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Volatiles Composition of Fresh Aroma and Hydrodistilled Volatile Oil of *Chrysopogon Zizanioides* Roots Growing in Egypt Along with the Cytotoxic Activities of the Hydrodistilled Oil

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Abstract

Chrysopogon zizanioides oil (CZO) is obtained from the roots of the *C. zizanioides* plant and it is frequently employed as a major odour contributor in the fragrance and aromatherapy industries. The hydrodistilled CZO was analyzed by GC/MS and compared to fresh sample aroma which was analyzed by headspace GC/MS method. The main constituents of the hydrodistilled oil were Khusimol (16.48%), aristol-1(10)en-9-ol (12.5%) and cyclocopacamphenol (7.69%). While headspace-GC/MS analysis identified bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl (14.17%), sativene (11.52%) and valencene (9.71%) as the major contributors to its fresh aroma. CZO was found to be active against two human cancer cell lines of lung (A549) and hepatocellular (HepG2) with IC50 32.2 and 37.6 µg/mL respectively using MTT assay. The results showed great similarity between the Egyptian oil and those available in the international market. Further studies are necessary for the full standardization and biological evaluation of the oil.

Keywords: *Chrysopogon zizanioides*; roots; oil; GC/MS; headspace; cytotoxic activities.

1. Introduction

Cancer is a serious issue for public health as it is the second leading cause of death in the globe [1]. However, the prevalence of this disease is increasing more quickly in Africa, Asia, and Central and South America, which account for roughly 70% of all cancer-related fatalities globally [2]. Around the world, 18 million cases of cancer were reported in 2018, affecting 9.5, 8.5 million for men and women, respectively[3].

Globally, lung cancer stands as the foremost contributor to cancer-related fatalities. Lung cancer is one of the deadliest forms of cancer for both men and women, with a growing number of fatalities each year and a 5-year survival rates of 10-20% in most countries [4], [5]. Its mortality reached 1.8 million deaths in 2018 [6]. Liver cancer is ranked third in the number of -cancer-related mortality worldwide [6]. The prevalence of liver cancer is higher in regions

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where hepatitis B virus infection is widespread, such as Asian nations [7].

The lack of efficient cancer treatments, the high expense of chemotherapy, and the serious side effects of anticancer medication significantly contribute to high cancer mortality [8]. Due to past success, many research efforts are directed to uncover natural anticancer substances to combat, impede, or even halt the development of cancer [9]. Cancer patients often use herbal remedies to manage the side effects of chemotherapy or radiation treatment, prevent depression and anxiety, and enhance chances of recovery [10].

Chrysopogon zizanioides L., also known as vetiver grass or Khus, is a Poaceae family perennial grass that is unique to South East Asia [11]. Vetiver grass can reach heights of up to 2 meters, and its robust root system, which frequently exceeds 3 meters, can help prevent sheet erosion [12]. Vetiver oil is produced from roots by steam distillation and utilized in traditional medicine, cosmetics, aromatherapy, and the perfume industry. The root is also used in traditional medicine for headache, rheumatism, sprain and malarial fever and as carminative, stimulant and diaphoretic [13]. The root essential oil (CZO) is traditionally used as stimulant, refrigerant and diaphoretic [14]. Many researchers looked in to the cytotoxicity and protective abilities of CZO using *in vitro* cancer models. CZO showed cytotoxicity against several cancer cell lines as LLCMK2 adherent epithelial cells [15], breast tumor cancer lines [16], [17], human epithelial cervical cancer cells [18] and WiDr colon cancer cells [16]. Reports from various geographic locations highlighted the chemical complexity of CZO which encompasses more than 200 compounds [19].

According to a recent research by [20], sesquiterpene hydrocarbons and their oxygenated derivatives were the main volatile components in different locations. To the best of our knowledge, reports are not available on the anticancer activity of CZO against lung (A549) and hepatocellular (HepG2) cell lines. Therefore the current study aims to assess the cytotoxic potential of CZO against two human cancer cell lines A549 and HepG2. We further compared the chemical profile of the hydrodistilled CZO to the fresh aroma of the root utilizing GC-MS and head space HS-GC-MS.

2. Materials and methods

2.1. Plant material

C. zizanioides roots(CR) were dug out from the medicinal and aromatic plants farm, Gzerat El-Sheer in El-Qanater El-Khayreya where sample's authentication was confirmed by Ass. Prof. Dr. Soaad Mohamed from the Botany Department. The plant's voucher specimen was deposited in the herbarium of Faculty of Pharmacy of Cairo University with a voucher number of (C 1.12.2022).

2.2. Essential oil isolation

Fresh parts of CR (1000 g) were subjected to hydrodistillation method using a medium scale Clevenger-type apparatus [21] for 3 hrs. The volatile sample was dried over anhydrous sodium sulphate and kept in a refrigerator at 4-6 °C.

2.3. GC/MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted utilizing a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA) integrated with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The analytical column was TR-5 MS (30 m x 0.32 mm i.d., 0.25 μm). Helium was employed as the carrier gas at a flow rate of 1.0 mL/min. Sample was injected with a split ratio of 1:10. The temperature program involved an initial 1-minute hold at 60°C, followed by a ramping over 55 min at 4.0°C/min to 240°C, where it was maintained for one minute. At 210 ^{o}C, the injector and detector

were maintained. Diluted samples (1:10 hexane, v/v) were prepared and 1 µL was injected. Using a spectral range of *m/z* 40-450, mass spectra were produced by electron ionisation (EI) at 70 eV.The spectra underwent deconvolution utilizing AMDIS software (available at www.amdis.net) and were characterized by their retention indices (relative to n-alkanes C8-C22) and mass spectrum alignment with the Wiley spectral library collection and NIST library database.

2.4. Headspace GC/MS for volatile analysis

Headspace GC/MS analysis was done using Shimadzu GCMS-QP2020 (Tokyo, Japan) and Shimadzu HS-20. Ten grams of the fresh and crushed root sample were placed into a 20 mL headspace vial which was immediately sealed with silicone rubber septa and aluminum caps. The sample vial was heated at 80 °C for 20 minutes with agitation. Subsequently, the headspace was directly injected into the GC injector, with the loop and transfer line temperatures set at 150 °C.The GC was equipped with Rtx-1MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) and a split–splitless injector. After being maintained at 45 °C for two minutes, the temperature of the column was programmed to rise to 300 °C at a rate of 5 °C per minute then kept constant for five minutes. Injector temperature was 250 °C. The carrier gas was helium, flowing at a rate of 1.41 mL/min. All mass spectra were recorded with the following criteria in place: (Equipment current): ion source temperature of 200 °C; ionisation voltage of 70 eV; filament emission current of 60 mA. By comparing the fragmentation pattern with those documented in the Wiley and NIST Mass Spectral Library data, numerous constituents could potentially be identified.

2.5. Cell culture

The cytotoxic activity bioassay of CZO was evaluated against two human cancer cell lines, lung (A549) and hepatocellular (HepG2) obtained from Bioassay-Cell Culture Laboratory, National Research Center, Cairo, Egypt. Cells were suspended in Dulbecco's Minimum Essential Medium (DMEM) for A549 and Roswell Park Memorial Institute (RPMI) medium for HepG2 supplemented with 1% antibiotic-antimycotic mixture (10,000 U/ml potassium penicillin, 10,000 µg/mL streptomycin sulfate and 25 µg/mL Amphotericin B) (Sigma) and 1% L-glutamine (Sigma) at 37 ºC under 5% CO_2. After batch culture for 10 days, cells were seeded into 96-well microtiter plastic plates at 37 ^{o}C for 24 hours at a concentration of 10x103 cells/well in fresh complete growth mediaunder 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). A laminar flow cabinet biosafety class II level(Baker, SG403INT, Sanford, ME, USA) was used as a sterile area for all aforementioned procedures.

2.6. Cytotoxicity assay

The mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma) to purple formazan was used to measure the vitality of the cells [22]. The media was removed, and fresh medium (without serum) was introduced. Cells were then incubated either alone (as a negative control) or with varying concentrations of CZO, resulting in a final concentration range of 100 to 3.125 µg/mL. Following a 48-hour incubation period, the media was aspirated, and 40 µL of MTT salt (2.5 µg/mL) was added to each well, followed by additional four-hour incubation under 5% CO2 at 37°C. 200 µL of 10% sodium dodecyl sulfate (SDS) in deionized water was added to each well and incubated overnight at 37°C to terminate the reaction and dissolve the formed crystals. Doxorubicin was used as positive control [23], [24]. The experiments were conducted in triplicate, and the absorbance was

subsequently assessed using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm, with a reference wavelength set at 620 nm. The plant extracts were dissolved in DMSO, resulting in a final concentration on the cells of less than 0.2%. The percentage change in viability was determined using the formula: ((Reading of extract / Reading of negative control) - 1) x 100.

2.7. Statistical analysis

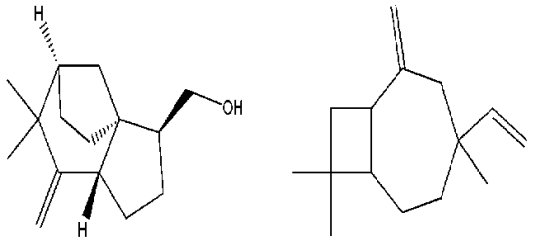
Independent t-test by SPSS 11 program was used to assess a statistical significance between sample and negative control (cells with vehicle). Additionally, probit analysis was performed to determine the IC50 using the SPSS 11 program.

3. Results and discussion

3.1. Chemical composition

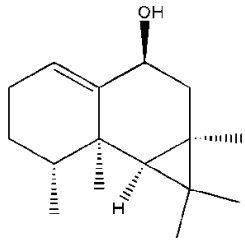
Vetiver has been recently acclimatized in Egypt; therefore it was interesting to compare the chemical composition of its oil to those reported in literature from different geographical regions. Additionally, we wanted to understand the effect of hydrodistillation on the volatile constituents of the CZO by comparing hydrodistilled oil (HDO) to the fresh sample aroma (FSA) utilizing headspace technique (HS). HS analysis provides an accurate profile of the natural aroma of the plant as it is applied to fresh samples at lower temperature compared to hydrositllation. Moreover, HS is simple, rapid and solventless process [25].In contrast to HS, hydrodistilled oil is prone to contain many artifacts due to decomposition of molecules by effect of heat, oxidation and hydrolysis. Only one study had utilized HS to study composition of commercial vetiver oil[26].Other studies focused on injection of diluted hydrodistilled oil. These studies were recently reviewed[27].To the best of our knowledge, no previous study analysed the composition of the fresh plant volatiles. Qualitatively, 58and 48 volatile compounds were identified in the fresh sample aroma and the distilled oil, respectively (**Table S1**). The identified compounds belong to six chemical classes, namely, monoterpenes, oxygenated monoterpenes, sesquiterpenes, sesquiterpene ethers, sesquiterpene alcohols and sesquiterpene ketones. Only one sesquiterpene aldehyde, isovalencenal, was detected in HDO. As shown in **Table S1**, sesquiterpene hydrocarbons were the chief components of HS aroma amounting to ca. 79.7% of the total aroma followed by sesquiterpene alcohols (14.14%) then sesquiterpene ketones (2.12%) and finally sesquiterpene ethers (1.3%). Other chemical classes represented less than 1% of the total HS aroma. In contrast, sesquiterpene alcohols were the chief constituents of HDO amounting to ca. 63.44% followed by sesquiterpene ethers (8.43%) then sesquiterpene ketones (8.12%). Sesquiterpene hydrocarbons represented only 2.47% in HDO vs 79.7% in HS. These findings indicated that hydrodistillation resulted in oxidation and isomerization of vetiver volatile constituents. Only five compounds were found in both HS and HD, namely, limonene, α-guaiene, α-muurolene, cedr-8-en-13-ol and khusimol. The sesquiterpene hydrocarbon, bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl, was the major constituent of the FSA (14.17%) followed by sativene (11.52%) then valencene (9.71%) and allo-aromadendrene (5%), while cedr-8-en-13-ol was the main sesquiterpene alcohol (5.74%). In the HDO sample, khusimol was the major constituent (16.48%) followed by aristol-1(10)en-9-ol (12.5%), then cyclocopacamphenol (7.69%). These three components constituted more than one third of the HDO composition indicating close similarity between the Egyptian CZO and the worldwide commercial

chemotype [27]. Sesquiterpene ethers were the second major chemical class in HDO with aristolene epoxide and ç-Gurjunenepoxide-(2) identified as the major components of this class at 3 and 2.25%, respectively. The major ketones in HDO were alpha-vetivone, khusimone and epizizanone, which were commonly found in vetiver oil samples [27]. The presence of vetivone indicated that Egyptian sample was similar to the worldwide commercial chemotype where Alpha-vetivone and khusimone are the major contributors to CZO aroma and are often considered parts of its fingerprint but [27]. The major hydrocarbons in HDO was alpha-guaiene which representing (0.94%) compared to previously investigated Chinese samples [28], Egyptian CZO had more constituents, 48 *vs* 21 components. Moreover, the composition of the two oils was different on both qualitative and quantitative levels. Chinese CZO contained cedr-8-en-13-ol (26.54%), guaiene (15.31%) and cycloisolongifolene (11.09%) as the major components [28], while khusimol (16.48%), aristol-1(10)en-9-ol (12.5%) and cyclocopacamphenol (7.69%) were the major components of Egyptian oil sample. Compared to several oil samples worldwide, sesquiterpene alcohols were the major class in Egyptian (63.44%) , Javanese (47.6%) , Indian (47%), and Chinese (41%) samples [20]. However, while sesquiterpene ethers were the second major chemical class in Egyptian CZO (8.43%), they were not detected in Javanese, Indian and Chines samples[20]. [27]indicated that the ratio of sesquiterpene alcohols in CZO varies between 18-49%, however our investigation detected higher ratios of this class at 63%. Sesquiterpene hydrocarbons represented approximately 2.5% of HDO similar to aldehydes in this study, but their ratio has generally fallen between 17-28% in CZO[27].The major hydrocarbons detected in Egyptian sample were alpha-guaiene, alpha-vatirenene and alpha-muurolene while beta-vetivenene, beta-vetispirene and alpha-amorphene were the major hydrocarbons in other worldwide oil samples [20]. No sesquiterpene acids or esters were detected in the HDO similar to Javanese and Indian samples where acids were not detected and esters were detected as minor constituents in some [20]. **Figure 1** showed the structures of major compounds identified in CZO.

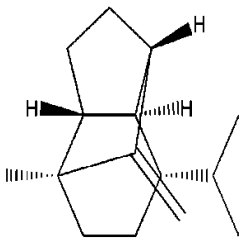


Khusimol (16.48%)

Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl (14.17%)



Aristol-1(10)-en-9-ol (12.51%)



Sativene (11.52%)

Figure 1: Major compounds identified in *C. zizanioides* root oil.

3.2. Cytotoxic activity

In the present study, we determined the cytotoxicity effect of CZO against two human cancer cell lines A549 and HepG2 using MTT assay and in comparison with drug doxorubicin In general, CZO showed moderate activity as seen in **Table S2, Figure S1**. These results indicated moderate cytotoxic effect of the oil sample against A549 and HepG2 cells with IC_{50} value of 32.16 and 37.63 µg/mL respectively. Meanwhile, doxorubicin showed better activity against A549 and HepG2 cells with IC_{50} value of 28.30 and 21.60 µg/mL respectively. Carvone, the major monoterpene found in HDO (7.07%) was reported to detoxify glutathione *S*-

transferase enzyme in liver and lung cancer cells [29] as well as inhibit nitrosamine carcinogenesis in pulmonary adenoma [30]. Also sativene, one of the major non-oxygenated sesquiterpenes identified in HS (11.52%) was reported as an inhibitory agent against lung cell line (A549) with IC_{50} value of 33.2 µmol [31]. Sesquiterpene alcohols, the major compounds identified in HDO sample were previously reported to induce cytotoxicity in HepG2 cells via a Fas- and mitochondrial-related pathway [32]. More generally, sesquiterpenes have demonstrated their efficacy against A594 and HepG2 cell lines [33]. Another study reported that sesquiterpenes caused cytotoxicity on A594 and HepG2 cells with IC_{50} values of 110.5 and 120.8 µg/ml respectively [34].

4. Conclusions

The present work details the chemical compositions of CZO obtained by hydrodistillation of Egyptian *Chrysopogon zizanioides* roots in comparison to its fresh aroma. We concluded that CZO is enriched in sesquiterpene compounds but alcohols represent the major constituents of HDO probably due to oxidation during the hydrodistillation process. CZO demonstrated anti-proliferative effects on human lung adenocarcinoma (A549) and human hepatocarcinoma (HepG2) cells. Further investigation of the molecular mode of action of CZO and its constituents is necessary in order to enable their future use as potential adjuvant therapy against certain types of cancers.

5. Conflicts of interest

There are no conflicts to declare.

6. Funding resources

This work did not receive any grants fromfunding agencies in the public, commercial, or nonprofit sectors.

7. References

[1] C. Rao *et al.*, "KSR1-and ERK-dependent translational regulation of the epithelial-to-mesenchymal transition," *Elife*, vol. 10, p. e66608, 2021.
[2] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2020," *CA. Cancer J. Clin.*, vol. 70, no. 1, pp. 7–30, 2020.
[3] A. Saini, M. Kumar, S. Bhatt, V. Saini, and A. Malik, "Cancer causes and treatments," *Int. J. Pharm. Sci. Res.*, vol. 11, no. 7, pp. 3121–3134, 2020.
[4] C. Allemani *et al.*, "Global surveillance of cancer survival 1995–2009: analysis of individual data for 25 676 887 patients from 279 population-based registries in 67 countries (CONCORD-2)," *Lancet*, vol. 385, no. 9972, pp. 977–1010, 2015.
[5] D. Chae, E. Ban, Y. S. Yoo, E. E. Kim, J. Baik, and E. J. Song, "MIR-27a regulates the TGF-β signaling pathway by targeting SMAD2 and SMAD4 in lung cancer," *Mol. Carcinog.*, vol. 56, no. 8, pp. 1992–1998, 2017.
[6] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA. Cancer J. Clin.*, vol. 68, no. 6, pp. 394–424, 2018.
[7] J. L. Petrick *et al.*, "International trends in hepatocellular carcinoma incidence, 1978–2012," *Int. J. cancer*, vol. 147, no. 2, pp. 317–330, 2020.
[8] D. J. Newman, G. M. Cragg, and K. M. Snader, "Natural products as sources of new drugs over the period 1981– 2002," *J. Nat. Prod.*, vol. 66, no. 7, pp. 1022–1037, 2003.
[9] S. Vinogradov and X. Wei, "Cancer stem cells and drug resistance: the potential of nanomedicine," *Nanomedicine*, vol. 7, no. 4, pp. 597–615, 2012.
[10] S. Damery *et al.*, "The use of herbal medicines by people with cancer: a cross-sectional survey," *Br. J. Cancer*, vol. 104, no. 6, pp. 927–933, 2011.
[11] G. Gao, Z. Lu, H. Xu, X. Zheng, and Y. Yang, "Chemical constituents from the aerial parts of Vetiveria zizanioides," *Chem. Nat. Compd.*, vol. 48, pp. 128–129, 2012.
[12] P. Burger *et al.*, "Vetiver Essential Oil in Cosmetics: What Is New?," *Medicines*, vol. 4, no. 4, p. 41, 2017.
[13] A. Pareek and A. Kumar, "Ethnobotanical and pharmaceutical uses of *Vetiveria zizanioides* (Linn) Nash: a medicinal plant of Rajasthan," *Int J Life Sci Pharm Sci*, vol. 50, pp. L12–L18, 2013.
[14] B. Bhushan, S. S. Kumar, S. Tanuja, S. Lalit, and A. Hema, "*Vetiveria zizanioides* (Linn.) Nash: a pharmacological overview," *Int Res J Pharm*, vol. 4, no. 7, pp. 18–20, 2013.
[15] T. A. S. Oliveira *et al.*, "Antibacterial, antiparasitic, and cytotoxic activities of chemical characterized essential oil of *Chrysopogon zizanioides* roots," *Pharmaceuticals*, vol. 15, no. 8, p. 967, 2022.
[16] M. Hanifa, R. Wulandari, U. M. Zulfin, E. P. Nugroho, S. Haryanti, and E. Meiyanto, "Different Cytotoxic Effects of Vetiver Oil on

Three Types of Cancer Cells, Mainly Targeting CNR2 on TNBC," *Asian Pacific J. cancer Prev. APJCP*, vol. 23, no. 1, p. 241, 2022.
[17]C. N. Powers *et al.*, "Antifungal and cytotoxic activities of sixty commercially-available essential oils," *Molecules*, vol. 23, no. 7, p. 1549, 2018.
[18]S. S. Fadhillah, A. Q. Aini, S. Mualifah, A. Bowolaksono, and A. Dwiranti, "Effects of *Vetiveria zizanioides* on Cytotoxicity of Human Epithelial Cervical Cancer Cells," *J. Hunan Univ. Nat. Sci.*, vol. 49, no. 2, 2022.
[19]J. J. Filippi, E. Belhassen, N. Baldovini, H. Brevard, and U. J. Meierhenrich, "Qualitative and quantitative analysis of vetiver essential oils by comprehensive two-dimensional gas chromatography and comprehensive two-dimensional gas chromatography/mass spectrometry," *J. Chromatogr. A*, vol. 1288, pp. 127–148, 2013.
[20]M. Grover, T. Behl, T. Virmani, S. Bhatia, A. Al-Harrasi, and L. Aleya, "*Chrysopogon zizanioides*—a review on its pharmacognosy, chemical composition and pharmacological activities," *Environmental Science and Pollution Research*, vol. 28, no. 33. Springer, pp. 44667–44692, 2021.
[21]E. Pharmacopoeia, "The english text of Arab Republic of Egypt," *Minist. Heal. Popul. 4th Ed. Cent. Adm. Pharm. Aff. Minist. Heal. Popul.*, p. 1295, 2005.
[22]T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *J. Immunol. Methods*, vol. 65, no. 1–2, pp. 55–63, 1983.
[23]M. I. Thabrew, R. D. Hughes, and I. G. McFarlane, "Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay," *J. Pharm. Pharmacol.*, vol. 49, no. 11, pp. 1132–1135, 1997.
[24]B. S. El-Menshawi, W. Fayad, K. Mahmoud, S. M. El-Hallouty, and M. El-Manawaty, "Screening of natural products for therapeutic activity against solid tumors," *Indian J. Exp. Biol.*, vol. 48, pp. 258–264, 2010.
[25]Z. Triaux, H. Petitjean, E. Marchioni, D. Steyer, and C. Marcic, "Comparison of headspace, hydrodistillation and pressurized liquid extraction of terpenes and terpenoids from food matrices—Qualitative and quantitative analysis," *J. Anal. Chem.*, vol. 76, pp. 284–295, 2021.
[26]A. Nikiforov, G. Buchbauer, L. Jirovetz, B. Remberg, and G. Remberg, "Headspace constituents of vetiver oil," *Zeitschrift für Naturforsch. B*, vol. 47, no. 3, pp. 439–440, 1992.
[27]E. Belhassen, J. Filippi, H. Brévard, D. Joulain, and N. Baldovini, "Volatile constituents of vetiver: A review," *Flavour Fragr. J.*, vol. 30, no. 1, pp. 26–82, 2015.
[28]A. David *et al.*, "Chemical composition, antioxidant, and antimicrobial activities of *Vetiveria zizanioides* (L.) nash essential oil extracted by carbon dioxide expanded ethanol," *Molecules*, vol. 24, no. 10, p. 1897, 2019.
[29]G. Zheng, P. M. Kenney, and L. K. T. Lam, "Anethofuran, carvone, and limonene: Potential cancer chemopreventive agents from dill weed oil and caraway oil," *Planta Med.*, vol. 58, no. 4, pp. 338–341, 1992.
[30]L. W. Wattenberg, V. L. Sparnins, and G. Barany, "Inhibition of N-Nitrosodiethylamine Carcinogenesis in Mice by Naturally Occurring Organosulfur Compounds and Monoterpenes," *Cancer Res.*, vol. 49, no. 10, pp. 2689–2692, 1989.
[31]H.-L. Ai, M.-S. Yang, S.-H. Zi, and H.-C. Guo, "Three new sativene sesquiterpenoids from cultures of endophytic fungus *Bipolaris eleusines*," *J. Asian Nat. Prod. Res.*, vol. 17, no. 10, pp. 982–987, 2015.
[32]W. Chen *et al.*, "α-Bisabolol induces dose-and time-dependent apoptosis in HepG2 cells via a Fas-and mitochondrial-related pathway, involves p53 and NFκB," *Biochem. Pharmacol.*, vol. 80, no. 2, pp. 247–254, 2010.
[33]L. Chen, Y. Liu, Y. Li, W. Yin, and Y. Cheng, "Anti-Cancer Effect of Sesquiterpene and Triterpenoids from Agarwood of *Aquilaria sinensis*," *Molecules*, vol. 27, no. 16, p. 5350, 2022.
[34]C. Wang, L. Wang, C. Li, C. Hu, and S. Zhao, "Anti-proliferation activities of three bioactive components purified by high-speed counter-current chromatography in essential oil from ginger," *Eur. Food Res. Technol.*, vol. 246, pp. 795–805, 2020.