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REVIEW PAPER

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Biological properties of *Cistus species*

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ABSTRACT

Aim. This paper presents a review of scientific studies analyzing the biological properties of different species of Cistus sp.

Materials and methods. Forty papers that discuss the current research of Cistus sp. as phytotherapeutic agent were used for this discussion.

Literature analysis. The results of scientific research indicate that extracts from various species of Cistus sp. exhibit antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, cytotoxic and anticancer properties. These properties give rise to the possibility of using Cistus sp. as a therapeutic agent supporting many therapies.

Keywords. biological properties, Cistus sp., medicinal plants

Introduction

Cistus species (family *Cistaceacea*) are perennial, dicotyledonous flowering shrubs in white or pink depending on the species. Naturally growing in Europe mainly in the Mediterranean region and in western Africa and Asia.[1-3] These plants are capable of growing in difficult climatic and soil conditions.[4,5] For many years, plants of the genus *Cistus sp*. were used in folk medicine mainly in Mediterranean regions as infusions, extracts and as a resin Ladano in the treatment of many diseases.

Modern scientific research has focused on the isolation and identification of compounds present in extracts, and resins from various species of *Cistus*. Studies have also analyzed their biological and pharmacological activity which elicit healing properties. Phytochemical studies using chromatographic and spectroscopic techniques have shown that *Cistus* is a source of active bioactive compounds, mainly phenylpropanoids (flavonoids, polyphenols) and terpenoids.

These compounds determine the medicinal properties of *Cistus* such as anti-inflammatory, antibacterial, antifungal, antiviral, anti-allergic and strengthening the body's resistance and an analgesic effect which allows their use as therapeutic agents in a wide range of diseases.[6-19]

This article presents the biological and pharmacological properties of various species of Cistus sp., with

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Fig. 1. Dried herb leaves *Cistus incanus* (photograph by Agnieszka Ewa Stępień)

particular emphasis on *Cistus creticus*, *Cistus creticus subspecies cretenicus L., Cistus creticus subspecies creticus, Cistus creticus subspecies eriocephalus, Cistus incanus L., Cistus inacus, Cistus inacus subspecies creticus, Cistus inacus subspecies tauricus, Cistus monspeliensis L., Cistus libanotis, Cistus villosus, Cistus villosus L., Cistus monspeliensis, Cistus ladanifer, Cistus populifolius, Cistus salviifolius, Cistus parviflorus, and Cistus laurifolius.* Their antioxidant, antibacterial, antifungal, antiviral, cytotoxic and anti-cancer properties have been particularly emphasized.

Cistus sp. species are a rich source of natural compounds with antioxidant properties, mainly flavonoids and polyphenols. The ability of antioxidants to capture toxic oxygen free radicals is very important. Oxygen reactive forms such as peroxides, superoxide, peroxy and hydroxy radicals play an important role in oxidative stress contributing to the development of many diseases including diabetes and Alzheimer's disease.

It was confirmed that the species *Cistus laurifolius* is characterized by antioxidant properties.[20] In the extract from leaves and small branches of *Cistus laurifolius* the presence of 16 bioactive compounds was determined by ^{1}H and ^{13}C NMR techniques and EI-MS mass spectrometry. The following compounds from *Cistus laurifolius* have shown the ability to capture free radicals: 3-*0*-methyl quercetin (**1**), 3,7-*0*-dimethyl quercetin (**2**), ellagic acid (**8**), quercetin 3-*0*-α-rhamnoside (**10**), 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-α-L-rhamnopyranoxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**12**), olivil 9-*O*-β-D-xyloside (**13**), berchemol 9-*O*-rhamnoside (**14**) and (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol 9'-*O*-α-L-rhamnoside (major isomer) (**16**).[20] It was observed that *Cistus incanus* and *Cistus parviflorus* both show high activity of scavenging free toxic radicals.[21]

In subsequent studies, it was observed that the content of antioxidant phenolic compounds in extracts of *Cistus populifolius* is higher than for *Cistus ladanifer*. Also, analysis of the ability of extracts to inhibit the formation of lipid peroxyl radicals confirmed that *Cistus populifolius* has higher antioxidant activity than *Cistus ladanifer*.[22]

It was also confirmed that other *Cistus* species including *Cistus incanus* L., and *Cistus monspeliensis* L., contain numerous compounds with antioxidant potential, among others polyphenols and flavonoids.[23,24] Their antioxidative capacity was also examined, due to the presence of phenols, flavonoids and tannins of the obtained ethanol, hexane and water extracts from the leaves of *Cistus monspeliensis* and *Cistus salvifolius*. The highest antioxidant activity was demonstrated by ethanol extracts from both *Cistus* species. The research results indicate that the proper selection of the type of solvent affects obtaining of an extract with a high content of antioxidants.

Research by Loizzo et al. (2013) indicated that the essential oils of the species *Cistus creticus*, *Cistus salvifolius*, *Cistus libanotis*, *Cistus monspeliensis* and *Cistus villosus* exhibit antioxidant properties.[16] They indicated that the greatest antioxidant properties have essential oils derived from *Cistus monspeliensis* and *Cistus libanotis*. They also analyzed their acetylcholinesterase and butyrylcholinesterase (BChE) inhibitory activity. *Cistus salvifolius* species were characterized by the highest activity against AChE, while *Cistus libanotis*, *Cistus creticus*, *Cistus salvifolius* had good inhibitory activity against BChE.There were 26 types of antioxidant present in the leaves and flower buds of *Cistus salviifolius*. The highest concentration of phenols and flavonoids were recorded in extracts from flower buds, while tanning agents and anthocyanins were found in leaf extracts. The obtained results indicated that the leaf extract was characterized by a higher inhibitory activity against the AChE enzyme as compared to the extracts from flower buds. This activity is probably due to the higher content of anthocyanins in the *Cistus salviifolius* leaf extract that may have a significant effect on the inhibition of this enzyme. The results of the above research work emphasize that extracts from the above *Cistus* species are a source of compounds with high antioxidant potential and can be used in therapy for many diseases caused by oxidative stress and may be helpful in the prevention and treatment of Alzheimer's disease.

It has been determined that polyphenol compounds with antioxidant properties present in extracts also have antiviral properties. Activity against the influenza virus from extracts derived from *Cistus incanus spp. tauricus* was found without negative side effects.[13]

Researchers also point to the valuable antibacterial and antifungal properties of *Cistus* species that result from their antioxidant activity, i.e. phenolic compounds.[18] Antibacterial and antifungal properties of leaf extracts of *Cistus villosus L. (= incanus)* and *Cistus monspeliensis L.* were determined against Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and the fungi *Candida glabrata*, *Candida krusei* and *Aspergillus fumigatus*. Extracts from *Cistus villo-*

sus showed higher activity than those from *Cistus monspeliensis* against *Staphylococcus aureus* and *Candida glabrata*. However, *Candida krusei* and *Aspergillus fumigatus* were characterized as the most resistant among all tested microorganisms for both extracts. The tests were carried out using chloramphenicol, amoxicillin and amphotericin B as standard antibiotics for comparison.[27]

In the extracts from the species *Cistus ladanifer*, the presence of a large group of ellagic acid with phenolic compounds punicalagin and gallate were determined.[12] The compound elagitanine is attributed to the effect on the strong inhibition of the growth of *Candida albicans*, *C. glabrata* and *C.parapsilosis*-inducing fungi causing infection in immunocompromised individuals.

It has been shown that methanolic *Cistus monspeliensis* flower extract has a more inhibitory effect than the leaf extract on the growth of Staphylococcus epidermis Gram-positive *Staphylococcus bacteria.*[28] Further phytochemical analysis of the extract obtained from *Cistus monspeliensis L.* leaves determined the structures of biologically active compounds by ^{1}H and ^{13}C NMR spectroscopy. It was determined that this inhibitory activity resulted from the interaction of clerodane (+)-19-acetoxy-cis-clerodan-3-ene-15-oic acid an isolated diterpene.[29] Phytochemical analysis of *Cistus creticus* essential oil showed the presence of ten different volatile diterpenes of the labile type by GC-MS.[30] In vitro studies of the impact of these diterpenes on *Borrelia burgdorferi sensu stricto (Bbss*) bacteria that cause borreliosis were carried out. It was found that the diterpenes manoyl oxide, 13-epi-manoyl oxide, 3-acetoxy-manoyl oxide, and the monoterpene carvacrol with 3-hydroxy-manoyl oxide determined the antibacterial effect of this oil. The interaction of aqueous extracts, hexane and ethyl acetate extracts from *Cistus* leaves was also analyzed, but only the aqueous extract did not inhibit the growth of microorganisms.

Barrajón-Catalán et al. in their studies determined that the aerial extract of *Cistus ladanifer* inhibits the growth of Gram-positive bacteria *Staphylococcus aureus*. The extract obtained from *Cistus populifolius* reveals a high growth inhibitory activity against Gram-negative bacteria strain *Escherichia coli*.[22]

Mahmoudiet et al. studied the activity of aqueous extracts obtained from *Cistus monspeliensis* and *Cistus salvifolius* leaves against pathogenic Gram-negative bacteria (*Escherichia coli ATCC 8739, Salmonella typhimurium NCTC 6017,* and *Pseudomonas aeruginosa ATCC 27853*), two gram-positive bacteria (*Staphylococcus aureus ATCC 29213* and *Enterococcus faecalis*) and two fungal/yeast species (*Aspergillus niger* and *Candida albicans*). Both extracts showed cytotoxic activity against each pathogen. The highest activity was demonstrated by *Cistus salvifolius* extract against *P. aeruginosa* and *A. niger*. In contrast, the extract from *Cistus monspeliensis* has demonstrated a high inhibiting ability against *E. coli, P. aeruginosa* and *C. albicana*.[25] Scientific research indicates that the extracts of *Cistus sp*. can be potentially used as an adjuvant for the treatment of inflammation caused by the undesirable effects of microorganisms.

Scientific research indicates and confirms the presence in many plants, including *Cistus sp*, of compounds with anticancer properties, among others *Cistus sp*. The anti-cancer compounds cause changes in the cancer cell cycle invoking a mechanism in the cell whose main element is the apoptotic pathway. The pathway involved the apoptotic process, i.e. the programmed death of a cell that causes cell contraction, changes in the cell membrane, and in chromatin resulting in the death of the apoptotic cell.[31,32] The observed changes are a decrease in the proliferation index (cytostatic effect), and a decrease in cell survival resulting from the induction of apoptosis (cytotoxic effect). The Dimas research team identified nine labdan diterpenes in the *Ladano Cistus creticus subspecies cretenicus* (L.) resin.[33] Subsequently, studies were conducted to assess their impact on human leukemic cell lines: T-ALL / lymphoblasts (CCRF-CEM), acute T cell lymphoid (MOLT3), T cell lines (H33AJ-JA13), T-cell lymphoma / lymphoblast (HUT78), lymphoma (H9), childhood B acute lymphoblastic (KM3), Burkitt's lymphoma (NAMALWA), Burkitt's lymphoma (DAUDI SDK), Burkitt's lymphoma (JIYOYE), acute lymphoblastic (CCRF-SB), promyelocytic leukemia / lymphoblast (HL60), CML / lymophoblast (K562) and monocytes (U937) indicating their cytotoxic activity. The test results showed cytotoxic activity of the compound (13E) -labd-13-ene-8a15-diol against 13 tested cell lines, while (13E) -labd-7,13-dienol was only active in the HL60 line. In the course of further research, Dimas et al. in 2001 determined the presence of other labile diterpenes in leaf and fruit extracts of *Cistus creticus subsp. creticus*. Isolated sclareol (1) and ent-3α-hydroxy-13-epi-manoyl oxide (2) belong to the labdane type diterpenes and the derivative of compound 2 thiomidazolidine (3), and their activity against human leukemic cell lines: T cell lines (H33AJ-JA1) and lymphoid (MOLT-3). They indicated that compounds 1 and 3 induce the death of apoptotic cells in human leukemia lines, disrupting their cell cycle.[8]

Isolated from the leaf extract *Cistus creticus subsp. eriocephalus* diterpenes of the labile type were the compounds: labd-13 (E) -ene, -8α, 15-diol (1) and labd-13 (E) -ene, -8α, 15-yl acetate (2) and 19-acetoxy-cis- clerodane-3-ene-15-oic acid (3) from *Cistus monspeliensis L*. The structure of these compounds was confirmed by the technique of ^{1}H and ^{13}C NMR and GC/ MS. These compounds were tested for cytostatic and cytotoxic properties in human leukemic cell lines: T-ALL / lymphoblasts (CCRF-CEM), acute T cell lymphoma / suspension (MOLT4), T-cell lymphoma (HUT78), myeloma / suspension. (RPMI 8226), promyelocytic leukemia / lymphoblast

(HL60), CML / lymophoblast (K562), camptothecin resistant: cross resistant to etoposide daunorubicin, doxorubicin (CCRF-CEM / C2), mitoxantrone resistant: cross resistant to etoposide and doxorubicin (HL60 / MX1, HL60 / MX2) mitoxantrone resistant: cross resistant to etoposide, daunorubicin and doxorubicin). It was determined that the highest cytostatic and cytotoxic activity among these diterpenes against the tested leukemic human cell lines was compound 1, followed by compound 2. In contrast, compound 3 showed no activity of this type. The least susceptible to cytostatic and cytotoxic compounds 1 and 2 were the HUT78 and K562 cell lines. Studies indicate that these diterpenes induce apoptosis in these tumor cell lines via a mechanism that regulates the c-myc gene, without affecting the expression of the anti-apoptotic protein bcl-2.[34]

Also, the extract from *Cistus creticus ssp. creticus* is characterized by cytotoxic activity in relation to cancer cells. Ethanol extracts of *Cistus creticus ssp. creticus* showed inhibitory effects on the development of line cell human cancer cervix (HeLa), breast (MDA-MB-453) and melanoma (FemX). It was determined that the effect of the labdan type of diastpenes in the Cistus extract is responsible for this effect on these cell lines.[35] In the extract of *Cistus incanus subsp. creticus* labd-14-ene-8,13-diol (sclareol) was identified by 1H NMr and GC/MS. The effect of sclareol against the MN1 (p53-expressing) and MDD2 (p53-defective) derived from the parental cell line MCF7 was evaluated. It has been shown that this sclareol was able to inhibit DNA synthesis in the cell apoptosis cycle independently of p53, and thus induce cell cycle arrest. The course of breast cancer cell apoptosis induced was assessed by detecting DNA fragments. It was also indicated that sclareol strengthens the activity of the anticancer drugs doxorubicin, etoposide and cisplatinum against human breast cancer cell lines MDD2. Sclareol is also now a certified drug used in cancer therapy on breast cancer.[36]

In subsequent studies, Dimas et al. in 2000 analyzed the cytotoxic and cytostatic activity of antioxidant flavonoids: 3,7,4 ', 5'-tetramethyl ether of myricetin (1) isolated from the hexane extract of *Cistus monspeliensis L.* and its 3', 5- diacetyl derivative (2) synthesized from 1 and myricetin (3). Their proliferation index (cytostatic effect) and cell survival (cytotoxic effect) were assessed against the human leukemic cell lines lymphoblasts (CCRF-CEM), lymphoid (MOLT4), T-cell lymphoma (HUT78), B lymphocyte (RPMI 8226), promyelotic (HL60), proerythrocytes (K562), multidrug resistant (MDR-CCRF-CEM / C2), and in mitoxantrone-selected HL60 (HL60 / MX1, HL60 / MX2). Compound 2 showed a higher inhibitory effect on the growth of all tested cell lines than compound 1. On the other hand, compound 3 showed lack of cytostatic and cytotoxic activity against these cell lines. This indicates that the acetylation of myceritin increases the cytostatic and cytotoxic effect of flavonoids. However, the lowest cytotoxic and cytostatic activity of compounds 1 and 2 were found in the K562 cell line.[37]

In in vitro experiments, extracts from *C. ladanifer* and *C. populifolius* have been analyzed for their cytotoxicity to human tumor cells. Extracts from *C. populifolius* and *C. ladanifer* have demonstrated the ability to inhibit pancreatic cancer cell proliferation (M220) and in breast cancer cells (MCF7 / HER2 and JIMT-1). The leaves of these plants are the source of water-soluble polyphenol extracts enriched with ellagitannins with antioxidant effect, and their effects of cytotoxicity against cancer cells deserves attention.[22] In the studies of Vitali et al., the effect of extracts of *Cistus incanus L.* and *Cistus monspeliensis* L on human tumor cell lines was determined. They showed activity against human prostate cells (PZ-HPV-7 and PNT1A) and lung fibroblast cell line (V79-4). Cytotoxic effects on these lines were observed, acting to inhibit their growth and significantly reduce cell viability. It indicates that the antioxidants present in the *Cistus incanus L.* and *Cistus monspeliensis L.* extracts may prove to be very helpful in the treatment of benign prostatic hyperplasia (BPH).[38] Human melanoma cell lines (A-375) introduced into cultures were compared to human breast cancer cells (MCF-7) extracts from *Cistus libanotis, C. villosus* and *C. monspeliensis*. The analysis of the results showed greater antiproliferative activity of these extracts against melanoma (A-375) than against breast (MCF-7).[39]

El Euch et al. in their studies analyzed the anticancer activity of leaf extract and flower buds from *Cistus salviifolius*.[26] They determined that the flower bud extract showed cytotoxic activity against ovarian carcinoma cell (OVCAR) and breast (MCF-7). And the leaf extract showed a lack of cytotoxic activity against both tumor lines. Antitumor activity results from the high content of polyphenols and flavonoids in the obtained flower bud extract. Subsequent researchers undertook a study to evaluate the antioxidant activity of *Cistus incanus L.* and pomegranate peel (*Punica granatum L.*) rich in polyphenolic compounds.[40] Incubation of human cancer line breast (MCF-7) and colon (LOVO) cultures with pomegranate and cistus extracts resulted in a slowing down of the growth of tumor cells of both lines.

Research indicates that purified extracts can complement human cancer treatment. However, it requires research to understand its effects and interact with the recommended drugs.

Summary

Species of the genus *Cistus* exhibit a number of medicinal properties resulting from the presence of compounds with biological activity. The presented research

results in our article indicate that the antioxidant properties of *Cistus sp*. affect its antibacterial and antifungal properties. This draws attention to the possibility of using extracts and biologically active compounds isolated from *Cistus* in the treatment of inflammation caused by pathogenic microorganisms and the strengthening of antibiotic therapy. The biological activity of the *Cistus* herbaceous plants against tumor cell lines indicates that they can be considered as potential therapeutic agents in the treatment of neoplastic diseases.

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