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Investigation of Antimicrobial and Cytotoxic Activities of Palmarosa (*Cymbopogon martinii*) Essential Oil

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ABSTRACT

In this study; It was aimed to investigate the cytotoxic activities and antimicrobial effects of Palmarosa essential oil obtained from *Cymbopogon martinii* plant. Content analyzes of Palmarosa essential oil were made by Gas Chromatopraphy-Mess Spectrometry (GS-MS). The antimicrobial effects of Palmarosa essential oil were investigated using Disk Diffusion and Minimum Inhibition Concentration (MIC) methods. Cytotoxic effects of essential oil at different concentrations in breast cancer (MCF-7), prostate cancer (DU-145) and healthy human fibroblast (WI-38) cell lines XTT (2,3-bis-(2-methoxy-4-nitro-5) -sulfophenyl)-2H-tetrazolium-5-carboxanilide) test. In the disc diffusion method of Palmarosa; against *K. pneumoniae*, *S. aureus*, and *E. coli*, it was observed that the first concentrations formed zone diameters very close to the standard. It has been determined that the antifungal effect against *C. albicans* is present in the first two concentrations (200-100 µg/mL). Palmarosa, in the MIC method; Showed the highest antibacterial effect against *B. cereus* (MIC: <1.56 µg/mL). It has reached effective MIC values against other bacteria and fungi. In our cytotoxic activity studies; The IC50 value for DU-145 cells was 3.14 ±0.126, 6.29 ±0.56 for MCF-7 and 20.06 ±1.02 for WI-38. The antitumor activity of Palmarosa essential oil was found to be more effective in DU-145 cells, but it was observed that there was no toxicity in WI-38 cell line.

Keywords: Antimicrobial, *Cymbopogon martinii*, Cytotoxicity, Essential oil.

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Introduction

Medicinal and aromatic plants have been used by people for many years to prevent and cure diseases [1]. Essential oils are volatile and aromatic compounds obtained from plants. These oils are found in special cells or cell groups such as leaves and stems of the plant [2]. 300 of the approximately 3000 essential oils known today are used in different industries such as medicine, food, cosmetics and perfume. In particular, some essential oils show medicinal properties and are used in the treatment of various systemic diseases [3]. Essential oils contain phytochemicals known as monoterpenes, sesquiterpenes and their oxygen derivatives. These phytochemicals (thymol, anethol, menthol, carvacrol, phenolic acids, flavones, etc.) show activity against bacterial and fungal species, and research has been carried out in this area for a long time [4,5]. Phenolic compounds constitute the main group of plant secondary metabolites with antioxidant effects. There are many studies on the beneficial properties of these phytochemical compounds on human health [6]. Secondary metabolites are compounds of natural origin that are not required for plant growth, but have biological and pharmacological activity, produced by different pathways. The source of natural antioxidants is phenolic compounds that can occur in all organs of plants. The main ones among these antioxidant compounds are flavonoids, phenolic acids, lignans, terpenes, tocopherols, phospholipids and organic acids [7].

Plants of the genus *Cymbopogon* belonging to the Poaceae family are economically very valuable as they are frequently preferred in the perfumery and pharmaceutical sectors with their characteristic odor in their essential oils. The genus *Cymbopogon* consists of about 140 species containing a wide variety of phytochemicals and their essential oils are obtained by steam distillation. Essential oils of the genus *Cymbopogon* contain components such as citral, geraniol, citronellol, citronellal, which have antibacterial, antifungal, insecticidal and insect repellent activities [8]. *Cymbopogon* species have important activities such as anthelmintic, anti-inflammatory, analgesic, antiageing, pesticide, antimicrobial, antifungal, antioxidant effects [9]. However, studies to determine the biological and pharmacological importance of these essential oils have increased rapidly in recent years; Besides its anticancer effects, many beneficial biological activities have been observed. Phytochemicals in the essential oil obtained from the *Cymbopogon* genus, It offers excellent biological activities and therefore can be used in the treatment of various types of cancer [8]. *C. flexuosus, C. nardus var.* nardus*, C. citratus, C. pendulus, C.winterianus* and *C. martinii var.* motia and sofia are among the other types with commercial value. Essential oils obtained from these species are widely used in many areas thanks to their typical lemon and rose-like aromas [9].

C. martinii is a perennial herb native to India with a height of 5-8 meters. Wide and leaves with an intense smell, long slender stems and structures with terminal flowers on the upper part are its distinctive physical properties. GC/GC-MS analysis studies reveal that *C. martinii* essential oil; geraniol, geranylacetate, farnesol, terpinene, myrsen, caryophyll, humulene, selinenes, linalool, nerolidol and limonene components [9]. *C. martinii* essential oil, known as Palmarosa, is particularly rich in geraniol [10]. Geraniol and geranylacetate compounds constitute approximately 75-90% of the total essential oil [11].

Microorganisms can gain resistance to drugs produced by utilizing antimicrobial properties over time. Due to this increase in resistance, studies on the development of new generation drugs in the fight against infections gain great importance [12]. In addition, it is observed that microorganisms do not acquire resistance against plants despite antibiotic resistance. This situation inevitably increases the importance of herbal drugs or plant extracts [13]. Antibiotic effects are the most widely known properties of essential oils because they are highly active against bacteria, viruses and protozoa. It is known that 60% of essential oils inhibit fungal growth and 30% inhibit bacterial growth [14].

In cancer treatment; various treatments are applied according to the location of the tumor in the body, the physiological condition of the patient and the structure of the tumor. Chemotherapy and radiotherapy are among the methods mostly used in the treatment of cancer in recent years. In radiotherapy, ionizing radiation is preferred to damage and destroy harmful tumors. Radiotherapy is locally effective in tumor treatment and is applied directly on the mass. The beam used in radiotherapy damages the DNA of the cell and causes its death [15]. If we look at the data in recent years; Cancer has a higher death rate than any other disease. Species observed more than others are; lung, breast, prostate, rectum and colon [16]. This has given great importance to research on cancer treatment.

The main feature that an antimicrobial agent should have is selective toxicity. The concept of selective toxicity was first introduced by Paul Ehrlich. Antimicrobial substances used in chemotherapy are required to be effective especially at low concentrations and to have extremely low toxic properties. In order to observe such a result; The antimicrobial agent must select microorganism cells as targets rather than mammalian cells. Bacteria are prokaryotic cells while mammalian cells are eukaryotic. Antimicrobial agents (penicillins, cephalosporins, sulfonamides) targeting a molecule in prokaryotic cells but not in eukaryotic cells have extremely selective toxicity [17].

In this study, it was aimed to investigate the antitumor activities of Palmarosa essential oil obtained from *Cymbopogon martinii* plant on MCF-7 breast cancer and DU 145 prostate cancer cells, cytotoxic activities on WI-38 human fibroblast cell line and antimicrobial effects on various bacteria and fungal cells.

Materials and Methods

Essential Oil Sample

Palmarosa essential oil was supplied by Art de Huile.

GC-MS (Gas Chromatopraphy-Mess Spectrometry)

GC-MS analyzes for the determination of the components in the Palmarosa essential oil sample were commissioned by Art de Huile, which supplied the oil. The average values of the essential oil composition are given in (Table 1). These components are geranyl acetate, beta caryophyllene, cis beta ocimene, geranial, geraniol, limonene, linalol, myrcene, neral, nerol, trans beta ocimene, trans trans farnesol, geranyl hexanoate. When the GC-MS results were examined, it was determined that the content of Palmarosa essential oil had the highest rate of geraniol (81,41%) and the lowest rate of myrcene (0.15%). Geraniol was determined as the major essential oil component.

Table 1. Components of Palmarosa essential oil

Name of Component	Reference	GC-MS
Geranyl acetate	7,0-16,0	% 8,71
Beta caryophyllene	0,7-2,5	% 1.98
Cis beta ocimene	0,2-0,6	% 0,35
Geranial	0,2-6,0	% 0,43
Geraniol	72,0-86,0	% 81,41
Limonene	1,3	% 0,33
Linalol	1,0-5,5	% 2,79
Myrcene	5,0	% 0,15
Neral	0,5	% 0,30
Nerol	0,2-0,5	% 0,32
Trans beta ocimene	0,5-3,0	% 1,33
Trans trans farnesol	0,2-1,5	% 0,65
Geranyl hexanoate	0,4-0,8	% 0,50

Determination of Antimicrobial Activity

Disc diffusion (Kirby-Bauer) and microdilution broth methods were used to determine the antimicrobial activity of Palmarosa essential oil.

Disc Diffusion

In the disk diffusion test, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (ATCC 13883), *Bacillus cereus* (ATCC 14579), C*andida albicans* (ATCC 10231) strains were used. Dilutions of Palmarosa essential oil (200-1.56 µg/mL) were absorbed into blank discs with 20 µl. Mueller-Hinton Agar (MERCK) and Sabouraud Dextrose Agar (Neogen) agars were inoculated with 0.5 McFarland turbidity bacterial and yeast solutions using sterile swab sticks. Essential oil impregnated discs were placed. Antibiotic discs were used for positive control (OXOID). Bacterial plaques were incubated at 37 ± 0.1°C for 24 hours, and yeast plaques at 25 ± 0.1°C for 48 hours. At the end of the expected time, the diameters of the zones observed in the medium were measured in mm [18].

Minimum Inhibition Concentration (MIC)

For the experiment, 96-well microtiter plates with U-type wells were used. MHB was used for bacteria and SDB was used for yeast. 10 µl essential oil (200 µg/mL) was applied to the first well, followed by serial dilutions. Bacteria and yeasts adjusted to McFarland 0.5 turbidity were diluted to 5 x10^5 CFU/mL for bacteria and 0.5-2.5 x10^3 CFU/mL for yeasts and 50 µl was added onto the wells [19, 20]. Plates were incubated for 24 hours at 37 °C. In order to better observe the growth at the end of the incubation, 50 µl of 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) solution of 2 mg/mL was applied to all. Incubation process complete at 37 °C for 2 hours. The first well in which a decrease in the color of Formazan due to the presence of live microorganisms in the wells was observed was accepted as the MIC. MIC results according to reference sources; It was evaluated as effective (MIC <100 µg/mL), Moderate (100 <MIC ≤ 625 µg/mL), Weak (MIC >625 µg/mL) [21, 22].

Cytotoxic Activity

In our study, MCF-7, DU-145, WI-38 cell lines were used for cytotoxic activity determination. Essential oils were prepared at different concentrations (200-1.56 µg/mL) and used. Cells were incubated in 5% CO_2 at 37°C. 1% penicillin (100 U/mL) and streptomycin (100 µg/mL) and 10% fetal bovine serum (FBS) were added to all wells.

Cytotoxicity was interpreted according to the XTT method. Cells were added to the wells in the appropriate medium, test compounds were applied in varying proportions and incubated in CO_2 medium at 37°C for 24 hours. When the time was up, 100 µL of XTT solution was added to all wells and incubated for an additional 2 hours and optical density values were determined at 475 nm [23].

Results and Discussion

According to the results of the disc diffusion test (Table 2), Palmarosa essential oil formed an inhibition zone against *E. coli, S. aureus* and *K. pneumoniae* bacteria at 1,2,3 and 4 concentrations (200-25 µg/mL) and against *C. albicans* yeast at 1st (200 µg/mL) and 2nd (100 µg/mL) concentrations.

Table 2. Disc diffusion results of Palmarosa essential oil

Palmarosa Essential Oil Disc Diffusion Zone Diameters (mm) Concentrations (µg/mL)											
Mikroorganizma	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**	**Positive Control**	**Negative Control**
Escherichia coli	10	10	10	9	7	7	7	7	1	9	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	1	7	-
Staphylococcus aureus	12	10	6	6	-	-	-	-	1	9	-
Klebsiella pneumoniae	20	16	12	9	-	-	-	-	2	2	-
Bacillus cereus	-	-	-	-	-	-	-	-	2	0	-
Candida albicans	10	6	-	-	-	-	-	-	1	8	-

The antimicrobial test results (MIC) of Palmarosa essential oil obtained from the *Cymbopogon martinii* plant are shown in (Table 3). Palmarosa essential oil produced the best antibacterial activity in *B. cereus* bacteria compared to the others (MIC: <1.56 µg/mL). While it was moderately effective against *P. aeruginosa*, it reached MIC values effective against other bacteria and fungi.

Table 3. MIC results of Palmarosa essential oil

Microorganisms	Palmarosa Essential Oil
Escherichia coli	12,5
Pseudomonas aeruginosa	200
Staphylococcus aureus	>6,25
Klebsiella pneumoniae	3,125
Bacillus cereus	<1,56
Candida albicans	3,125

The color change caused by the addition of XTT solution to the wells in the microplate and caused by different concentrations of essential oils was measured in ELISA at a wavelength of 450 nm. % Inhibition was determined by making the calculations specified in the reference sources and the relevant tables and figures were obtained.

In studies with Palmarosa essential oil on DU-145 and MCF-7 cell lines, Palmarosa essential oil was observed to be more effective (Table 4) on DU-145 cell line. It has been observed that Palmorosa essential oil has no toxicity in healthy WI-38 cell lines at the effective doses.

Table 4. IC50 values of Palmarosa essential oil

IC_{50}(µg/mL)	MCF-7	DU-145	WI-38
Palmarosa	6,29 ±0,56	3,14 ±0,12	20,06 ±1,02

Chemotherapy drugs used in cancer treatment show severe side effects depending on the treatment. Methods such as radiotherapy, surgical treatment and hormone therapy, which are used in cancer treatment apart from existing cancer drugs, have increased the search for other methods that can be used in treatment because of the low probability of success in the treatment result and their side effects [24]. The importance of introducing alternative and complementary drugs and treatment methods for the treatment of cancer is emphasized in clinical, epidemiological and experimental studies. Plants are the leading natural resources preferred in the traditional treatment of different types of cancer diseases. About 60% of the sources used for anticancer treatment consist of plants, seafood or microorganisms [9].

Jain et al.; as a result of their examination in *C. martinii* oil with a chromatography device; they determined that it contains geraniol, geranilacetate, farnesol, terpinene, myrsen, caryophyll, humulene, selinenes, linalool, nerolidol and limonene [9]. *C. martinii* plant, known as Palmarosa, gives an essential oil especially rich in geraniol [10]. According to another study; The geraniol and geranyl acetate compounds of the essential oil obtained from the *C. martinii* plant constitute approximately 75-90% of the total essential oil [11].

Numerous studies have been conducted on the antimicrobial activities of essential oils.

According to the study of Verma R. et al.; the essential oil obtained from the *Cymbopogon martinii* plant was observed to provide broad-spectrum antibacterial effects with medium to very good effects against Gram-positive and Gram-negative strains [25].

From the works of Ganjewala D; essential oils of *Cymbopogon* species have emerged as having superior antifungal activities and significant antibacterial activities [8].

Bassole I.H.N. et al. in their study; essential oils distilled from *Cymbopogon citratus* and *Cymbopogon giganteus* plants were tested on nine bacteria by disc diffusion and microdilution methods. *C. giganteus* essential oil provided antimicrobial effects to all microorganisms used, while *C. citratus* essential oil could not inhibit *Pseudomonas aeruginosa* [26].

In a study by Khan and Ahmad in 2011; The effects of *Cymbopogon martinii* together with other essential oils on Aspergillus fumigatus and *Trichophyton rubrum* were investigated. As a result of this study, it was determined that the essential oil of *Cymbopogon martinii* showed high antimicrobial activity and therefore it could be evaluated as a new resource in the pharmaceutical industry [27].

Sharma R. et al.; investigated the effects of essential oil from *Cymbopogon flexuosus* on twelve human cancer cells. As a result; 502713 (colon), IMR-32 (neuroblastoma), Hep-g-2 (liver) and SiHa (cervix) showed significant cytotoxic activity against all cell lines, more specifically [28].

Thangam R. et al. *Cymbopogon citratus* in their study on the anticancer activities of essential oil; concluded that the essential oil provided cytotoxic and apoptotic activity in cancerous tissues [29].

Bayala B. et al. in their study; The reason for this study was to determine the cytotoxic activities of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils on cancerous cells. For this purpose, Antioxidant, potential anti-inflammatory effect and cytotoxic effect were studied on different prostate cancer and glioblastoma cell cultures. *C. citratus* essential oil gave significant results in prostate cell line PC-3 (IC50 ¼ 32.1 mg/mL) and glioblastoma cell lines (SF-767 (IC50 = 45.13 mg/mL) and SF-763 (IC50 = 172.05 mg/mL) [30].

According to the GC-MS analysis in our study, geranyl acetate, beta caryophyllene, cis beta ocimene, geranial, geraniol, limonene, linalool, myrcene, neral, nerol, trans beta ocimene, trans trans farnesol, geranyl hexanoate components were determined in the content of Palmarosa essential oil. It was determined that geraniol (81.41%) was in the first place and geranyl acetate (8.71%) was in the second place in the composition of our essential oil. In our study; The content of our Palmarosa essential oil sample is very similar to the results obtained from studies conducted by different researchers in the literature.

As a result of the disc diffusion experiment studied at diverse dilutions of Palmarosa essential oil; first concentration (200 µg/mL) formed a zone diameter (20mm) very close to the standard against *K. pneumoniae* bacteria. Against *S. aureus* and *E. coli* bacteria, It was determined that Palmarosa concentrations between 200-25 µg/mL formed zone diameters close to the standard. It was concluded that the first and second dilutions had a positive effect on *C. albicans* (200 µg/mL-10 mm, 100 µg/mL-6 mm).

As a result of the microdilution method studied with 8 different dilutions of Palmarosa essential oil, the strongest activity against *B. cereus* was determined (MIC: <1.56 µg/mL). While Palmarosa was moderately effective against *P. aeruginosa,* it reached MIC values effective against other bacteria and fungi.

To show the effect of essential oil on MCF-7 breast cancer and DU-145 prostate cancer cell lines, it was prepared at concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.56 µg/mL and the cells were found to be viable by the XTT method.

It has been determined that Palmarosa essential oil has a major activity on MCF-7 cells in the first 6 dilutions (200-6.25 µg/mL). At the first concentration (200 µg/mL), more than 100% effect was seen. Even at the sixth concentration (6.25 µg/mL), the rate of killing cancer cells, which is 92.79%, is quite high. Since the IC50 value is 6.29 µg/mL, it is possible to see the effectiveness of Palmarosa essential oil even at low concentration.

It has been determined that Palmarosa essential oil has a major activity on DU-145 cells in the first 6 dilutions (200-6.25 µg/mL). At the first concentration (200 µg/mL), more than 100% effect was seen. Even at the lowest concentration (1.56 µg/mL), it has a very high killing rate of 75.43% cancer cells. Since the IC50 value is 3.14 µg/mL, it is possible to see the effectiveness of Palmarosa essential oil even at very low concentrations.

In studies with Palmarosa essential oil on DU-145 and MCF-7 cell lines, it was observed that Palmarosa essential oil was more effective particularly on DU-145 prostate cancer cell line than MCF-7 breast cancer cell line. No antitumor activity study of the *Cymbopogon martinii* species was found in the studies. However, according to reference sources, antitumor activity has been detected in different species belonging to the genus *Cymbopogon*.

Eight different concentrations of Palmarosa essential oil were tested on the WI-38 cell line, which is a normal human lung fibroblast cell, and the cytotoxic activity results were evaluated as % cell viability.

Although a very low cell viability (5.81%) was observed at the first concentration of Palmarosa essential oil (200 µg/mL), an increasing percentage of cell viability was obtained at other concentrations. Cell viability reached 100% at the last 5 dilutions (25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.56 µg/mL).

It has been observed that Palmorosa essential oil has no significant toxicity in healthy WI-38 cell lines at the effective doses.

According to the data we obtained as a result of research, there are no adequate publications on the

antitumor activity of Palmarosa essential oil. In order to investigate the antitumor effect of similar essential oils on other cancer cell lines and to determine whether they have any toxic effects on normal human cells, research can also be carried out on different human cell lines.

As a result; Palmarosa essential oil has been shown to have the capacity to be used as an another product in anticancer treatments. In the next stage, it may be suggested that these two essential oils be tested in animal experiments and then directed to clinical research and developed as a chemotherapy drug.

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Conflicts of interest

All authors declare that they have no conflict of interest.

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