Geraniol and citral: recent developments in their anticancer credentials opening new vistas in complementary cancer therapy

1 Introduction

The leading cause of death worldwide, particularly in developing nations, is cancer [1]. According to a World Health Organisation (WHO) report, 80% of the population in underdeveloped nations receives their primary medical care from traditional medicine [2]. The current cancer therapies such as targeted drug therapy, immunotherapy, and personalized and precision medicines, which were not commonly used a few years ago, are now extensively utilized in the treatment of various cancers [3]. Among them, chemotherapy demonstrated to greatly improve patient survival rates and significantly lower tumor recurrence. Chemotherapy drugs play a significant role in treating advanced-stage cancers when there are no other treatment options. Anand et al. have provided a concise overview of the current advancements in chemotherapy and assessed the effectiveness of the enrolled drugs/pharmaceuticals. They have also extensively explored the emerging role of targeted therapies to improve the survival rate and overall success in cancer patients [3]. However, serious adverse effects and drug resistance associated with chemotherapeutics have undermined their efficacies and applications [4, 5]. Hence, it is highly advantageous to investigate new alternative cancer therapies. A repertoire of natural compounds derived from microbes, plants, or other natural sources has been identified as having enormous potential to target stress response pathways, triggering an exaggerated response that leads to the death of cancer cells. These natural compounds or agents, utilized as chemotherapeutic drugs, offer several advantages compared to synthetic counterparts, including lower toxicity, cost, and a simpler production/extraction process. Numerous phytochemicals have already been identified in plants to possess anticancer, antioxidant, anti-inflammatory, proapoptotic, and pro-autophagic properties. These compounds have shown the potential to prevent many processes involved in the development of cancers by effectively controlling oxidative stress [6]. Furthermore, many new phytochemicals are regularly being discovered yet only limited compounds of them possess these bioactive...
properties. Phytochemicals that target multiple signaling pathways that underlie cancer progression will be of paramount importance for the development of new therapeutics for cancer therapy. In this context, many monoterpenes belonging to a class of isoprenoids which demonstrated a wide spectrum of biological properties mainly anticancer, antiproliferative, and cytotoxic, have emerged as promising cancer therapeutic agents [7]. They are colorless, lipophilic, and volatile compounds and are widely used in flavor, fragrance, perfumery, cosmetics, food, and pharmaceuticals. Mainly, they are major constituents of essential oils, floral scents, and defensive resins of many aromatic plants, to which they confer characteristic aromas [8].

In recent years, two monoterpenes, geraniol, and citral have emerged as potent anticancer agents. In plants, they are biosynthesized via the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway [9, 10]. Recent reports revealed that both exhibit potent anticancer activity against breast, lung, colon, prostate, pancreatic, and liver cancers [7, 11–13]. They also have other significant activities such as antimicrobial, anti-inflammatory, antioxidant, antiulcer, and neuroprotective (Figure 1) [14–16]. They are classified as the generally recognized as safe (GRAS) by the Flavour and Extract Manufacturers Association (FEMA) and the Food and Drug Administration (FDA), USA, so they are completely safe for humans [17]. These monoterpenes control multiple signaling pathways. It is involved in various biological processes like the cell cycle, cell survival, and proliferation, apoptosis, autophagy, and metabolism [18–20]. Considering these features, they have the potential to be used to treat complex diseases, such as cancer, and are less vulnerable to adaptive resistance [20–22].

The C10 citral consists of a racemic mixture of two stereoisomers the trans-geranial (E-isomer, or citral A) and the cis-neral (Z-isomer, or citral B). It is mainly found in lemongrass (Cymbopogon citratus and Cymbopogon flexuosus), lemon balm (Melissa officinalis), lime, and oranges. Other sources of citral include plants like verbena (Verbena officinalis), lemon verbena (Lippia citriodora), ginger (Zingiber officinale), aromatic litsea (Litsea cubeba) also known as May Chang, or Makaui in Asia. Furthermore, it is also reported from Cinnamomum wilsonii, Citrus aurantifolia (key lime), Backhousia citriodora, Leptospermum petersonii, M. officinalis. Citral exhibits wide pharmacological properties such as antimicrobial, anticancer, anti-inflammatory, antipyretic, antispasmodic, analgesic, diuretic, and sedative [23, 24]. Recent advances in anticancer properties substantiated their use as lead molecules for the development of new therapies and supplemental cancer treatments [20]. In the present study, we analyzed an increasing number of reports on the anticancer activity of citral and geraniol and presented key data about the effects of these compounds and their promising role in alternative cancer therapies in the future. The target molecules and pathways involved in the mechanisms of cancer progression have also been discussed. In the end, we discussed other activities, which are strongly correlated to cancer such as anti-inflammatory and antioxidant activities.

Figure 1: Various bioactivities of geraniol and citral.
We explored popular search platforms PubMed, Science Direct, DOAJ, and Google to collect the reports published on anticancer activity in the past 5 years or so. Initially, we selected 1244 research papers from these databases. Of 1244, only 95 articles about biological properties were included, refined by the Wiley database. All duplicate articles were removed. Finally, based on titles, abstracts, and whole papers only 86 articles were selected (Figure 2).

2 Major cancers studied to evaluate anticancer effects

Worldwide, more than 10 million people are diagnosed with cancer every year. This genetic disease is the second major cause of mortality worldwide after cardiovascular disease [25]. In the USA cancer has been a leading cause of death accounting for nearly one in every four deaths. It is predicted that 14.6 million people may die due to cancer by 2035. There are many biochemical, molecular, and physiological processes of the human body involved in the progression of tumors starting with multistep carcinogenesis. This complex interplay of various processes makes cancer highly complicated to treat [26, 27]. In the initial stage, cancer is restricted to specific tissues/organs, and then it may metastasize to other tissues, which makes its treatment difficult. Reports show that the prevalence of cancer may escalate by 70% of the current rate, particularly in developing countries. Cancer ranks as the second most prevalent ailment in the Indian subcontinent. The most prevalent cancers in India are lung, breast, colon, rectum, stomach, and liver cancers [28, 29]. Our previous study indicated that females in the age group 25–50 years are at high risk of cervix cancers. Unlikely, males in the age group 50–75 years are most susceptible to cancers. The prevalence varied with age and the major risk factors associated were tobacco and food style, particularly non-vegetarian [23].

Despite several drugs and therapies available for the treatment of cancers, the survival rate of cancer patients is still poor. Therefore, cancer remains a major havoc for human populations. Many oncologists, biochemists, and medicinal chemists have been working to develop effective medicines and therapeutics for the complete cure of cancers from plant-derived phytochemicals. In this context, geraniol and citral have emerged as excellent anticancer agents as they exhibited positive effects against breast, hepatic, colon, skin, prostate, and endometrial cancers. They exert anticancer effects via modulating molecular pathways associated with cancer growth and progression.
3 Breast cancer

Breast cancer is the most common malignancy in women and is the second leading cause of cancer mortality worldwide [30]. The World Health Organization foresees that the global incidence of breast cancer will increase rapidly by 2030. At present, several modern treatment modalities such as radiotherapy, surgical methods, hormonal therapy, and chemotherapeutic drugs are available. However, several adverse effects are associated with these modalities like drug reactions, therapeutic resistance, metastasis, or cancer reoccurrence, which lead to high mortality [31]. Many case studies have indicated that tumor recurrence due to chemoresistance has been a major cause of cancer deaths. Given these facts, elucidation of the mechanisms underlying drug resistance in breast cancer cells may be crucial to the better management of this disease. Nowadays, endocrine therapy has greatly improved the survival of breast cancer patients. In addition, phytochemicals such as vinblastine, vincristine, resveratrol, curcumin, paclitaxel, silybin, quercetin, genistein, and epigallocatechin gallate from plants possessed chemopreventive properties show the potential to treat breast cancer. The majority of these phytochemicals have antioxidant, antiproliferative, and proapoptotic effects on several cancers [32].

4 Colon cancer

Colon cancer, also known as bowel or colorectal cancer (CRC) is one of the most dominant types of gastrointestinal cancers [5, 33]. In the past two decades, dramatic changes in human lifestyles have been responsible for an increased prevalence of colon cancer worldwide [34, 35]. Other risk factors including inflammatory bowel disease, obesity, processed foods, smoking status, alcohol consumption, genetic predisposition, diabetes, and sedentary lifestyle further increased the prevalence of colon cancer [34, 35]. Literature shows that colon cancer is one of the predominant factors for cancer-related morbidity and mortality among malignant tumors [36]. Women are more prone than men to colorectal cancer but the death rate is approximately 25% lower in women than in men [37].

Currently, colon cancer is treated by surgery such as right colectomy, sigmoid colectomy, and total abdominal colectomy with ileorectal anastomosis and chemotherapy using 5-fluorouracil (5-FU) alone or in a combination of adjuvants such as oxaliplatin and avastin [34, 37, 38]. Limited advanced diagnostic facilities are the major cause of the high mortality rate of colon cancer patients. Therefore, the development of advanced diagnostic tools to detect cancer in the initial stages can completely cure this disease.

5 Prostate cancer

In recent years, the increasing incidence of prostate cancer has drawn the attention of many researchers and the medical fraternity to investigate molecular targets of the disease for the development of novel treatment plans to overcome the disease. The prevalence of prostate cancer in western countries is very high compared to Asian countries [39]. However, in Asia, it is the most prevalent among the Korean male population [40]. According to the Korean National Cancer Incidence Database, the age-standardized incidence report suggested that the annual percent change in prostate cancer was 11.4% which is the second-largest cancer observed following thyroid cancer [41].

At present, limited treatments are available for prostate cancer mainly radical prostatectomy and chemotherapy. However, these are not satisfactorily effective treatments due to the patient’s poor response and adverse effects [42]. Over the period, researchers have identified many plant bioactive compounds, which may be very useful as complementary medicines to prevent the progression of the disease with little or no side effects [43]. Nevertheless, diagnosis in the early stage is crucial in the treatment and in reducing the mortality rate of prostate cancer patients [44].

6 Liver cancer

Liver or hepatocellular carcinoma (HCC) is the sixth most prevalent cancer compared to other carcinomas. Global cancer statistics report indicated that it is the third leading cause of cancer-related death in the world [45]. Furthermore, only 18% of liver cancer patients survive after 5 years, which makes it the second most lethal malignant tumor after pancreatic cancer [46]. Major risk factors associated with this disease are mainly alcohol consumption, smoking, carcinogenic chemicals, hepatitis B and C virus, type 2 diabetes, and age. A compound nitroso-diethylyamine present in betel leaves, cigarettes, cheddar cheese, fried meals, and cosmetics products has been identified as the major factor for inducing hepatic carcinoma. Treatment options available for liver cancer include surgery, radiotherapy, and chemotherapy [47]. Presently, many chemotherapeutic drugs such as sorafenib, lovastatin, adriamycin, and mitoxantrone are used to treat liver cancer but they show poor tissue selectivity in terms of targeting the tumor cell.
7 Anticancer effects of geraniol and citral

In recent times, geraniol has been at the center of cancer research for its potential role against a variety of human cancers. Several studies have confirmed its anticancer action against breast, liver, melanoma, endometrial, colon, prostate, and tongue cancers \textit{in vitro} and \textit{in vivo}, as well as on pre-tumor lesions (Table 1) [48–51]. This trend is opening new opportunities for geraniol as a lead molecule for the development of effective complementary medicine. It could provide symptomatic relief to cancer patients by inducing the production of reactive oxygen species (ROS) that trigger apoptosis of cancer cells. Previously, Cho et al. reported that geraniol could sensitize tumor cells to commonly used chemotherapy agents by controlling a variety of signaling molecules and pathways the hallmarks of tumors [7]. However, the worth of geraniol in the management of cancer progression is a long deal unless researchers plan intensive clinical studies on the candidate drug.

So far, only a few studies have investigated the anticancer role and the mode of action of geraniol in human lung and skin cancer. A study by Fatima et al. demonstrated the antiproliferative action of geraniol on different organ-specific human cancer cell lines, including PC-3, AS49,
A431, MDA-MB-231, K562, and HEK-293 [11]. As per the study, geraniol modulates the molecular targets of initiation (DHFR, Tubulin), promotion (LOX-5 and COX-2), and progression stage (ODC ornithine decarboxylase, CATD, and HYAL hyaluronidase) of carcinogenesis in A431 and A549 cells. Further, it inhibits the proliferation of PC-3, A431, and A549 cells and suppresses the activity of ornithine decarboxylase (ODC) and hyaluronidase in A549 cells, LOX-5, and hyaluronidase in A431 cells. Structurally, geraniol consists of two isopentenyl diphosphate units, and one of these functionalized with one hydroxyl group at the tail end has been responsible for the antiproliferative activity. Additionally, geraniol possesses all the drug-like properties and, hence, can be used as a new prototype to develop a novel anticancer agent.

Several essential oils characterized by the presence of geraniol and citral show anti-proliferative and anti-estrogenic properties, which could be useful as an alternative therapy for treating estrogen-dependent cancers. The essential oil of lemongrass dominated by citral and geraniol has anticancer effects [62]. They affect the HSP90 gene and protein expression in MCF-7 cells compared to the normal HEK-293 cells. The HSP90 is one of the most important chaperones involved in the proper folding of oncproteins. Similarly, the essential oil of Lippia alba showed an antitumor effect on breast and gastric carcinoma credited to the major constituent geraniol [12]. The cytotoxicity of geraniol inhibits the growth of AGS cells [12]. In another study, geraniol was found to protect against acute kidney injury in male Wistar rats induced by methotrexate [13]. This effect was mediated through the cytoplasmic repressor Kelch-like, ECH-associated protein 1 (KEAP1) and nuclear factor-erythroid two p45-related factor-2 (NRF2, also called Nfe2l2) and mitogen-activated protein kinase (MAPK/NF-κB) pathways [13]. These pathways are the major signaling cascade responsible for the resistance of metabolic, oxidative stress, inflammation, and anticancer effects [13]. Recent reports suggest that NRF2 is aberrantly activated in cancer, implicating the NRF2/KEAP1 pathway in cancer cell proliferation and tumorigenesis through metabolic reprogramming [63]. Although methotrexate is an anti-metabolite drug used for cancer and autoimmune conditions, it causes renal injury. However, the co-administration of methotrexate with geraniol helps attenuate methotrexate-induced acute kidney injury [13].

According to a new report, an acetylated derivative of geraniol namely 1-acetoxy-3,7-dimethyl-7-hydroxy-octa-2E5E-dien-4-one (Figure 3) displayed cytotoxic effects on the cancer cell lines HUVEC, MDA-MB-231, and MCF-7 cells [64]. Geraniol was also found to be highly effective against human

![Figure 3: Effectiveness of geraniol, geraniol derivatives, and nanolipid carriers against various cancers.](image)
gastric adenocarcinoma AGS cells. Qi et al. reported that geraniol and geranyl acetate (Figure 3) exhibited anticancer effects on Colo-205 colon cancer cells via induction of apoptosis, DNA damage, and cell cycle arrest. However, geraniol was comparatively more effective as compared to geranyl acetate. The low cost and non-toxicity of geraniol to humans are ideal characteristics for the development of new therapeutics for the treatment of colon cancer. Kuzu et al. studied the apoptotic action of geraniol in Ishikawa cells. Another study revealed its promising positive effects on oral cancer using oral squamous cell carcinoma (OSCC) cells. They used the UM1 xenograft mouse model for evaluating the antitumor effects in vivo and observed that geraniol substantially reduced OSCC cell proliferation and migration in a time and dose-dependent manner. This effect is mediated by concurrent induction of OSCC apoptosis and blocking phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling. Most importantly, the study did not observe any adverse effects of geraniol even at the dose of 100 and 250 mg/kg of the body weight of tumor-bearing mice.

In recent years, self-assembled nanopolymers have emerged as excellent drug delivery tools. They can increase the solubility of hydrophobic drugs, prolong blood circulation and improve anti-cancer efficacy. Duan et al. synthesized a multi-responsive (pH/GSH/HAase) nanosystem (HSGNPs) that can deliver the drug to cancer cells and monitor target drug release in cancer cells. It is based on hyaluronic acid-mediated targeting (via the CD44 receptor). Nanopolymers of geraniol improve its anticancer activity facilitating the enhanced intracellular accumulation, which induces apoptosis of cancer cells. Functionalization of nanopolymers with hyaluronic acid (HA) improved internalization efficiency in HepG2, Huh7 cells, and H22 tumor-bearing mice, and was also superior to HCCG nanopolymers and geraniol in suppressing tumor growth in vitro and in vivo showed a marked advantage. Combined with precise targeting, controlled release, and high cytotoxicity, the creative HSGNPs hold promise for targeted therapy and controlled delivery systems in clinical applications.

Geranyl isovalerate (Figure 3), one of the active components of Argyreia nervosa, also exhibited anticancer activity against the HCT116 cell line, affecting cell viability in a dose- and time-dependent manner. This compound was able to induce oxidative stress and affect the mitochondrial membrane potential, which led to the induction of apoptosis. Furthermore, it may suppress the expression of anti-apoptotic genes such as BCI2 and PARP and induce the expression of pro-apoptotic genes such as caspase-3 and 9. Geranyl isovalerate thus may be a viable lead or supplementary molecule for treating colorectal cancer (CRC). Sheikh et al. for the first time, reported antiproliferative and apoptosis-inducing activity of citral in colorectal cancer cell lines HCT116 and HT29. This effect was dose- and time-dependent and did not induce cytotoxicity in CDD841-CoN normal colon cells. Dolghi et al. for the first time reported the anti-CRC properties of three essential oils from Hippophae rhamnoides, C. citratus, and Ocimum basilicum against two human colorectal adenocarcinoma cells (Caco-2 and HT-29). Thus, these oils may be highly useful as potential chemo-prophylactic or chemo-therapeutic alternatives in the management of CRC. The cytotoxic effect of C. citratus (lemongrass) essential oil was attributed to its major compound β-citral. Given the rapidly increasing CRC incidence (GLOBOCAN data), this alternative approach may be further investigated for detailed clinical trial studies. Previously, Gomes et al. reported the antitumor effect of C. citratus improving chemotherapy activity in prostate cancer cells. Many plant extracts with a high percentage of citral demonstrated tocolytic or anti-inflammatory effects, which may be utilized to prevent preterm births. Because of the rapidly increasing preterm birth cases, this effect is promising in preventing preterm births. Citral can inhibit prostaglandin F-2α (PGF-2α) induced contractions, elevate the level of myometrial cAMP, decrease lipopolysaccharide (LPS)-induced TNFα and IL-1β production, and significantly increase IL-10 production in a dose-dependent manner. Among these, the cAMP level has been associated with anti-inflammatory and tocolytic effects. Thus, it could be a safe and effective adjuvant to control preterm births and obstetric and gynecological problems. Mota et al. observed that citral reduced nociception, pre-nociceptive and pro-inflammatory signaling in the spinal cord, and systemic oxidative stress in rats with arthritis. Similarly, Citrus limon essential oil significantly reduced the expression of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, and counteract oxidative stress, which was induced by LPS in murine and human macrophages.

These days, anticancer phytochemicals and nano-therapy are rapidly becoming viable treatment options for breast cancer, which has become one of the major causes of cancer death in females globally. We are witnessing, the increasing significance of nano-therapy like the self-nano emulsifying drug delivery systems (SNEDDS), and nano-lipid-carrier (NLC) in the treatment of cancer because they deliver drugs specifically to the tumor cells. The NLC system was primarily developed to deliver citral in cancer cells. Such type of system releases drugs sustainably without inducing toxicity. The self-nano emulsifying drug delivery system is a class of liquid lipid nanocarriers that direct the delivery of insolubilized drugs or therapeutic bioactive compounds such
as citral. They are ideally suited for drugs with low water solubility. Further, they provide better uniformity, and dosing with a low-risk effect [75]. Izham et al. synthesized and characterized citral (0.5%)-loaded-SNEDDS formulation and evaluated its antiproliferative effects on colorectal cancer cell lines (HT29 and SW620) [77]. These results indicated that CIT-SNEDDS is an excellent tool permitting the sustainable release of citral and, thus, highly effective against colorectal cancer cells.

Despite the astonishing anticancer effects of citral, its poor solubility in water has serious limitations. Many researchers are therefore trying to increase its water solubility and bioavailability upon in vivo administration [69]. Recently developed SNEDDS have shown promise in overcoming the water solubility of citral and efficient delivery at the tumor site [76]. Nordin et al. have carried out notable work in this direction. They synthesized nanostructured lipid carriers, which can deliver citral at the tumor site [75]. The incorporation of citral into a nanostructured lipid carrier (NLC-citral) improves solubility and delivery without abrogating its toxic effects in vivo [58, 75]. The NLC-citral showed positive effects in MDA MB-231 cells in vitro by reducing the tumor weight and size in the 4T1-induced murine breast cancer model. Further, they evaluated the anti-tumor and anti-metastatic effects of NLC-citral in triple-negative breast cancer in the 4T1-induced breast cancer mouse model [78]. Another similar study reported the synthesis of solid lipid nanoparticles from citral with anti-inflammatory and anti-cancer activities [57]. Another study indicated that citral could specifically target drug-resistant breast cancer cells [79]. The study was conducted in MDA-MB-231 cells in vitro by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay, and developed spheroids of MDA-MB-231 breast cancer cells. Citral inhibited the growth of MDA-MB-231 spheroids with lower IC50 values compared to monolayer cultures of DA-MB-231 cells [79]. The results of this study supported the role of citral in eliminating drug-resistant breast cancer cells in a spheroid model. In cancer-related studies, the in vitro tumor spheroids method was used to culture cancer cells in 3-dimensional form. The literature revealed that breast cancer spheroids are resistant to tamoxifen and this characteristic has been attributed to the overexpression of aldehyde dehydrogenase-1 (ALDH1) and wingless/integrated (Wnt) proteins [80–82]. Therefore, ALDH isoforms seem to be an attractive target for preventing breast cancer.

Over the years, researchers have discovered many phytochemicals that target ALDH isoforms and suppress the capacity to form secondary tumors. Natural products such as pro-epigallocatechin gallate from green tea [83] and resveratrol from red wine [84] have the power to inhibit ALDH activity in triple-negative breast cancer cells in vitro. Likewise, citral can inhibit ALDH1A3 in a patient-derived tumor xenograft. Many drugs currently available for breast cancer treatments, such as 3-hydroxy-dl-kynurenine, daidzin, gossypol, kynurenic acid, and pargyline work on a similar mechanism involving apoptosis and targeting ALDH isoforms. Specific ALDH isoforms such as ALDH1 play important roles such as stemness cell markers, and drug-detoxifying enzymes (associated with activation of Wnt/β-catenin signaling), in self-protection, differentiation, and expansion [85]. Isozymes such as ALDH1A3, ALDH1A1, and ALDH2 serve as novel markers of breast cancer stem cells (CSCs) [56], hence, they have been proposed as molecular targets in cancer drug discovery. Nigeh et al. reported the ALDH isozyme-mediated antitumor effect of citral against breast cancer cells, and the ability to form secondary tumors using a 4T1 mouse model [59]. Triple-negative (4T1) breast cancer cells are widely used as an animal model representing highly oncogenic, metastatic, and aggressive human breast cancer [86].

Citral exhibited cytotoxic and genotoxic effects in human cultured cells HepG2 and leukocytes because it can induce DNA damage, especially after being metabolized by cells with active liver enzymes [60]. However, precautions are needed at the time of application of citral. Krishnan et al. observed the effectiveness of citral against hepatocellular carcinogenesis induced by N-nitrosodiethylamine [87]. Citral as a natural chemopreventive agent helps in the restoration of the antioxidants and phase II xenobiotic-enzyme levels. Recently, Taskin Senol et al. have also demonstrated the protective effects of geraniol and vitamin C on liver cancer using a hepatocellular carcinogenesis (HCC) model [88]. The carcinogenesis in the FLR3B hepatocyte cell line was induced by diethylnitrosamine (DENA). Diethylnitrosamine is often used as a carcinogen in experimental applications, as it causes changes in the expression of many genes that none of the other hepatotoxins regulate [87].

In the literature, we found a few studies on the positive effects of citral on melanoma cells. Sanches et al. studied the cytotoxic effects of citral on B16F10 murine melanoma cells and revealed that this effect was exerted by the modulation of cellular oxidative status and/or intracellular signaling [89]. This study showed that citral reduced ERK1/2 protein and inhibited the translocation of this protein to the nucleus, thereby blocking further cell signaling [89]. Interestingly, the effect of citral was not specific to cancer cells, although HaCaT (human skin keratinocytes) and NIH-3T3 cells (mouse fibroblasts) underwent apoptosis and necrosis only at high concentrations. It has obvious toxic effects on neoplastic cells. Citral (1%) was found extremely effective in skin
cancer by suppressing ultraviolet-B (UVB) induced skin carcinogenesis in hairless mice [90]. Citral can increase antioxidant status because of which it can prevent cells from the cytotoxic effects of hydrogen peroxide.

Rhabdomyosarcoma (RMS) is a rare type of soft tissue sarcoma most commonly found in pediatric patients, which is not completely treatable by currently available options. During the course of the drug discovery program for the treatment of RMS patients, researchers found that citral was highly effective against this cancer using RD and RH30 cells [91]. Another interesting study revealed that citral and metformin a drug used in diabetes work antagonistically when taken together by the patient. Metformin obtained from French lilac reduces the incidence of cancer in diabetic patients. Clinically citral is a promising anti-tumor therapy but it needs to be used carefully in diabetic patients who are on metformin medication. It is also found immensely effective in human endothelial cells by preventing them from hydrogen peroxide-induced oxidative stress [61].

8 Mechanisms of anticancer action

Despite geraniol and citral demonstrating promising anticancer effects on various cancers, the molecular mechanism underlying this effect is yet not fully understood. Studies to date revealed that they both exert anticancer effects by inducing apoptosis of cancer cells like several chemotherapeutic drugs [92]. Their general mechanism of action of the anticancer effect is presented in Figure 4. Apoptosis is a common form of programmed cell death. In various cancers, deregulation of apoptosis has been associated with tumor initiation, progression, and metastasis [93]. In addition to apoptosis, citral, and geraniol arrest the cell cycle progression and prevent the cancer cells from developing into tumors and spreading to other parts of the body [94]. Several anti-cancer drugs are available that block the cell cycle progression by targeting specific proteins resulting in clusters of cancer cells. The reactive oxygen species (ROS) is another important factor associated with anticancer action, which plays an important role in regulating key apoptosis-related pathways mediated by death receptors, mitochondria, and the endoplasmic reticulum [95]. The ROS damages DNA and inhibits the G2/M cell cycle. According to published reports, the accumulation of ROS caused oxidative bursts and DNA damage. Furthermore, the inhibition of tubulin in a colchicine-like manner, polymerization, promotion of microtubule, and de-polymerization is associated with an inhibition of the microtubule affinity-regulating kinase MARK4 enzyme. Geraniol and citral also target ALDH isozymes to suppress the tumor [19].

It is evident from the literature, that citral compared to geraniol has been more intensely investigated for anticancer effects and its mechanism of action. Based on the reports, they both display anticancer effects through a similar mechanism involving apoptosis and cell cycle arrest (Figure 4). Yang et al. have done a lot of work on the mode of anticancer action of geraniol using human gastric adenocarcinoma AGS cells [94]. They observed that geraniol initiates cell growth inhibition and apoptosis through mitochondrial ROS production. Besides, p38, MAPK, JNK, and ERK1/2 signaling pathways were inhibited and the expression of caspase-9, Bcl-2 (B-cell lymphoma 2), Bax (BCL2-Associated X Protein), and caspase-3 decreased significantly (Figure 5). Another study reported a similar anticancer mechanism (Figure 5) in Ishikawa cells characterized by a significant increase in the expression of Bax, caspase-3, caspase-8, cytochrome C, and Fas, and a decrease in the expression of Bcl-2 [53]. Qi et al. suggested that geraniol and geranyl acetate trigger mitochondrial ROS production, which plays a crucial role in the apoptosis of colon cancer Colo-205 cells [54].

Citral has a similar mode of action via mitochondrial-mediated apoptotic cell death by the p53 and ROS in colorectal cancer cell lines HCT116 and HT2933. According to the study, mitochondria-mediated apoptosis proceeds through the activation of caspase-3 by the modulation of Bcl-2 family members and an increased ratio of Bax/Bcl-2 and Bax/Bcl-xL (Figure 6). Citral activates p53, which increases the expression of Bax and decreases the expression of Bcl-2 ultimately.
favoring apoptosis [36]. Apoptosis of human prostate cancer cells (PC3) by citral is induced through the activation of AMPK phosphorylation and downregulation of the key genes involved in lipogenesis such as fatty acid synthase, acetyl-CoA carboxylase, 3-hydroxy-3-methylglutaryl-coenzyme-A reductase, and sterol regulatory element-binding protein-117. Apoptosis of PC3 cells is triggered by the upregulation of Bax and downregulation of Bcl-2 expression [18]. Studies have found citral as a safe potent drug for the treatment of prostate cancer. Recently, Taskin Senol et al. have also found that citral and vitamin C tend to decrease the expression of Bax, caspase-3, COX-2, NFkB, growth arrest, and DNA damage-inducible gene 153 (GADD153), apoptosis-inducing factor (AIF), and glucose regulating protein 78 (GRP78) [87]. Application of citral and vitamin C increased the expression of Bcl-2. In Candida albicans, citral derivatives induce apoptosis by arresting cell growth and inhibiting the activities of antioxidant enzymes [55]. In the case of breast cancer, citral inhibits tumor growth by targeting ALDH1A3, which regulates gene expression through the retinoic acid (RA)
signaling pathway and plays an important role in cancer progression and chemo-resistance [56]. In 4T1 breast cancer cells and MDA-MB-231 spheroid cells, anticancer action has manifested via the downregulation of ALDH and activation of the apoptotic process (Figure 7) [79]. Apart from this citral reduces the self-renewal capacity of spheroid cells and downregulates the Wnt/β-catenin pathway (Figure 7). The nano-lipmyelocytomatosis and causes apoptosis of breast cancer cells [78]. In most human cancers, this oncogene is overexpressed accounting for 40% of tumors [96]. Its activity usually increases in tumor cells because of its own mutation, but more commonly due to induction of c-Myc expression via upstream oncogenic pathways [97, 98]. Several other metastasis-related genes such as matrix metalloproteinases-9 (MMP-9), intercellular adhesion molecule 1 (ICAM1), inducible nitric oxide synthase (iNOS), NF-kB, granulocyte colony-stimulating factor (G-CSFα), etoxacin, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), interleukin-1α (IL-1α), and macrophage colony-stimulating factor (M-CSF) have also been involved in the anti-metastatic effect of citral (Figure 7). Among all 20 MMPs, MMP-9 is the most studied metalloproteases, which play important roles in many biological processes and is associated with the pathology of cancers including invasion, metastasis, and angiogenesis [98]. It serves as a biomarker in different cancers, along with the development of novel MMP-9 biosensors [99]. The expression of iNOS increases in a number of tumors and is responsible for the poor survival of patients [100]. Under normal physiological conditions, NOS catalyzes the production of NO, which plays an important role in various stages of carcinogenesis [100].

Cytokines (interleukins) and chemokines (etoxacin) have either tumor-promoting or suppressing activity. The expression of pro- or anti-inflammatory cytokines can lead to cancer progression [101]. The VEGF is a potent angiogenic factor that is upregulated in many tumors [102]. Similarly, macrophage colony-stimulating factor (M-CSF), also known as colony-stimulating factor-1 (CSF-1), is found in a variety of tumor tissues and cancer cells. However, its expression markedly varied in various cancers [103].

A study revealed that apoptosis increases with a decrease in oxidative stress and pro-inflammatory cytokines (IL-1β, IL-4, IL-10, IL-23, TNF-α, and IFNγ) in UVB-induced skin carcinogenesis in hairless mice [90]. Tumor necrosis factor alpha (TNF-α) is a cytokine that has pleiotropic effects on various cell types [90]. Interferon (IFN) receptor signaling initiates the Janus kinase (JAK)-signal transducer and activator of the transcription 1 (STAT1) pathway, which leads to the production of traditional interferon-stimulated genes with important immune effector activities. As a result, IFN may have significant consequences for autoimmune, metabolic illnesses, atherosclerosis, neurological diseases, and immune checkpoint blockade therapy for cancer [104].

The intrinsic potency of citral has been severely constrained by several restrictions, most notably poor stability, low bioavailability, and inability to discriminate between tumor and non-tumor cells. Bioavailability and anticancer activity of citral are improved by new stable formulations created employing cyclodextrins, biodegradable polymers, or different nanostructured particles. As of now, the biggest challenge researchers are facing in using these monoterpenes for cancer therapy is the inability to selectively target tumor cells. However, the Food and Drug Development Administration (FDA), USA has approved citral as a

Figure 7: Molecular mechanisms of anticancer action of citral in HBC/4T1 breast cancer cells/MDA-MB231 spheroid cells. ALDH1A3, Aldehyde dehydrogenase 1 family, member A3; bFGF, Basic fibroblast growth factor; C-myc, Cellular myelocytomatosis oncogene; ELF3, ETS related transcription factor; G-CSFa, Granulocyte colony-stimulating factor; ICAM, Intercellular adhesion molecule; IFNγ, Interferon-gamma; iNOS, Nitric oxide synthase; M-CSF, Macrophage colony-stimulating factor; MMP-9, Matrix metallopeptidase 9; NF-kB, Nuclear factor kappa B; RA, Retinoic acid; RARRES1, Retinoic acid receptor responder 1; RARβ, Retinoic acid receptor beta; VEGF, vascular endothelial growth factor.
safe food additive because of its health-promoting effects on humans and animals.

9 Conclusions

Given their wide-spectrum biological activities, geraniol and citral have been labeled as ‘the most skilled bioactive agents’. We conducted a literature review on their anticancer potential to explore their applications in cancer therapy. These acyclic monoterpenes are currently emerging as powerful anticancer compounds offering alternative cancer treatment plans. Because of the limited treatment options, mostly chemotherapy, which is both hazardous and expensive, these monoterpenes offer an attractive alternative or complementary treatment in cancer therapy. Certainly, they are environmentally friendlier, biocompatible, and more cost-effective options than the current chemotherapeutics. Combining these phytochemicals with nano-therapy could prove highly effective in treating cancers. In this direction, self-nano emulsifying drug delivery systems (SNEDDS) and nano-lipid-carrier (NLC) are gaining importance in cancer treatment due to their ability to target tumor cells. A combination of Citral and SNEDDS has already shown promise in the sustained release of citral, making it highly effective against colorectal cancer cells. However, before they can be applied to humans, extensive in vitro and in vivo studies are needed to understand the toxicological and genotoxic effects of these monoterpenes against a wide range of cancers. At present, due to a lack of preclinical data, their applications in cancer therapy are limited. Therefore, further research is needed to collect extensive clinical data. In addition, researchers should also focus on the production of new derivatives of geraniol and citral to enhance their anticancer potency. Despite these challenges, there is potential for these monoterpenes to become novel complementary and alternative cancer therapeutics. However, much work is still required to improve their stability and efficacy.

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