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ORIGINAL ARTICLE

Phytochemical characterization and assessments of antimicrobial, cytotoxic and anti-inflammatory properties of *Lavandula coronopifolia* Poir. volatile oil from Palestine

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Abstract Since the dawn of time, people have relied on herbal medicine to heal many ailments. Lavandula coronopifolia (LC) is an aromatic plant that has been utilized in ethnomedicine since ancient times. This investigation aimed to figure out the chemical constituents of the volatile oil (VO) of the LC herb from Palestine for the first time and determine its microbicidal, anti-inflammatory, and cytotoxic effects. The LC aerial parts VO phytochemical constituent characterizations were performed utilizing gas chromatography linked to a mass spectrometric system. While, the effects of LC-VO's ability to suppress microbial growth were established against selected bacterial and fungal strains by a broth microdilution technique. Besides, an in vitro cyclooxygenase enzyme (COX) suppressant bioassay kit was utilized to estimate the anti-inflammatory effect against bovine cyclooxygenase (COX) type 1 and 2. In addition, the colorimetric MTS test was employed to determine the cytotoxic activity against the cervical adenocarcinoma (HeLa) tumor cell line. Linalyl acetate (41.65 ± 0.91%) and linalool (41.41 ± 0.77%) were identified as major components of LC-VO, and oxygenated monoterpenoid was the dominant phytochemical class of this VO. The LC-VO demonstrated potent bactericidal and fungicidal effects, especially on methicillin-

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resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Proteus vulgaris, and Candida albicans (MIC = 0.33 ± 0.03, 0.63 ± 0.1, 1.25 ± 0.81, and 0.16 ± 0.06 μg/mL), respectively. Besides, LC-VO suppressed COX enzymes type-1 and −2, with an inhibition percentage at 50 μg/mL of 95.00 and 88.02 percent, respectively, in comparison with the positive control, ketoprofen non-steroidal anti-inflammatory drugs (NSAID), at the same concentration with an inhibition percentage of 96.25 and 65.02%, respectively. In addition, the LC-VO inhibited the growth of HeLa cells dose-dependently, with an IC_{50} dose of 0.71 ± 0.01 μg/mL. The current study demonstrates that LC-VO is a promising alternative for the healing or preventing various infectious diseases, cancer, and chronic inflammation. These results reveal that LC-VO has beneficial health benefits and has the potential to be utilized for therapeutic purposes.

1. Introduction

Ancient cultures and civilizations used a wide variety of plant species as medicine, nutraceutical ingredients, flavoring, food preservatives, and cosmetic ingredients. Nowadays, the use of herbal medicines in primary health care is in increasing demand in the developed and developing worlds due to their fewer side effects, safety, and efficacy than synthetic pharmaceuticals (Tilburt and Kaptchuk, 2008, Yuan et al., 2016). Plants' secondary metabolic chemicals have a wide range of biological actions, and many of the pharmacological formulations currently in use have been derived from plants (Wink, 2015).

Volatile oils (VOs) are mixtures of aromatic, volatile, secondary metabolic compounds derived primarily from plants. They possess a variety of pharmacological actions, including anthelmintic, anti-inflammatory, digestive enzyme inhibitory, anticancer, antimicrobial, and antioxidant capabilities (Dhifi et al., 2016, Hong et al., 2021, Hong et al., 2022). However, due to their economic features, VOs are used commercially in the production of cosmetics, perfumes, pharmaceuticals, soaps, household products, beverages, food flavoring agents, hygienic products, and other industrial products (Sarkic and Stappen, 2018).

Inflammatory conditions are among the most prevalent sources of pain associated with tissue damage. The aims of drugs that are utilized to treat or reduce inflammation are usually pointed toward the normalization of pain sensitivity. Actually, available medications that are currently being used to solve this critical problem usually have severe negative consequences and poor pharmacological potency. Non-steroidal anti-inflammatory drugs (NSAIDs) are considered effective tools for the treatment of different diseases associated with inflammation, and compared with steroids; they have fewer adverse and side effects (Bellomo et al., 2017).

NSAIDs work by inhibiting the formation of prostaglandins from arachidonic acid by binding to the cyclooxygenase (COX) enzymes. The COX enzyme exists in two isoforms: COX-1 and COX-2. COX-2 selective inhibitors and NSAIDs have recently been utilized to treat various types of conditions caused by inflammation (Zarghi and Arfaei, 2011). In fact, much evidence has revealed that recently available drugs with COX-2 inhibitory activity are linked to an increased risk of stroke and myocardial infarction. Recently, the reputation of NSAIDs and selective COX-2 inhibitors has decreased due to their harmful side effects on the cardiovascular and gastrointestinal systems, which has led to the search for more potent drugs that can suppress inflammation with fewer side effects than NSAIDs (Arora et al., 2020). Therefore, plant products, such as VOs, are considered excellent sources of anti-inflammatory compounds for the next generation of pharmaceuticals (Parsa, 2012, An et al., 2013).

The World Health Organization (WHO) reported that cancer is one of the most common and costly diseases in the world, even in wealthy countries. Cancer is no longer considered an incurable illness thanks to early identification techniques and medication advances. Ovarian cancer is one of the most often diagnosed tumors in females and one of the top causes of cancer-related mortality (Zhao et al., 2017). Recent studies indicate that supplementary drugs derived from herbal substances can significantly enhance treatment outcomes for many cancer patients with few side effects (Yin et al., 2013). Besides, many kinds of VOs have been shown to have antiproliferative and cytotoxic properties (Gautam et al., 2014). In fact, the discovery of anticancer drugs based on naturally occurring molecules, such as silibinin, vinblastine, paclitaxel, and vincristine, has been facilitated by studying natural products. It is crucial to continue searching for novel cytotoxic agents derived from phytochemicals to investigate potent anticancer medications.

The discovery of antibiotics has prevented the deaths of many people worldwide by treating various infectious diseases and fundamentally improving life expectancy. Unfortunately, the irrational prescription and frequent use of these medications have resulted in the creation of multiple types of microbial resistance, making infectious diseases difficult to cure. Recently, antibiotics have often been used in combination therapy to increase their effectiveness with a greater spectrum of activity and to cope with infections resulting from multidrug-resistant bacteria. The development and discovery of effective drugs are increasingly needed to resolve this alarming problem, which constitutes a major public health issue. Furthermore, natural products like botanical VOs include many phytochemicals that might be used as safe antimicrobial alternatives to combat microbial resistance (Ventola, 2015).

The genus *Lavandula* (Lamiaceae) has 39 species. The majority of them are found in southeastern India, southwestern Asia, North Africa, the Mediterranean basin, and Europe (Messaoud et al., 2012). The species *Lavandula coronopifolia* Poir (Lamiaceae) is an aromatic woody perennial herb with a pleasant odor due to the presence of VO (Abdelaziz et al., 2020). In addition, it contains phenolic compounds such as protocatechuic, rosmaric acid, chlorogenic acid, quercetin, pinocembrin, and luteolin (Messaoud et al., 2012, Ait Said et al., 2015, Farshori et al., 2015, Abdelaziz et al., 2020). Previous investigations have demonstrated that LC-VO has the potential to fight antibiotic-resistant bacteria, and its extract has hepatoprotective potential against ethanol-induced oxidative stress-mediated cytotoxicity in HepG2 cells (Ait Said et al., 2015, Farshori et al., 2015).

Therefore, the current research aims to identify and quantify the chemical components of the LC aerial parts VO from Palestine for the first time, as well as to assess its microbicidal, cytotoxic, and COX suppressive effects.

2. Material and methods

2.1. Plant collection and drying

From the lowest point on the earth, Jericho-Palestine, the LC aerial parts (leaves, flowers, and stems) were collected in July 2019. The botanical recognition of LC herb was performed

in the Laboratory of Natural Products by a specialist Dr. Nidal Jaradat. The sample was deposited in the same laboratory with a voucher specimen code of pharm-PCT-1367.

The collected aerial parts were deeply washed and dried for nine days in the shade at a humidity level of 50 ± 3% RH) and stable room temperature (24 ± 4 °C). After drying, the dried herbal material was ground roughly and stored in special containers for further use.

2.2. Extraction procedure

The VO of the LC herb was obtained by the water-distillation system described by (Jaradat 2016). In brief, about 100 g of the herb was mixed well with 1 L of distilled water, and the VO was extracted using a Clevenger apparatus operating at atmospheric pressure for 240 min at 100 °C. The extracted VO was chemically dried using magnesium sulfate and then kept in a refrigerator at 4–6 °C until further use.

2.3. Characterization of VO

The volatile compounds of the LC herb were recognized and quantified using gas chromatography connected with mass spectrometry (GC–MS), as described by (Jaradat et al., 2022). Briefly, the analysis was done by an HP 5890 series II gas chromatograph linked to a mass spectrometer, Perkin Elmer Elite-5-MS. However, the components were identified based on the comparison of their relative retention index and compared with the library mass spectral database (Wiley and NIST databases). The percentage composition of compounds was measured based on the peak area.

2.4. Bactericidal and fungicidal activity

Proteus vulgaris (ATCC 8427), *Klebsiella pneumonia* (ATCC 13883), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), and clinically diagnosed methicillin-resistant *Staphylococcus aureus* (MRSA) were the screened bacterial species. Moreover, *Candida albicans* (ATCC 90028) was the fungi screened strain in this study. The bactericidal and fungicidal activity of LC-VO were carried out by the broth microdilution method as described by (Balouiri et al., 2016). This procedure was conducted in triplicate. In addition, bactericidal and fungicidal activity was validated utilizing known antibiotics, namely ciprofloxacin, ampicillin, and fluconazole.

2.5. COX inhibitory assay

Utilizing a COX inhibitor screening test kit, the capacity of the LC-VO to prevent the conversion of arachidonic acid to PGH2 by bovine COX-1 and human recombinant COX-2 was assessed according to the manufacturer's instructions (Item No: 560131, Cayman Chemical, USA). The IC_{50} values (50 percent inhibitory concentration) of VO COX-1/COX-2 potentials were calculated using two concentrations (50 and 350 µg/mL). To assess the inhibition of the LC-VO, a standard curve of eight concentrations of prostaglandin, a non-specific binding sample, and a maximal binding sample were utilized, as indicated in the manual of the used kit, and the produced multiple regression best-fit line was employed. The percentage inhibition of the two concentrations was utilized to derive the IC_{50} values (Jaradat et al., 2020).

2.6. Cytotoxicity procedure

In (RPMI-1640) medium was mixed with a mixture of 10% fetal bovine serum, 1% Penicillin/Streptomycin antibiotics to avoid contamination, and 1 % l-glutamine, the HeLa (cervical adenocarcinoma) tumor cells were cultured in 96-well plates (2.6×10^4 cells) and grown in a humidified atmosphere containing 5% CO_2 at 37 °C. After two days, the cells were incubated with VO at various concentrations (1, 0.5, 0.25, 0.13 and 0.063 µg/mL) for 24 h. Cell viability was estimated by CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay following the directions of the producer (Promega Corporation, Madison, WI). At the end of the treatment, 20 µL of MTS solution per 100 µL of media was added to each well and incubated at 37 °C for two h. Finally, the absorbance was measured by spectrophotometer apparatus at 490 nm (Mosmann, 1983).

2.7. Statistical analysis

All conducted experimental works in this investigation were carried out in triplicate for the LC-VO. The results were expressed as means (±) standard deviation (SD). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Phytochemical composition

The hydro-distillation of the ground air-dried aerial parts of LC afforded a colorless aromatic smelling oil in 1.03% v/w. The VO components were identified by GC–MS analysis based on comparing the obtained Kovats retention index (RI) and MS fragmentation patterns to those of standard compounds and on matching with the NIST library. The identified components, their retention indices, and their percentages from the total oil are presented in Table 1.

3.2. Antimicrobial effect

The Microdilution broth technique was employed to estimate the microbicidal effect of LC-VO. The result showed that most of the microbial growth is inhibited by LC-VO, as illustrated in Table 2. Compared with pharmaceutical antifungal and antibacterial agents, including fluconazole, ampicillin, and ciprofloxacin. This test outcome showed that the LC-VO has a strong microbicidal effect against most of the tested strains, especially against *C. albicans, P. vulgaris*, *S. aureus*, and MRSA.

3.3. COX inhibitory activity

In the current investigation, the ability of the LC-VO to inhibit the COX-1 and COX-2 enzymes was established by employing a cyclooxygenase inhibitory screening assay kit, and the outcomes were compared with a commercial NSAID ketoprofen.

Table 1 Phyto-molecules included in the volatile oil of Lavandula coronopifolia.

Compounds	Retention time	Retention index	Area	% (Area), ±SD
α-Pinene	8.73	933	52,216	0.03 ± 0.002
Camphene	9.38	949	11,355	0.01 ± 0.001
β-Pinene	10.53	977	23,800	0.02 ± 0.002
Amyl ethyl ketone	10.81	984	179,229	0.12 ± 0.009
Actan-3-ol	10.98	988	545,446	0.35 ± 0.01
Myrcene	11.11	991	138,567	0.09 ± 0.001
Yomogi alcohol	11.52	1001	116,280	0.07 ± 0.006
Hexyl acetate	12.12	1016	527,658	0.34 ± 0.01
p-Cymene	12.51	1025	59,211	0.04 ± 0.001
Limonene	12.71	1030	1,959,132	1.26 ± 0.02
1,8-Cineole	12.85	1034	2,366,303	1.52 ± 0.04
cis-Ocimene	13.05	1039	69,235	0.04 ± 0.002
trans-Ocimene	13.48	1049	55,265	0.04 ± 0.001
cis-Linalool oxide	14.48	1073	45,821	0.03 ± 0.001
trans-Linalool oxide	15.26	1092	1,393,820	0.90 ± 0.02
Linalool	15.7	1103	64,386,684	41.41 ± 0.77
Camphor	17.45	1149	2,958,918	1.90 ± 0.02
Borneol	18.44	1174	422,834	0.27 ± 0.01
Terpinen-4-ol	18.75	1183	1,024,786	0.66 ± 0.03
α-Terpineol	19.32	1197	7,150,691	4.60 ± 0.31
Linalyl formate	20.4	1227	159,455	0.10 ± 0.01
Linalyl acetate	21.33	1253	64,772,228	41.65 ± 0.91
Geranial	21.99	1272	112,804	0.07 ± 0.001
Lavandulyl acetate	22.49	1286	490,911	0.32 ± 0.01
Neryl acetate	25.06	1361	303,231	0.20 ± 0.03
Geranyl acetate	25.71	1381	614,458	0.40 ± 0.02
β-Caryophellene	27.06	1422	1,648,998	1.06 ± 0.01
α-Caryopyllene	28.2	1458	57,957	0.04 ± 0.001
Caryophellene oxide	32.11	1588	3,509,676	2.26 ± 0.02
Total identified compounds			155,500,932	99.80
Phytochemical groups				
Monoterpene hydrocarbons				1.45
Oxygenated monoterpenoid				93.06
Sesquiterpene hydrocarbons				1.9
Oxygenated sesquiterpenoids				2.96
Miscellaneous				0.43
Total identified groups				99.80

Table 2 MIC values (μg/mL) of Lavandula coronopifolia volatile oil (LC-VO) and positive control antibiotics.

Tested samples	Microbial strains						
	MRSA	S. aureus	E. coli	K. pneumonia	P. vulgaris	P. aeruginosa	C. albicans
Fluconazole	–	–	–	–	–	–	1.56 ± 0.1
Ampicillin	R	3.12 ± 0.02	3.12 ± 0.02	1.1 ± 0.01	18 ± 0.12	R	–
Ciprofloxacin	12.5 ± 0.91	0.78 ± 0.01	1.56	0.13 ± 0.01	15 ± 0.11	3.12 ± 0.12	–
LC-VO	0.33 ± 0.03	0.63 ± 0.1	3.12 ± 0.06	2.5 ± 0.05	1.25 ± 0.81	6.25 ± 0.12	0.16 ± 0.06

Fig. 1 presents the inhibition percentage of both LC-VO and positive control ketoprofen at two different concentrations toward COX-1 and COX-2. It was clear that LC-VO has a potent inhibition percentage at both used concentrations. However, it was more potent against COX-2 *iso*-enzyme than the positive control, with values of inhibition of 88.02 and 65.02%, respectively.

3.4. Cytotoxic activity

The result of the cytotoxic effect of LC-VO on HeLa (cervical adenocarcinoma) was evaluated using the MTS assay. The treatment of HeLa cells with 1 μg/mL of LC-VO induced the best cytotoxic effect (95.14%) with an IC_{50} value of 0.71 ± 0.01 μg/mL. In addition, the growth inhibition according to each used dose was presented in Fig. 2 compared with positive control Dox (Doxorubicin).

4. Discussion

For many years, various VO-bearing herbs have been utilized for the treatment of a broad variety of pathological disorders. Several *Lavandula* genus plants are mainly dominated by linalool and linalool acetate (linalyl acetate), which are classified as

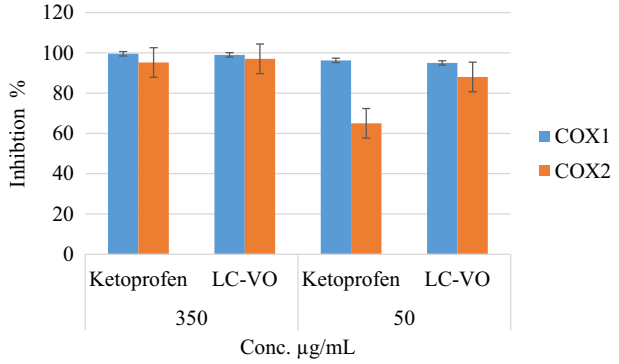

Fig. 1 Inhibition percentages of COX-1 and COX-2 by LC-VO and positive control ketoprofen (p values < 0.005).

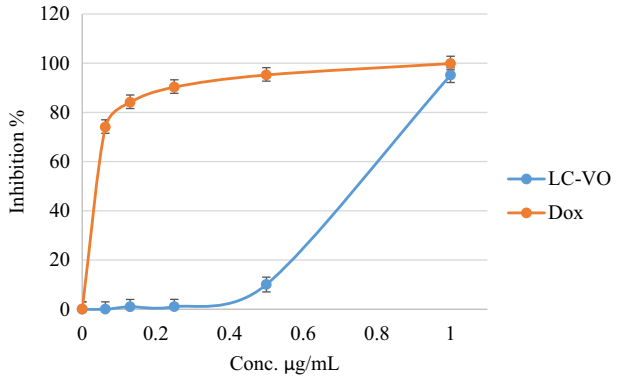

Fig. 2 Cytotoxic effects of the LC-VO in comparison with the positive control (Dox) against HeLa cancer cell line.

oxygenated monoterpenoid molecules. It was reported that linalool and linalool acetate are the abundant molecules of many aromatic species containing VOs, such as *Ocimum sanctum* L. (Lamiaceae), *Cymbopogon citratus* (DC.) Stapf (Poaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Melissa officinalis* L. (Lamiaceae), *Lavandula angustifolia* Mill. (Lamiaceae), and *Jasminum subtriplinerve* Blume (Oleaceae) (Souto-Maior et al., 2017).

Various plant species that produce linalool acetate and linalool are widely used in herbal folk medicine to treat and relieve a wide range of chronic and acute illnesses. Their therapeutic effects are attributed to the presence of alcohol (linalool) and its parallel ester compound (linalool acetate) (Peana et al., 2002).

4.1. Phytochemical components

GC–MS analysis recognized 29 components accounting for around 99.8% of the total VO content. The most LC-VO content was contributed by oxygenated monoterpenes (93.06%), of which linalyl acetate (41.65 ± 0.91%), linalool (41.41 ± 0.77%), and α-terpineol (4.60 ± 0.31%) were the main compositions of the LC-VO. Only 1.45% of total VO was made up of monoterpene hydrocarbons, with limonene (1.26 ± 0.02%) being the most abundant. The total amount of sesquiterpenes in the oil accounted for only 1.9% of the total amount of oil. A comparison of the reported volatile components extracted from LC by hydro-distillation revealed differences in the occurrence of major components, which could be attributed to environmental conditions, soil characteristics, harvest time, and drying methods (Moghaddam and Mehdizadeh, 2017).

Our outcomes are in relative agreement with those of Aburjai et al., who documented that oxygenated terpenes (80.60–85.59%) were the major constituents of VO of LC herb grown in Jordan, where linalool, camphor, terpinen-4-ol, 1,8-cineole, and borneol were found to be the major constituents. They discovered that linalool (41.2%) was the most abundant constituent in flowering tops and leaves, while 1,8-cineole (25.4%) was the most abundant constituent in the entire aerial parts oil. Most of the VOs components showed highly significant qualitative and quantitative differences for different geographical regions (Aburjai et al., 2005).

Messaoud et al. identified 29 compounds in LC-VO grown in Tunisia, with monoterpene hydrocarbons (46.2%) being the most abundant fraction, followed by oxygenated monoterpenes (27.6%). The abundant molecules were *trans*-β-ocimene (26.9%), carvacrol (18.5%), β-bisabolene (13.1%), myrcene (7.5%), and α-terpinolene (5.4%) (Messaoud et al., 2012).

4.2. Antimicrobial activity

Due to the presence of VOs, medicinal herbs can impede the growth of various harmful microbes. However, in the field of infectious diseases caused by microbial resistance, no one can deny the role of VOs in discovering new potential microbicidal therapeutics. 2-Isopropyl-5-methyl phenol, linalool, eugenic acid, menthols, and caryophyllene are among the significant compounds used so far to develop potent antimicrobial agents (Akthar et al., 2014, Osuntokun and Ogunleye, 2017).

However, the development of resistance to antibiotics against MRSA limits their effectiveness, which will only worsen in the future. Therefore, there is a lot of work to be done because there is an urgent need to discover novel alternative pharmaceuticals (Guo et al., 2020).

The obtained data from the microbicidal test revealed variability in the MIC of LC-VO. In fact, the antibacterial lowest MIC value was noticed against MRSA (0.33 ± 0.03 µg/mL) followed by S. *aureus* (0.63 ± 0.1 µg/mL), and both of them are gram-positive bacterial strains. The LC-VO exhibited a more potent effect than the commonly used positive antibiotic controls against these two virulent species. Furthermore, LC-VO inhibited the growth of *P. vulgaris* (Gramm-negative bacteria) significantly more than Ampicillin and Ciprofloxacin, with MICs of 1.25 ± 0.81, 18 ± 0.12, and 15 ± 0.11 µg/mL, respectively. Compared with the potent fluconazole antifungal pharmaceutical, the LC-VO showed remarkable fungicidal effects with MICs of 0.16 ± 0.06 and 1.56 ± 0.1 µg/mL, respectively. Indeed, linalool and linalool acetate-producing species have potent antimicrobial activity against both bacterial and fungal species.

Actually, linalool and linalool acetate are by far the most abundant acyclic monoterpenoid, accounting for approximately 70% of the isoprenoid content found in the scents of flowers. They were recognized in the VOs of 200 species of plants that belong to various families (Carson and Riley, 1995, Pattnaik et al., 1997).

The results of the Khayyat study demonstrated that linalool acetate strongly inhibited the growth of *Microsprum cani-*

s and *Microsprum gallinna* grown on solid media. Furthermore, it showed that all linalyl acetate and its derivatives effectively reduced the growth of tested fungi (Khayyat, 2020).

Besides, Nelson reported that L. angustifolia, which was rich in linalool, has potential activity against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium (Nelson, 1997).

In addition, our microbicidal study results agree with a study by Bogdan et al. in which they found that *L. angustifolia* VO rich in linalool and linalool acetate manifested remarkable antibacterial potential against *S. aureus* (12.5 v/v) and *E. coli* (25 v/v) and also has a potent fungicidal effect against *C. albicans* (12.5 v/v) (Bogdan et al., 2021).

4.3. Anti-inflammatory activity

Inflammation is a critical physiological reaction to activation of cellular damage, infectious diseases, or insufficient immune system. Excessive production of proinflammatory mediators can result in severe and chronic inflammation, resulting in various conditions, including rheumatoid arthritis, rheumatoid arthritis, asthma, multiple sclerosis, and atherosclerosis (Nathan, 2002).

The present investigation outcomes revealed that the LC-VO has inhibitory actions on COX-1 and COX-2. Indeed, the LC-VO COX inhibitory activity estimations demonstrated that this VO has a much more specific inhibitory effect on COX-2. Hence, it had higher COX-2 inhibitory activities at 50 μg/mL than ketoprofen.

The percentage of COX-2 inhibition increased as the concentration of VO used increased, rising from 88 to 97 percent when the VO concentration was increased from 50 to 350 μg/mL. Similarly, COX-1 inhibition increased as the VO concentration rose. For example, when the LC-VO concentration was increased from 50 to 350 μg/mL and the COX-1 inhibition rose from 95 percent to 99.55 percent.

An investigation conducted by Peana et al. indicated that linalool and linalool acetate play a vital role in the anti-inflammatory effect of the VOs containing these compounds in herbal species. Furthermore, their investigation proved that linalool and linalool acetate performing plant species are potent anti-inflammatory agents (Peana et al., 2002).

Moreover, an investigation established by Husseini et al. showed that L. officinalis hydroalcoholic extract inhibited the COX activity in a dose-dependent manner, where the inhibitory effects on COX type 1 and 2 activities were 33 and 45%, respectively (Husseini et al., 2016).

4.4. HeLa cytotoxic effect

Different kinds of cytotoxic examinations, such as colorimetric, dye exclusion, fluorometric, and luminometric assays, are utilized in toxicology and pharmacology (Gerets et al., 2009). The LC-VO has a moderate cytotoxic effect because in comparison with Doxorubicin. This potent anticancer commercial drug has cytotoxic activity against HeLa cancer cells with an IC_{50} dose of 0.17 ± 0.02 μg/mL. On the other hand, the LC-VO displayed approximately four folds' lower cytotoxicity against HeLa cancer cells with an IC_{50} dose of 0.71 ± 0.01 μg/mL.

L. angustifolia VO and its main components, linalool, and linalyl acetate, were found to have strong cytotoxic activity against DU145 and PC-3 cell lines, with IC_{50} values of (0.19 9 ± 0.026 percent and 0.037 ± 0.011 percent (v/v)), (7.22 ± 0.28 μM, 11.74 ± 0.62 μM), and (3.06 ± 0.22 μM and 4.98 ± 0.31 μM), respectively (Zhao et al., 2017). Furthermore, Al-Sheddi discovered that MDA-MB-231 cells' survival reduced in a concentration-dependent manner when exposed to ethanol, chloroform, ethyl acetate, and petroleum ether extracts of LC plant (Al-Sheddi 2019).

Finally, we would say that the LC screened plant was collected from the Jericho region, which is located near the north of the Dead Sea in Palestine. Jericho is considered the lowest area on earth and has a unique climatic zone (Juaidi et al., 2016). It is among the Palestinian areas that are popular for its variety of bioactive plant species used by the local population for diverse goals. As expected, the Jericho region has very different ecological and environmental conditions than other places (Qumsiyeh and Abusarhan, 2021). This is why the composition of the VOs in this study is very different from other studies. Moreover, to reach the cytotoxic dose on HeLa cancer cell lines, we need about ten folds of the effective concentration dose of bacterial strains (MIC) or COX enzymes inhibitory activities. The cytotoxic effects of plants or active agents against HeLa cancer cell lines could be used instead of normal cell lines to evaluate the cytotoxicity (Hawash et al., 2020).

5. Conclusion

The GC–MS fingerprinting results revealed that the LC-VO contains high amounts of linalool acetate and linalool. The VO's major phytochemical group was oxygenated monoterpenoids. The LC-VO study demonstrated potent bactericidal and fungicidal effects, especially against C. albicans, P. vulgaris, S. aureus, and MRSA strains. LC-VO also has a potential COX inhibitory effect compared with ketoprofen, and it is an active cytotoxic product on HeLa cancer cells compared with Doxorubicin. Further in vivo and preclinical therapeutic screenings are needed for approval or disproval of these effects for the possible use of LC-VO in the treatment and prevention of infectious diseases, chronic inflammations, and cancer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All authors have agreed to take responsibility for the entire content of this manuscript and have approved its submission.

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