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ANTICANCER PROPERTIES OF PLANTS OF THE FAMILY OF CARAWAYS

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Abstract. Caraway is a famous medicinal plant in various pharmaceutical, food, and cosmetic industries. This study aimed to investigate the chemical composition, antioxidant, antimicrobial, and anticancer activities of this plant's essential oil (EO). Caraway EO was obtained from dried caraway seeds using the hydrodistillation process. The composition of caraway EO was inspected by gas chromatography- mass spectrometry (GC–MS) analyses. The antioxidant activity of caraway EO was determined by three different *in vitro* antioxidant assays: 2,2-diphenylpicrylhydrazyl ($DPPH^{\bullet}$), 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) scavenging activity and reducing power. The agar well diffusion method was used to assess the antimicrobial action. The cytotoxic activity was evaluated using the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay, and the data were expressed as the half-maximal inhibitory concentration (IC_{50}).

Keywords: antimicrobial, antioxidant, caraway, carvone, cytotoxicity, preservative.

INTRODUCTION

Plant-derived molecules have long been recognized in medicine and pharmacy [1] for their wide range of biological activities, including antioxidant [2], antimicrobial [3,4], cytotoxicity [5], antiacetylcholinesterase [6,7], and anti-inflammatory [5]. The number and types of these compounds vary among species and individuals within the same plant group [8]. They shield plants from biotic (such as bacteria, fungi, nematodes, insects, or

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animal grazing) and abiotic (such as heat, salinity, drought, and heavy metal) stresses. Owing to their high economic worth, humans employ them mostly as chemicals for medications, flavors, perfumes, insecticides, and colors [9]. Essential oils (EOs) are potent plant-based medicines that have long been used to both prevent and treat a wide range of illnesses [10]. In terms of chemistry, herbs ' secondary metabolites, including EOs, serve various functions, such as defense against herbivores, pests, and bacteria that interact with other plants of the same species and signal inside the plant in response to external stimuli [11]. To protect itself from a certain predator or set of predators, every plant species or subspecies creates its own 'signature' blend of EO chemical components [12]. In various fields, including the pharmaceutical, cosmetic, and food sectors, plant EOs and the bioactive elements they contain that are derived from spices and herbs are gaining more and more attention [13]. EOs with a high potential for scavenging free radicals may help prevent diseases, including cancer, cardiovascular disease, cognitive dysfunction, and ageing of the immune system, along with the dramatically increased consumer interest in using natural remedies to cure a variety of conditions [2].

MATERIALS AND METHODS

C. carvi extracts contained terpenoids, alkaloids, and tannins [2]. Caraway oils are present in every part of the plant, but when extracted from the seeds using a hydrodistillation technology, the seeds contain the maximum oil concentration. Approximately 95% of an EO's components comprise the two primary ingredients, carvone and limonene. D-carvone (50–65%) and (þ)-limonene (up to 45%) are the two main EOs found in caraway seeds, which have an overall EO content of 3%. Caraway's primary chemical, D-carvone, gives it its distinctive aroma [2]. Additionally, caraway EO has been demonstrated to have cancer-chemopreventive properties against dimethylhydrazine-induced colon premalignant damage [3]. Caraway EO's antimicrobial, antioxidant, anti-acetylcholinesterase, and antidiabetic properties have also been reported by Dadkhah *et al.* [4]. According to Laribi *et al.* [5], carvone, limonene, b-myrcene, and a-selinene made up most of the Egyptian chemotype's chemical components (61.6, 29.1, 3.9, and 10.9%, respectively). Owing to their active antioxidant components, such as phenolic compounds, which are substantially more significant than the typical antioxidant compounds, and their antibacterial properties, caraway seeds have antioxidant properties [6]. Therefore, this work aimed to study the phytochemicals of Egyptian caraway (*C. carvi L.*) EO extracted by a hydrodistillation method. The antioxidant activity of caraway EO has been evaluated *in vitro* using different assays. The antimicrobial activity of caraway EO on some pathogenic and spoilage microorganisms and anticancer effect against three cells [colon cell line (HCT-116), liver cell line (HepG-2), and Caucasian breast adenocarcinoma (MCF-7)] were also studied.

Overall, 5 kg of caraway seeds (*C. carvi L.)* was purchased from the Medicinal and Aromatic Plants Research Department of Horticulture Institute, Agriculture Research

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Center, Dokki, Giza, Egypt. The media were purchased from Hi-Media and Difco. All chemicals were of analytical grade and were purchased from Sigma-Aldrich.

RESULTS AND DISCUSSION

Hasika *et al.* [3] used the broth dilution method to assess the least inhibitory concentration [minimum inhibitory concentration (MIC)] and the minimum bactericidal and fungicidal concentrations (MBC and MFC) of caraway EO. For establishing the MIC, the EOs with the highest antibacterial activity in the agar- well diffusion assay were chosen. A loop was used to collect one colony of each microbial strain, which was subsequently inoculated into 25 ml of broth medium. After 18–24 h of incubation at 37°C, 10^9 CFU/ml of bacterial suspension was obtained. Each stock solution was diluted with buffered peptone water (Oxoid) to yield suspended bacterial cultures at a concentration of 10^5 CFU/ml. In a test tube, 0.5–5 µl/ml dilutions of EOs in broth medium were combined with bacterial suspensions to yield a volume of 4 ml and a final concentration of around 5×10^4 CFU/ml. Earlier specified temperatures were used to incubate the final solutions. The MIC is the lowest concentration of EO that inhibits observable microbial growth. The MBC and MFC were measured by subculturing 100 µl from each negative test tube onto PCA plates. MBC or MFC was defined as the lowest concentration yielding a negative subculture or a single colony following incubation. The experiments were conducted in duplicate four times.

MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) was used to investigate the cytotoxicity of caraway EO on colon cell line (HCT-116), liver cell line (HepG-2), Caucasian breast adenocarcinoma (MCF-7), and normal kidney cells (VERO) [4]. In 96-well plates, 1×10^5 cells were seeded in 0.2 ml of medium in each well. Following incubation, the media from the wells was removed carefully for the MTT experiment. Each well was rinsed two to three times with MEM (without FCS), and then 200 µl of MTT (5 mg/ml) was added. In a 5% CO_2 incubator, the plates were incubated for 6–7 h to test for cytotoxicity. After incubation, 1 ml of DMSO (a solubilizing agent) to each well was added, micropipette-mixed, and left for 45 s. Owing to forming of formazan crystals, the presence of live cells was shown by developing a purple hue. The suspension was placed in a spectrophotometer cuvette, and the OD (optical density) readings were measured at 595 nm using DMSO as a blank. The concentration required for a 50% inhibition of viability (IC_{50}) was visually estimated after measurements were conducted. Using the concentration of the EO on the X-axis and relative cell viability on the Y-axis, a Standard Graph was constructed.

Cell viability(%) = Mean OD/Control OD × 100 Where mean OD is the mean of optical density for different concentrations of EO on treated cells and control OD is the mean of optical density for different concentrations of EO on control cells.

Statistical analysis

Analysis was done using the statistical analysis system software for Windows

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(Statistical Analysis System, Version 9.1.3, SAS Institute Inc. Cary, NC, USA) [9]. The data were given as mean±SD. Analysis of variance was used for statistical analysis, and Duncan's multiple range tests were used to compare the experimental findings ($P \geq 0.05$) [5].

Chemical composition of caraway essential oil

The GC analysis of caraway EO revealed the presence of 25 compounds, representing 99.33% of the EO's total composition. Carvone (56.52%) was the major compound of caraway EO, followed by limonene (39.49%). The chemical class characterization showed that caraway EO is mainly composed of monoterpene ketones (56.71%), represented by carvone and camphor, and monocyclic terpenes (39.63%), with limonene as the main constituent, followed by small proportions of groups of bicyclic terpenes, aliphatic hydrocarbon, aromatic hydrocarbon, oxides, alcohols, esters, aldehydes, sesquiterpene, and unknown compounds. These findings are consistent with those of Siwar *et al*. [6], who discovered that oxygenated monoterpenes (59.6%), monoterpenehydrocarbons (39%), and phenylpropanoids (0.1%) were the main components of caraway EO. The most prevalent chemicals were carvone (58.2%) and limonene (38.5%).

In vitro antioxidant activity of caraway essential oil Antioxidant activity is an extraordinarily complex mechanism, encompassing a variety of processes, including free radical scavenging, inhibition of hydrogen abstraction, reducing capacity, and binding of transition metal ions [7]. EOs are complex combinations of bioactive molecules with multifunctional capabilities as their functional groups and chemical activity vary [9]. Therefore, the antioxidant activity of caraway EO was using three different *in vitro* antioxidant assays to evaluate (DPPH$^{\bullet}$ scavenging assay, ABTS$^{\bullet+}$ scavenging assay, and reducing power test) and compared to a widely used synthetic antioxidant, BHA.

The DPPH values of EO caraway (IC_{50}=32.46 ±0.75 µg/ml) were marginally lower than those of the antioxidant BHA (IC_{50}=11.55±0.53 µg/ml), and the ABTS results followed a similar pattern, although with better efficiency (IC_{50}=2.44±0.44 µg/ml) compared with the antioxidant BHA (IC_{50}=1.50 ±0.29 µg/ml). However, in the reducing power assay, caraway EO could convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), and its IC_{50} value (17.65±0.70 µg/ml) was only 1.31 fold lower than that of the positive control, BHA (23.19±0.78 µg/ml). In all antioxidant assays, caraway EO showed significant antioxidant activities. These results are consistent with those reported by Hajlaoui *et al.* [4]. The high antioxidant capacity of caraway EO may be attributable to its high terpene concentration. As a pre-aromatic monoterpene hydrocarbon, the non-phenolic molecule terpinene has exhibited antioxidant action and is also capable of inhibiting lipid peroxidation [8]. Edible lipids could benefit significantly from significant increases in their oxidative stability and shelf-life by adding EO rich in terpinene. Similar to past studies, the presence of

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carvonein, especially at high concentrations, was found to be responsible for *C. carvi'*s potent free radical scavenging abilities. Carvone comprises conjugated double bonding and has significant antioxidant activity [9]. In addition, carvone has been proved to be a lipid peroxidation inhibitor [4].

DPPH radical scavenging assay

According to the approach proposed by Mighri et al. [9], DPPH radical scavenging activity was evaluated. Overall, 1 ml of each EO dilution in methanol (290–4640 μg/ml) was combined with 1 ml of DPPH methanolic solution (0.04%, wt/vol). The mixtures were vortexed and left in the dark at room temperature for 30 min. The absorbance was then determined at 517 nm.

The study of natural products as potential anticancer agents has garnered significant attention in recent years. Among these, plants belonging to the Apiaceae family (commonly known as the caraway family) have shown promising potential due to their diverse bioactive compounds. This family includes well-known plants like caraway (Carum carvi), cumin (Cuminum cyminum), fennel (Foeniculum vulgare), and dill (Anethum graveolens). These plants are traditionally used for culinary and medicinal purposes, and modern research is uncovering their potential in cancer prevention and therapy.

The anticancer properties of caraway family plants are largely attributed to their phytochemical profile, which includes:

Terpenes and Terpenoids: These compounds, such as carvone and limonene, are known to exhibit cytotoxic effects on cancer cells. Limonene, for instance, induces apoptosis (programmed cell death) in breast and prostate cancer cells.

Flavonoids: Plants like fennel and cumin are rich in flavonoids, which possess strong antioxidant properties. These antioxidants neutralize free radicals, reducing oxidative stress—a key contributor to cancer progression.

Phenolic Acids: Compounds such as caffeic acid and ferulic acid inhibit tumor growth by preventing the formation of new blood vessels (angiogenesis) that supply nutrients to cancer cells.

Essential Oils: The essential oils extracted from caraway and dill exhibit antimicrobial and anticancer activities. They disrupt the cellular structure of cancer cells and trigger mechanisms like DNA damage and apoptosis.

Mechanisms of Anticancer Action

The anticancer effects of the Apiaceae family plants are achieved through several mechanisms:

Induction of Apoptosis: Compounds such as carvone and flavonoids activate apoptosis pathways, selectively targeting cancer cells while sparing healthy cells.

Inhibition of Proliferation: Essential oils and phenolics in these plants inhibit the rapid division of cancer cells, which is a hallmark of tumor growth.

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Anti-inflammatory Effects: Chronic inflammation is a precursor to cancer development. The anti-inflammatory properties of caraway family plants, mediated by compounds like limonene, reduce the risk of cancer formation.

Antioxidant Defense: By reducing oxidative stress, these plants prevent DNA damage and mutations that can lead to cancer.

Chemoprevention: Regular consumption of caraway and fennel has shown potential in preventing cancers of the colon, liver, and skin by modulating detoxifying enzymes and enhancing the body's natural defense mechanisms.

Evidence from Scientific Studies

Several studies support the anticancer properties of the caraway family:

A study on Carum carvi demonstrated its ability to induce apoptosis in colon cancer cells through oxidative stress modulation.

Cuminum cyminum extract was shown to reduce the size of tumors in animal models of breast cancer, attributed to its high levels of antioxidants.

Research on Foeniculum vulgare highlighted its inhibitory effects on liver and lung cancer cells due to its rich essential oil composition.

Applications and Future Prospects

The anticancer properties of the caraway family plants present opportunities for developing natural, plant-based therapies. These plants could be used as:

Dietary Supplements: Their incorporation into the daily diet can serve as a preventive measure against cancer.

Adjunct Therapies: Combined with conventional treatments, these plants may enhance efficacy and reduce side effects.

Pharmaceutical Development: Isolated bioactive compounds can be synthesized and developed into targeted anticancer drugs.

Challenges and Considerations

Despite promising findings, the translation of these properties into clinical applications requires overcoming certain challenges:

Standardization: Variability in plant composition due to geographic and environmental factors can affect efficacy.

Toxicity Studies: Although generally safe, high doses of certain compounds may have adverse effects, necessitating thorough toxicity evaluations.

Clinical Trials: More human studies are needed to validate the anticancer potential observed in vitro and in animal models.

Caraway EO showed effective inhibitory activity against all tested cultures with an inhibition zone ranging from13.73±0.26 mm for *B. cereus* to 26.40 ±0.25 mm for *L. monocytogenes.* The MIC values of the caraway EO varied from 1.0 and 3.0 µl/ml for the five tested bacterial strains. The lowest MIC value was recorded for both *S. aureus*

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and *L.monocytogenes*, whereas *B. cereus* showed the highest MIC value. The MIC values of caraway EO for *A. niger* and *Candida sp* were 2.00 and 1.50, respectively.

These results are in harmony with the results reported by many authors. Katarzyna et al. [4] discovered that caraway EO had moderate antibacterial properties, with carvone identified as an active component. Simic et al. [5] showed that caraway oil's MIC inhibited fungal growth at 2.5 mg/ml, but they did not compare the observed results to oil composition. Jaripa et al. [6] discovered that caraway EO was tested for antibacterial effectiveness against 10 pathogenic bacteria and six phytopathogenic fungi. Even at 2 µl/ disc, the EO exhibited a good inhibitory effect against all test microorganisms. The EO's MIC (100–300 ppm) and MBC (200–400 ppm) were determined. The EO's antifungal screening revealed that at 100 ppm, it inhibited 100% of the test fungi's radial mycelial growth. The MIC and MFC values range between 50 and 300 ppm and 200 and 400 ppm, respectively.

In vitro cytotoxicity

Understanding the biological and pharmacological characteristics of caraway EOs requires determining the effective concentration (IC_{50}) that reduces the cell growth inhibitory concentration. The proliferation rates of the colon cell line (HCT-116), liver cell line (HepG-2), and Caucasian breast adenocarcinoma (MCF-7) were compared with normal kidney cells (VERO) by studying the IC_{50} value of eight different concentrations (78.1, 156.2, 312.5, 625, 1250, 2500, 5000, and 1000 µg/ml) of caraway EO.

The results indicated that caraway EO has a toxic effect on the cancer cells under study, as the increase in the concentration used increased the properties corresponding to the rise in the toxic effect on cancer cells, as the effect of the oil was greater on the colon cell line (HCT-116, IC_{50}=390.12±0.41 µg/ ml), followed by liver cell line (HepG-2 IC_{50}=589.75 ±0.60 µg/ml) and Caucasian breast cancer (MCF-7 IC_{50}=843.52±0.75 µg/ml), compared with untreated cells from each of the above cell types (% viability=100%, %toxicity=0%) Additionally, caraway EO has been tested on normal cells to ensure the safety of using caraway. These results show the potential of caraway EO on cancer treatment because it contains biologically active compounds such as carvone and limonene. This opinion is consistent with Kamaleeswari and Nalini [4], who stated that oils recovered from caraway seeds might help to reduce tumor volume and occurrence. It made resistant tumor cells exposed to free radical attack, which resulted in a decrease in cancerous cell propagation. The activation of antioxidant enzymes scavenges the free radicals in colon cancer rats. Caraway EO may have anticancer benefits owing to the presence of carvone, a monoterpene with cancer-preventive and anthelmintic qualities [2]. Owing to its ability to trigger apoptosis by upregulating pro-apoptotic factors and downregulating anti-apoptotic issues, limonene has also been discovered to be an anticancer drug [9].

These results indicate that caraway EO can be used safely in the prevention and treatment of colon, liver, and breast cancers.

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CONCLUSION

In this work, carvone and limonene were identified as the main compounds in caraway EO. This EO demonstrates significant antimicrobial action against a variety of pathogenic and food spoilage pathogens, with large widths of growth inhibition zones and low minimal inhibitory doses for nearly all tested strains. The anticancer activity of caraway EO was investigated. Using a Vero and three tumor cell lines of human hepatocarcinoma (HepG-2), human breast carcinoma (MCF-7), and colon cell line, the most interesting biological effect of EO on the cell proliferation stimulating activity at extremely low doses was demonstrated (HCT-116). At low doses, 50% of cancer cells were found to be killed by the EO, demonstrating its potency.

REFERENCES

1 Sachan AK, Das DR, Kumar M. *Carumcarvi*-an important medicinal plant. J Chem Phar Res 2016; 8:529–533.

2 Senapati S, Mahanta AK, Kumar S. Controlled drug delivery vehicles for cancer treatment and their performance. Sig Transduct Target Ther 2018; 3:7.

3 Malhotra SK. Handbook of herbs and spices. In: Peter KV, editor. Woodhead Publishing Series in Food Science, Technology and Nutrition. 2nd ed. Cambridge, UK: Woodhead Publishing 2012. 2.

4 Bakari S, Ncir M, Felhi S, Hajlaoui H, Saoudi M, Gharsallah N, Kadri A. Chemical composition and in vitro evaluation of total phenolic, flavonoid, and antioxidant properties of essential oil and solvent extract from the aerial parts of Teucriumpolium grown in Tunisia. Food Sci Biotechnol 2015; 24:1943–1949.

5 Dadkhah A, Allameh A, Khalafi H, Ashrafihelan J. Inhibitory effects of dietary caraway essential oils on 1,2-dimethylhydrazine-induced colon carcinogenesis is mediated by liver xenobiotic metabolizing enzymes. Nutr Cancer 2010; 63:46–54.

6 Laribi B, Kouki K, Bettaieb T, Mougou A, Marzouk B. Essential oils and fatty acids composition of Tunisian, German and Egyptian caraway (*Carum carvi L.*) seed ecotypes: a comparative study. Ind Crop Prod 2013; 41:312–318.

7 Rasooli I, Allameh A. Caraway (*Carum carvi L.*) essential oils. In: Preedy VR, editor. Essential oils in food preservation, flavor and safety. San Diego, CA: Academic Press 2016. 287–293

8 Maisonneuve SA. Sainte Ruffine European Pharmacopoeia, vol 1. Canada:Maisonneuve; 1983.

9 Snoussi M, Noumi E, Punchappady-Devasya R, Trabelsi N, Kanekar S. Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*. J Food Sci Technol 2018; 55:2824–2832.

10 Mighri H, Hajlaoui H, Akrout A, Najjaa H, Neffati M. Antimicrobial and antioxidant activities of Artemisia herba-alba essential oil cultivated in Tunisian arid

ISSN:1624-1940
http://magellanes.com/

zone. CRC Chimie 2010; 13:380–386.

11 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 1999; 26:1231–1237.

12 Iordache TA, Vlase L, Istudor V, Gîrd CE. Researches regarding obtaining selective extracts with hypoglycemiant properties from vegetal indigenous products (*Cichoriiherba* and *Fraxini folium*). Note III. Phenolic compounds analysis from Fraxini folium. Farmacia 2013; 61:625–631.

13 Deans S, Ritchie G. Antibacterial properties of plant essential oils. J. Food Microbiol 1978; 5:165–180.