Essential oils: from prevention to treatment of skin cancer

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The increasing incidence of cutaneous malignancies signifies the need for multiple treatment options. Several available reviews have emphasized the potential role of various botanical extracts and naturally occurring compounds as anti-skin-cancer agents. Few studies relate to the role of chemoprevention and therapeutic activity of essential oils (EOs) and EO components. The present review summarizes an overview of chemopreventive, anti-melanoma and anti-nonmelanoma activities of EOs from various plants and EO components in *in vitro* and *in vivo* models with special emphasis on skin cancer. Also, the mechanisms by which EOs and EO components exert their effects to induce cell death are presented.

Introduction

Skin cancer is the common malignancy in humans [1] which arises due to UV-B radiation. The two types of skin cancer are cutaneous malignant melanoma (CMM) and nonmelanocytic skin cancer (NMSC). Basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) are the two major types of NMSC [1,2]. Malignant melanoma is a treatment-refractory and metastasis-prone malignancy of the skin [3]. UV radiation causes specific mutations in keratinocytes leading to the development of NMSCs, which represent the most common type of skin cancer in humans [2]. Actinic keratosis (AK) (solar keratosis) is described as the earlier stage of SCC *in situ* [4]. BCC spreads locally with little or no metastasis and accounts for 80–85% of all NMSC [5,6]. SCC can progress to invasive SCC (showing a 2–6% risk of metastasis) [7,8] and accounts for 15–20% of all NMSC [2,5]. Currently, surgical removal, radiation therapy, chemotherapy and/or cryosurgery are used for skin cancer treatment. Topical drugs used in chemotherapies for superficial BCC and SCC *in situ* include 5-fluorouracil and imiquimod, whereas imiquimod only is approved for topical therapy of CMM. Further, α-difluoromethylornithine [9], T4 endonuclease 5 (T4N5) [10], monoterpene perillyl alcohol (POH) [11,12] and DL-α-tocopherol [13] are the fresher therapeutics under evaluation. DL-α-tocopherol and α-difluoromethylornithine recently failed to show protective effects [9,13].

Prevention and therapeutic strategies for skin cancer

The growing incidence of cutaneous malignancies indicates the need for multiple treatment options. Although surgical modalities remain the basis for treatment, effective strategies are still required to reduce morbidity and mortality. Therefore, development of effective chemotherapeutic agents and complementary approaches are needed to treat skin carcinoma. One such approach is to utilize the potential of natural products to be effective in cancer chemoprevention and cancer therapy, especially those natural products derived from plants [14]. Phytochemical compounds from plant extracts have shown promising potential as anticancer drugs and as lead molecules in the synthesis of new drugs [15]. Ng *et al.* [16] recently reviewed the role of phytochemicals, namely polyphenolic compounds, phenolic acids, flavonoids, polyflavonoids, polystilbene, terpenoids and organosulfur, for their anticancer activities against skin carcinoma. Wang *et al.* [17] reviewed various phytochemicals found in the diet, such as broccoli, garlic, green tea, capsaicin (pepper), resveratrol (grapes), curcumin (turmeric), genistein (soyabean), caffeic acid (coffee), silymarin, among others, for their chemopreventive effects against cutaneous carcinoma.

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Plant essential oils (EOs) are complex mixtures of components such as monoterpenes, sesquiterpenes, alcohols, ethers, aldehydes, esters and ketones [18]. Of these, the largest group of chemical entities in EOs are the monoterpenes, sesquiterpenes and oxygenated derivatives [19]. Gautam *et al.* [20] reviewed the cytotoxic effect of EOs and their major components: carvacrol, D-limonene, geraniol, myrcene, POH, α-humulene, thymol and citral, on various cancer cell lines and in *in vivo* studies. A Pubmed database search was carried out with the terms: skin chemopreventive AND 'essential oil', 'essential oil' AND melanoma apoptosis, 'essential oil' and skin epidermoid cancer, 'monoterpenes AND skin cancer', 'sesquiterpenes AND skin cancer' to obtain the data. This review discusses the chemopreventive and chemotherapeutic activity of EOs and EO components against skin carcinoma. Chemical structures of EO components exhibiting these activities are shown in Fig. 1.

Chemopreventive activity of EOs and EO components against skin carcinogenesis

Many phytochemicals can prevent, halt or reverse the process involved in development of skin cancer. This section discusses the chemopreventive activity of EOs and EO components against skin cancer. Turmeric essential oil (TEO) significantly delayed and prevented the incidence of skin papilloma development in 7,12-dimethylbenz[a]anthracene (DMBA) and croton-oil-treated mice. In *in vitro* studies, TEO exhibited inhibition of isoforms of cytochrome P450 enzymes (CYP1A1, CYP1A2, CYP2B1/2, CYP2A, CYP2B and CYP3A), indicating its mechanism for anticarcinogenic activity [21]. Topical application and pretreatment of sandalwood oil significantly reduced skin papilloma incidence, multiplicity and TPA-induced ornithine decarboxylase (ODC) activity in CD-1 mice suggesting its chemopreventive effects [22,23].

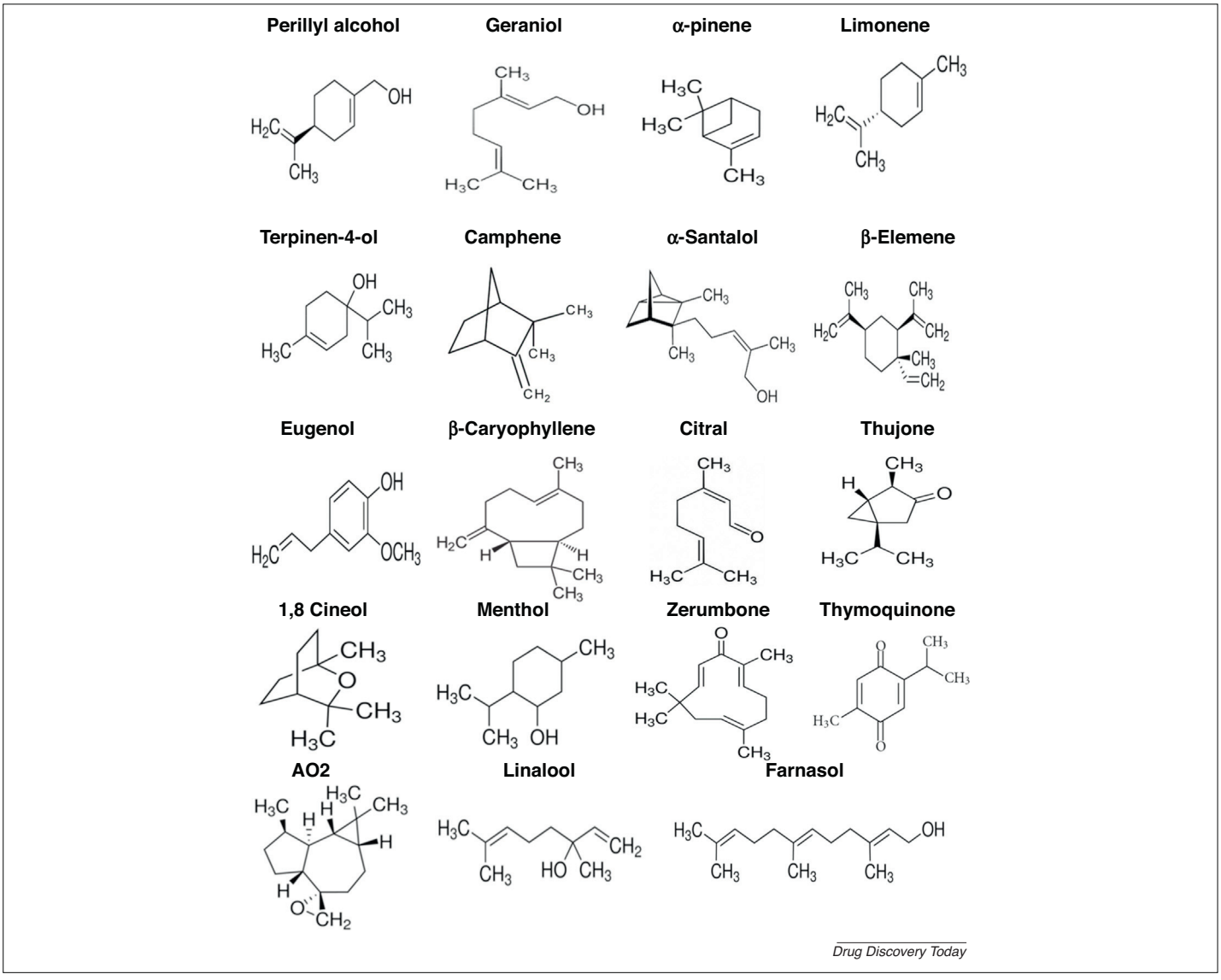

FIGURE 1

Structure of various essential oil (EO) components exhibiting anticancer activity against cutaneous malignancies.

POH is mainly found in small concentrations in EOs from lavendin, peppermint, spearmint, sage, cherries, cranberries, perilla (*Perilla frutescens*), lemongrass, wild bergamot, gingergrass, savin, caraway and celery seeds. The chemopreventive and antitumor properties of POH studied preclinically indicated that topical application of POH inhibits UVB-induced murine skin carcinogenesis (squamous cell tumor models) and DMBA-induced murine melanoma. POH inhibited photocarcinogenesis in a nonmelanoma model of mouse skin carcinogenesis and inhibited UVB-induced activator protein-1 (AP-1) transactivation in mouse skin and human keratinocytes [11]. Topical application of POH significantly inhibited tumor incidence, average tumor size and tumor burden in mice without any noticeable toxicity. POH (10 mM) exhibited delay in the appearance of tumors and showed 25–35% reduction in melanoma incidence. *In vitro* POH treatment reduced the levels of Ras protein, UV-induced reactive oxygen species (ROS), inhibited mitogen-activated protein kinases (MAPKs) and Akt signaling in melanoma cells [24]. Interestingly, Farazuddin *et al.* [25] demonstrated the efficacy of poly-lactic glycolic acid (PLGA)-based microparticle formulation of POH against a skin epidermoid cancer cell line (A253) and in DMBA-induced skin papilloma in Swiss albino mice. Their studies showed that POH–PLGA microparticles enhanced the expression of p21/waf1 and Bax at 12–24 h post-incubation, whereas POH in its free form was not very efficient. POH-based microparticles when administered to tumor-bearing animals caused greater tumor regression and increased survival rate (~80%) compared with the group receiving the free form of POH (survival rate 40%).

A Phase I clinical trial by Stratton *et al.* [26] showed that topical POH cream was well tolerated at a dose of 0.76% (w/w) in participants with normal-appearing skin. In a Phase IIa trial, topically administered POH at 0.76% twice-daily for 3 months showed modest reduction in nuclear chromatin abnormality in moderate-to-severe sun-damaged skin [12]. The chemopreventive potential of α-santalol (major component of sandalwood oil) by several investigators in 7,12-dimethylbenz[a]anthracene-12-*O*-tetradecanoylphorbol-13-acetate (DMBA–TPA) and UVB-induced skin carcinogenesis in mice have been reported. Results from their studies showed that topical application of α-santalol significantly decreased tumor incidence, tumor multiplicity and UVB-induced ODC activity in chemical-induced and UVB-induced skin cancer mouse models. These studies were reviewed by Santha and Dwivedi [27]. Pretreatment with combinations of α-santalol with honokiol and magnolol in SKH-1 mice notably decreased tumor multiplicity up to 75% more than the individual treatment of α-santalol, honokiol or magnolol alone [28].

A study was conducted on the effect of eugenol in DMBA–croton-oil in mice by Pal *et al.* [29], showing that chemopreventive activity was due to inhibition of *c-Myc* and *H-ras*, antiapoptotic *Bcl2*, upregulation of *Bax*, *p53* and activation of caspase-3. Kaur *et al.* [30], in their studies on the effect of eugenol on DMBA–TPA-induced carcinogenesis in murine skin, revealed that topical application of eugenol reduced hyperplasia and epidermal ODC activity, inhibited protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2), decreased levels of proinflammatory cytokines [i.e., interleukin (IL)-6, tumor necrosis factor (TNF)-α] and prostaglandin E2 (PGE2), and attenuated elevated levels of phospho-IκBα and nuclear factor (NF)-κBp65.

D-Limonene is a cyclic monoterpene present in oil from peels of citrus fruits. Chaudhary *et al.* [31] demonstrated that topical application of D-limonene (50 and 100 mg/kg bodyweight) reduced TPA-induced edema and hyperplasia, expression of COX-2, ornithine decarboxylase activity and [^{3}H]thymidine incorporation into DNA. Also, the levels of reduced glutathione, glutathione peroxidase, glutathione reductase, glutathione *S*-transferase, catalase and malondialdehyde were restored in TPA-treated mouse skin. D-Limonene exhibited significant reduction in the tumor burden and tumor incidence as compared with DMBA–TPA-treated mice. The DMBA–TPA-induced tumors treated with D-limonene showed a decrease in the expression levels of Ras, Raf and phosphorylation of extracellular-signal-regulated protein kinase 1/2 (ERK 1/2), Bcl-2 and increase in Bax. These findings reveal D-limonene exerts its chemopreventive activity through inhibition of inflammation, oxidative stress and Ras signaling. Menthol exhibited chemopreventive potential against DMBA–TPA-induced inflammation, oxidative stress and skin carcinogenesis in female ICR mice [32].

The chemopreventive potentials of farnesol [33], geraniol [34] and POH [35] were investigated by several investigators using DMBA-initiated TPA-promoted skin tumorigenesis in a Swiss albino mouse model. The studies showed that topical application of farnesol (doses 25, 50 and 100 mg/kg bodyweight), geraniol (50 and 100 mg/ kg) and POH (6 and 12 mg/kg) 30 min before TPA treatment significantly reduced the TPA-induced skin edema, hyperplasia, expression of COX-2 and oxidative stress response, decreased TPA-induced ODC activity and [^{3}H]thymidine incorporation, respectively. These monoterpenes showed regressed tumor incidence and tumor burden with an extension of latency period during the promotion phase. The chemopreventive effects of farnesol, geraniol and POH occurred via suppression of the Ras/Raf/ERK1/2 signaling pathway, alteration of the Bax:Bcl-2 ratio and induction of apoptosis in mouse skin tumors.

In another study conducted by Manoharan and Selvan [36], geraniol exhibited the chemopreventive activity in DMBA-induced mouse skin carcinogenesis through alteration of phase II detoxification agents and via its free-radical scavenging ability. Oral administration of geraniol at a dose of 250 mg/kg significantly prevented tumor formation as well as reinstating the status of phase II detoxification agents, lipid peroxidation byproducts and antioxidants to the near-normal range in DMBA-treated mice.

The photochemopreventive activity of linalool against UVB-mediated photocarcinogenesis in mouse skin was demonstrated [37]. Linalool treatment [topical or intraperitoneal (i.p.)] prevented acute UVB-induced hyperplasia, edema formation, lipid peroxidation and antioxidant depletion in mouse skin. The treatment prevented UVB-induced overexpression of COX-2 and ODC in mouse skin. Linalool pretreatment (before each UVB-exposure) significantly prevented the expression of NF-κB, TNF-α, IL-6, COX-2, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β1, Bcl-2 and mutated p53 in mouse skin and decreased the tumor incidence in mouse skin. Topical and i.p. linalool treatment prevented the development of SCC in the chronic UVB-exposed mouse skin. Gunaseelan *et al.* [38] demonstrated the photoprotective activity of linalool against UVB-induced damage in human dermal fibroblasts via modulation of MAPK and NF-κB signaling.

1,8-Cineole is a natural monoterpene cyclic ether found in eucalyptus. Lee *et al.* [39] reported that 1,8-cineole inhibited phosphorylation of ERK1/2, its upstream kinases, c-Src and epidermal growth factor receptor (EGFR). 1,8-Cineole suppressed UVB-induced expression of aryl hydrocarbon receptor (AhR) and cyp1a1. Also, topical treatment of 1,8-cineole on mouse skin delayed tumor incidence, reduced tumor numbers and inhibited COX-2 expression *in vivo*. Zerumbone is a sesquiterpene found in ginger, its skin chemopreventive and therapeutic activity in various animal models reported by investigators have been reviewed [40]. Daaboul *et al.* [41,42] investigated the effect of β-2-himachalen-6-ol (HC) a major sesquiterpene from *Daucus carota* (L) oil extract and Lebanese wild carrot in a DMBA and TPA skin carcinogenesis Balb/c murine model. HC treatment (IP 25 mg/kg and topical 5%) exhibited a significant decrease in papilloma yield, incidence and volume.

Anti-melanoma activity of EOs

This section deals with activity of crude EOs against malignant melanoma. Table 1a shows the mechanism of cell death induced by EOs in various cell lines and animal models. EOs from various plants have been investigated for their growth-inhibitory activity on murine (B16B16) and human (A375, A2058) melanoma cell lines by several investigators [43]. Loizzo *et al.* [44] reported the cytotoxic activity of *Platycladus orientalis* (L.) Franco, *Prangos asperula* and *Cupressus sempervirens* ssp. *pyramidalis* EOs and identified the active components involved in growth inhibition of human cancer cell lines. *C. sempervirens* ssp. *pyramidalis* leaf oil exerted the highest cytotoxic activity with an IC_{50} value of 104.90 μg/ml against amelanotic melanoma C32, followed by activity of *P. orientalis* with an IC_{50} of 330.04 μg/ml. Linalool, β-caryophyllene and α-cedrol, the identified components, were found to be active on C32 melanoma cells. EOs from *Salvia bracteata* Bank & Sol and *Salvia rubifolia* Boiss were investigated for inhibitory effect on M14 human melanoma cells by Cardile *et al.* [45]. EO from *S. rubifolia* was significantly more active compared with the EO from *S. bracteata* in inducing apoptotic cell death.

The effect of EO from *Tridax procumbens* L on lung metastasis developed by B16F10 melanoma cells in C57BL/6 mice was studied by Manjamalai *et al.* [46]. The EO showed cytotoxicity at 50 μg and *in vivo* oil treatment significantly inhibited tumor nodule formation by 71.7% compared with untreated mice. Formation of tumor-directed new blood vessels was also found to be inhibited by 39.5%. A significant increase in the number of apoptotic positive cells, increase in p53 and caspase-3 expression was also found to be greater in the EO-treated group than the normal and cancer groups. These results showed the effect of EO from *T. procumbens* in preventing lung metastasis induced by melanoma cells.

In another study, Manjamalai and Grace [47] assessed the anticancer activity of the EO from *Plectranthus amboinicus* (Lour) on the B16F10 melanoma cell line injected into C57BL/6 mice. The EO-treated mice showed an increase in p53 and caspase-3

TABLE 1A

Anti-melanoma activity of EOs

EOs	Major constituents	Cell lines or animal model used	Concentration/dose and mode of administration	Mechanism	Refs
Cupressus sempervirens L	α-Pinene, 3-carene, cedrol, terpinolene and sabinene	Amelanotic melanoma C32	IC_{50} = 104.90 μg/ml	Growth inhibitory effect	[44]
Salvia rubifolia Boiss.	ý-Muurolene, 1-epi-cubenol, trans-pinocarvyl acetate, thujone, α-Pinene and p-cymene	M14	IC_{50} = 12.5 μg/ml	Growth inhibitory effect	[45]
Tridax procumbens (L.)	α-Pinene, ß-pinene, phellandrene, sabinene	B16F10 melanoma cells injected in C57BL/6 mice	50 μg/dose Intraperitoneal	Increase in p53 and caspase-3 expression	[46]
Plectranthus amboinicus (Lour.) Spreng	Carvocrol, thymol, *cis*-caryophyllene, *trans*-caryophyllene, and p-cymene	B16F10 melanoma cells injected into C57BL/6 mice	50 μg/dose Intraperitoneal	Increase in p53, caspase-3 expressions, inhibition of tumor nodule formation and blood vessel formation	[47]
Pituranthos tortuosus (Coss.) Maire	Sabinene, α-pinene, limonene, and terpinen-4-ol	B16F10 murine melanoma B16F10 injected in Balb/c mice	100 mg EO/kg/d Intraperitoneal	Downregulation of FAK, Src, ERK, p130Cas and paxillin, decrease in the expression level of p190RhoGAP, Grb2 and apoptosis Tumor growth suppression	[48]
Zornia brasiliensis Vogel.	*trans*-Nerolidol, germacrene D, transcaryophyllene, α-humulene, and farnesene	Mice inoculated with B16F10 mouse melanoma	50 and 100 mg/kg Intraperitoneal	Tumor growth inhibition	[49]
Annona vepretorum Mart	Bicyclogermacre e, spathulenol, (E)-β-ocimene, α-phellandrene, o-cymene, germacrene D and α-pinene	Mice inoculated with B16F10 mouse melanoma cells	EO (50 mg/kg) and EO complexed with β-cyclodextrin (50 mg/kg) Intraperitoneal	*In vivo* tumour growth inhibition	[50]

expression and an increase in the number of apoptotic cells. Also, EO-treated mice showed inhibition of tumor nodule formation (68.69%) and tumor-directed blood vessel formation (24.76%) compared with the cancer-induced group, thereby indicating the anti-lung metastatic effect of the EO.

Pituranthos tortuosus (Coss.) Maire was studied for its anticancer effects on B16F10 melanoma cancer cells *in vitro* as well as *in vivo* [48]. *In vitro* studies showed EO-induced apoptosis, inhibited migration and invasion processes. The mice treated with 100 mg EO/kg/d (for 27 days) exhibited tumor weight reduction by 98% compared with mice without EO treatment. Costa *et al.* [49] reported the antitumor activity of EO from *Zornia brasiliensis* Vogel. in mice inoculated with B16F10 mouse melanoma cells. EO treatment at 50 and 100 mg/kg showed tumor growth inhibition rates of 1.68 and 38.61%, respectively.

Further to the encapsulation study of POH by Farazuddin *et al.* [25], Bomfim *et al.* [50] reported the effect of *Annona vepretorum* Mart. leaf EO alone (50 mg/kg) and EO complexed with β-cyclodextrin (50 mg/kg) in a microencapsulation. Their efficacies were determined in mice inoculated with B16F10 mouse melanoma cells. *In vivo* tumor growth was inhibited by the treatment with the EO (inhibition of 34.46%), whereas microencapsulation of the EO increased *in vivo* tumor growth inhibition by 62.66%. From the above-mentioned encapsulation studies, it is noted that therapeutic efficacies of EO and EO components are enhanced and, hence, can be useful in skin cancer therapies.

Anti-melanoma activity of EO components

Figure 2 shows the schematic view of EO components acting on various cellular targets, thereby activating the apoptotic pathways. Table 1b outlines the mechanism-of-action leading to apoptotic cell death.

Perillyl alcohol

POH, also called *p*-metha,1,7-diene-6-ol, is a monoterpene, composed of two isoprene units. POH showed growth-inhibitory activity in murine B16 melanoma cells with an IC_{50} value of 250 μmol/l [51].

Geraniol

Geraniol is an unsaturated monoterpenic alcohol and is a major constituent of rose and palmarosa EO (85%). It is also present in the EOs of geranium species (25–50%) and lemongrass (30%).

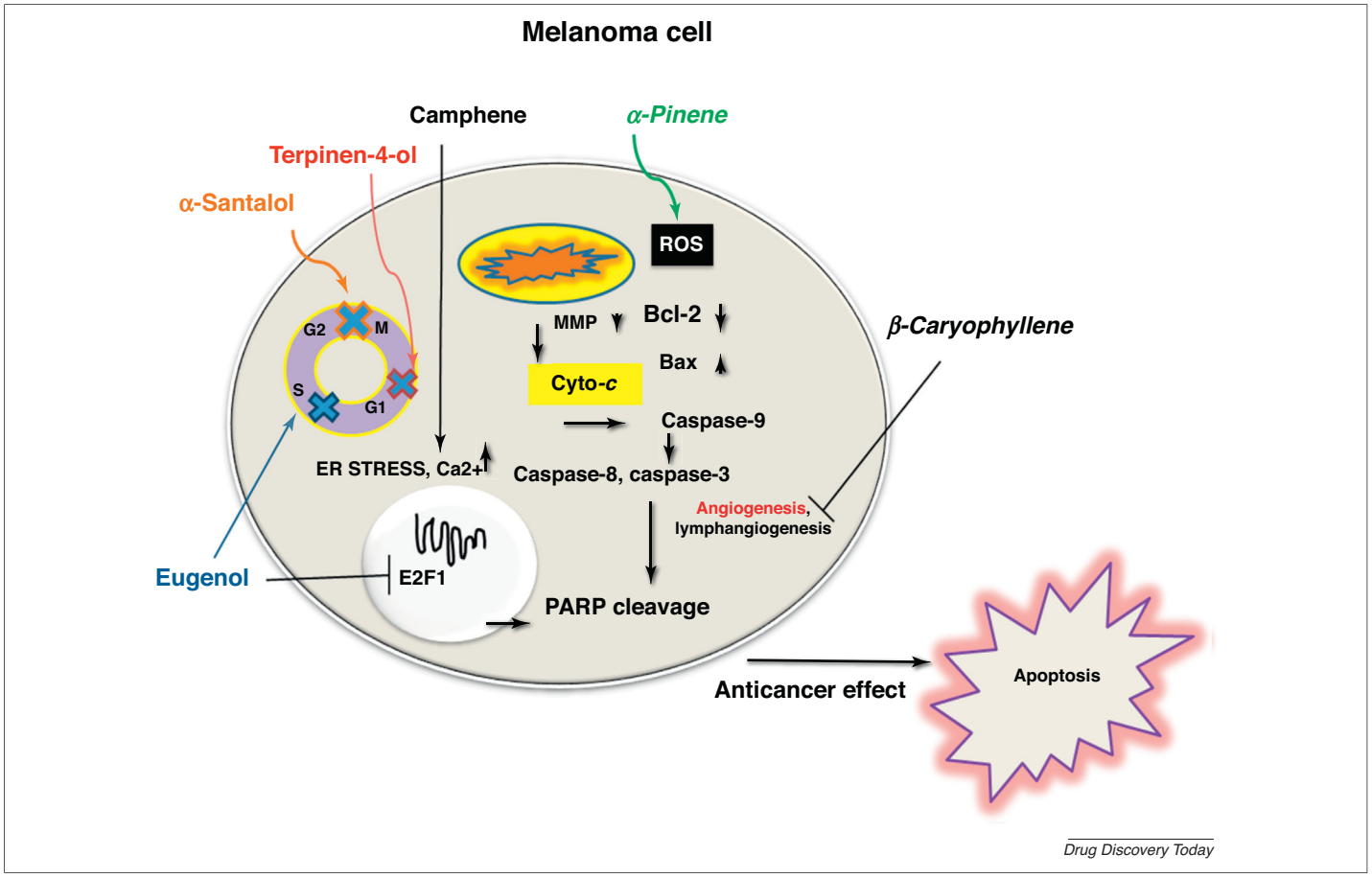

FIGURE 2

A schematic overview of anti-melanoma activity by various essential oil (EO) components. Individual EO components act on various cellular targets thereby causing cell death by apoptosis. Colored arrows (→) indicate the activity exerted by individual EO components on different cell cycle phases causing cell cycle arrest, *viz.*, G2/M phase arrest by α-santalol, S phase by eugenol and G1 phase by terpinen-4-ol. Camphene induces endoplasmic reticulum (ER) stress and increase in Ca^{2+}. α-Pinene increases intracellular reactive oxygen species (ROS) level. Symbol ⊣ indicates suppression/inhibition activity. β-caryophyllene suppresses angiogenesis, lymphangiogenesis and eugenol inhibits E2F1 transcription factor.

TABLE 1B

Anti-melanoma activity of EO components

EO components	Cell lines and animal model used	Concentration/dose and mode of administration	Mechanism	Refs
Perillyl alcohol	B16 murine melanoma	IC_{50} = 250 μmol/l	Growth inhibitory activity	[51]
Geraniol	Female C57BL mice	Dose 6.5 and 65 mmol/kg diet	Tumor growth suppression	[52]
α-Pinene	B16F10-Nex2 cells B16F10 injected in C57Bl/6 mice	100 μg/ml 10 mg/ml (100 μl dose) Intraperitoneal doses	Disruption of the MMP, reactive oxygen species, increase in caspase-3 activity, DNA fragmentation and exposure of phosphatidyl serine and reduced number of lung tumor nodules	[53]
Terpinen-4-ol	M14 WT, resistant M14 ADR melanoma cells	0.02–0.03%	Caspase-dependent apoptosis	[54]
	AE17 mesothelioma and B16 murine melanoma	0.01% 0.04%	Necrosis and G1 cell cycle arrest	[55]
Camphene	B16F10-Nex2 Cells B16F10-Nex2 cells injected in male C57Bl/6 mice	70 μg/ml Peritumoral doses of 300 μg (total 10 mg/kg)	Endoplasmic reticulum (ER) stress, release of $Ca^{(2+)}$, HmgB1 and calreticulin, loss of mitochondrial membrane potential and upregulation of caspase-3 Inhibition of subcutaneous tumor growth	[56]
α-Santalol	UACC-62	50–75 μM	G2/M arrest and altered expressions of cyclin A, cyclin B1, Cdc2, Cdc25c, p-Cdc25c and Cdk2	[57]
β-Elemene	B16F10 C57BL/6 mice	468.8 μM 20 and 50 mg/kg Intraperitoneal	Inhibition of cell proliferation by suppression of VEGF-mediated angiogenesis Tumor size reduction, downregulation of CD34 expression	[58]
β-Elemene	B16F10 B16F10 cells injected in subretinal region of C57BL/6J mice	332.4 μM 2 μl of β-elemene Intravitreal injection	Downregulation of uPA, uPAR, MMP-2 and MMP-9 Tumor growth inhibition via downregulation of uPA, uPAR, MMP-2 and MMP-9	[59,60]
Eugenol	G361	Not available (NA)	S phase arrest, release of cytochrome-*c*, cleavage of PARP and DFF45, AIF, downregulation of pro-caspase-3, −9 and decrease in levels of cyclin A, cyclin D3, cyclin E, cdk2, cdk4 and cdc2 expressions	[61]
Eugenol	WM1205Lu Female B6D2F1 mice with B16 melanomas	1 μM 125 mg of eugenol/kg of bodyweight twice a week Intraperitoneally	S phase arrest, deregulation of the E2F1 Tumor growth delay and size reduction	[62]
β-Caryophyllene	HFD-induced melanoma progression in male C57BL/6N mice	HFD (60 kcal% fat) containing BCP (0, 0.15 or 0.3%) for 16 weeks	Suppression of melanoma progression via reduction in expression of VEGF-A, VEGF-R2, VEGF-C, VEGF-D, VEGF-R3, CCL19, CCL21 and CCR7 levels	[63]
Citral	B16F10 murine melanoma	1.04 μM	p53 nuclear translocation; and depletion of nitric oxide levels and reduction in nuclear factor kappa B, ERK1/2 and AKT levels	[64]
Thujone	B16F10 melanoma injected in C57BL/6 mice	1 mg/kg/bodyweight Prophylactically and simultaneously with tumor induction	Inhibition of tumor nodule formation via downregulation of tumor necrosis factor-α, interleukin (IL)-1β, IL-6 and granulocyte-monocyte colony-stimulating factor, MMPs, VEGF, ERK-1, ERK-2, TIMPs, nm23 TIMP-1 and TIMP-2 expressions	[65]
Zerumbone	A375	20 μM	Downregulation of Bcl-2 gene protein levels, upregulation of Bax and cytochrome c gene and protein levels and activated caspase-3	[66]
Zerumbone	CHL-1	4 μg/ml	Increase in cellular ROS levels, reduction in matrix membrane potential, decreased in ATP and mitochondrial DNA levels and mitochondrial transcription factor A mRNA levels	[67]
Menthol	A375	11.8 μM	Reduction of TRPM8 transcript level, increase in cytosolic Ca^{2+} levels	[68,69]

Geraniol showed dose-dependent impact on the growth of B16 melanomas. Dietary geraniol (0.65, 6.5 and 65 mmol/kg diet) fed to female C57BL mice for 14 days before and for 21 days after tumor transplant showed tumor growth suppression at 6.5 and 65 mmol geraniol/kg diet [52].

α-*Pinene*

α-Pinene is an alkene and found in the oils of many species of coniferous trees, notably the pine. It is also found in the EO of rosemary (*Rosmarinus officinalis*). α-Pinene isolated from *Schinus terebinthifolius* Raddi EO showed anticancer effects on malignant melanoma cells. Disruption of the mitochondrial potential, exposure of phosphatidylserine, production of ROS, increase in caspase-3 activity and DNA fragmentation were the mechanisms of apoptosis induction by α-pinene. Further α-pinene exhibited significant antimetastatic effects in an experimental metastatic melanoma model by reducing the number of lung tumor nodules [53].

Terpinen-4-ol

Terpinen-4-ol, also known as *p*-menth-1-en-4-ol, is a monoterpene found in all spices. Calcabrini *et al.* [54] revealed the antiproliferative activity of *Melaleuca alternifolia* Cheel. tea tree oil (TTO). The TTO and its main active component terpinen-4-ol were able to induce caspase-dependent apoptosis of M14 WT melanoma cells and this effect was more-evidently seen in a M14 ADR (adriamycin-resistant) population. The anticancer effect of TTO and terpinen-4-ol occurred through their interaction with the plasma membrane and reorganization of membrane lipids. Another study demonstrated the anticancer activity of TTO and terpinen-4-ol in murine tumor cells lines, AE17 mesothelioma and B16 melanoma. TTO and terpinen-4-ol significantly inhibited their growth through G1 cell cycle arrest [55].

Camphene

Camphene is a minor ingredient of many EOs such as turpentine, cypress oil, camphor oil, citronella oil, neroli, ginger oil and valerian. It is a bicyclic monoterpene containing two fused cycloheptane rings with a pungent smell. Girola *et al.* [56] isolated monoterpenes from the EO of *Piper cernuum* Vell. (Piperaceae) leaves and evaluated the crude oil and the individual monoterpenes for cytotoxicity on human tumor cell lineages and B16F10-2 murine melanoma. Among the EO components, camphene induced apoptosis by the intrinsic apoptotic pathway on B16F10-2 murine melanoma. Camphene exerted antitumor activity *in vivo* by inhibiting tumor growth of highly aggressive melanoma cells in a syngeneic model.

α-*Santalol*

α-Santalol is a major component (61%) of sandalwood oil (*Santalum album* Linn, Indian sandalwood) and has been traditionally used in the treatment of various skin ailments. The effect of α-santalol on the cell cycle progression of p53 wild-type human melanoma UACC-62 cell line was studied [57]. α-Santalol at 6 h treatment induced G2/M phase arrest and caused depolymerization of microtubules in UACC-62 cells.

β-*Elemene*

β-Elemene belongs to a class of elemane sesquiterpenoids. β-Elemene, a sesquiterpenoid, exhibited an antiangiogenic effect *in vitro* and *in vivo*. It also inhibited melanoma growth and metastasis by suppressing EGF-mediated angiogenesis. *In vitro* studies showed β-elemene at 20 and 50 μM inhibited VEGF-induced sprouting vessels of the rat aortic ring and microvessel formation of the chick embryo chorioallantoic membrane. *In vivo*, it significantly reduced tumor size and downregulated CD34 expression of primary melanoma in mice. It also caused a decrease in metastatic melanoma colonies, melanin content and VGEF expression in the lungs of β-elemene-treated mouse groups [58]. β-Elemene inhibited the metastasis of B16F10 melanoma cells and the growth of intraocular melanoma in a C57B/6L mouse model through downregulation of urokinase-type plasminogen activator (uPA), uPA receptor (uPAR), matrix metalloproteinase (MMP)-2 and MMP-9 [59,60].

Eugenol

Eugenol is the active component of EO from clove (*Syzigium aromaticum*). Eugenol exhibited growth-inhibitory effects in the human melanoma G361 cell line by inducing S phase cell cycle arrest and apoptosis [61]. A study performed by Ghosh *et al.* [62] in a B16 xenograft model showed that eugenol treatment demonstrated a significant tumor growth delay and decrease in tumor size by 40% compared with the animals in the control group. Their studies showed that the antiproliferative effect of eugenol in WM1205 Lu (human malignant melanoma cell line) was through S phase arrest leading to apoptosis. Eugenol also caused deregulation and inhibited transcriptional activity of E2F1.

β-*Caryophyllene*

β-Caryophyllene (BCP, also known as β-c) is a natural bicyclic sesquiterpene found in many essential oils *viz.*, basil, cinnamon, black pepper, cannabis, cloves, lavender, rosemary and oregano. Jung *et al.* [63] examined the inhibitory activity of BCP in high-fat diet (HFD)-induced melanoma progression in male C57BL/6N mice. Their study showed that the inhibitory effects of BCP on HFD-stimulated melanoma progression could occur by suppression of cell proliferation, angiogenesis, lymphangiogenesis, adipocytes and by blocking CCL19/21-CCR7 expression. Their findings demonstrated that BCP administration inhibits solid tumor growth by the inhibition of cell proliferation and induction of apoptosis. Dietary BCP suppressed expression of HFD-induced CD31, VE-cadherin (vascular endothelial cadherin), VEGF-A (Vascular endothelial growth factor A) and VEGF-R2 (Vascular endothelial growth factor receptor 2) expression, thereby inhibiting tumor angiogenesis. Also, it suppressed lymphangiogenesis by reducing the expression of VEGF-C (Vascular endothelial growth factor C), VEGF-D (Vascular endothelial growth factor D) and VEGF-R3 (Vascular endothelial growth factor receptor 3). Further, BCP inhibited adipocyte accumulation, reduced the expression of CCL19 (Chemokine (C-C motif) ligand 19) and CCL21 (Chemokine (C-C motif) ligand 21) levels in the lymphnode, and CCR7 (C-C chemokine receptor type 7) expression in tumors. In the B16F10 cell line, BCP showed inhibitory effects on monocyte migration, secretion of monocyte chemo-attractant protein-1 (MCP-1) and CCR7 mRNA expression.

Citral

Citral exerted antiproliferative and cytotoxic effects in B16F10 murine melanoma cells through oxidative stress and downregulation of signaling pathways required for cell proliferation and survival. Their studies revealed that human skin keratinocytes (HaCaT cells) and murine fibroblasts (NIH-3T3 cells) were more resistant to citral treatment than B16F10 cells [64].

Thujone

Thujone, a component of several EOs, is a ketone and a monoterpene. Siveen and Kuttan [65] reported the antimetastatic potential of thujone. Administration of thujone (1 mg/kg), prophylactically and simultaneously with tumor induction, inhibited tumor nodule formation in the lungs by 59.45% and 57.54%, respectively, with an increase in the survival rate (33.67% and 32.16%) of the metastatic-tumor-bearing animals. *In vitro*, thujone treatment inhibited the activity of MMP-2 and MMP-9 and inhibited the invasion and migration of B16F10 melanoma cells across the collagen matrix in a Boyden chamber. These results indicate that thujone can inhibit the lung metastasis of B16F10 cells through

inhibition of tumor cell proliferation, adhesion and invasion in metastatic animals.

Zerumbone

Zerumbone exerts a chemotherapeutic effect in human melanoma A375 cells through a mitochondria-mediated pathway [66] and inhibits proliferation and migration of human melanoma CHL-1 cells via alteration of mitochondrial functions [67].

Menthol

Menthol is a component found in peppermint oil or mint oil. Menthol suppressed the growth of melanoma cells through activation of transient receptor potential melastatin 8 (TRPM8) [68,69].

Anti-nonmelanoma activity of EO

In brief, the anti-nonmelanoma activities of various EOs and components are shown in Table 2a. The cytotoxic effect of EO from rosewood *Aniba rosaeodora* Ducke (REO) on A431 epipermoid carcinoma and precancerous HaCaT cell lines, HEK001 (transformed normal keratinocytes) and on NHEK (primary normal keratinocytes), was investigated by Sœur *et al.* [70]. Their studies showed that REO selectively killed A431 and HaCaT cells but had only a minimal cytotoxic effect on HEK001 and NHEK cells. Intrinsic and extrinsic apoptotic pathways were implicated in REO-induced cell death. TEO inhibited the growth of A431 cells by apoptosis [71]. EO from *Zanthoxylum bungeanum* Maxim inhibited the proliferation of HaCaT cells, through intrinsic and extrinsic apoptotic pathways [72]. Li *et al.* [73] reported on the effect of bitter apricot EO (BAEO) on HaCaT cells. Their study showed that BAEO induced apoptosis through death receptor, mitochondrial pathways and by reduction in Rel/NF-κB levels. EO from *Pamburus missionis* (Wight) Swingle was potent in inducing cell death in A431 and precancerous HaCaT cell lines through intrinsic (mitochondrial) and extrinsic apoptotic pathways. An *ex vivo* study showed that EO inhibited growth of multicellular tumor spheroids of A431 and HaCaT cell lines [74]. East Indian sandalwood oil (EISO) induced cell death of HaCaT cells by cleavage of LC3 and induction of autophagy and not via apoptosis. This effect suggests that EISO prevents the development of AK and skin cancer [75]. These results suggest that the above EOs can be used as chemotherapeutic agents for treatment of precancerous and epidermoid skin cancer cells.

Anti-nonmelanoma activity of EO components

The anti-nonmelanoma activity of various EO components is shown in Fig. 3 and the mechanism-of-action of cell death is summarized in Table 2b.

α-*Santalol*

Anticancer activity of α-santalol has been studied by Kaur *et al.* [76] in human epidermoid carcinoma A431 cells. Treatment of A431 cells with α-santalol resulted in a concentration- and time-dependent apoptosis via mitochondrial, caspase-dependent and -independent pathways. In another study performed by Zhang *et al.* [57], the effect of α-santalol on the cell cycle progression of A431 cells was studied. Santalol treatment at 6 h (50–75 μM) induced G2/M phase cell cycle arrest and decreased cell viability at 24 h.

Thymoquinone

Das *et al.* [77] reported that thymoquinone (TQ) from black cumin (*Nigella sativa*) inhibited cell proliferation and induced apoptosis by the caspase, JNK and Akt (Ser^{473}) pathway in the A431 cell line.

Aromadendrene oxide 2

Aromadendrene oxide 2 [AO-(2)] is an oxygenated sesquiterpene found as a chemical component of EOs. A study showed that AO-(2) induces G0/G1 cell cycle arrest and apoptosis through a ROS-mediated mitochondrial pathway in A431 and HaCaT cell lines [78].

TABLE 2A

Anti-nonmelanoma activity of EOs

EOs	Main constituents of EOs	Cell lines and animal model used	Concentration IC_{50} values	Mechanism	Refs
Aniba rosaeodora Ducke	Linalool	A431, HaCaT	300 nl/ml	Reactive oxygen species (ROS), phosphatidylserine externalization, depolarization of the mitochondrial membrane potential (MMP)(ΔYm) and caspase-dependence	[70]
Curcuma longa (L)	r-Turmerone, α-turmerone, α-phellanderen, terpinolen, α-zingiberene, β-sequiphellanderen,	A431	5–80 mg/l	Increased caspase-3, -9 expression	[71]
Zanthoxylum bungeanum Maxim	D-Limonene, β-myrcene, trans-β-ocimene, terpinen-4-ol and γterpinene	HaCaT	99.2 μg/ml	S phase arrest, increased expression of cleaved caspase-8/9/3, poly ADP ribose polymerase (PARP) and Bax, decreased Bcl-2 levels	[72]
Prunus armeniaca L. *var. ansu* Maxim	Benzaldehyde, benzoic acid and mandelonitrile	HaCaT	142.45 μg/ml	G0/G1 cell cycle arrest, early- and late-stage apoptosis, caspase-3/8/9, PARP activation and reduction in Rel/NF-κB levels	[73]
Pamburus missionis (Wight) Swingle	β-caryophyllene, 4(14),11-eudesmadiene, aromadendrene oxide-(2) and phytol	HaCaT A431	50 μg/ml 100 μg/ml	Early and late apoptosis, ROS accumulation, loss of mitochondrial membrane potential, increase in Bax: Bcl-2 ratio, caspase activation (-3,-8,-9), PARP cleavage and DNA fragmentation	[74]

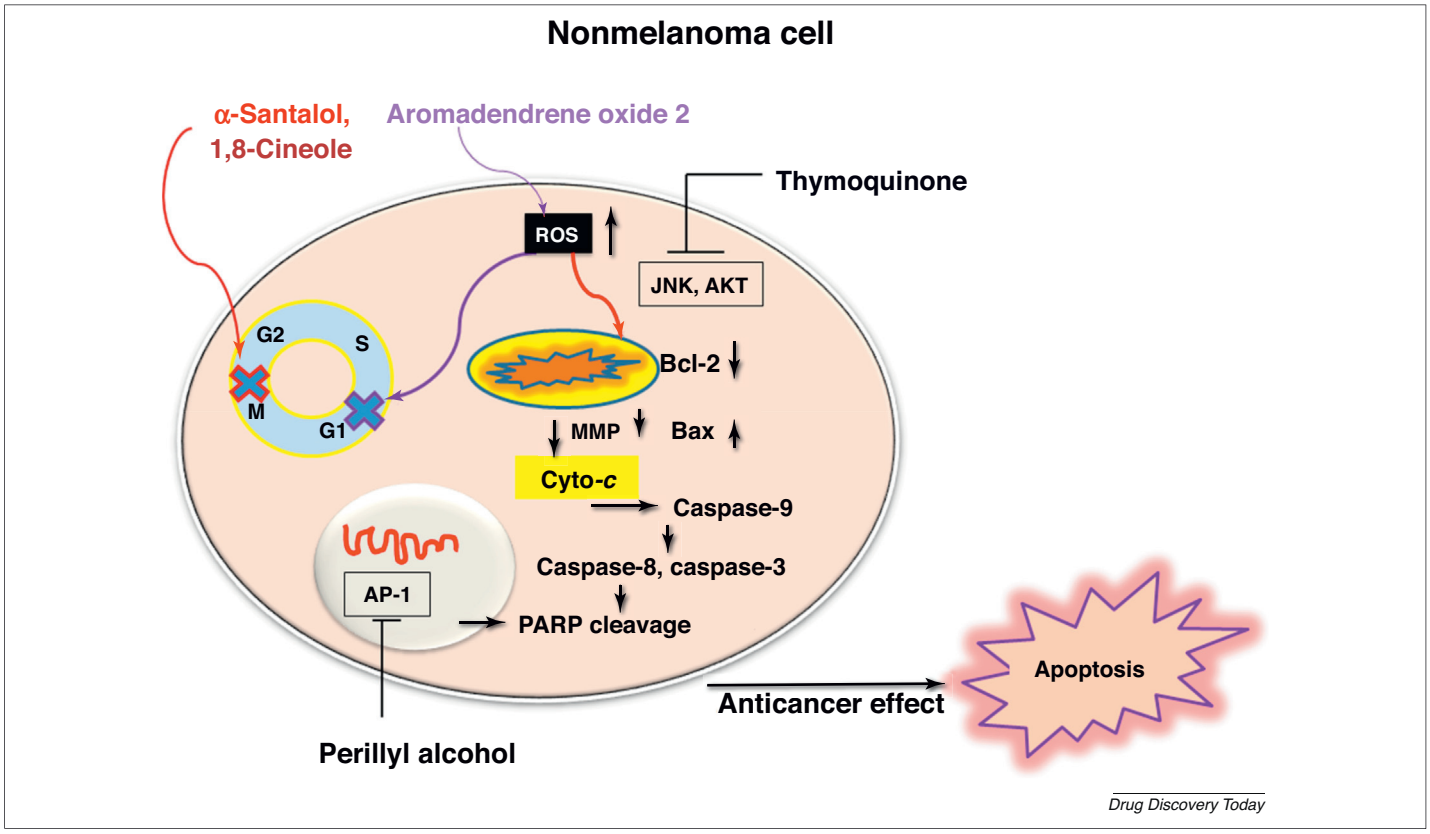

FIGURE 3

A schematic overview of anti-nonmelanoma activity by various essential oil (EO) components. Individual EO components act on various cellular targets, thereby causing cell death by apoptosis. Colored arrows (→) indicate the activity exerted by individual EO components on different cell cycle phases causing cell cycle arrest, *viz.*, G2/M phase arrest by α-santalol and 1,8-cineole. Aromadendrene oxide 2 increases intracellular reactive oxygen species (ROS) and causes G1 phase arrest. Symbol ⊣ indicates suppression/inhibition. Perillyl alcohol inhibits activator protein-1 (AP-1) transcription factor. Thymoquinone inhibits Akt and JNK phosphorylation.

1,8-Cineole

1,8-Cineole, known as eucalyptol, is a monoterpene commonly found in several aromatic plants. Sampath *et al.* [79] reported the anticancer activity of 1,8-cineole from the hexane leaf extract of *Callistemon citrinus* (Curtis) Skeels on skin carcinoma cells. Their results showed that 1,8-cineole effectively induced apoptosis and G2/M phase arrest in A431 cells by increasing the expression of p53.

Combinations of EO components

Studies on combinations of EO components by investigators revealed that combinations of EO components exhibited significant anticancer effects compared with individual components. α-Santalol with honokiol and magnolol retarded the proliferation and enhanced apoptosis in A431 cells [28]. TQ in combination with diosgenin (DG) [(i) 10 μM:10 μM (TQ:DG); (ii) 10 μM:20 μM (TQ:DG); (iii) 20 μM:10 μM (TQ:DG); and (iv) 20 μM:20 μM (TQ:DG)] reduced the viability of A431 cells, increased apoptosis, reduced tumor volume and reduced mass by synergistic effects [77]. The combination of β-c/AO-(2) and β-c/phytol (β-C/P) exhibited synergistic interactions, whereas AO-(2)/P exhibited an additive effect in induction of apoptosis in A431 and HaCaT cell lines [80].

Morphological effects of EOs and EO components on skin cancer cells

Several investigators showed that exposure of skin cancer cells to EOs and EO components caused morphological changes such as cell shrinking, loss of connection and adherence, rounded morphology, membrane blebbing, shrinking of cytoplasm, nuclear condensation and bead formation [53,54,62,73,74].

Adverse effects of EOs and EO components on skin

The field of dermatology has come across a rise in the frequency of allergic reactions to EOs, secondary to the rising popularity of topical use of EOs. Adverse effects caused by EOs include local irritation, allergenic effects and phototoxicity (e.g., limonene, turpentine, bergamot oil). Toxicity of EOs and their active constituents to a large extent depends on their concentration, chemical structure, physicochemical properties, route of exposure and dose. Based upon their lipophilicity, EOs are readily absorbed by intact skin, the respiratory or gastrointestinal tract and, therefore, can also provoke systemic toxicity. Application of excessive amounts of highly concentrated oils to a large surface of the skin or on broken skin can result in significant systemic absorption and increase the chance of serious side effects, such as convulsion [81].

TABLE 2B

Anti-nonmelanoma activity of EO components

EO components	Cell lines and animal model used	Concentration, dose and mode of administration	Mechanism	Refs
α-Santalol	A431	25–50 μM	Induction of apoptosis: caspase-3, -8, -9 activation and PARP cleavage, disruption MMP and release of cytochrome c	[76]
α-Santalol	A431	50–75 μM	G2/M phase arrest, upregulation of p21 expression and suppression of expressions of mutated p53	[56]
Thymoquinone (TQ)	A431 and Sarcoma180-induced tumors *in vivo*	10 μM 10 mg/kg/day Tail vein injections	Increased Bax:Bcl-2 ratio, activation of caspases and cleavage of PARP, inhibition of Akt and JNK phosphorylation Reduction in tumor size and weight, decrease in Ki-67 and CD31-positive cells	[77]
Aromadendrene oxide 2 [AO-(2)]	A431 HaCaT	50 μM 76 μM	G0/G1 arrest, ROS generation, loss of MMP, increase in Bax: Bcl-2 ratios, cytochrome c release, activation of caspases (cleaved caspase-3 and caspase-9) and PARP cleavage	[78]
1,8-Cineole	A431	10 μg/ml	G2/M arrest, increase in expression of p53, Bax/Bcl-2, Cyt-c, caspase-9 and caspase-3	[79]
β-2-Himachalen-6-ol	HaCaT-ras II-4 epidermal squamous cells	8 μg/ml	Decrease in p53 and Bcl-2 protein levels and increase in p21 and Bax, decrease in p-Erk and p-Akt protein levels	[41,42]
α-Santalol/honokiol α-Santalol/magnolol	A431	50 μM + 50 μM 50 μM + 75 μM	Decreased proliferation and enhanced apoptosis	[28]
Thymoquinone (TQ)/ diosgenin (DG)	Sarcoma 180-induced tumors	TQ (10 mg/kg/day) + DG (20 mg/kg/day) Tail vein injections	Reduction in tumor size and weight, increase in DNA fragmentation and disorganization of F-action filaments, reduction in Ki-67 and CD31-positive cell	[77]
β-C/AO-(2), (β-C/P) and (AO-(2)/P)	A431 HaCaT	12.5/6.25 μM, 25/21.75 μM and 25/43.5 μM, respectively 12.5/9.25 μM, 25/17.5 μM and 38/35 μM, respectively	Phosphatidylserine externalization, ROS generation, loss of mitochondrial membrane potential ($\Delta\Psi$m), increase in Bax:Bcl-2 ratios, cytochrome c release, activation of caspases (cleaved caspase-3, -8, -9) and PARP cleavage	[80]

EOs are known sensitizers, and there is proof linking them to cases of contact allergy and allergic contact dermatitis. Photosensitizer oils include cumin, dill, sandalwood oil, lemon oil ylang ylang, lime oil and eucalyptol. Limonene, linalool, citral, cinnamyl alcohol and furocoumarins are EO components that are prone to cause sensitization. Some sensitizers have been shown to interact with other molecules. For example, cinnamaldehyde interacts with proteins causing immunogenic effects. Linalool is the most sensitizing component in many EOs. Photosensitization mainly depends upon the amount of product applied and the area of exposure. Citrus EOs are photosensitive oils (e.g., bergamot, grapefruit, lemon, lime, tangerine and wild orange). These oils are UV-sensitive and induce irritation and darkening of skin upon exposure to sunlight. Clinical studies on dermal exposure patterns are needed for the reliable use of EOs [82,83].

Concluding remarks

The studies carried out by several investigators in *in vitro* and *in vivo* models demonstrate that EOs and EO components prevent the initiation of carcinogenesis and possess the therapeutic ability to control the proliferation of cancer cells at different stages via cell cycle arrest, alter various signaling pathways and induce cell death by apoptotic pathways. This review provides an up-to-date and concise review on efficacy of EOs and EO components as anti-melanoma and as anti-nonmelanoma agents. Owing to their lipophilic nature, diverse therapeutic potential makes them ideal and promising agents for topical use in treatment of melanoma and nonmelanoma skin cancer. Further studies are required to develop evidence-based medicine through clinical trials to enable their therapeutic use in humans.

Conflicts of interest

The authors declare that there are no conflicts of interest to disclose.

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