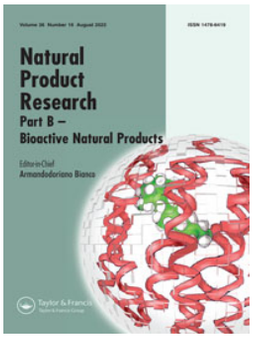

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Fungal biotransformation of carvone and camphor by *Aspergillus flavus* and investigation of cytotoxic activities of naturally obtained essential oils

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Fungal biotransformation of carvone and camphor by *Aspergillus flavus* and investigation of cytotoxic activities of naturally obtained essential oils

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ABSTRACT

In this study, the biotransformation of carvone and camphor by *Aspergillus flavus* and the products were investigated. The biotransformation reaction of carvone by *A. flavus* resulted in the production of neodihydrocarveol, dihydrocarvone, 2-cyclohexene-1-one,2-methyl-5-(1-methylethenyl), limonene-1,2-diol, trans-p-mentha-1(7),8-dien-2-ol, p-menth-8(10)-ene-2,9-diol, and the biotransformation reaction of camphor resulted in the production of 2 -campholenic acid, 2-cyclohexene-1-one,2-hydroxy-4,4,6,6-tetramethyl, α-campholene aldehyde. The naturally produced essential oils by biotransformation of carvone and camphor were observed to be cytotoxic to breast cancer cells while no significant inhibition was seen in the healthy cell line. Additionally, biotransformation products had the highest inhibition (74%) against aflatoxin B1. The bioactivities of biotransformation products are promising, and they can be further investigated for their therapeutic potential as active agents.

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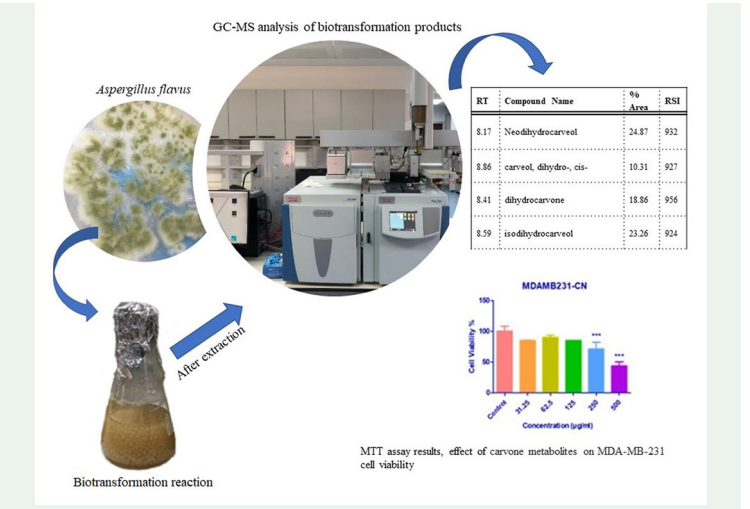

1. Introduction

Plants synthesize many organic compounds and metabolites which are linked with vital activities such as growth, cell division, respiration, photosynthesis, reproduction, and storage. Metabolites involved in basic activities are primary metabolites whilst those which are involved in other functions are secondary metabolites (Hussain et al. 2012). Secondary metabolites are also known as 'specialized metabolites', 'secondary products', or 'natural products' (Demain and Fang 2000). The value of secondary metabolites has not been sufficiently understood for many years, i.e. they were considered the final product of primary metabolism or metabolic waste, and were thought to have no specific function. However, in recent years their important role in the treatment of many diseases has been well established and studies are increasing (Ghasemi et al. 2019). Terpenes occupy a large place in this group of compounds.

Terpenes are a hydrocarbon group with the formula $(C_5H_8)n$ found in the structure of plants which constitute the largest and most diverse class of secondary metabolites. Terpenes consist of units having five carbons that are called isoprene (Huang et al. 2012). They have various therapeutic effects and can easily show their biological activities by crossing the cell membrane; therefore, terpenes can be used in modern pharmaceutical manufacturing (Bhatla 2018).

Commercially useful chemical building blocks and pharmaceutical stereoisomers can be produced by the biotransformation of terpenes. Biotransformation provides a method of producing regioselective and stereoselective compounds under temperate conditions, while the biotransformation products are still considered 'natural'. Enzymes and extracts originated from bacteria, cyanobacteria, microalgae, fungi, and plants are used for the biotransformation of terpenes (de Carvalho and da Fonseca 2006a). Another purpose of biotransformation is to give activity to a molecule that does not show biological activity (Abdella et al. 2018). The biotransformation of plant-derived molecules provides a variety of potential anticancer products which are generally less

toxic to healthy cells when compared to conventional chemotherapy (Muhamad and Na-Bangchang 2020).

Terpenes and terpenoids, along with their microbially biotransformed products, are broadly evaluated for their antitumor, antimicrobial, anti-inflammatory, antihyperglycemic effects, and potential medical uses. In this regard, carvone and camphor are two promising terpene compounds. Carvone has both antibacterial and antifungal activity, and it has proven to be effective against many pathogens such as *Aspergillus niger, Saccharomyces cerevisiae, Candida albicans, Campylobacter jejuni, Listeria monocytogenes, Enterococcus faecium, Escherichia coli* (de Carvalho and da Fonseca 2006b). Likewise, the antitumor activity of carvone has been shown against breast cancer cells, e.g. R-(-)-carvone was shown to potentiate doxorubicin's antitumor activity *in vitro* (Patel and Thakkar 2014; Abbas et al. 2020). Camphor has also been shown to be a promising molecule in medical applications and is widely used in traditional medicine (Zuccarini 2009; Zielińska-Błajet and Feder-Kubis 2020). Besides its significant antimicrobial effects, the antitumor activity of camphor is under investigation. It was shown that with the topical application of camphor to a mouse model of non-melanoma skin cancer, the total tumor burden was observed to decrease by 90% (Greenberg et al. 2016).

As camphor and carvone have anti-tumor activities, the compounds derived from them can also have similar, or even stronger effects. A study showed that a ferrocene-containing camphor sulfonamide molecule has a cytotoxic effect on breast cancer (Schröder et al. 2019). In another study, the biological effects were determined of (R)-(+)-limonene and (-)-alpha-pinene, a derivative of carvone, biotransformation products on human colon tumor cells and normal colonic epithelium cells, and normal colonic epithelium cells, reporting that the obtained biotransformation showed their *in vitro* effects against tumor cells at lower concentrations when compared to their monoterpene precursors (Paduch et al. 2016).

In this study, two natural essential oil mixtures were obtained by the biotransformation of carvone and camphor by *Aspergillus flavus*. The obtained essential oils were investigated for their aflatoxin inhibitory activities along with cytotoxic effects against a human breast cancer cell line. This method for obtaining the biotransformation products of *A. flavus* is a promising alternative to the challenging methods of classical organic synthesis.

2. Results and discussion

2.1. Analytical results by GC-MS

Aspergillus species are widely used fungi in biotransformation studies, especially in the biotransformation of terpenes and steroids. In a previous study, Okuno et al. investigated the biotransformation of (+)-isofraxinellone by *Aspergillus niger* (Okuno et al. 2019). In their study, isofraxinellone was transformed into only one compound and they identified that the structure of the compound was (-)-(4S)-4-hydroxyisofraxinellone. In another study, microbial transformation of 20(R)panaxatriol by the fungus *Aspergillus flavus* was performed (Li et al. 2019). The obtained compounds after biotransformation were determined to be 3,6-dioxo-22β-hydroxyl-20(*R*)-panaxatriol, 3,6,23-

trioxo-20(*R*)-panaxatriol, a hydroxylated derivative of 20(*R*)-panaxatriol, and 24α-hydroxy- 20(*R*)-panaxatriol. In addition, the cytotoxic effects of the metabolites on the growth of human cancer cell lines (K562/ADR, Du-145, Hela, MCF-7, and HepG2) were evaluated. Among them, 15β-hydroxy-20(*R*)-panaxatriol (**4)** significantly inhibited the proliferation of human leukemic progenitor cells K562/ADR by arresting cell cycle.

In this study, the GC-MS total ion chromatograms of the carvone and camphor fungal biotransformation extracts are shown in the supplementary file. The identification of the compounds was done by comparing the mass spectra with the corresponding standard in the NIST library data on GC-MS. The monoterpenes of biotransformed products of carvone by *A. flavus* are listed in Table S1. As seen in the table, the monoterpenes of biotransformation products of carvone were neodihydrocarveol (T: 8.17), dihydrocarvone (RT:8.41), 2-cyclohexene-1-one,2-methyl-5-(1-methylethenyl) (RT:9.18), limonene-1,2-diol (RT: 11.3), trans-p-mentha-1(7),8-dien-2-ol (RT: 14.08), p-menth-8(10)-ene-2,9-diol (RT:14.40). The monoterpenes of biotransformed products of camphor by *A. flavus* are listed in Table S2 and they are α-campholenic acid (RT:11.2), 2-cyclohexene-1-one,2-hydroxy-4,4,6,6-tetramethyl (RT: 11.8), α-campholene aldehyde (RT:12.8).

2.2. Metabolic pathways

Adding a hydroxy group to an aromatic or nonaromatic ring skeleton by organic synthesis is a multi-step process. However, with biotransformation, this process can be done in one step (Sultana and Saify 2013; Winkler et al. 2021). Biotransformation has a number of advantages when compared with the corresponding chemical methods. Many biotransformations not only are regio- and stereospecific but are also enantiospecific, allowing the production of chiral products from racemic mixtures. The conditions for biotransformations are mild and in the majority of cases, they do not require the protection of pre-existing functional groups (Hegazy et al. 2015).

A possible biotransformation pathway is proposed in Figures 1 and 2 based on the structures of the metabolites obtained. In Figure 1, it is seen that mostly hydrogenation reaction occurs, considering the structures of the metabolites obtained as a result of the biotransformation of carvone. **2** and **7** were formed by hydrogenation of the double bonds of the carvone molecule from the A and F routes, while **3** was formed by the hydrogenation of the carbonyl group of its B route (Maczka et. al., 2018; Santos et al. 2018). **4** was formed by dehydrogenation of **3** from the C route, and it is seen that **5** was formed by acetylation of **4** from the D route. As a result of the hydroxylation reaction of **1** and **3** at different positions, the conversion of **6** and **8** occurred from the E and G routes. It is also seen that the carbonyl group turns into a hydroxyl group with the formation of **8**.

In the proposed biotransformation pathway of camphor (**9**) (Figure 2), it is seen that a series of rearrangement reactions take place in addition to the classical organic reactions. In the transformation of **10**, the bond of the carbonyl group with the bridgehead carbon was broken and it was converted to carboxylic acid as a result of an oxidation reaction, while on the other hand, a double bond was formed as a result of dehydrogenation from the same bridgehead carbon by the H route. After this reaction, it was converted to **11**, which contains aldehyde, following the I route by

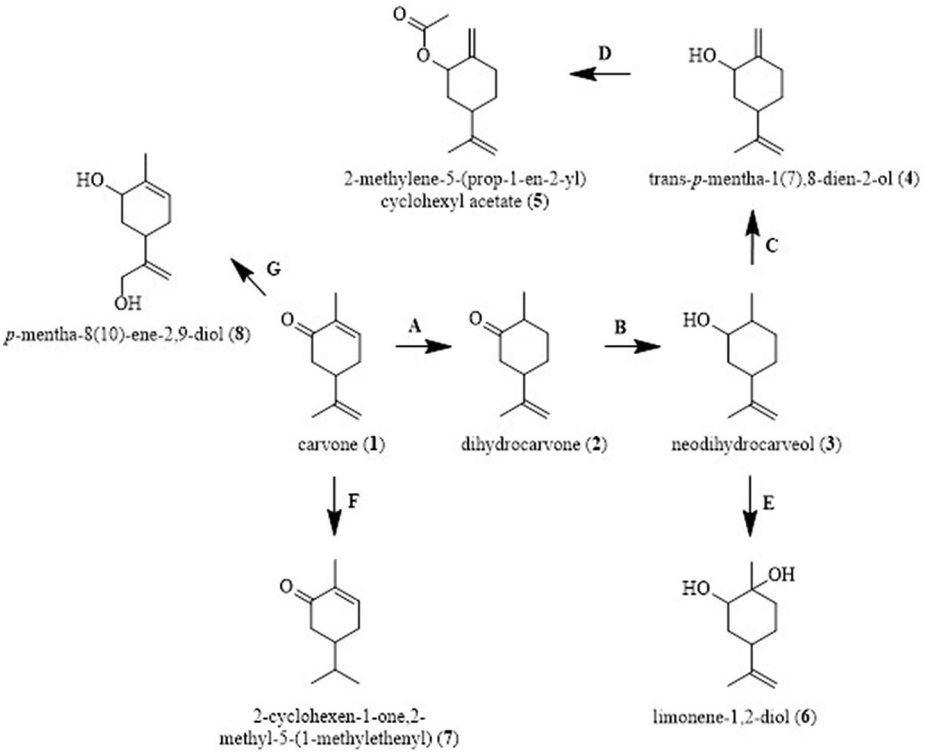

Figure 1. Possible pathway for the biotransformation of carvone by *Aspergillus flavus*.

reduction of the carboxylic acid group. It can be said that the biotransformation pathway is more complex for **12** (Nakahashi and Miyazawa 2011). Because, in this transformation, reactions such as bond breaking of the bridgehead carbons, repositioning of the methyl groups as a result of the rearrangement reaction, and hydroxylation and dehydrogenation reactions from the alpha position take place. When the structures of the compounds obtained as a result of biotransformation of *d*-limonene in the study by Houjin et al. are examined, it is seen that these transformations follow reactions such as hydrogenation, hydroxylation, and cleavage of C-C bonds (Demirci et al. 2001; Houjin et al. 2006; Eaton and Sandusky 2009). The results obtained are coherent with the present study, and the transformations proceed through similar reactions.

2.3. Aflatoxin inhibitory activities

The results against the absorption of the control group were calculated and the varying fluorescence intensities due to aflatoxin inhibition are given in Figure S13. The essential oil, which was obtained by biotransformation of camphor, exhibited the strongest anti-aflatoxigenic activity with low absorbance in this assay. These results showed that essential oil biotransformation products had the highest inhibition (74%) against aflatoxin. It should be borne in mind that there is a gradual increase in inhibition due to the increased concentration of the essential oils tested.

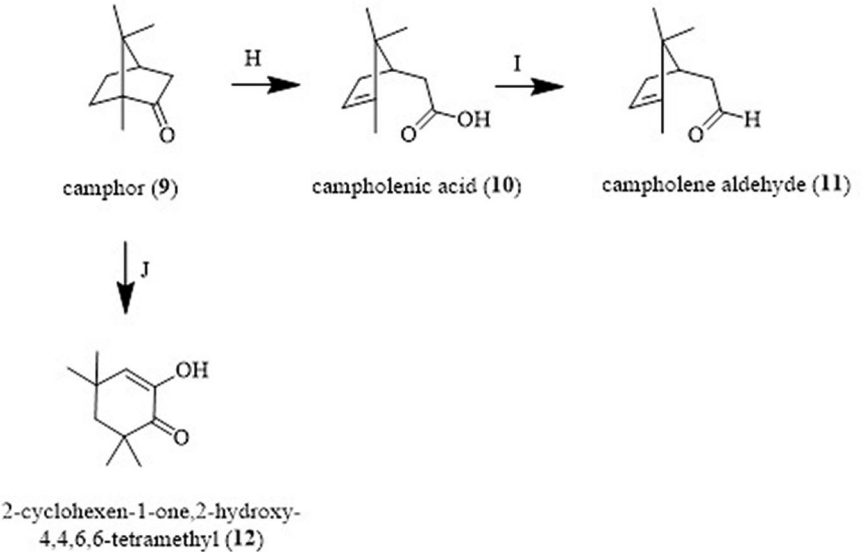

Figure 2. Possible pathway for the biotransformation of camphor by *Aspergillus flavus*.

Natural products including plant extracts have been identified as potential inhibitory candidates active against aflatoxin (Deabes et al. 2011). Deabes et. al. showed that each essential oil fraction showed significant inhibition against the production of *A. flavus*-originated AFB1.

2.4. Determination of cytotoxic activity

According to the MTT cytotoxicity test results, the metabolites were not toxic to the healthy cells, and although not too strong, the metabolites were found to be more cytotoxic to the cancer cells. The obtained essential oil by carvone biotransformation (CN) showed moderate but statistically significant cytotoxicity against MDA-MB-231 breast cancer cells (Figure S14). CN was observed to be active in terms of cytotoxicity against breast cancer cells showing an IC_{50} value of 446.9 μg/ml while the IC_{50} value recorded for carvone standard (CNS) was >500 μg/ml. 43.62% cell viability in the breast cancer cell line was determined after CN treatment while no inhibition was seen in the healthy cell line. Accordingly, the most active substance in terms of cytotoxic activity was CN (inhibiting cell viability 56.38%). In a study investigating L-carvone's effects on breast cancer cell lines, it was found that the active substance inhibited cell migration and cell proliferation of MDA-MB-231 cells (IC_{50} 1.0 mM) and induced apoptosis along with oxidative stress and genotoxicity, and our results are in accordance with their results (Patel and Thakkar 2014).

The obtained essential oil by camphor biotransformation (CM) at the tested high concentrations also showed moderate but statistically significant cell viability inhibition in the breast cancer cell line while not affecting the viability in the healthy cell line, although the IC_{50} values for CM and camphor standard (CMS) were both found to be >500 μg/ml. Furthermore, CM inhibited cell viability in the 250 μg/ml group in breast cancer cells while CMS did not have a significant effect, namely showing an inhibitory

effect on cell viability at a lower concentration. A recent *in vivo* study investigating the effects of camphor white oil indicates tumor regression through cytotoxic T cell-dependent mechanisms (Moayedi et al. 2019). Considering these results, the produced metabolites in this study can be potential therapeutic alternatives suitable for further *in vivo* studies. Indeed, *in vitro* cytotoxicity studies are mandatory for proceeding to *in vivo* and clinical practices which are necessary for safety assessment and screening of substances. They are widely used for the purpose of investigating the bioactivities of isolated and synthesized substances, providing information for research and development (Tolosa et al. 2015). In this study, the experiment results showed that the metabolites are not toxic to the healthy cell line having the potential to be used for therapeutic purposes. The evaluation results of the metabolite's potential for being alternative anticancer agents to see if the metabolites were stronger compared to the standard molecules are given. Hereby, the metabolites' cytotoxic effects against breast cancer cells were observed to be slightly higher when compared to the standards' effects.

3. Experimental

3.1. Instrument and reagents

The fungal strain *Aspergillus flavus Link var. flavus (ATCC® 22547)* was purchased from American Type Culture Collection. Carvone and camphor were purchased from Sigma Corporation, USA. Dimethyl sulfoxide (DMSO) and ethyl acetate were from Merck. Cell culture reagents were from Gibco, USA. MTT reagent was from Biomatik, CA.

The analysis of the biotransformation products was carried out using gas chromatography-mass spectrometry (GC-MS). The GC-MS apparatus was equipped with Thermo Scientific, 6890 N Network GC System, 5973 Network Mass Selective Detector, 7683 Series Injector, and G1701 DAMSD Chemstation. The microplate reader BioTek, Synergy H1 was used for the cytotoxicity analysis of the products.

3.2. Culture media

Potato Dextrose Agar, for the growth of *Aspergillus flavus*, contained: potato extract (4 g/L), agar (15 g/L), and D(+) glucose (g/L). Sabouraud %2 Dextrose Broth, for the biotransformation experiments, contained: peptone (10 g/L) and D(+) glucose (20 g/L). The liquid medium was autoclaved at 121 °C for 70 minutes.

3.3. Cell culture

MDA-MB-231 human breast cancer and CCD-1079Sk human healthy skin cells from ATCC were used. DMEM-F12 medium containing 10% FBS (fetal bovine serum) with 1% penicillin-streptomycin-amphotericin was used for cell culturing medium at 37 ◦C with 5% CO_2 and 92% humidity. Passaging the cells was with trypsinization when 70-80% confluency was reached.

3.4. Biotransformations

100 mL of medium was added to 250 mL erlenmeyer flasks in a sterile safety cabinet. Produced in a solid environment, *A. flavus* spores were added to each flask using a sterile loop. The erlenmeyers were incubated at 37 °C on a rotary shaker at 200 rpm. After 3 days, for each flask, 50 mg of camphor was dissolved in 1 mL of DMF and added to 3 flasks through a 0.22 μm membrane filter. At the same time, 50 mg of carvone was dissolved in 1 mL of DMF and added to the other 3 flasks through a 0.22 μm membrane filter. The flasks were taken back to an incubator for biotransformation and left to incubate for 3 days at 37° C in a 200 rpm shaker. The flasks were shaken in the incubator for 3 days for biotransformation and were removed from the incubator at the end of the 3rd day. The solutions in the flasks were divided into clean falcon tubes and were centrifuged. After centrifugation, filtering was done with filter paper.

3.5. Extraction of the metabolites

After biotransformation was completed, ethyl acetate was added to the filtered solutions twice their amount each time. The solution was shaken and extraction was performed, with the tap and lid of the separating funnel closed. After waiting for the separation of the phases for a while, the two phases were bottled separately. The ethyl acetate phase was evaporated using an evaporator. After the ethyl acetate was evaporated almost completely, the remaining solution was taken into the glass vial for chromatographic analysis.

3.6. GC-MS analysis

The structures of metabolites formed after biotransformation were revealed by GC-MS, an analytical chemistry method that uses gas chromatography and mass spectrometry properties together. GC-MS analyzes were performed with the Thermo Scientific Trace GC device in the Bezmialem Vakıf University Pharmaceutical R&D Center. Detector: Zebron Phase 2B-5 MS, Colon: 30 m x ID= 0.25 mm 0.25 μm were selected as. Temperature program: injector 250°C; detector 280°C; oven temperature start at 50°C, programmed from 50 to 250°C, hold for 3 min. Carrier gas: helium (1 mL/min), injection mod: splitless, injection volume: 1 μL, scan: m/z 50-350 (31) were determined. The samples were dissolved in dichloromethane and taken into vials and 1 μL was injected into the device.

3.7. Extraction of aflatoxin B1 from A. flavus cultures

Flasks with mycelia were filtered using pre-weighed Whatman filter no. 1 and then washed with distilled water. The mycelia were placed in falcon tubes and were allowed to dry at 40 °C for 24 h. The net dry weight of mycelia was determined**.** Then, dry mycelia were homogenized using a tissue homogenizer and then extracted by 100 mL of methanol: water (70:30). After filtration, the filtrate was used for the determination of aflatoxin B1.

3.8. Determination of aflatoxin B1 inhibition

Aflatoxin B1, produced by *Aspergillus flavus* is a toxic compound and takes place in the group 1 carcinogen list of IARC (Su et al. 2022). The aim of this study was to evaluate the growth and aflatoxin B1 production by *Aspergillus flavus* and to investigate the produced essential oils effects to aflatoxin B1 production. A Hitachi F-2700/F-2710 fluorescence spectrophotometer was used for fluorescence spectra recording of AFB1 with a scan rate of 1200 nm min^{-1}. The excitation and emission slits were both 5 nm and all measurements were performed in a quartz cell at room temperature. The excitation and emission wavelengths were 360 and 450 nm, respectively (Durmus et al. 2020).

The inhibition was determined according to the decrease of the fluorescence intensity due to aflatoxin B1 inhibition. The following equation was used for the calculation:

$$\% \text{ inhibition} = (\text{control} - \text{treatment}/\text{control} \times 100)$$

3.9. Determination of cytotoxic activities

Cytotoxic activity studies were performed on MDA-MB-231 human breast cancer and CCD-1079Sk human healthy skin cells. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used for measuring cell viability (Mosmann 1983). Before seeding the cell to the 96 well-plates, the trypan blue method (Strober 2001) was used to count the cells: An equal amount of 0.4% trypan blue dye was added onto cell suspension, and cells were counted using a hemocytometer under a microscope. 10^4 cells/well were seeded and incubated for 24 h in a 96 well plate before treatment with the test compounds. The cells were exposed to the obtained essential oils from the biotransformation process. After 24 h treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and the cells were kept in the dark for 3 h at 37 °C with 5% CO_2 and 92% humidity. After 3 h incubation, the formazan crystals were dissolved in 100 µL DMSO and the absorbance was measured at 590 nm. The percentage of cell viability was calculated by dividing the absorbance of the experimental groups by the solvent control cells. IC_{50} values were calculated by transferring the data of concentration-dependent cell viability to the Graphpad Prism program.

3.10. Statistical analysis

Experiments were conducted in triplicate. The results were given as the mean ± SD and the comparison of experimental groups was by the one-way ANOVA test followed by the Tukey's test with the GraphPad Prism Software. p-Value < 0.05 was accepted statistically significant.

4. Conclusion

Compared to classical chemical synthesis methods, the method used in this study provides many advantageous properties including microbial biotransformation, mild reaction conditions, regional and stereoselectivity, and environmental friendliness. Obtaining substances by biotransformation from carvone and camphor via classical organic synthesis follows very difficult methods, however, in this study, these compounds were obtained without using these challenging methods. Promising results were recorded according to the cytotoxicity and anti-aflatoxin studies of natural essential oil mixtures obtained by fungal biotransformation, and further research can be done for their therapeutic potential as active substances. Purification from the ethyl acetate extract and more detailed *in vitro* bioactivity studies, including the evaluation of each biotransformation product observed in the GC-MS analysis, that can potentially lead to *in vivo* experiments are planned in our future studies.

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Disclosure statement

The authors declare that they don't have any conflict of interest.

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References

Abbas MM, Kandil Yİ, Abbas MA. 2020. R-(-)-carvone attenuated doxorubicin-induced cardiotoxicity in vivo and potentiated its anticancer toxicity in vitro. Balkan Med J. 37(2):98–103.

Abdella A, El-Baz AF, Ibrahim IA, Mahrous EE, Yang ST. 2018. Biotransformation of soy flour isoflavones by *Aspergillus niger* NRRL 3122 β-glucosidase enzyme. Nat Prod Res. 32(20): 2382–2391.

Bhatla SC. 2018. Plant Physiology, Development, and Metabolism. Singapore: Springer. Chapter 33, Secondary metabolites; p. 1099–1166.

Deabes MM, A, El-Soud NH, El-Kassem LTA. 2011. Inhibition of growth and aflatoxin B1 production of strain (ATCC 16872) by various medicinal plant essential oils. Maced J Med Sci. 4(4): 345–350.

de Carvalho CCR, da Fonseca MMR. 2006a. Biotransformation of terpenes. Biotechnol Adv. 24(2): 134–142.

de Carvalho CCR, da Fonseca MMR. 2006b. Carvone: why and how should one bother to produce this terpene. Food Chem. 95(3):413–422.

Demain A, Fang A. 2000. The natural functions of secondary metabolites. Adv Biochem Eng Biotechnol. 69:1–39.

Demirci F, Kirimer N, Demirci B, Noma Y, Başera KHC. 2001. Screening of biotransformation products of carvone enantiomers by headspace-SPME/GC-MS. Z Naturforsch C J Biosci. 56(1–2): 58–64.

Durmus ZZ, Kurt B, Gazioglu I, Sevgi EK, Hancer C. 2020. Spectrofluorimetric determination of aflatoxin B1 in winter herbal teas via magnetic solid phase extraction method by using metal-organic framework (MOF) hybrid structures anchored with magnetic nanoparticles. Appl Organomet Chem. 34(3):e5375.

Eaton RW, Sandusky P. 2009. Biotransformations of 2-methylisoborneol by camphor-degrading bacteria. Appl Environ Microbiol. 75(3):583–588.

Ghasemi S, Habibi Z, Mohajeri M, Yousefi M. 2019. Biotransformation of two furanocoumarins by the fungi species *Aspergillus* sp. PTCC 5266 and *Aspergillus niger* PTCC 5010. Nat Prod Res. 33(6):835–842.

Greenberg SA, Moayedi Y, Jenkins BA, Marshall K, Owens DM, Lumpkin EA. 2016. 524 antitumor activity of camphor oil in non-melanoma skin cancer and its effects on keratinocyte homeostasis. J Invest Dermatol. 136(5):S93.

Hegazy MEF, Mohamed TA, ElShamy AI, Mohamed AH, Mahalel UA, Reda EH, Shaheen AM, Tawfik WA, Shahat AA, Shams KA, et al. 2015. Microbial biotransformation as a tool for drug development based on natural products from mevalonic acid pathway: a review. J Adv Res. 6(1):17–33.

Houjin L, Wenjian L, Chuanghua C, Yipin Z, Yongcheng L. 2006. Biotransformation of limonene by marine bacteria. Chin J Anal Chem. 34(7):946−−950.

Huang M, Lu J, Huang MQ, Bao J, Chen X, Wang Y. 2012. Terpenoids: natural products for cancer therapy. Expert Opin Investig Drugs. 21(12):1801–1818.

Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ, Saeed M. 2012. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci. 4(1):10–20.

Li JL, Ding P, Jiang B, Yang M, Ren J, Song Y, Chen G. 2019. Biotransformation of 20 (R)-panaxatriol by the fungus *Aspergillus flavus* Link AS 3.3950. Nat Prod Res. 33(10):1393–1398.

Mączka W, Sołtysik D, Wińska K, Grabarczyk M, Szumny A. 2018. Plant-mediated biotransformations of S(+)- and R(–)-carvones. Appl Sci. 8(12):2605.

Moayedi Y, Greenberg S, Jenkins B, Marshall K, Dimitrov L, Nelson A, Owens D, Lumpkin E. 2019. Camphor white oil induces tumor regression through cytotoxic T cell-dependent mechanisms. Mol Carcinog. 58(5):722–734.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65(1-2):55–63.

Muhamad N, Na-Bangchang K. 2020. Metabolite profiling in anticancer drug development: a systematic review. Drug Des Devel Ther. 14:1401–1444.

Nakahashi H, Miyazawa M. 2011. Biotransformation of (-)-camphor by *Salmonella typhimurium* OY1002/2A6 expressing human CYP2A6 and NADPH-P450 reductase. J Oleo Sci. 60(10): 545–548.

Okuno Y, Marumoto S, Tsurumi J, Miyazawa M. 2019. Biotransformation of (+)-isofraxinellone by *Aspergillus niger* and insect antifeedant activity. Nat Prod Res. 33(10):1518–1521.

Paduch R, Trytek M, Król SK, Kud J, Frant M, Kandefer-Szerszeń M, Fiedurek J. 2016. Biological activity of terpene compounds produced by biotechnological methods. Pharm Biol. 54(6): 1096–1107.

Patel P, Thakkar V. 2014. L-carvone induces p53, caspase 3 mediated apoptosis and inhibits the migration of breast cancer cell lines. Nutr Cancer. 66(3):453–462.

Santos RAMD, Souza FDO, Pilau EJ, Porto C, Gonçalves JE, Oliveira AJBD, Gonçalves RAC. 2018. Biotransformation of (+)-carvone and (−)-carvone using human skin fungi: a green method of obtaining fragrances and flavours. Biocatal Biotransform. 36(5):396–400.

Schröder M, Yusein-Myashkova S, Petrova M, Dobrikov G, Kamenova-Nacheva M, Todorova J, Pasheva E, Ugrinova I. 2019. The effect of a ferrocene containing camphor sulfonamide DK-164 on. Anti-Cancer Agents Med Chem. 19(15):1874–1886.

Strober W. 2001. Trypan blue exclusion test of cell viability. Curr Protoc Immunol. 21(1):A–3B.

Su C, Lu Y, Li J, Wang Y, Pan L, Zhang M. 2022. Effects of bile acids on aflatoxin B1 bioaccumulation, detoxification system, and growth performance of Pacific white shrimp. Food Chem. 371: 131169.

Sultana N, Saify ZS. 2013. Enzymatic biotransformation of terpenes as bioactive agents. J Enzyme Inhib Med Chem. 28(6):1113–1128.

Tolosa L, Donato MT, Gómez-Lechón MJ. 2015. Protocols in In Vitro Hepatocyte Research. Methods in Molecular Biology (Methods and Protocols). Vol. 1250. New York (NY): Humana Press. Chapter 26, General cytotoxicity assessment by means of the MTT assay; p. 333–348.

Winkler CK, Schrittwieser JH, Kroutil W. 2021. Power of biocatalysis for organic synthesis. ACS Cent Sci. 7(1):55–71.

Zielińska-Błajet M, Feder-Kubis J. 2020. Monoterpenes and their derivatives—recent development in biological and medical applications. Int J Mol Sci. 21(19):7078.

Zuccarini P. 2009. Camphor: risks and benefits of a widely used natural product. J Appl Sci Environ Manag. 13(2):69–74.