

Analysing the Concentration, Dosage and Effect of Limolene Extract-From Eucalyptus Oil on Cancer -A Scientific Scoping Review

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Abstract:

➤ *Background:*

Limonene, a monocyclic monoterpene abundantly found in Eucalyptus oil and citrus fruits, has gained increasing attention for its potential anticancer properties. Limonene may be safe and well-tolerated while suppressing tumor development, inducing apoptosis, and modulating many molecular pathways, according to preclinical and clinical studies. It is a promising candidate for cancer prevention and treatment due to its natural origin, bioavailability, and selective accumulation in lipid-rich tissues. With an emphasis on its modes of action, safety profile, and therapeutic potential across a range of cancer models, the current review attempts to assess the concentration, dosage, and anticancer effects of limonene extract from eucalyptus oil.

➤ *Materials and Methods:*

A thorough search was conducted using databases such as PubMed, Scopus, and Google Scholar to gather pertinent studies on limonene's anticancer effects. In vitro, in vivo, and clinical investigations were analyzed to determine its bioactivity, tissue distribution, effective dosage ranges, and molecular interactions. The collected data was examined and presented to provide a full understanding of the role of limonene in cancer treatment.

➤ *Results:*

Limonene has shown considerable anticancer action in a dose-dependent manner by inducing apoptosis, autophagy, and arresting the G2/M cell cycle. It affects key pathways like caspase activation, Ras signaling, p53 regulation, and oxidative stress mitigation. Clinical investigations have shown its safety, with little gastrointestinal symptoms noted at therapeutic levels. Combination therapy with drugs like curcumin and andrographolide improves efficacy, whereas derivatives like perillyl alcohol improve potency and selectivity.

➤ *Conclusion:*

Limonene is a safe, bioactive, multifunctional chemical with powerful anticancer properties. However, variations in concentration, dose, and formulation indicate the need for more standardized clinical trials to optimize delivery methods and establish their therapeutic significance in cancer treatment.

Keywords: Limonene, Eucalyptus oil, Anticancer, Apoptosis, Autophagy, Bioavailability.

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I. INTRODUCTION

Cancer has become one of the most concerning diseases globally over the past few decades. The significant increase in cancer cases can be linked to changes in dietary habits, tobacco and alcohol consumption, persistent infections, exposure to harmful radiation and chemicals, or more generally to shifts in lifestyle and environment. It is a complex disease that causes unchecked growth and invasion of abnormal cells, resulting in tumor formation. GLOBOCAN in the year 2020, the estimated lifetime risk of cancer among 185 countries from the time of birth to death was 25.10%; for men, the risk was 26.27%, and for women, 23.96%. [1] In terms of mortality, ischemic heart disease is the top cause of death globally, accounting for 8.97 million deaths; however, cancer is anticipated to overtake it in 2060 with around 18.63 million deaths. [2]. The treatment methods for cancer often include radiotherapy, chemotherapy, and surgical excision. These drugs are designed to target rapidly growing and dividing cells in various tumors. However, these synthetic compounds also impact normal cells in our body that divide rapidly, resulting in some significant irreversible side effects. Every treatment approach has drawbacks and related issues, which may lower the quality of life by increasing mortality and morbidity.

This highlights the need to explore alternative medicines, as well as traditional medicine, as potential sources for identifying new chemical compounds for cancer therapy. In contrast to conventional methods, products derived from plants are anticipated to cause fewer side effects compared to synthetic medications. The efficacy and safety of natural remedies for the prevention or treatment of cancer are currently under evaluation in various clinical trials. A compound that occurs naturally in the essential oils of citrus fruits and eucalyptus, d-limonene (1-methyl-4-isopropyl-cyclohexene), has been shown to possess chemopreventive and anticancer properties. Various in vitro studies have demonstrated that d-limonene can trigger apoptosis in tumor cells and exhibits cytotoxic effects on cancerous cells. Additionally, studies on animals indicate that d-limonene may offer therapeutic benefits for cancers of the breast, liver, kidney, and skin [3]. Among its sources, Eucalyptus essential oils are recognized as significant sources of bioactive limonene, which contribute to their diverse therapeutic properties. The analysis revealed that EGEO comprises several monoterpenes, with 1,8-cineole (63.1%), p-cimene (7.7%), α -pinene (7.3%), and α -limonene (6.9%) being the predominant constituents [4].

Many cancer drugs have smaller difference between the effective and toxic doses. Limonene is a promising option for treating cancer because of its apoptotic, anti-inflammatory, and tumour-inhibitory properties. [5] however, its therapeutic efficacy and safety largely depend on the concentration, dosage, and mode of administration, according to certain

rodent research, male rats developed kidney cancers after consuming extremely high levels of limonene [6]. This seeks the need to examine the concentration, dosage, and overall effect of limonene on cancer to guarantee its efficacy and safety. It is essential to evaluate toxicity profile of limonene to find the possible hazards such liver toxicity or metabolic imbalances, or whether excessive or insufficient doses can result in ineffectiveness, medication resistance, or negative effects. It is essential to comprehend limonene's toxicity profile to evaluate its long-term safety and possible combinations with other cancer treatments. Additionally, different cancer kinds may react differently to limonene, which calls for research into which cancer types are most vulnerable to its effects and whether it may be customized for individualized cancer treatment. While studies have demonstrated the anticancer effects of limonene but, a comprehensive analysis of its optimal concentration, dosage and mechanism of action remains underexplored. This review aims to analyze the existing literature on the concentration, dosage, and effects of limonene extract from Eucalyptus oil on cancer in order to determine the optimal dosage of limonene required to achieve a significant therapeutic effect while minimizing toxicity and unwanted side effects.

II. MATERIALS AND METHODS

➤ Information Sources

This study is a scoping review designed to systematically assess the existing literature on the concentration, dosage, and effects of limonene extract in cancer treatment.

The following electronic databases were searched from the time of their conception until 2024, in compliance with PRISMA guidelines: PubMed, Research gate, and SpringerLink.

➤ Search Strategy

The keywords used include “limonene” AND “cancer”, “limonene” OR “D-limonene”, “concentration” OR “dosage” OR “effect” AND “limonene”, “limonene” AND “anticancer” OR “antitumour”, “cancer prevention” AND “limonene”.

Boolean operators (AND, OR) were applied to refine and expand the search results as needed.

➤ Inclusion Criteria

We included research conducted on cancer diagnosed patients given limonene orally, to determine induced changes in systemic and tissue biomarkers, clinical trials on assessing toxicity, the maximum tolerated dose (MTD) and pharmacokinetics. Included were original research studies that had full texts available and were published in English. Included only human studies.

➤ *Exclusion Criteria*

Research that was determined to be duplicate or irrelevant was not included. Articles with a simple abstract

and those published in other languages were excluded. Animal study were also excluded.

III. METHODOLOGY

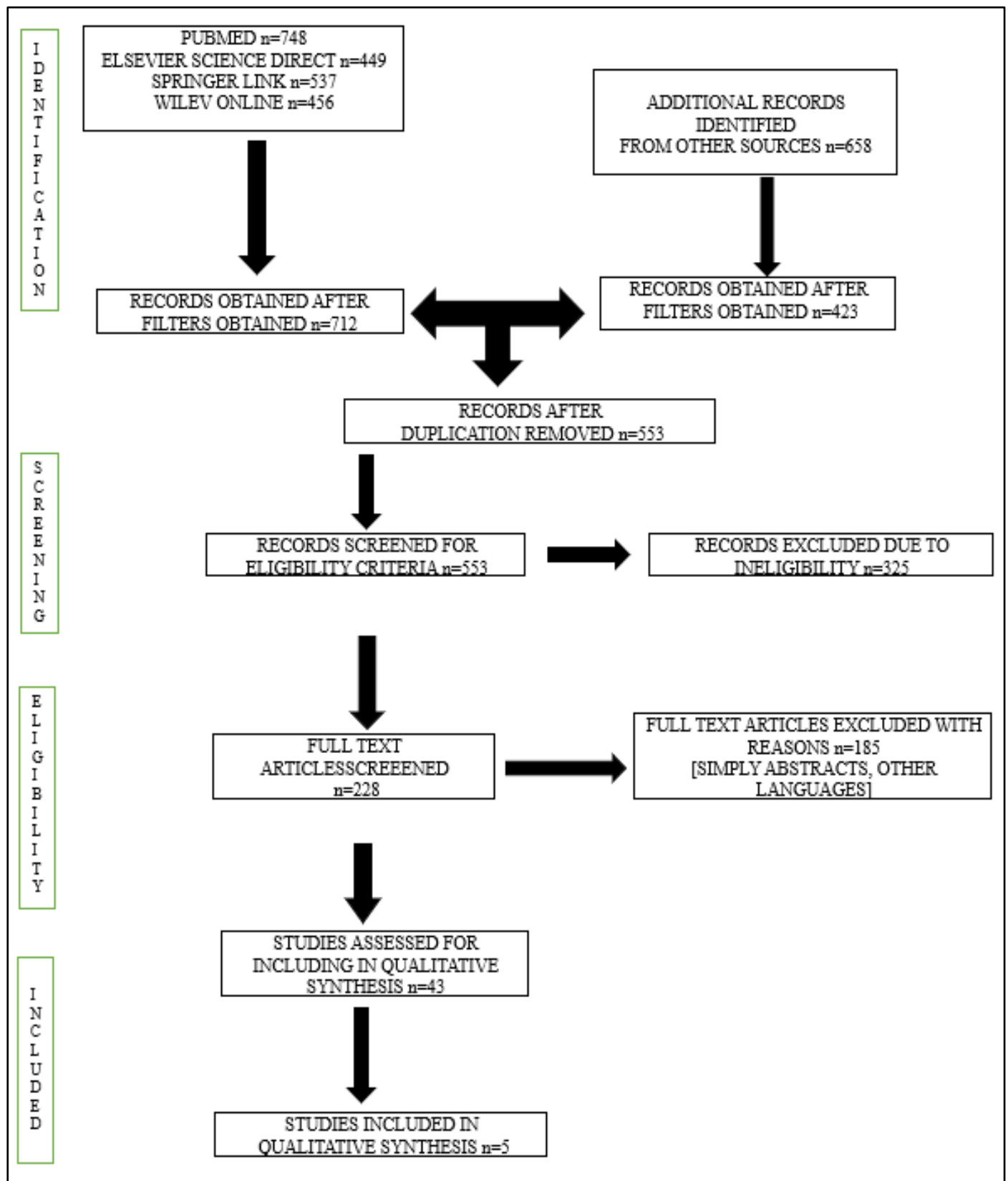


Fig 1 Shows Flow Diagram Illustrating the Number of Papers Found, Screened, Evaluated for Eligibility, Omitted, and Included in the Systematic Review.

IV. RESULT

Table 1 Characteristics of the Study Included in the Systematic Review

AUTHOR	YEAR	STUDY DESIGN	SAMPLE	INTERVENTION	RESULT
Jessica et al	2013	Phase I clinical trial with pre-surgical intervention	The study included 43 women diagnosed with early-stage breast cancer	Participants took 2g of limonene capsules daily over a window period of 2-6 weeks. At surgery, the grossly normal tissue was collected for drug concentration determinant. The level of limonene in serum and tissues were estimated by gas chromatography-mass spectrometry. Immunohistochemistry (IHC) Assay was used for Tissue Biomarkers.	Limonene accumulated preferentially in breast tissue, with an average concentration of 41.3 µg/g (about 332 µM). Despite the high tissue concentration, no significant changes were seen in systemic indicators of proliferation or apoptosis, though cyclin D1 expression was reduced in breast tumor tissue, indicating a possible anti-proliferative action.
David et al	1997	Phase I clinical and pharmacokinetic study	The study included 43 patients with advanced solid tumors, most of whom had received prior treatments.	Phase 1: Patients received D-limonene for 21 days. Cohorts of three to five patients were entered at each of eight dose levels in an escalating schedule ranging from 0.5 to 12 g/m ² per day. Phase 2: each tissue sample (200mg) was added to 2ml 50mM sodium phosphate. Samples in glass tubes were ground using a Potter homogenizer. After centrifugation analyzed by LC-MS as previously described.	D-limonene was well tolerated up to 8 g/m ² /day, with dose-dependent plasma concentrations of D-limonene and its metabolites. No severe toxicities were reported, and disease stabilization was noted in some patients, indicating potential antitumor activity.
Zhaohua et al	2020	An in vitro experimental study	The study used T24 human bladder cancer cell lines to evaluate the anticancer effects of limonene in vitro.	T24 cells were treated with limonene at different doses, and the effects on apoptosis, cell cycle progression, and metastasis were assessed using molecular and cellular tests.	Limonene decreased cell proliferation, activated caspases, produced cell cycle arrest in the G2/M phase, and greatly lowered the metastatic potential of T24 bladder cancer cells
Jessica et al	2012	exploratory clinical study	women - 18–65 years, who had cancers within past 5 years	NAF was collected into small capillary tubes and then immediately diluted in phosphate buffered saline (1:10). participants underwent their massage for four weeks	Daily topical administration of 0.1 mL limonene per breast for 4 weeks in humans (n=8) resulted in 100% compliance and no side effects. Still, there was no discernible rise in limonene levels in plasma or nipple aspirate fluid. Both topical and oral administration of limonene resulted in approximately 2.0 µmol/g in the mammary tissue of mice, indicating good animal absorption.

Helena et al	2020	In vitro experimental study	strains: Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Acinetobacter baumannii, etc. Cell lines: Human lung cancer (A549) and normal fibroblast (HFF-2) Virus: Herpes Simplex Virus type 1 (HSV-1)	Eucalyptus camaldulensis hydroalcoholic extract was used in the study to test its antiviral, antibacterial, and anticancer properties in vitro. To evaluate cytotoxicity, minimum inhibitory concentration (MIC), and viral inhibition, the extract was applied to a variety of bacterial strains, the HSV-1 virus, and cancer (A549 lung carcinoma) and normal (HFF-2) cell lines.	HSV-1 replication was markedly inhibited ($IC_{50} = 40-80 \mu\text{g/mL}$). cytotoxic to A549 cancer cells, suggesting a possible anticancer effect; at higher doses, it also affects normal cells.
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Table 2 Results as Given in the Included Studies

AUTHOR	YEAR	OUTCOME
Jessica et al	2013	The limonene intervention significantly reduced breast tumor cyclin D1 expression while having negligible effects on cell proliferation and apoptotic indicators.
David et al	1997	D-Limonene is well tolerated in cancer patients and may have therapeutic activity.
Zhaohua et al	2020	Limonene induced cytotoxic effects and reduced cell viability of T24 human bladder cancer cells showing an IC_{50} value of $9 \mu\text{M}$
Jessica et al	2012	mammary tissue disposition of limonene with no clinical signs of toxicity
Helena et al	2020	The <i>Eucalyptus camaldulensis</i> extract demonstrated promising anticancer, antibacterial, and antiviral activity in vitro. It effectively inhibited bacterial growth, reduced HSV-1 infection, and showed cytotoxic effects on lung cancer cells, suggesting its potential as a natural therapeutic agent.

Table 3 Risk of Bias Assessment (Limonene Studies)

Author	Random Sequence Generation	Allocation Concealment	Blinding of Participants & Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	No Other Bias	Overall Risk
Jessica A. Miller et al (2013)	Some Concerns	Some Concerns	Some Concerns	Low Risk	Low Risk	Low Risk	Low Risk	Some Concerns
Vigushin et al (1997)	High Risk	High Risk	High Risk	Some Concerns	Low Risk	Some Concerns	Low Risk	High Risk
Ye et al (2020)	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Miller et al (2012)	Some Concerns	Some Concerns	Some Concerns	Low Risk	Low Risk	Low Risk	Low Risk	Some Concerns

➤ Table:3 Risk of Bias Assessment as Included in the Studies

- Green -Low Risk - No or minimal risk of bias; study methods are appropriate and well reported
- Yellow - Some Concerns - Some aspects are unclear or not fully reported, possibly affecting validity
- Red - High Risk - Major methodological flaws or high potential for bias

V. DISCUSSIONS

This scientific scoping review aims to thoroughly assess the connection between limonene extract from eucalyptus oil's anticancer properties, dosage, and concentration. In order to comprehend how varied limonene concentrations affect its biological activity against various cancer types, this study will examine the preclinical and clinical data currently

available. It also looks for the ideal range of dosages that yield the greatest therapeutic benefit with the least amount of harm.

The scoping review finds limonene as a promising phytochemical with substantial anticancer properties, owing to its ability to accumulate in fatty tissues and impact tumor suppression pathways. The findings of several research show that limonene has promise anticancer effects, with concentration, dose, and mode of administration all playing important roles in determining therapeutic efficacy and safety.

Limonene, a naturally occurring monoterpene found in eucalyptus oil and citrus, has shown promising anticancer activity due to its selective accumulation in lipid-rich tissues and acceptable safety profile. Clinical investigations reveal that orally administered limonene concentrates in breast adipose tissue, where it alters tumor biomarkers and induces apoptosis in early-stage breast cancer [7]. Topical application as a massage oil has also been found to be safe and well-tolerated, with no significant local or systemic adverse effects [10,11]. Phase I trials further confirm that doses up to 12 g/day are generally tolerated, with minor gastrointestinal effects, supporting the feasibility of long-term administration [14, 19].

According to experimental investigations, limonene inhibits cancer growth via a variety of routes, including cell cycle arrest, apoptosis, autophagy, and caspase activation. In vitro studies on bladder, lung, and gastric cancer models show that limonene can suppress cell growth, induce G2/M arrest, and diminish the metastatic potential. [9, 13, 21] Furthermore, limonene's broad-spectrum efficacy against a variety of tumor types is attributed to its modulation of oxidative stress responses, Ras signaling, and p53 pathways.[12]

It has also been demonstrated that limonene increases the effectiveness of other anticancer therapies. In comparison to single-agent therapy, studies combining limonene and 4-hydroxyandrostenedione showed better tumor shrinkage in chemically produced mammary tumors. [14,15] Additionally, limonene increased radiobiological responses and decreased DNA damage in colonic cancer cells when given in conjunction with dietary supplements like curcumin and andrographolide, suggesting possible synergistic effects in combination therapy. [17, 19].

Studies on limonene's chemical derivatives, including perillyl alcohol, have shown enhanced potency and selectivity against cancer cells. [18,21]. Limonene has the potential to be a lead chemical for new anticancer medications if its structure is altered or metabolite-based derivatives are created, which could enhance bioavailability, tissue targeting, and therapeutic efficacy [20, 21].

Despite convincing preclinical data and limited clinical proof, problems persist. Variability in extraction procedures, concentrations, and dosing protocols can influence repeatability and therapeutic effects. [11,12]. Additional randomized controlled trials are needed to determine ideal dosages, assess long-term effects, and confirm combination

techniques even if safety has been shown. To optimize limonene's anticancer potential across many tumor types, future research should concentrate on standardized formulations, tailored delivery systems, and nanoencapsulation. [20,21] Limonene, a naturally occurring substance present in Eucalyptus oil, shows significant anticancer properties while being safe for both oral ingestion and topical application. [7, 8, 10, 11, 20]. It generally gathers in adipose tissues, amplifying its therapeutic benefits, and operates in a dose-dependent fashion to impede tumor progression by encouraging apoptosis, autophagy, and cell cycle arrest across various cancer forms, including breast, bladder, lung, gastric, and colorectal cancers. [9, 13, 12, 22]. Limonene also affects important cellular processes, including the activation of caspases, Ras signaling, regulation of p53, and management of oxidative stress, and it can improve the efficacy of other compounds such as curcumin or 4-hydroxyandrostenedione. [14, 15, 17, 19]. In summary, limonene serves as a safe, multifunctional, and bioactive cancer-fighting agent, although additional research is required to optimize dosage, standardize extraction methods, and create efficient delivery systems for medical application. [18, 21].

VI. CONCLUSION

This systematic review highlights d-limonene as a potential phytochemical with strong anticancer properties, particularly when derived from natural sources such as Eucalyptus species. It has been shown to induce apoptosis, autophagy, and cell cycle arrest, with notable accumulation in fatty tissues like the breast, based on evidence from both in vitro and in vivo studies. While its bioavailability varies depending on the method of delivery, clinical studies have demonstrated a favorable safety profile at doses of up to 8 g/day with minimal side effects. When applied topically, absorption is limited but remains safe. Research further supports the antioxidant, antibacterial, and anticancer effects of eucalyptus extracts, particularly against lung and pancreatic cancers. However, due to the limited clinical data, recommended dosages, and variations in tissue absorption and effectiveness, most findings are confined to laboratory settings. Future research needs to emphasize clinical trials, standardization of doses, long-term safety assessments, and validation of mechanisms, as well as investigate the combined therapeutic effects of limonene with traditional treatments.

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