increased to 200^{o}C in 10^{o}C/minute increments and then increased to 300^{o}C with increase of 20^{o}C/min, at 300^{o}C maintained constant for 5 minutes), Injector temperature: 250 ^{o}C (constant), Detector temperature: 270 ^{o}C, energy 1.25 kV. Carrier gas: Helium Injection volume: 0.1 µl Column pressure: 70 kPa.

DETERMINATION OF CYTOTOXICITY

Cell Culture

In RPMI media having 100 g/mL streptomycin, 100 IU/mL penicillin and 10% v/v FBS, under humidified 5 percent CO_2 incubator, cells were cultivated (37^{0}C), HeLa cells were grown. The cells were extracted for 5-10 minutes with a 1 mL trypsin-EDTA solution after they had reached 80 percent confluency. Under an inverted microscope, the cells were examined, and 3 mL of complete growth media was added to the flask. In a new flask with fresh media, 0.5-1x 10^6 cells were subcultured.

Viability Assay

The vitality of the cells was determined using the MTT test. HeLa cells (2x10^3 cells/well) were seeded into 96-well culture plates and incubated at 37°C overnight in 5% CO_2. The cells were given an essential oil treatment for 48 hours. The wells were then filled with 100 mL MTT (0.5 mg/mL) and incubated at 37°C for four hours. MTT was taken off the plate. Each well received 100 mL of DMSO. The Microplate Reader was used to measure the absorbance at 550 nm.

RESULTS AND DISCUSSION

Essential oils have a wide range of chemical compositions based on the type of plant, climate, growing region, season, type of soil, extraction method, and plant part used. Therefore, in this research, the leaves was subjected to hydrodistillation to yield 0.0781 % (w/w). The ten primary ingredients of the essential oil discovered by GC–MS are listed in Table 1. Caryophyllene (27.65%) and Germacrene D (23.01%) had the highest mean content values, followed by Cyclodecadiene (23.01%).

Caryophyllene is a bicyclic sesquiterpene that is found in plants. This chemical has potent anticancer properties, inhibiting the growth and multiplication of a variety of cancer cells. Caryophyllene enhances the efficacy of traditional drugs by increasing their concentrations inside cells. Caryophyllene is a phytocannabinoid that binds to cannabinoid receptors with a high affinity. Caryophyllene has been demonstrated

Table 1: Lists the chemicals found in L. Camara essential oil.

Chemicals	Amount (%)	Retention Time (Min)
α-Pinene	04.04	3.32
o-Cymene	05.38	4.93
Cyclohexane	0.270138889	19.84
Caryophyllene	27.65	21.22
Humulene	0.277083333	22.59
Germacrene D	23.01	23.96
Naphthalene	0.264583333	24.45
Hexahydronaphthalene	0.223611111	25.69
Cyclodecadiene	11.26	27.04
Patchouli alcohol	06.15	30.56

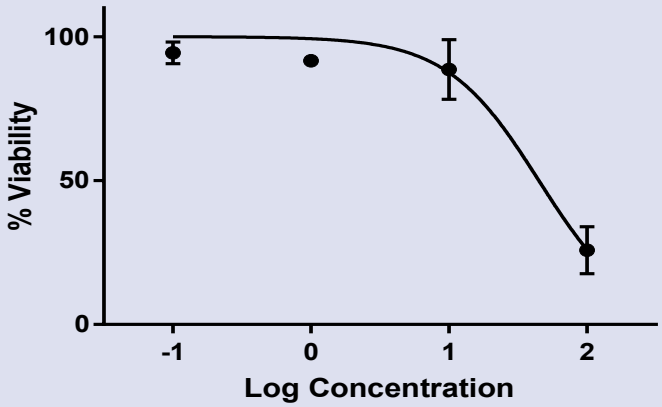

Figure 1: Essential oil of *L. camara* inhibits the viability of HeLa cancer cells. The averages ± standard deviations of three independent experiments make up the data.

to affect several important cancer pathways, including the mitogen-activated protein kinase pathway. (MAPK), PI3K/AKT/mTOR/S6KI, and STAT3 pathways.[19]

A brine shrimp was used to test the essential oil, which contains germacrene D, as found in Mugwort (*Artemisia vulgaris* L.) plants. Germacrene D was discovered to be the first major ingredient in the oils studied.[20]

To examine the effect of *L. camara* essential oil as an anticancer agent, HeLa cells were treated with essential oil (0-100 g/mL) for 48 hours and cell viability was determined using the MTT test. The IC_{50} is derived using a plot of cell viability (percentage) vs essential oil concentration. The treatments were tested at varied concentrations in the cell viability assay. HeLa cancer cells were decreased in a dose-dependent manner by essential oil with IC_{50}. The IC_{50} value was 44.86 g/mL ± 0.07 after 48 hours of incubation (Figure 1).

CONCLUSION

This study concludes that essential oils of *L. camara* contain the main chemical are Caryophyllene and Germacrene D and can be proposed as drug candidate as anticancer agent.

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CONTRIBUTIONS FROM THE AUTHOR

All authors contributed significantly to the conceptualization and design, data collection, data analysis, and data interpretation; participated in the drafting of the article or critically revised it for important intellectual content; agreed to submit the article to the current journal; gave final approval of the version to be published; and agreed to be responsible for all aspects of the work.

INTEREST CONFLICTS

There are no financial or other conflicts of interest among the authors of this study.

ETHICAL APPROVALS

There are no animal or human subjects in this study.

NOTE FROM THE EDITOR

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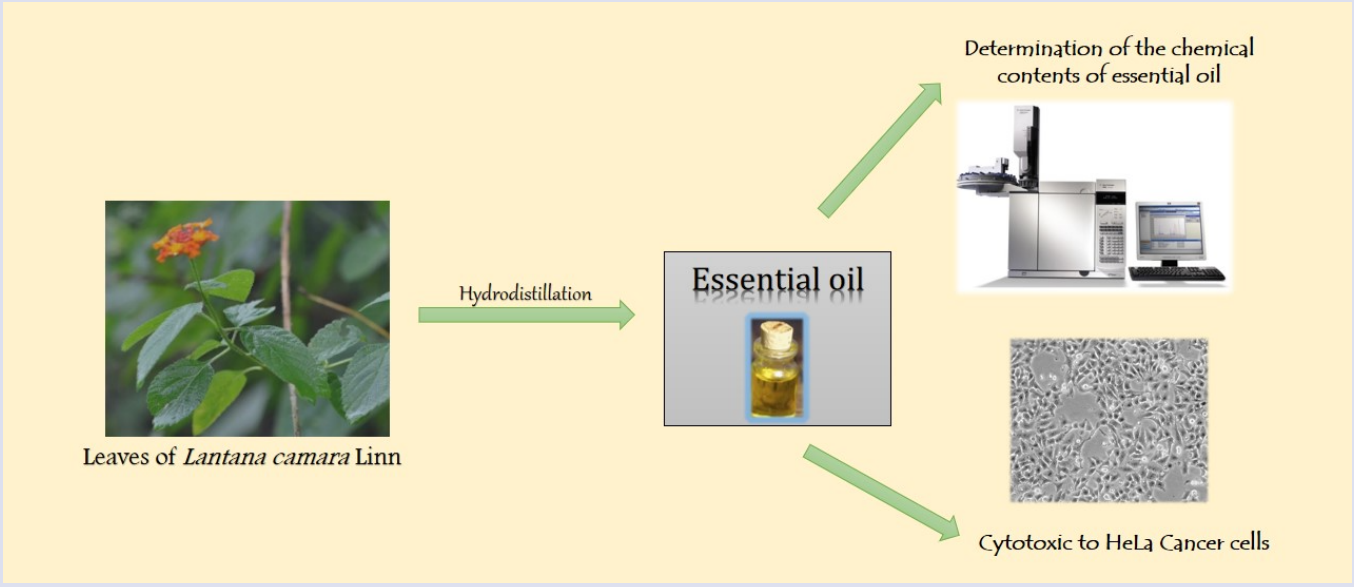
GRAPHICAL ABSTRACT



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