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Anticancer properties of beta−caryophyllene and *d*−limonene terpenes: A review

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ABSTRACT

Terpenes are a structurally diverse family of secondary metabolites found mostly in plants and microorganisms. Beta-caryophyllene and *d*-limonene are abundant in aromatic medicinal plants. Beta-caryophyllene can be sourced from clove and cannabis amongst others, and *d*-limonene is abundant in the *Citrus* genera. Apart from their use in agriculture, cosmetics, and food industries, these terpenes possess a wide range of therapeutic activities, including antimicrobial, analgesic, and anticancer activities. This review discusses the anticancer effects of these two compounds against malignant tumors including breast, lung, gastrointestinal, bone, blood, endometrial, and bladder cancer. Beta-caryophyllene induces apoptosis and prevents proliferation and metastasis through the downregulation of HSP60, HTRA, survivin, XIAP, Bcl-xL, and Bcl-2 and the upregulation of caspase 3, annexin V, p21, Bad, Bak, and Bax. The anticancer activity is also mediated by G_1/M arrest, ROS induction, and JAK1/STAT activation. *d*-Limonene exerts its anticancer effects by upregulating autophagy-linked genes, Bax, and caspase 3 and downregulating cyclin D1 and Bcl-2. These compounds also elicit synergistic effects upon co-administration with anticancer drugs and show great prospects as useful agents in the fight against cancer.

KEYWORDS: Anticancer; Beta-caryophyllene; *d*-Limonene; Terpenes; Drug discovery; Apoptosis

1. Introduction

Cancer is a type of malignant tumor that can develop in any part of the body and has the potential to spread from an initial site to other parts. It was responsible for 608 366 mortalities in the US in 2022 thereby requiring the discovery of new and better strategies for its treatment[1]. Unarguably, nature has been a huge contributor to the discovery of therapeutics for the treatment of different diseases including cancer. It is estimated to be the source or the blueprint for 50% of the commercially available pharmaceuticals globally[2]. One of the breakthroughs in early anticancer drug research was the discovery of paclitaxel from the Pacific yew tree[3]. This discovery

Summary

Question: What are the anticancer properties of beta-caryophyllene and *d*-limonene?

Findings: Beta-caryophyllene and *d*-limonene exhibit anticancer activities against malignant tumors including breast, lung, gastrointestinal, bone, blood, endometrial and bladder cancer. β-caryophyllene induces apoptosis and prevents proliferation and metastasis through the downregulation of HSP60, HTRA, survivin, XIAP, Bcl-xL, and Bcl-2 and the upregulation of caspase 3, annexin V, p21, Bad, Bak, and Bax, while *d*-limonene mediates its anticancer effects by upregulating autophagy-linked genes, Bax, and caspase 3 and downregulating cyclin D1 and Bcl-2.

Meaning: Beta-caryophyllene and *d*-limonene are potential therapeutic agents for cancer.

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paved the way for the development of other members of the taxane family: docetaxel, cabaxitaxel, and abraxane. Taxanes belong to the group of natural products called terpenes.

Terpenes are a highly structurally diverse family of secondary metabolites found mostly in plants and microorganisms. They have gained widespread use in agriculture, healthcare, cosmetics, and food industries. In this review, we focus on the anticancer potentials of two compounds belonging to this family—beta-caryophyllene, and *d*-limonene. Beta-caryophyllene ($C_{15}H_{24}$) is a bicyclic sesquiterpene found in essential oil-producing plants like cloves and cannabis[4,5]. Cloves have a long history of use in traditional medicine as a stimulant for nerves and for the treatment of gastrointestinal disorders[6]. One of the major constituents, beta-caryophyllene, elicits antimicrobial, analgesic, antineoplastic, and antioxidative activities[5]. *d*-Limonene ($C_{10}H_{16}$) is a naturally occurring monocyclic monoterpene with much abundance in different species of the genus *Citrus* including *Citrus aurantium* (bitter orange), *Citrus limon* (lemon), *Citrus sinensis* (sweet orange) and *Citrus reticulata* (mandarine)[7]. *Citrus* has been a crucial part of traditional medicine due to their constituent essential oils which possess a wide array of pharmacological activities[8]. *d*-Limonene is the primary constituent of *Citrus* essential oils and investigations have reported its anticancer, gastroprotective, antioxidant, anti-inflammatory, antinociceptive, and neuroprotective activities[9]. Apart from its use as a dietary supplement, it is used in the food industry as a flavoring and can be used in cakes, ice creams, fruit juices, and drinks[10].

The antineoplastic activities of these compounds *in vitro* and *in vivo* have been reported, revealing their importance in anticancer drug research. Interestingly, they have also been found to elicit potentiating characteristics when used in combination with taxanes. This review is a comprehensive discussion of the scientific findings about the prospects of beta-caryophyllene and *d*-limonene as anticancer agents.

2. Methods

We reviewed the anticancer properties of beta-caryophyllene and *d*-limonene by searching across databases including Google Scholar, PubMed, Scopus, and ResearchGate using keywords such as "*d*-limonene and anticancer" and "beta-caryophyllene and anticancer". Retrieved studies published between 1971 and 2024 were assessed for quality and 80 articles were eventually cited.

3. Chemistry and biosynthesis

Beta-caryophyllene, with the chemical name (1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-one, is a bicyclic sesquiterpene mostly abundant as *trans*-caryophyllene with a mixture of smaller amounts of its isomers, iso-caryophyllene and α-humulene, and its derivative, beta-caryophyllene oxide[5]. Commercially available beta-caryophyllene is presented at ≥90% purity. It has a relatively low water solubility but a high interaction with artificial phospholipid bilayer[11]. With lipophilicity being an important physicochemical property, this characteristic is expected to greatly influence the pharmacokinetic disposition of the substance in the biological system.

d-Limonene is the dextro isomer of limonene, a colorless liquid with an odor like that of lemon. The chemical name of limonene is 1-methyl-4-(1-methylethenyl) cyclohexane. The *D*-isomer occurs more abundantly in nature and is more commercially available than the *L*-isomer. Commercially available *d*-limonene has about 90%-98% purity[12]. *d*-Limonene is immiscible with water, a feature predictable from its structure.

The biosynthesis of both beta-caryophyllene and *d*-limonene (Figure 1) involves the mevalonate and the deoxyxylulose phosphate pathways. Geranyl diphosphate, a metabolite in the pathways undergoes cyclization to form limonene while farnesyl phosphate reacts with an isopentenyl pyrophosphate to form farnesyl pyrophosphate which ultimately undergoes enzyme-catalyzed cyclization to produce caryophyllene[13,14]. Since the reactions are not stereospecific, all possible isomers may be produced. However, beta-caryophyllene and *d*-limonene are the most abundant of all the isomers of caryophyllene and limonene, respectively[10,15].

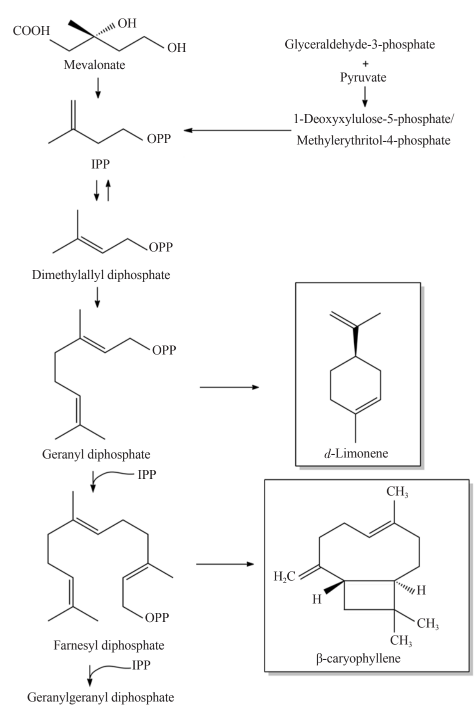

Figure 1. Biosynthetic pathway of β-caryophyllene and *d*-limonene (created with ChemDraw). OPP: pyrophosphate; IPP: isopentenyl pyrophosphate.

4. Extraction techniques for essential oils

Several techniques can be employed for extracting β-caryophyllene and *d*-limonene from plant materials. The usual first step is the extraction of essential oils containing the compound. Steam distillation, a technique that utilizes steam to volatilize essential oils from plant materials is the traditional extraction method. The steam carries volatile components into a condenser, where they are liquefied and collected as essential oil[16]. This method is simple, relatively inexpensive, and widely used; however, it requires large volumes of water and can lead to partial thermal degradation of the constituents.

A much less energy-consuming method is solvent extraction which involves immersing plant material in a suitable organic solvent, such as *n*-hexane, ethanol, or dichloromethane. The chosen solvent selectively dissolves non-polar components from the plant matrix[17]. Notably, this method offers greater control over selectivity compared with steam distillation and can be used at lower temperatures, thereby minimizing thermal degradation. However, the choice of optimal solvent is crucial, as some solvents may co-extract unwanted components, requiring further purification. Proper environmental considerations are also necessary for solvent disposal.

Supercritical fluid extraction is a more sophisticated technique, utilizing a supercritical fluid, typically carbon dioxide (CO_2), above its critical temperature and pressure. The supercritical CO_2 acts as a solvent, selectively extracting desired compounds without the need for traditional organic solvents[18]. This method is environment-friendly, produces high-purity extracts, and operates at lower temperatures compared with traditional extraction methods, thus, minimizing degradation. However, it requires specialized equipment which can be expensive. Also, the process parameters need careful optimization to efficiently extract the compounds of interest.

Fractional distillation method separates components in a mixture based on their different boiling points. Here, the essential oil or crude extract is heated under controlled pressure, and components with lower boiling points vaporize first and are collected as separate fractions. This method may be useful for concentrating and purifying beta-caryophyllene and *d*-limonene from complex mixtures. However, it requires careful temperature control to avoid thermal degradation, and repeated distillation steps may be necessary to obtain high-purity distillates.

Other techniques such as microwave-assisted extraction utilize microwave irradiation to accelerate solvent extraction. Compared with the traditional solvent extraction method, this method offers faster processing times, improved yields, and potentially reduced solvent consumption[19]. Ultrasound-assisted extraction, another viable technique, employs ultrasound waves to disrupt plant cell walls, enhancing the release of the volatile oil[20]. This method provides shorter extraction times and lower solvent usage compared with conventional methods.

Chromatography is a widely used method in the isolation of compounds from natural sources. Volatility and significant thermal stability make gas chromatography a commonly used technique for the analysis of beta-caryophyllene and *d*-limonene[21,22]. The strenuous process of isolating the compounds from the essential oil mixture for bioassays can be averted due to their commercial availability.

5. Pharmacokinetics

A few studies have established the pharmacokinetic and toxicological characteristics of beta-caryophyllene and *d*-limonene.

5.1. Beta–caryophyllene

The first pharmacokinetic study of caryophyllene was reported in 1986 by Asakawa *et al*[23]. (E)-caryophyllene isolated from commercial clove oil was used alongside a few other terpenes for the *in vivo* study of their metabolic properties in male rabbits. The study showed that the metabolism of caryophyllene led to its conversion to an intermediate metabolite, caryophyllene-5,6-oxide, which then is hydroxylated to 10S-(-)-14-hydroxycaryophyllene-5,6-oxide or caryophyllene-5,6-oxide-2,12-diol and their acetates (Figure 2)[23]. A recent study also revealed the distribution characteristics of inhaled beta-caryophyllene in the organs and tissues of mice[24]. This study reported its large distribution to the brown adipose tissues, lung, serum, heart, liver, kidney, olfactory bulb, epididymal fat, and brain. The distribution to the adipose tissue is quite justifiable due to the high lipophilic property of the compound. This property could also have played a role in its crossing of the blood-brain barrier. Moreover, beta-caryophyllene was found to increase the glutathione level in the liver, suggesting that it may influence metabolism in the liver[24]. In another study, it significantly inhibited the cytochrome p450 3A enzyme activity in human and rat liver *in vitro*[25]. More pharmacokinetic information on beta-caryophyllene was provided by Mödinger *et al*. in their study that aimed at comparing the oral bioavailability of beta-caryophyllene neat oil and one in a self-emulsifying drug delivery system, using human volunteers. The area under the curve, AUC_{0-12} and AUC_{0-24} were 260 ng/mL and 305.9 ng/mL × h (P<0.000 1) respectively, while the C_{max} was 58.22 ng/mL[26].

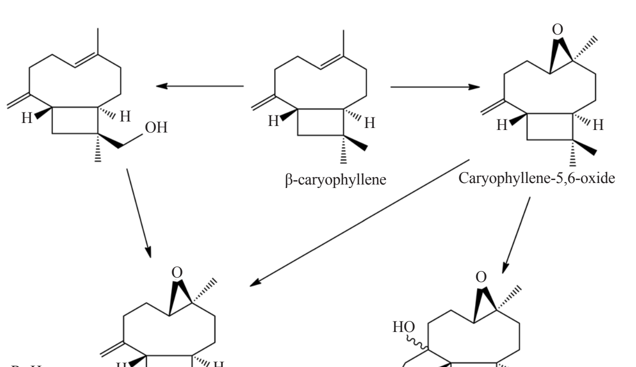

Figure 2. β-caryophyllene metabolism in rabbits[23] (created with ChemDraw).

5.2. *d*–Limonene

d-Limonene is rapidly absorbed following oral and dermal administration and also quickly eliminated[27,28]. The bioavailability is 43% when administered orally[29]. In a recent study by Chen *et al*., the major pharmacokinetic parameters following the oral and inhalational administration of *d*-limonene in mice were C_{max} of (97.150 ± 34.450) ng/mL and (4 336.415 ± 1 142.418) ng/mL, AUC of (162.828 ± 27.447) h·ng/mL and (2 085.721 ± 547.787) h·ng/mL, half-life of (3.196 ± 0.825) h and (0.989 ± 0.095) h respectively. *d*-Limonene was also distributed in different tissues, including the liver, kidney, heart, lung, and spleen, in the order of reducing distribution levels[29]. *d*-Limonene has exhibited different pharmacokinetic properties in different animal models. It is majorly excreted in the urine (almost 60%) in rats, while less than 5% of orally administered *d*-limonene is excreted in the feces[27]. In rabbits, 72% was found to be excreted, with 7% in urine 74 hours after oral administration[27]. Moreover, in male human volunteers, 52% -83% of the dose was excreted within 48 hours of administration[30]. Metabolic investigations of *d*-limonene in different animal models revealed several compounds as metabolites of *d*-limonene. Figure 3 shows an overview of the direct and downstream metabolic products of *d*-limonene[28,31]. Interestingly, as with beta-caryophyllene, *d*-limonene also alters metabolic pathways in biological systems[32].

6. Anticancer properties of β–caryophyllene

Beta-caryophyllene has shown great promise for anticancer activity. Its anti-tumorigenic properties, coupled with the possible mechanisms of action have been assessed in the liver, endometrium, colon, breast, oral and bone cancer.

6.1. Breast cancer

Tomko *et al*. reported the anticancer activity of beta-caryophyllene against the taxol-resistant cell lines[33]. MDA-MB-231 and MCF-7 breast cancer cells were cultured and made to develop resistance by continued exposure to increasing concentrations of paclitaxel. The cells were separately treated with beta-caryophyllene, nerolidol, ocimene, terpinolene, and β-myrcene. Among these compounds, beta-caryophyllene and nerolidol elicited the highest cytotoxic effect with beta-caryophyllene displaying a relatively low IC_{50} value of 27.6 μM and 4.4 μM in paclitaxel-sensitive and resistant MDA-MB-231 cells, respectively, and 2.5 μM and 10.8 μM in paclitaxel-sensitive and resistant MCF-7 cancer cells, respectively[33]. A similar study on caryophyllene oxide, a metabolite of beta-caryophyllene, revealed an IC_{50} of 69 μg/mL and 24 μg/mL against MDA-MB-231 and MCF-7 breast cancer cells, respectively[34]. The results showed that beta-caryophyllene elicited antiproliferative activity in paclitaxel-resistant cells and this activity was mediated by caspase-3 apoptosis and invasion disruption[33]. These results, however, need to be validated through animal or human studies or in HER2-positive cell lines.

Furthermore, beta-caryophyllene repelled cigarette smoke-induced chemoresistance in MDA-MB-468 breast cancer cell lines[35]. This suggests a way to reduce the harm of smoking in breast cancer patients. Hanifa *et al*. reported the anti-tumor activity of vetiver oil against breast cancer[36]. The IC_{50} values on 4TI and T47D breast

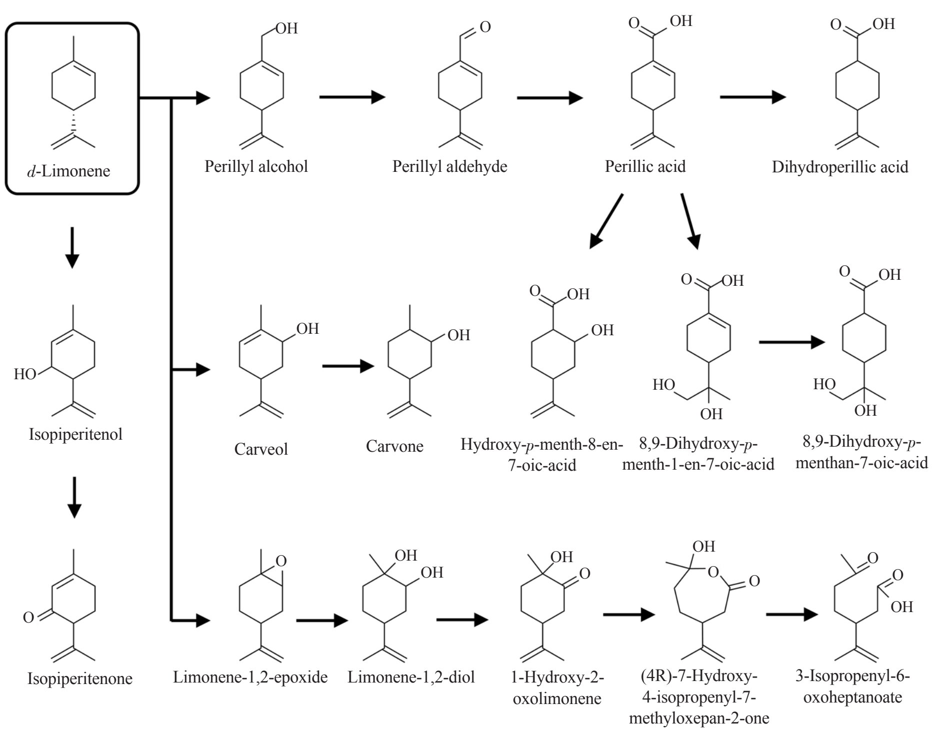

Figure 3. Metabolic products of *d*-limonene in the biological system[28,31] (created with ChemDraw).

cancer cell lines were 62 μg/mL and 112 μg/mL, respectively, showing that the oil had stronger activity against 4TI cancer cell lines. 4TI cell lines underwent a G_1/M arrest while for T47D, an increased G_1 population was observed. Upon analysis of the vetiver oil through gas chromatography, beta-caryophyllene was found to be the major constituent, implying that the anticancer activity of the oil could be attributed to this compound[36]. However, there is a need to validate this by isolating it from vetiver oil and testing its anticancer activity on the same cell lines. Another group has long reported the anti-proliferative activity of beta-caryophyllene against breast cancer (BT-20) cells with an IC_{50} of 3.92 μg/mL[37]. Together, these studies have presented beta-caryophyllene as a potential drug molecule for the treatment of breast cancer.

6.2. Colon/colorectal cancer

Dahham *et al*. reported the anti-proliferative activity of beta-caryophyllene using seven cancer cell lines. The compound elicited a selective activity against colon cancer cell line HCT 116 and HT29, with an IC_{50} of 19 μM and 63 μM, respectively. The activity was mediated by nuclear condensation and mitochondrial membrane potential disruption[38]. Moreover, the compound elicited low toxicity against normal cell lines. The selectivity indices (SI) for the compound were calculated as a ratio of the IC_{50} on normal cell lines to that of the cancer cell lines[38]. An SI value of 3 and above reveals a high selectivity for a test cell line[39]. Notably, beta-caryophyllene had an SI value of 32.2 μM and 9.7 μM in HCT116 and HT29 cancer cell lines, respectively[38]. As cancerous cell specificity is an important property in anticancer lead candidate discovery, this study reveals the selective cytotoxicity of beta-caryophyllene against colon cancer cells. With this foundation, their subsequent research established the dose-dependent anti-angiogenic property of beta-caryophyllene on rat aortic ring, *ex vivo* [IC_{50} = (15.6 ± 2.3) μM]. Their study also revealed the recovery of colorectal cancer-bearing mice following treatment with 100 mg/kg and 200 mg/kg of beta-caryophyllene. The compound blocked angiogenesis by the upregulation of p21 and the downregulation of HSP60, HTRA, survivin, and XIAP[40].

6.3. Endometrial cancer

Endometrial cancer results from the abnormal growth of the cells lining the uterus, known as the endometrium. Endometrial cancer is sometimes called uterine cancer[41]. In a study, the anticancer potential of ethanolic extract of *Sigesbeckia orientalis*, richly containing caryophyllene and caryophyllene oxide was investigated. Different cell lines including the endometrial cancer cell line RL95-2 were cultured and treated with the extract at different concentrations[42]. The cytotoxicity of the extract was then quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) reduction assay[43]. Chang *et al*.[42] found out that the *Sigesbeckia orientalis* extract induced cell arrest at G_2/M phase and caused the RL95-2 cell death through the upregulation of Bcl-2-associated death promoter (Bad), Bcl-2 homologous killer (Bak), and Bcl-2 associated X (Bax) protein expression, as well as the downregulation of B-cell lymphoma extra-large (Bcl-xL) and B-cell lymphoma 2 (Bcl-2) protein expression. The gas chromatography-mass spectrometric analysis of the extracts revealed caryophyllene and caryophyllene oxide to be two major constituents of the extract. The two compounds were suggested to be responsible for the cytotoxic activity of the extract against the endometrial RL95-2 cancer cells[42]. Although this draws attention to the anticancer properties of caryophyllene against endometrial cancer, the compound should be further isolated and tested as a singular entity against the endometrial cancer cell line *in vitro* and in animals.

6.4. Bone cancer

The *in vitro* anticancer activity of beta-caryophyllene has been studied using a human MG-63 cell line. The ability of beta-caryophyllene to induce oxidative stress, apoptosis, proliferation, and inflammation in the cells was investigated. At 20 μM, the compound promoted the generation of reactive oxidative species (ROS) and elicited an antiproliferative effect on bone cancer cells. Its anticancer action was possibly through the induction of the Janus kinase 1/signal transducer and activator of transcription 3 (JAK1/STAT3) signaling pathway[44]. In contrast, the inhibition of JAK1/STAT3 was also reported to induce apoptosis and reduce tumor cell invasion[45]. The underlying mechanisms need to be further investigated.

6.5. Liver cancer

Essential oil from *Murraya paniculata* exhibited selective anticancer activity against murine hepatoma cells[46]. Essential oil was extracted from the plant's fresh leaves by hydrodistillation. Gas chromatography-mass spectrometric analysis revealed that the major compound was beta-caryophyllene with 57.57% composition. The essential oil was tested for cytotoxicity against 3T3 fibroblasts or Hepa 1c1c7 cells. The essential oil displayed higher cytotoxicity against cancer cells (IC_{50} = 63.73 μg/mL) than in the normal fibroblasts (195.3 μg/mL)[46]. Since selectivity by antitumor agents is a challenge in cancer management[47], the preservation of normal cell lines by this essential oil requires further exploration.

6.6. Oral cancer

Oral squamous cell carcinoma is a common form of cancer and a leading cause of death in developing countries[48]. Ramachandhiran *et al*. recently did a study on the antiproliferative properties of beta-caryophyllene against squamous carcinoma KB (mouth) cell line. Using the MTT assay, beta-caryophyllene was found to show anticancer activity against this cancer cell in a dose-dependent manner with an IC_{50} of 40 μg/mL, while at a high concentration of 140 μg/mL, total cell death was observed. According to the study, beta-caryophyllene induced nuclear condensation, apoptosis, and oxidative DNA damage[49]. This research presents beta-caryophyllene as an active compound against oral cancer cell proliferation.

Furthermore, Alam *et al*. reported *d*-limonene and beta-caryophyllene as the major constituents of *Psidium guajava* fresh leaves with 38.01% and 27.98% composition, respectively[50]. The essential oil was tested against the human oral epidermal carcinoma (KB) cells. The oil showed significantly higher antitumor activity compared to doxorubicin, with an IC_{50} of 188.98 μg/mL[50]. The potential synergism between these major constituents could be studied to determine the plausibility of their co-administration in future research.

7. Anticancer properties of *d*–limonene

d-Limonene has shown great potential to be used for treatment against different cancers.

7.1. Breast cancer

d-Limonene is a fat-soluble compound that is distributed into the adipose tissues of the breast[51]. The absence of hydrophilicity-conferring functional groups on the chemical structure can be linked to its lipophilicity. The anticancer properties of *d*-limonene have been investigated both *in vitro* and *in vivo*, as well as in humans. In rats, dietary *d*-limonene prevented nitrosomethylurea-induced mammary carcinoma. *d*-Limonene was then suggested as an inhibitor of carcinoma development induced by a direct-acting carcinogen[52]. In the same model, 4-hydroxy androstenedione acted synergistically with *d*-limonene for the regression of the mammary tumor[53]. Moreover, *d*-limonene was chemopreventive against 7,12-dimethylbenz [a]anthracene-induced mammary lesion *in vitro*[54]. Its metabolic product, perillyl alcohol was reported to elicit a better tumor regression effect[55]. Metabolic products of drugs are sometimes intended to be pharmacologically active compounds, and this concept is employed in the development of inactive or less active pro-drugs which get metabolized into active compounds.

While the antitumor activity of *d*-limonene had been studied using animal models, the activity in humans was largely understudied. Miller *et al*. reported the activity of *d*-limonene on early-stage breast cancer patients. Forty-three women were recruited for the study and were given 2 g/day of *d*-limonene 2-6 weeks before their surgery. *d*-Limonene was observed to be preferentially distributed in the breast tissue, while its major metabolite, perillic acid was not. There was also a 20% reduction in the level of cyclin D1 (P=0.002) in the breast cancer tissue[56]. The cyclin D1 is a regulatory protein in the cell cycle and a reduction in its expression could arrest cell proliferation. The authors further investigated the influence of *d*-limonene on the plasma metabolic profile of early-stage breast cancer patients. The study showed a significant change in the plasma levels of adrenal steroids, bile acids, and collagen catabolic products. In addition, *d*-limonene administration was found to influence glucose metabolism. Notably, metabolic changes that were linked to a decrease in cyclin D1 were observed in the breast tumor tissues[32]. This confirms the cyclin D1 expression-reducing activity of *d*-limonene reported in their previous research. The reduction of steroid levels is important as the breast takes up certain steroids as precursors of androgen and estrogen, which when converted, are implicated in cell proliferation[57]. Vigushin *et al*. had earlier recruited 32 breast cancer patients in a similar human study, and a partial antitumor effect that lasted 11 months was observed in one of the volunteers[58].

7.2. Lung cancer

The effect of *d*-limonene on lung cancer and its mechanism of action were studied using xenograft animal models and five lung cancer cell lines *in vitro*. A dose-dependent inhibition of cell proliferation in A549 and H1299 cell lines was reported. Both cells were treated with 0.5 mM and 0.75 mM of *d*-limonene respectively, for 24, 48, and 72 h, and cell viability was assessed using the CCK-8 assay. The treatment yielded a significant inhibition of the colony formation of the cells. The three other cell lines PC9, H520, and H1975 also showed similar results[59]. This indicates the *in vitro* antiproliferative activity of *d*-limonene in lung cancer. Furthermore, mice were given a subcutaneous injection of Matrigel® for 2 weeks and then treated with *d*-limonene by gavage for 4 weeks at a daily dose of 400 or 600 mg/kg. The tumor size was measured every four days for four weeks. After the fourth week, tumor sizes in two treatment groups (P<0.05) were observed to be smaller than those in the control group. A dose-dependent reduction in tumor sizes (577.08, 867.44, and 1 251.99 mm^3 in the high dose, low dose, and control groups respectively) and weights (0.31, 0.48, and 0.77 g in the high dose, low dose, and control groups respectively) was also observed. *d*-Limonene exerted antiproliferative effects against lung tumors *via* upregulating the expression of apoptosis and autophagy-linked genes[59]. Moreover, apoptosis induction was suggested to have mediated the antineoplastic activity of nanoparticles loaded with *d*-limonene-abundant Elemi essential oil, against A549 human lung adenocarcinoma cell lines[60]. This suggests the potential of nanoparticles in the efficient delivery of essential oils to cancer cells to suppress neoplasm.

7.3. Bladder cancer

d-Limonene elicits a dose-dependent antiproliferative activity against bladder cancer cells. Ye *et al*. reported the inhibitory activity of *d*-limonene against T24 bladder cell growth with an IC_{50} of 9 μM. In addition, cell apoptotic percentage was increased by *d*-limonene treatment in a dose-dependent manner. The observed upregulation of Bax and caspase-3 and the downregulation of Bcl-2 expression further confirmed this result[61]. *d*-Limonene is a potential candidate for bladder cancer treatment and should be further investigated *in vivo* for its cytotoxicity.

7.4. Gastric cancer

Zhang *et al*. investigated the anticancer effect of berberine and *d*-limonene and observed a dose-dependent inhibition of human

Table 1. Summary of the anticancer effects of β-caryophyllene and *d*-limonene.

Terpene	Cancer type	Model used	Key findings	Reference
Beta-caryophyllene	Breast	Human MDA-MB-231 and MCF-7 cell lines	IC_{50} of 27.6 µM and 4.4 µM in paclitaxel-sensitive and resistant MDA-MB-231 cells, respectively, and 2.5 µM and 10.8 µM in paclitaxel-sensitive and resistant MCF-7 cancer cells, respectively. Anticancer activity was mediated by caspase-3 apoptosis and invasion disruption.	[33]
		Human MDA-MB-468 cell line	It repelled cigarette smoke-induced chemoresistance in MDA-MB-468 breast cancer cells.	[35]
		Mouse 4TI and Human T47D cell lines	Beta-caryophyllene-abundant vetiver oil displayed IC_{50} values of 62 and 112 µg/mL against 4TI and T47D breast cancer cells, respectively. 4TI cell lines underwent a G_1/M arrest while an increased G_1 population was observed in T47D.	[36]
		Human BT-20	IC_{50} of 3.92 µg/mL.	[37]
	Colon/colorectal	Human HCT116 and HT29 cell lines	IC_{50} of 19 µM and 63 µM, respectively.	[38]
		Orthotopic mice	IC_{50} of (15.6 ± 2.3) µM; it blocked angiogenesis by the upregulation of p21 and the downregulation of HSP60, HTRA, survivin, and XIAP.	[40]
	Endometrial	Human RL95-2 cell line	It induced cell arrest at G_2/M phase, upregulated Bad, Bak and Bax, and downregulated Bcl-xL and Bcl-2.	[42]
	Bone	Human MG-63 cell line	Generation of reactive oxygen species at 20 µM.	[44]
	Liver	Human Hepa 1c1c7 cell lines	Beta-caryophyllene-abundant essential oil shows an IC_{50} of 63.73 µg/mL against Hepa 1c1c7 cells.	[46]
	Oral	Human KB (mouth) cell line	IC_{50} of 40 µg/mL; it induced total cell death at 140 µg/mL concentration and also nuclear condensation, apoptosis, and oxidative DNA damage.	[49]
		Human KB (mouth) cell line	IC_{50} of 188.98 µg/mL.	[50]
d-Limonene	Breast	Rats	It prevented nitrosomethylurea-induced mammary carcinoma.	[52]
		7,12-Dimethylbenz [a] anthracene-induced mouse mammary lesion	Chemopreventive against mammary lesion.	[54]
		Breast cancer patients	It induced a 20% reduction in the level of cyclin D1 (P = 0.002) in the breast cancer tissue.	[56]
		Breast cancer patients	Partial antitumor effect that lasted 11 months.	[58]
	Lung	Human A549, H1299, PC9, H520 and H1975 cell lines	Significant inhibition (P < 0.001) of the colony formation of the cells.	[59]
		Matrigel®-induced mouse tumor	It exerts antiproliferative action by apoptosis and autophagy induction.	[59]
	Bladder	Human T24 cell line	IC_{50} of 9 µM; upregulation of Bax and caspase-3 and downregulation of Bcl-2.	[61]
	Gastric	Human MGC803 cell line	Dose-dependent inhibition, and synergism when administered with beberine.	[62]
		Nude mice	Inhibition rate of 47.58% (P < 0.05).	[63]
	Blood	K562 cell lines	Cell arrest at the G_1 phase.	[64]
		K562 and HL-60 cell lines	IC_{50} of 0.75 mmol/L; downregulation of *Bcl–2* and upregulation of *Bax* genes in HL-60 cells and K562 cells respectively; downregulation of *Bcl–2* gene expression in K562 cells.	[65]
		K562 tumor xenograft	IC_{50} of 3.6 mM and 3.29 mM 24 and 48 h after administration, respectively.	[66]
		K562 tail vein injection and chick chorioallantoic membrane models	At 0.5 mg/kg, it significantly reduced white blood cell and neutrophil counts, without a significant impact on red blood cells count and hemoglobin; it also reduced angiogenesis at 1, 5, and 10 µg/implant.	[67]
	Oral	Human KB (mouth) cell line	IC_{50} of 132.52 µg/mL.	[72]
		Human KB (mouth) cell line	*d*-Limonene-abundant essential oil showed an IC_{50} of 188.98 µg/mL.	[50]

gastric carcinoma MGC-803 cell growth when the compounds were administered alone, and a synergistic effect when administered together[62]. In an *in vivo* study, *d*-limonene significantly reduced tumor weight in nude mice compared to the control group, with an inhibition rate of 47.58% ($P<0.05$)[63].

7.5. Blood cancer

d-Limonene has shown anticancer activity against leukemia both *in vivo* and *in vitro*. The application of *d*-limonene to K562 cells caused a morphological change and a dose-dependent reduction of proliferation[64]. It also caused cell arrest at the G_1 phase and also induced apoptosis[64]. Guo *et al*. reported a dose-dependent antiproliferative effect of this compound against K562 and HL-60 cells with an IC_{50} of 0.75 mmol/L[65]. Moreover, *d*-limonene downregulated *Bcl–2* and upregulated *Bax* genes in HL-60 cells and K562 cells, respectively, and downregulated *Bcl–2* gene expression in K562 cells in a dose-dependent manner[65]. This *in vitro* trend against K562 was the same in a study by Shah *et al*[66]. Notably, *d*-limonene elicited no cytotoxicity against normal mouse hepatocytes while doxorubicin reduced cell viability with an IC_{50} of 14.13 μM. Also, they took a step further to investigate this in an *in vivo* tumor xenograft model. Immunocompromised mice were injected subcutaneously with K562 cells and treated with *d*-limonene orally. Tumor size reduction was recorded in a concentration-dependent manner like the *in vitro* studies[66]. This tumor cell selectivity of *d*-limonene is an advantage that should be maximized in chronic myeloid leukemia management.

In another study, the tail injection model of K562 cells was used to evaluate the hematological effects of *d*-limonene in immunocompromised C57BL/6 mice[67]. At a dose of 0.5 mg/kg, *d*-limonene significantly reduced white blood cell and neutrophil counts, without a significant impact on red blood cell count and hemoglobin content. The compound showed a similar effect at 1.5 mg/kg dose except for the significant increase in red blood cell count and hemoglobin content[67]. Moreover, *d*-limonene was found to reduce angiogenesis at 1, 5, and 10 μg implant. These results show the potential of *d*-limonene in the treatment of chronic myeloid leukemia. Further research into the anti-angiogenic effect will help establish its mechanism of action.

7.6. Oral cancer

Squamous cell carcinoma is the leading type of oral cavity carcinoma as most head and neck cancers are linked to it[68,69]. Consequently, cytotoxicity studies targeting oral cancer have been carried out on the human epithelial carcinoma cell line (KB cell line). Essential oils extracted from plants such as *Artemisia capillaris*, *Solanum spirale* Roxb, *Curcuma longa*, and *Ocimum basilicum*, have been studied against KB cell lines[70,71]. Using the same cell lines, *Aegle marvels* (L.) Corea essential oil with 63.71% limonene exhibited anticancer activity in a dose-dependent manner with an IC_{50} of 132.52 μg/mL[72]. Several studies have also supported the antineoplastic activity of *Aegle marvels* (L.) Corea

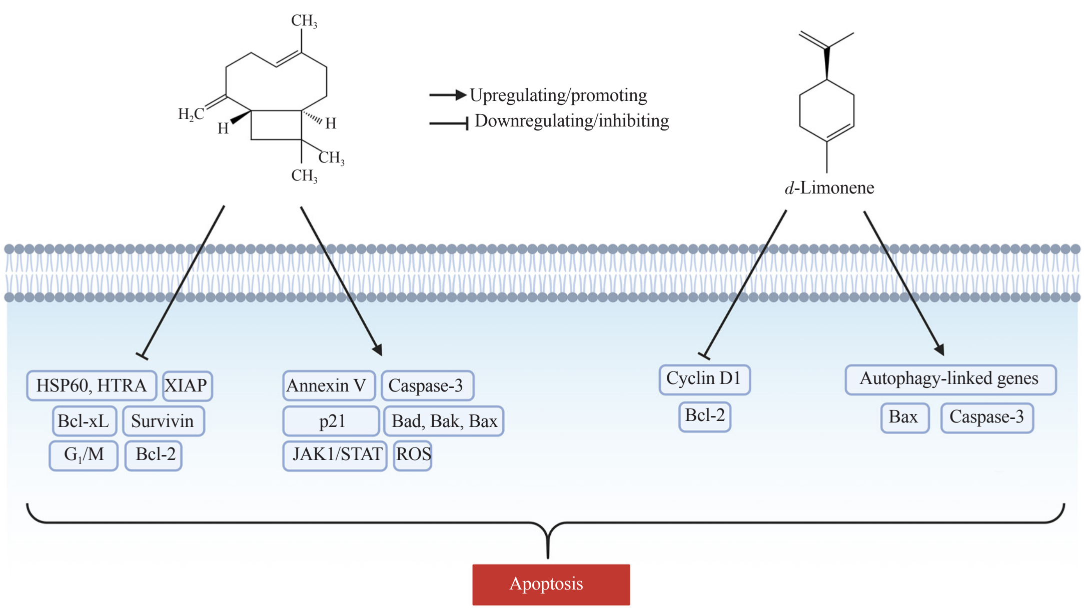

Figure 4. Mechanisms of anticancer activities of β-caryophyllene and *d*-limonene. β-caryophyllene induces apoptosis and prevents proliferation and metastasis through the downregulation of HSP60, HTRA, survivin, XIAP, Bcl-xL, and Bcl-2, and the upregulation of caspase 3, annexin V, p21, Bad, Bak, and Bax. The anticancer activity is also mediated by G_1/M arrest, ROS induction, and JAK1/STAT activation. The anticancer activity of *d*-limonene is mediated by the upregulation of apoptosis and autophagy-linked gene, Bax, and caspase 3 and the downregulation of cyclin D1 and Bcl-2 (Image created with Biorender.com).

essential oil against other cell lines[72]. In addition, *d*-limonene and beta-caryophyllene were the major constituents of the essential oil extracted from *Psidium guajava* (L.) leaves, which exhibited anticancer activity against the KB cells with an IC_{50} of 188.98 µg/mL[50]. The reported antineoplastic activities of beta-caryophyllene and *d*-limonene are summarized in Table 1.

8. Potentiating effect of β–caryophyllene and *d*–limonene on conventional anticancer agents

Blowman *et al*. highlighted the potentiating effect of beta-caryophyllene and *d*-limonene when used with conventional antineoplastic agents[73]. Beta-caryophyllene increased the potency of paclitaxel in human breast cancer (MCF-7), human colorectal adenocarcinoma (DLD-1), and mouse fibroblast (L-929)[74]. Also, *d*-limonene, when co-administered with paclitaxel, reduced the IC_{50} from 2.8 nM to 1.9 nM[75]. A synergism between metformin and *d*-limonene in the promotion of apoptosis of breast and liver cancer cell lines has also been reported[76]. Moreover, beta-caryophyllene enhanced the cytotoxicity of doxorubicin in three human cancer cell lines: Caco-2, CEM/ADR5000, and CCRF/CEM[77]. These indicate the potentiating ability of these compounds when co-administered with commercially available antitumor agents. Since a major concern of current conventional anticancer drugs is their dose-dependent adverse effects, the potentiating effects of these compounds can be useful in reducing the therapeutic doses of these drugs.

9. Pharmacological comparison between β–caryophyllene and *d*–limonene

Beta-caryophyllene and *d*-limonene have some properties in common. However, although both compounds are terpenes, they differ in some pharmacological properties, some of which can be attributed to their chemistry. The Swiss ADME web tool highlights the pharmacokinetic differences between the two compounds. According to the tool, beta-caryophyllene (Log P = 4.24) is more lipophilic than *d*-limonene (Log P = 3.37), and both have low gastrointestinal absorption and a bioavailability score of 0.55[78,79]. Water solubility is an important property for oral absorption. Highly lipophilic compounds like beta-caryophyllene and *d*-limonene have low solubility in water, which impacts their oral absorption. In addition, beta-caryophyllene has the potential to inhibit CYP 2C19 and CYP 3A4 enzymes while *d*-limonene does not[25,78].

Beta-caryophyllene and *d*-limonene have both been investigated in different cancers *in vitro* and *in vivo*, and their activities reported. Different models and laboratory conditions have been used in these bioassays. By implication, an unbiased comparison of the cytotoxic activities of beta-caryophyllene and *d*-limonene may not be established. However, concerning their mechanisms of action, they both upregulate caspase 3 and Bax and downregulate Bcl-2 (Figure 4).

10. Conclusion and future perspectives

d-Limonene and beta-caryophyllene present versatility and potency against different types of cancer. These terpenes elicit their activity through different mechanisms, including an arrest of cell division and the up or down-regulation of pathways that impact cancer growth. *In vitro* and *in vivo* studies have highlighted the anticancer properties of beta-caryophyllene and *d*-limonene and their effects in humans have also been investigated. An advantage is their abundance in aromatic medicinal plants, thereby easing their sourcing – both are also commercially available. Evidence from the literature, including their potentiating effects, supports the usefulness of these compounds in the chemotherapy of cancer. They are worth further exploration for clinical trials. However, additional studies are needed to ascertain their mechanisms of action and specificity. While this is open to exploration, nanoparticles as their delivery mechanisms may present a targeted and improved activity of *d*-limonene and beta-caryophyllene against specific cancers. The role of niosomes, nanoparticles used for site-specific drug delivery, in the sustained delivery of essential oil has been investigated. Ahmad *et al*. analyzed essential oil from *Ocimum basilicum* which contained a predominant amount of caryophyllene and naringenin, and loaded it into niosomes[80]. These loaded niosomes elicited higher anticancer activity against K562 and HEK293 than free essential oil at a concentration range of 15-1 920 µg/mL[80]. A co-formulation with approved anticancer drugs may also provide insight into their synergistic potentials following their site-specific release.

Conflict of interest statement

The authors declare no competing interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

OSG conceptualized and designed the study. OSG, EDO, and FAA wrote and edited the manuscript. All authors approved the final version of the manuscript.

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