Proposal for Hippolyt Orthomedequ

ZISTROSE (CISTUS)

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CONTENT

I. SUMMARY

Cistus (german: Zistrose) is a genus of flowering plants in the rockrose family Cistaceae in the Mediterranean area. The leaves are evergreen, covered with glands secreting essential oils and resins (Ladano).

Major constituents of aqueous extracts of *Cistus* species are bioflavonoids. Among the compounds identified so far are quercetin, flavan-3-ols, kaempferol and kaempferol-3-methyl ether, aesculin, myrecitin and labdane-type diterpenes. These compounds exert several pharmacological effects including anti-inflammatory, anti-oxidant, anti-bacterial, anti-viral, anti-fungal, anti-cancer, anti-diabetic, anti-thrombotic, and neuroprotective effects.

Mechanism-based evidence for pharmacological effects of cistus-derived extracts has been reported for *Cistus incanus* L. (*hybrid of C. albidus* L. and *C. crispus* L.), *Cistus creticus, Cistus ladanifer, Cistus laurifolius, Cistus monspeliensis, Cistus populifolius,* and *Cistus salviifolius*. The majority of peer-reviewed information about pharmacological effects is available for *Cistus incanus* extracts.

Cistus incanus extracts are described to exert a) anti-inflammatory effects , b) anti-oxidant effects, c) gastro-protective effects, d) anti-bacterialeffects, e) anti-viral effects, f) and anti-cancer effects. Furthermore, Cystus-sud from *Cistus incanus* has shown activity as heavy metal remover.

Cistus creticus extracts are described to exert a) anti-leishmanial effects, and b) anti-cancer effects.

Cistus ladanifer extracts are described to exert a) anti-oxidant effects, b) anti-bacterial effects, and anti-cancer effects. Furthermore, bee pollen *Cistus ladaniferus* extract has an anabolic effect on bone metabolism in rats in vitro and in vivo.

Cistus laurifolius extracts are described to exert a) anti-inflammatoy effects, b) anti-oxidant effects, c) anti-nociceptive effects, d) gastro-protective effects, and e) anti-*Helicobacter pylori* effects.

Cistus monspeliensis extracts are described to exert a) anti-leishmanial effects, b) anti-bacterial effects, c) anti-fungal effects, and d) anti-oxidant effects.

Cistus populifolius extracts are described to exert a) anti-bacterial effects, b) anti-oxidant effects, and c) anti-cancer effects.

Cistus salviifolius extracts are described to exert a) anti-oxidant effects, and b) inhibitory effects with regard to α-amylase and α-glucosidase, indicating the potential of the extract for controlling hyperglycemia.

II. INTRODUCTION

The family Cistaceae consists of 8 genera and 180 species, with 5 genera native to the Mediterranean area: *Cistus*, *Fumara*, *Halimium*, *Helianthemum*, and *Tuberaria*.

Cistus (from the Greek *kistos*) is a genus of flowering plants in the rockrose family Cistaceae, The leaves are evergreen, covered with glands secreting essential oils and resins. The common Greek name for the resin since antiquity is Ladano (labdanum or ladanum).

The main *Cistus* species found in the Mediterranean basin include *C. albidus*, *C. creticus*, *C. crispus*, *C. parviflorus*, *C. monspeliensis*, *C. populifolius*, *C. salviifolius*, *C. ladanifer*, *C. laurifolius*, and *C. clusii*. *Cistus incanus* is a hybrid of *C. albidus* and *C. crispus*.

The resin, ladano, secreted by the glandular trichomes of certain *Cistus* species contains a number of phytochemicals with antioxidant, antibacterial, antifungal, and anticancer properties. Furthermore, total leaf aqueous extracts possess anti-influenza virus activity. All these properties have been attributed to phytochemicals such as terpenoids, including diterpenes, labdane-type diterpenes and clerodanes, phenylpropanoids, including flavonoids and ellagitannins, several groups of alkaloids and other types of secondary metabolites (for a review, see Papaefthimiou et al., 2014).

Traditionally, a number of *Cistus* species have been used in Mediterranean folk medicine as herbal tea infusions for healing digestive problems and colds, and as extracts for the treatment of different diseases. Data from recent studies provided a rational basis to understand the beneficial effects of *Cistus* extracts in inflammatory or infective diseases by demonstrating their strong gastric antiulcer activity (e.g., Yesilada et al., 1994; Attaguile et al., 1995), antiproliferative and cytotoxic activity (e.g., Chinou et al., 1994; Dimas et al., 1998), and antibacterial, antifungal and antiinflammatory properties (e.g., Chinou et al., 1994; Yesilada et al., 1997; Demetzos et al., 1997; Lendeckel et al., 2002).

Major constituents of aqueous extracts of *Cistus* species are bioflavonoids. Among the compounds identified so far are quercetin, flavan-3-ols, kaempferol and kaempferol-3-methyl ether, aesculin, myrecitin and labdane-type diterpenes (Demetzos et al., 1990; Chinou et al., 1994; Danne et al., 1994; Anastasaki et al., 1999). Compounds belonging to the latter group were shown to be highly gastroprotective (Attaguile et al., 1995; Yesilada et al., 1997), antibacterial and cytotoxic against human leukemic cell lines (Chinou et al., 1994; Demetzos et al. 1997; Dimas et al., 1998).

Mechanism-based evidence for pharmacological effects of cistus-derived extracts has been reported for *Cistus incanus* L. (*hybrid of C. albidus* L. and *C. crispus* L.), *Cistus creticus, Cistus ladanifer, Cistus laurifolius, Cistus monspeliensis, Cistus populifolius,* and *Cistus salviifolius.*

Extracts of all of these *Cistus* species contain pharmacologically active polyphenols such as kaempferol, quercetin, apigenin, and various catechines, as well as other interesting compounds such as labdanum, borneol, zineol, diterpenes, triterpnes and ledol (Danne et al., 1993; Demetzos et al., 1990, Petereit et al., 1991). Among these *Cistus* species, *Cistus incanus* L. provides the highest content of polyphenols (for a review, see Gabele 2008).

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III. CISTUS INCANUS EXTRACTS

III.1. Components in C. incanus extracts

III.1.1. **Chromatographic analyses**

Analysis by Demetzos et al. (1990)

Chromatographic analysis of dried leaves of *C. incanus* demonstrated the presence of 7 labdane-type diterpenoids, together with quercetin, myricetin, kaempferol, kaempferol-3-methyl ether, apigenin, luteolin, and aesculin,

Analysis by Petereit et al. (1991)

Chromatographic analysis of dried aerial parts of *C. incanus* demonstrated the presence of 4 monomeric and 7 oligomeric flavonoids.

- Flavan-3-ols are (+)-catechin, (+)-gallocatechin, the novel (+)-gallocatechin 3-gallate and the rarely occurring (+)-catechin 3-O-α-L-rhamnoside.
- Proanthocyanidins are procyanidins B, and B_3, gallocatechin-(4α→8)-gallocatechin, its novel (4α→6)-regioisomer, gallocatechin-(4α→8)--catechin, and the trimer gallocatechin-(4α→8)-gallocatechin-(4α→8)-catechin.

III.2. Pharmacological effects of selected components in C. incanus extracts

III.2.1. **Myricetin**

Myricetin is a member of the flavonoid class of polyphenolic compounds, with antioxidant properties (Ong and Khoo, 1997). It is commonly derived from vegetables, fruits, nuts, berries, tea, and is also found in red wine. Myricetin is structurally similar to fisetin, luteolin, and quercetin and is reported to have many of the same functions as these other members of the flavonol class of flavonoids (Ross and Kasum, 2002).

Antiinflammatory effects. Myricetin, along with other lipoxygenase- and cyclooxygenase-blocker flavonoids have significant anti-inflammatory characteristics, demonstrated by their ability to reduce edemas caused by carrageenan and croton oil (Ong and Khoo, 1997). The anti-inflammatory nature of myricetin lies in its ability to inhibit the amplified production of cytokines that occurs during inflammation. Testing on various types of macrophage cells as well as on human synovial sarcoma cells, demonstrated the inhibition of several kinds of cytokines, such as interleukin-12 and interleukin-1β, through down-regulation of transcription factors and mediators involved in their production (Li and Ding, 2012). Other studies suggest that myricetin's anti-inflammatory nature could also potentially be dependent upon interfering in inflammatory signal pathways by inhibiting various kinases and, consequently, the function of tumor necrosis factor alpha (Gupta et al., 2014).

Antiviral effects. Myricetin demonstrated antiviral activity against a number of viruses including Moloney murine leukemia virus, Rauscher murine leukemia virus, and the human immunodeficiency virus. Its effects against the proliferation of viruses is thought to be a consequence of myricetin's ability to inhibit the proper functioning of reverse transcriptase. Myricetin was identified as a competitive inhibitor of the reverse transcriptase of Rauscher murine leukemia virus (Ong and Khoo, 1997).

Anticarcinogenic effects. Myricetin has been shown to be effective in protecting cells from carcinogenic mutation. Myricetin reduced the risk of skin tumorigenicity caused by polycyclic aromatic hydrocarbons like benzo(a)pyrene, a highly carcinogenic compound. Myricetin provided protection against the formation of skin tumors in mice models after tumor initiating and tumor promoter agents were applied to the skin (Ong and Khoo, 1997).

Antithrombotic effects. Polyphenols such as myricetin may prevent oxidative stress-induced platelet activation/aggregation. In addition to offering protection by neutralizing peroxide radicals and effecting thromboxane production via the PTGS1 pathway, polyphenols such as myricetin may target other platelet activation pathways, limiting fibrinogen's ability to bind platelet surface receptors (Santhakumar et al., 2013).

Antidiabetic effects. Several *in vitro* and animal studies have indicated the antidiabetic capabilities of myricetin. However, the evidence in clinical trials is less convincing. The flavonoid has been demonstrated to have a hypoglycemic effect by increasing the ability of adipocytes, as well as cells of the soleus muscle and liver of rats, to uptake glucose (Ong and Khoo, 1997; Li and Ding, 2012). This insulinomimetic effect is hypothesized to be a consequence of myricetin's either direct or indirect interaction with glucose transporter 4 (GLUT4), however, no analysis has produced concrete conclusions detailing exactly from where this effect is derived.

Neuroprotective effects. Myricetin is also effective in protecting neurons against oxidative stressors. PC12 cells treated with hydrogen peroxide (H_2O_2) as an oxidative stressor experienced cell death due to apoptosis. When treated with myricetin, these oxidatively stressed cells displayed statistically significant increased cell survival (Dajas and Rivera-Megret, 2003). It has been suggested that myricetin not only has oxygen radical scavenging abilities, but also inherent, specific cell-survival capacities

III.2.2. **Kaempherol**

Kaempferol is a natural flavonol, a type of flavonoid, found in a variety of plants and plant-derived foods. Kaempferol acts as an antioxidant by reducing oxidative stress. Many studies suggest that consuming kaempferol may reduce the risk of various cancers, and it is currently under consideration as a possible cancer treatment

Anticancer effects. *In vitro* studies along with some animal testing has demonstrated the wide range of potential anti-cancer properties of kaempferol. It has been shown in malignant cancer cells to interrupt cell growth, limit angiogenesis, induce apoptosis, and to reduce their available energy and ability to metastasize (Calderon-Montano et al., 2011).

- A case controlled study found that "consumption of kaempferol-containing foods was associated with a reduced gastric cancer risk" (Calderon-Montano et al., 2011).
- In A549 lung cancer cells, kaempferol up-regulated pro-apoptotic bax,, while it down-regulated anti-apoptotic bcl-2 and bcl-xL expression. This resulted in an increase in apoptosis of the cancer cells Kim and Choi, 2013).
- An eight-year study found the consumption of three flavonols (kaempferol,

quercetin, and myricetin) correlated with a lower risk of pancreatic cancer among current smokers, but not non-smokers or ex-smokers (Nöthlings et al., 2007).

- Kaempferol has been shown to reduce growth in pro-myelocytic leukemia cells through altering the cell cycle Jaganathan and Mandal, 2009).

Antioxidant effects. Kaempferol has been shown to have an array of antioxidant effects in vitro and in vivo. At low concentrations, its acts as a superoxide scavenger, specifically against the highly reactive hydroxyl radical and peroxynitrite species. At high concentrations it increases the activity or expression of antioxidant enzymes such as superoxide dismutase, catalase, and heme oxygenase-1. Kaempferol can prevent the oxidation of low-density lipid proteins indicating a potential protective role in atherosclerosis (Calderon-Montano et al., 2011).

Anti-bacterial effects.In a four-week study, kaempferol and its glycosides decreased the number of *Helicobacter pylori* colonies in gerbils (small mammals of the order Rodentia) (Calderon-Montano et al., 2011).

Anti-viral effects. Kaempferol has been shown to inhibit or decrease the activity of enzymes that partake in viral infection such as reverse transcriptase, viral proteases and neuraminidase (Calderon-Montano et al., 2011).

III.2.3. **Apigenin**

Apigenin (4′,5,7-trihydroxyflavone), found in many plants, is a natural product belonging to the flavone class.

Anticancer effects. Through effects on cell signaling, inflammation, cell cycle, and protease production, apigenin has demonstrated effectiveness against a wide range of cancer types, while not showing toxicity to normal cells (Shukla and Gupta, 2010; Srivastava and Gupta, 2007).

Neuronal differentiation. Apigenin also stimulates adult neurogenesis in rats by promoting neuronal differentiation (Taupin 2009).

Apigenin readily crosses the blood-brain barrier and has not demonstrated toxicity at high doses (Venigalla et al., 2015).

III.2.4. **Luteolin**

Luteolin is a plant flavone. Synonyms: Luteolol, Flacitran, Luteoline, Salifazide, Digitoflavone

Anti-inflammatory effects: Luteolin reduces lipopolysaccharide-induced lethal toxicity and expression of proinflammatory molecules in mice (Kotanidou et al., 2002).

Anti-viral effects. Two small molecules, tetra-O-galloyl-beta-D-glucose (TGG) and luteolin, exhibited anti-SARS (severe acute respiratory syndrome)-corona virus (CoV) activities as confirmed by using a wild-type SARS-CoV infection system (Yi et al., 2004).

Anticancer effects. In nude mice with xenografted tumors using HAK-1B hepatoma cells, luteolin significantly inhibited the growth of the tumors in a dosage-dependent manner (Selvendiran et al., 2006).

In another study, luteolin greatly sensitized TNF-alpha-induced apoptotic cell death in a number of human cancer cell lines; including colorectal cancer COLO205, HCT116 cells and cervical cancer HeLa cells (Shi et al., 2004).

III.2.5. **Quercetin**

Quercetin is a plant polyphenol from the flavonoid group, found in many fruits, vegetables, leaves, and grains. It can be used as an ingredient in supplements, beverages, or foods.

Anti-inflammatory activity. *O*-methyl quercetin showed efficient prostaglandin (PG) inhibitory activity (Sadhu et al., 2006). PGs are well-known mediators of inflammatory reactions, and the compounds affecting their receptors, such as EP1–4, may act to reduce inflammation, pain, fever, etc. (Funk, 2001). Furthermore, PGE2 receptors play a key role in the pathogenesis of rheumatoid arthritis (McCoy et al., 2002).

In another study, quercetin has been demonstrated to attenuate TNF-induced inflammation by inhibiting the NF-κB pathway (Granado-Serrano et al., 2012). NF-*κ*B is a transcription factor that regulates inflammation, immunity, apoptosis, cell proliferation, and differentiation after binding to DNA and activating gene transcription. NF-*κ*B is activated by a wide variety of inflammatory stimuli, including tumor necrosis factor *α* (TNF), interleukin-1 (IL-1), lipopolysaccharide (LPS), or H_2O_2.

Anticancer effects. Using published food-composition data for flavonoids, an inverse association between intake of quercetin and risk of lung cancer (P for trend = 0.7) was found that appears consistent with association of its foud sources (Le Marchand et al., 2000).

Another study demonstrated that quercetin induced the cytotoxicity and apoptosis in both A549 and H1299 lung carcinoma cells in a concentration-dependent manner (Kuo et al., 2004).

However, there is no reliable clinical evidence that quercetin can prevent or treat cancer in humans (Ades 2009).

Anti-oxidant activity. 3-*O*-methyl quercetin shows significant anti-oxidant activity determined as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Sadhu et al., 2006).

In another study, quercetin has been demonstrated to decrease oxidative stress in streptozotocin-induced diabetic rats (Dias et al., 2005).

Evaluation of toxicity. A critical review of the data related to the safety of quercetin has demonstrated lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Therefore, it may be concluded that quercetin, at estimated dietary intake levels, does not produce adverse health effects (for a review, see Harwood et al., 2007).

III.2.6. **Catechins**

(+)-Catechin and the steroisomer (-)-epicatechin as well as their gallic acid conjugates are ubiquitous constituents of vascular plants, and frequent components of traditional herbal remedies, such as the Chinese medicine plant *Uncaria rhynchophylla* and others.

Epigallocatechin gallate (EGCG). EGCG (also known as epigallocatechin-3-gallate), the ester of epigallocatechin and gallic acid, has been the subject of basic and clinical research studies investigating its potential use as a therapeutic for a broad range of disorders (for a review, see Fürst and Zündorf., 2014).

In 1993, (-)epigallocatechin gallate and theaflavin digallate (1-10 μM) have been reported to inhibit the infectivity of both influenza A virus and influenza B virus in Madin-Darby canine kidney (MDCK) cells in vitro. The findings of this study suggested that tea polyphenols bind to the haemagglutinin of influenza virus, inhibit its adsorption to MDCK cells, and thus block its infectivity (Nakayama et al., 1993).

However, as of 2017 there are no approved health claims for EGCG in the United States or Europe (USA 2016; Europe 2011).

III.2.7. **Labdane-type diterpenes**

Several labdane-type diterpenoids, isolated from the leaves of Cistus incanus subsp. creticus; exhibited anti-bacterial and anti-fungal activities (Chinou et al., 1994).

III.3. Anti-inflammatory effects of C. incanus L. extracts

Short-boiled aqueous extract from leaves of *Cistus incanus* L. *ssp*. *incanus* dose-dependently inhibited the enzymatic activities of alanyl aminopeptidase) and dipeptidylpeptidase IV This inhibition was not reversible and very likely resulted from a covalent binding of reactive compounds to the enzymes (Lendeckel et al., 2002).

Local sites of inflammation are naturally infiltrated by monocytes, macrophages, neutrophils and activated T cells. Proteases either released by these cells or expressed at their plasma membrane play a crucial role in the regulation of the local immune response by activating or degrading neuropeptides, cytokines, growth factors and the corresponding receptors as well as matrix proteins. Although, these proteolytic activities normally are tightly controlled by endogenous inhibitors, imbalances in the proteolytic/antiproteolytic system are frequently observed in chronic inflammation and may also compromise wound healing after acute inflammation. In these cases both the inhibition of proteolytic activity and the reduction of the number of infiltrating immune cells could be beneficial.

The important function of e.g. matrix metalloproteases in wound repair and intestinal inflammation has been demonstrated by a number of recent studies (Madlener et al.,

1998; Witte et al., 1998). Alanyl aminopeptidase (APN, CD13, EC 3.4.11.2) and dipeptidylpeptidase IV (DP IV, CD26, EC 3.4.14.5) are both membrane-bound ectopeptidases the expression of which is significantly enhanced in the course of T cell activation (Schön et al., 1987; Riemann et al., 1997).

III.4. Anti-oxidant effects of C. incanus L. extracts

Recent studies on the antioxidant properties of aqueous *cistus incanus* extracts have indicated polyphenols to be the most active compounds.

In a recent study, chemical characterisation of polyphenolic compounds in leaves of *Cistus incanus* (*C. incanus*) has been performed. Three different polyphenolic enriched extracts, namely EAC (ethyl acetate fraction), and two aqueous fractions (AF1 and AF2) were obtained from a crude ethanolic leaf extract. The results indicated that the EAC, enriched in flavonols, exhibited a higher antiradical activity compared to the tannin enriched fractions AF1 and AF2 (Gori et al., 2016).

III.5. Gastro-protective effects of C. incanus L. extracts

In rats, short-boiled aqueous extract from aerial parts of *Cistus incanus* L. showed antiulcer activity against gastric lesions induced by necrotizing agents (1 **N** HCl and absolute ethanol), indomethacin, serotonin and reserpine. The extract, containing bioflavonoids, orally administered in the range from 0.25 to 0.50 g/ kg, was found to have significant dose-related protective effects in all these experimental models, and was more effective against reserpine- and serotonin-induced mucosal congestion and haemorrhagic ulcers (Attaguile et al., 1995).

III.6. Anti-bacterial effects of C. incanus extracts

Six labdane-type diterpenoids, isolated from the leaves of Cistus incanus subsp. creticus; exhibited anti-bacterial and one of these labdane-type diterpenoids exhibited anti-fungal activities (Chinou et al., 1994).

The antimicrobial studies showed that from seven isolated labdane-type diterpenes 6 compounds were active against *K pneumoniae*, *S. aureus* and *P. aeruginosa*, while only one compound was active against fungus *Candida albicans*.

III.7. Anti-viral effects of C. incanus L. extracts

Influenza A virus. A plant extract from *Cistus incanus* L. that is rich in polymeric polyphenols, exhibited antiviral activity against a highly pathogenic avian influenza A virus (H7N7) in cell culture and in a mouse infection model (Droebner et al., 2007).

In MDCK cells, a 90% reduction of plaque numbers on cells pre-incubated with the plant extract was achieved. For *in vivo* experiments we used a novel monitoring system for influenza A virus-infected mice that allows measurement of body temperature and gross motor-activity of the animals. Mice treated with CYSTUS052 did not develop disease, showed neither differences in their body temperature nor differences in their gross motor-activity and exhibited no histological alterations of the bronchiolus epithelial cells. *In vitro* and *in vivo* treatment was performed with an aerosol formulation, because the bioavailability of high molecular weight polyphenols is poor (Droebner et al., 2007).

Comparable results were reported for the highly pathogenic avian influenza viruses of the H5N1 subtype (Ehrhardt et al., 2007).

Human immunodeficiency virus (HIV).
Cistus incanus (Ci) extract inhibited clinical HIV-1 and HIV-2 isolates *in vitro*, and, importantly, a virus isolate with multiple drug resistances, confirming broad anti-HIV activity. Antiviral activity was highly selective for virus particles, preventing primary attachment of the virus to the cell surface and viral envelope proteins from binding to heparin (Rebensburg et al., 2016).

Bioassay-guided fractionation indicated that Ci extract contains numerous antiviral compounds and therefore has favorably low propensity to induce virus resistance. Indeed, no resistant viruses emerged during 24 weeks of continuous propagation of the virus in the presence of Ci extracts (Rebensburg et al., 2016).

Other viral pathogens. *Cistus incanus* (Ci) extracts also inhibited infection by virus particles pseudotyped with Ebola and Marburg virus envelope proteins, indicating that antiviral activity of Ci extract extends to emerging viral pathogens (Rebensburg et al., 2016).

These results demonstrate that Ci extracts show potent and broad *in vitro* antiviral activity against viruses that cause life-threatening diseases in humans and are promising sources of agents that target virus particles.

III.8. Anticancer effects of C. incanus L. extracts

Three labdane-type diterpenoids, isolated from the leaves of Cistus incanus subsp. creticus; exhibited cytotoxic activity for murine leukemia P-388 (3PS) cells, KB (human rhinopharynx cancer) cells and NSCLCN6 (human bronchial epidermoid carcinoma) cells (Chinou et al., 1994).

III.9. Aluminium detoxification with C. incanus L. extracts

Polyphenols can strongly complex with metal ion to form stable complexes (Hynes and Coinceanainn, 2001). Interactions of aluminum with catechins in vitro have been reported by Tang et al. (2004).

In another study with volunteers, confirmed cigarette smokers, the therapeutic potential of Cystus-Sud (Cistus incanus ssp. tauricus) as a heavy metal remover was evaluated. The results that were obtained indicated that the herbal medicine Cystus-Sud efficiently lowered the heavy metal concentration (accumulated due to cigarette smoking) from the subject's blood (Wan Mohd Azhar bin Ibrahim et al., 2012).

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IV. CISTUS CRETICUS EXTRACTS

Cistus creticus subsp. *creticus* (L.) is a native of the Mediterranean region. The leaves and stems secret a resin (Ladano), which has been used in traditional medicine.

IV.1. Components in C. creticus (subsp. creticus) extracts

Analysis by Anastasaki et al. (1999)

In this study, the content of labdane diterpenes in methanolic and hexane extracts and in the essential oils (leaves and fruits) of *C. creticus* subsp. *creticus* was investigated. The essential oils from the leaves and fruits from *C. creticus* subspecies and from the resin Ladano, from subsp. *creticus* were obtained by hydrodistillation for 3 h to 130 ^{o}C. The compounds isolated from the methanolic extract of fruits of *C. creticus* subsp. *creticus* were used as standards to identify the compounds detected in the extracts from *Cistus creticus*.

The analyses showed that the seven labdane diterpenes isolated from the methanolic extract of the fruits of *C. creticus* subsp. *creticus*, were also present in the hexane extracts from air-dried and powdered leaves and fruits, but not in the essential oils.

IV.2. Anti-leishmanial effects of C. creticus extracts

Leishmaniasis is a zoonotic disease caused by a protozoal parasite of the genus Leishmania. There are more than 30 known species of Leishmania that vary with region.

The protozoa are endemic in many tropical and subtropical regions in both the Eastern and Western hemispheres, where it is well described in people and dogs. Dogs are the most commonly affected domestic species and may act as a reservoir for disease. They can have visceral, eye, and cutaneous lesions. In endemic areas all breeds of dogs are affected, while in the US the disease has been most significant in the foxhound population.

Signs include fever, anemia, diarrhea, darkening of the skin, spleen and liver enlargement, and lymphadenopathy.

IV.2.1. **Leishmaniasis in horses**

Equine Cutaneous Leishmaniasis has been documented in horses around the world. Lesions were most commonly observed as nodules on the head, external ear, scrotum, legs, and neck. These nodules can ulcerate and are often mistaken for aural plaques or sarcoids. Visceral lesions have not been widely reported in the horse.

L. infantum has been reported as the causative agent of cutaneous leishmaniasis in horses in Germany, Spain, and Portugal. Recently a report from central Europe identified *L. siamensis*, a species previously reported as a cause of visceral human leishmaniasis, in four horses. In South America, *L. braziliensis* has been identified as the causative organism in horses. In all mammals affected with leishmaniasis, the mode of transmission is believed to be by various species of sandflies. In the Eastern Hemisphere, these are *Phlebotomus spp*, while *Lutzomyia spp* are predominant in the Western Hemisphere. The protozoa are transmitted by the female sandfly bite during salivation that occurs during blood feeding.

Leishmaniasis in horses has been recognized sporadically in the US, mainly in horses with a history of international transportation. Recently, however, two horses in Florida were diagnosed with cutaneous leishmaniasis due to *L. siamensis*. With increasing international transportation of horses, leishmaniasis should be considered in any horse with cutaneous nodule. While the disease is not fatal to horses, they can be infected with species of leishmania that are capable of transmission to humans.

IV.2.2. **Anti-leishmanial activity of *Cistus creticus* extracts**

As demonstrated in the study of Fokialakis et al. (2006), eleven *cis*-clerodane diterpenes, seven labdane type diterpenes and one triterpene isolated from the resin "Ladano" of *Cistus creticus* subsp. *creticus* exhibited activity against *Leishmania donovani* promastigotes. Among these natural compounds, the most poten ones were three *cis*-clerodane diterpenes with IC_{50} values of 3.3, 3.4 and 5.0 μg/ml.

IV.3. Anticancer effects of C. creticus extracts

Nine labdane-type diterpenes isolated from the plant *Cistus creticus* subsp. *creticus* and from the resin "Ladano" which is excreted on the surface of the leaves and stems of this plant, were examined for their in vitro cytotoxic activity against14 human leukemic cell lines.

Compound (13E)-.labd-13-ene-8α,15-diol, exhibited cytotoxic activity against 13 of the cell lines tested, while compound (13E)-labd-7,13-dienol, was active only against H160 cells (Dimas et al., 1998).

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V. CISTUS LADANIFER EXTRACTS

Cistus ladanifer is an aromatic shrub that is widespread in the Mediterranean region. It is especially abundant in the Iberian Peninsula and northwestern Africa.

V.1. Components in C. ladanifer extracts

Cistus ladanifer produces different types of secondary metabolites. Among them phenols, terpenes, alkaloids, polyacetylenes, fatty acids and steroids have been reported in the literature. Some of these compounds have been partly identified before (Chaves et al., 2001a, b; Dias and Moreira, 2002; Andrade et al., 2009). The phenolic and flavonoid fraction has been related to the reported antioxidant activity of *C. ladanifer* extracts (Andrade et al., 2009). The essential oil extraction and composition are well documented in the literature (Mariotti et al., 1997; Ramalho et al., 1999; Teixeira et al., 2007).

Analysis by Chaves et al. (2001a)

Eleven allelochemicals (ferulic acid, cinnamic acid, 4-hydroxybenzoic acid, hydroxycinnamic acid, methyl propionate, oxalic acid, methylmalonic acid, *p*-anisic acid, butyric acid, 3-hydroxybutyric acid, and azulene) were identified in the exudate of *Cistus ladanifer* L.

Analysis by Andrade et al. (2009)

The ethanol and acetone/water extract of *Cistus ladanifer* was characterised concerning the total phenolic and flavonoid contents, presenting relatively high values when compared with other species described in the literature.

Analysis by Fernandez-Arroyo et al. (2009)

From 57 peaks in the base peak chromatogram of *an aqueous Cistus landanifer extract*, 36 compounds have been characterized. Many well-known compounds present in *Cistus ladanifer* were characterised, such as flavonoids, phenolic acids, ellagitanins, hexahydroxydiphenoyl and derivatives, and other compounds.

V.2. Anti-oxidant effects of C. ladanifer extracts

Study of Andrade et al. (2009)

In this study, the antioxidant activity of an acetone/water extract of *Cistus ladanifer* was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method in terms of EC_{50}, using trolox as standard reference. The extract showed scavenging activity for the DPPH radical.

Study of Barrajón-Catalán et al. (2010).

In this study, phenolic and tannin content of *C. ladanifer* leaves aqueous extracts were determined and their antioxidant and antimicrobial activity were studied by several in vitro assays. Their major compounds were identified and quantitated by high-performance liquid chromatography with diode array detection coupled to electrospray ion-trap mass spectrometry. *C. ladanifer* extract showed significant antioxidant activity in electron transfer reaction based assays, but was even more effective to inhibit peroxyl radicals. The major compounds were identified as ellagitannins,

especially punicalagins derivatives. The results of this study are comparable to those previously reported by Dudonni et al. (2009).

V.3. Anti-bacterial effects of C. ladanifer extracts

Study of Barrajón-Catalán et al. (2010)

The antibacterial activity of an aqueous *C. ladanifer* extract was measured with Gram-positive (*Staphylococcus aureus*) and a Gram-negative (*Escherichia coli*) bacteria. The concentrations of the extract corresponding to 50% bacterial growth inhibition (MIC_{50}) were 0.90 mg of dry extract/ml for *E. coli* and 0.15 mg of dry extract/ml for *S. aureus*.

V.4. Anti-cancer effects of C. ladanifer extracts

Study of Barrajón-Catalán et al. (2010)

The cytotoxic activity of an aqueous *C. ladanifer* extract for several cancer cell lines, including pancreatic, colon and breast cancer cells was determined. The antitumor activity was measured on subconfluent (80–90% confluent) cells as the capacity of the extracts to inhibit cell proliferation.

Pancreatic cancer cells HS-766T and M186 were somehow resistant to the extracts, whereas pancreatic cancer cells M220 were highly sensitive, showing CC50 values of 0.49 mg of dry extract/ml. Regarding breast cancer cells, MCF7/HER2 and JIMT-1 cells were the most sensitive to this Cistus extract showing CC50 values within the range of 0.5–1 mg of dry extract/ml for MCF7/HER2 and 1.6–2 mg of dry extract/ml for JIMT-1 cells. SKBr3 breast cancer cells and HT29 colon cancer cells were more resistant to this Cistus extract.

V.5. Anabolic effects of C. ladanifer bee pollen extracts

Bee pollen extract. The powder of bee pollen (5 g) is suspended in distilled water (20 ml) and mixed vigorously. The suspension is centrifuged at 10,000 x g in a refrigerated centrifuge for 20 min. Tthe supernatant is filtered and freeze-dried. The powder of the water-solubilized extract is dissolved in ice-cold distilled water for use in experiments (Hamamoto et al., 2006 b).

Study of Yamaguch et al. (2006). This study demonstrates that bee pollen *Cistus ladaniferus (C. ladaniferus)* extract has an anabolic effect on bone metabolism in rats in vitro and in vivo. The extract stimulates bone calcification as potently as propolis (bee glue), a resinous mixture produced by honey bees.

Study of Hamamoto et al. (2006a). The bee pollen *Cistus ladaniferus* extract has stimulatory effects on bone formation and inhibitory effects on bone resorption in vitro.

Study of Yamaguch et al. (2007c). The anabolic effect of bee pollen *Cistus ladaniferus* extract has also been demonstrated in osteoblastic MC3T3-E1 cells.

Study of Yamaguch et al. (2007a + b). The intake of bee pollen *Cistus ladaniferus* extract has been demonstrated to have preventive effects on bone loss in rats induced in the diabetic state (Yamaguchi et al., 2007a) and in ovariectomized rats (Yamaguchi et al., 2007b), suggesting a useful role in the prevention of osteoporosis with aging.

Study of Hamamoto et al. (2006b). The active component of bee pollen *Cistus ladaniferus* extract which stimulates bone formation and inhibits osteoclastic bone resorption, has been shown to be a fraction with a molecular weight of less than 1000 (Hamamoto et al., 2006b).

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VI. CISTUS LAURIFOLIUS EXTRACTS

In Turkish folk medicine, *Cistus laurifolius* is reputed to be effective against a broad range of disorders either internally or externally.As internal remedy, infusion or decoction of leaves is used to treat diarrhoea and hypoglycaemia, while flower decoction is used to treat gastric pain (Yesilada et al., 1995). Moreover, a potent anti-ulcerogenic activity was reported against various *in vivo* peptic ulcer models previously (Yesilada et al., 1997a).

The leaves of the plant are also used externally as an effective remedy against several inflammatory ailments such as rheumatic pain, high fever and urinary inflammations. For relieving rheumatic pain a warm decoction of leaves is used as a bath or wilted leaves are externally applied on joints.

VI.1. Components in C. laurifolius extracts

Analysis by Sadhu et al. (2006)

In this study, the dried leaves and small branches of *Cistus laurifolius* (1.93 kg) were extracted with 14 liter of MeOH at room temperature to give the first extract (258.8 g). The residue was further extracted with 8 liter ofMeOHto obtain the second extract (196.4 g).

Chromatographic analysis yielded various compounds identified as 3-*O*-methyl quercetin, 3,7-*O*-dimethyl quercetin, genkwanin, 3,7-*O*-dimethyl kaempferol, 3,4'-*O*-dimethyl quercetin, apigenin, 3,4'-*O*-dimethyl kaempferol, ellagic acid, β-sitosterol-3-*O*-β -glucoside, quercetin 3-*O*-β-rhamnoside, 5-*O*-*p*-coumaroyl quinic acid methyl ester, 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-α-l-rhamnopyranoxypropyl)-2-methoxyphenoxy]-1,3-propanediol, and 2,3-dihydro-2-(4'-α-l-rhamnopyranosyloxy-3'-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol.

Analysis by Küpeli and Yesilada (2007)

Extracts and fractions from the leaves with nonwoody branches of *Cistus laurifolius* L. (Cistaceae) were analyzed by column chromatography. The chemical structures of three isolated compounds were elucidated as flavonoid derivatives being as 3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin and 3,7-*O*-dimethylkaempferol.

VI.2. Anti-inflammatory effects of C. laurifolius extracts

Study of Yesilada et al. (1997b).

In this study, *in vitro* inhibitory effects of extracts or fractions of *Cistus laurifolius* L. leaves on interleukin-1 (IL-1 α, IL-1β) and tumor necrosis factor (TNF-α) biosynthesis was demonstrated.

The cytokines IL-1α, IL-1β and TNF-α are also considered to play a key role in inflammatory and immune responses, based on their occurrence at inflammatory sites and their ability to induce many of the hallmarks of the inflammatory response.

Study of Sadhu et al. (2006)
This study shows prostaglandin (PG) inhibitory activity of a leaf extract of *Cistus laurifolius* which has been used traditionally to treat inflammatory and rheumatic disorders. The leaf extract showed inhibitory effects at 300 µg/ml on PGE1- and PGE2-induced contractions in guinea pig ileum.

PGs are well-known mediators of inflammatory reactions, and the compounds affecting their receptors, such as EP1–4, may act to reduce inflammation, pain, fever, etc. (Funk, 2001). Furthermore, PGE2 receptors play a key role in the pathogenesis of rheumatoid arthritis (McCoy et al., 2002).

The separation guided by the activities provided sixteen compounds. Known compounds were identified as 3-*O*-methyl quercetin, 3,7-*O*-dimethyl quercetin, genkwanin, 3,7-*O*-dimethyl kaempferol, 3,4'-*O*-dimethyl quercetin, apigenin, 3,4'-*O*-dimethyl kaempferol, ellagic acid, β-sitosterol-3-*O*-β -glucoside, quercetin 3-*O*-β-rhamnoside, 5-*O*-*p*-coumaroyl quinic acid methyl ester, 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-α-l-rhamnopyranoxypropyl)-2-methoxyphenoxy]-1,3-propanediol, and 2,3-dihydro-2-(4'-α-l-rhamnopyranosyloxy-3'-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol. Among the isolated compounds, 3-*O*-methyl quercetin showed most efficient PG inhibitory activity.

Study of Küpeli and Yesilada (2007).
Effects of the extracts and fractions from the leaves with nonwoody branches of *Cistus laurifolius* L. (Cistaceae) were studied using two *in vivo* models of inflammation in mice. Model one was based on observed potent inhibitory activity against carrageenan-induced hind paw oedema and the second model used was acetic acid-induced, increased vascular permeability model.

Two types of extracts (aqueous and ethanolic) were prepared and assayed. The aqueous extract did not show any remarkable effect against carrageenan-induced hind paw oedema model, while the EtOH extract was significantly active both in 250 and 500 mg/kg doses. A comparable result was obtained against the other inflammatory model based on the inhibition of increased vascular permeability, induced by the intraperitoneally injection of acetic acid.

Further analyses revealed that all three isolated flavonoids (3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin and 3,7-*O*-dimethylkaempferol) were found to possess almost equally potent anti-inflammatory activity in carrageenan-induced paw oedema model.

VI.3. Anti-oxidant effects of C. laurifolius extracts

Study of Sadhu et al. (2006)
This study shows anti-oxidant activity of a leaf extract of *Cistus laurifolius* determined as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect.

The results indicate that the catechol structure of the B ring is essential for the radical scavenging effect of the flavonoids.

Among the isolated compounds, 3-*O*-methyl quercetin showed most efficient anti-oxidant activity.

VI.4. Anti-nociceptive effects of C. laurifolius extracts

Study of Küpeli and Yesilada (2007). The antinociceptive activity of the extracts and the three major flavonoid compounds (3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin and 3,7-*O*-dimethylkaempferol) were studied by using *p*benzoquinone-induced writhing model in mice. A similar activity pattern was observed as that of anti-inflammatory activity, i.e. EtOH extract, CHCl3 and EtOAc fractions inhibited the writhes remarkably. All three isolated compounds (3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin and 3,7-*O*-dimethylkaempferol) demonstrated potent antinociceptive without induced any apparent gastric lesions (Küpeli and Yesilada, 2007).

VI.5. Gastro-protective effects of C. laurifolius extracts

Study of Yesilada et al. (1997a).

In this study, the anti-ulcergenic activity of the ethanol-precipitated part from the aqueous extract of flowers and flower buds of *Cistus laurifolius* L. are demonstrated in various ulcer models in rats and mice. This fraction was active against gastric lesions induced by pylorus ligation, absolute ethanol, indomethacin, and indomethacin plus HCl(ethanol). This fraction was also active against duodenal lesions induced by cysteamin, but was ineffective against gastric lesions induced by sertonin. The active fraction showed its activity not only after oral administration but also after subcutaneous injection.

VI.6. Anti-Helicobacter pylori effects of C. laurifolius extracts

Study of Yesilada et al. (1999).

This study demonstrates that a chloroform extract of *Cistus laurifolius* exhibits a significant anti-*Helicobacter pylori* activity.

Study of Ustün et al. (2006)

In this study, the active component(s) involved in the anti-*Helicobacter pylori* activity of a chloroform extract of *Cistus laurifolius* was isolated and defined through activity-guided fractionation procedures. The compound with the highest activity against *Helicobacter pylori* (MIC 3.9 µg/mL) was identified as quercetin3-methyl ether (isorhamnetin) by various spectroscopic techniques.

VI.7. References

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VII. CISTUS MONSPELIENSIS EXTRACTS

The genus *Cistus* L. (Cistaceae) comprises many medicinal plants, distributed primarily in the Mediterranean region. *Cistus monspeliensis* L., which is a compact, aromatic bush up to 1m tall, widespread in Greece and in Marocco, and one of the most common *Cistus* species in the Mediterranean basin.

VII.1. Anti-leishmanial effects of C. monspeliensis extracts

For background information on leishmaniasis in horses, see section IV.2.

Study of Fokialakis et al. (2006)

In the study, eleven *cis*-clerodane diterpenes, seven labdane type diterpenes and one triterpene isolated from *Cistus monspeliensis* exhibited activity against *Leishmania donovani* promastigotes. Among these natural compounds, the most poten ones were three *cis*-clerodane diterpenes with IC_{50} values of 3.3, 3.4 and 5.0 µg/ml.

VII.2. Anti-bacterial effects of C. monspeliensis extracts

Study of Bouamama et al. (2006)

This study demonstrates ant-bacterial activity of ethyl acetate extracts and butanol extrtacts of dried and finely powdered *Cistus monspeliensis* leaves with regard to Gram-positive (*Staphylococcus aureus* and *Enterococcus hirea*) and Gram negatice bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

VII.3. Anti-fungal effects of C. monspeliensis extracts

Study of Bouamama et al. (2006)

This study demonstrates ant-fungall activity of methanol extracts, ethyl acetate extracts and butanol extrtacts of dried and finely powdered *Cistus monspeliensis* leaves with regard to *Candida glabrata*. The extracts were less effective against *Candia albicans* and *Candida krusei*.

VII.4. Anti-oxidant effects of C. monspeliensis extracts

Study of Sayah et al. (2017)

In this study, the antioxidant α-amylase and α-glucosidase enzyme inhibitory effects of aqueous and hydromethanolic extracts from the aerial parts of Moroccan *Cistus monspeliensis* (CM) are demonstrated.

Antioxidant activity has been assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2 -azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals and ferric reducing/antioxidant power (FRAP) methods.

The α-amylase and α-glucosidase inhibitory activity has been assessed using an in vitro model.

The extract exhibited potent antioxidant activity in all used systems and possess strong inhibitory effect towards α-glucosidase (IC_{50}: 0.95to 14.58 μg/mL) and significant inhibitory potential against α-amylase (IC_{50}: 217.1 to 886.1 μg/mL).

The potential of the extracts to inhibit α-glucosidase enzyme and their significant inhibition of α-amylase indicate that CM extracts may be effective therapeutic agents for controlling hyperglycemia.

VII.5. References

Bouamama H., et al.: Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. J. Ethnopharmacology 104: 104-107; 2006.

Fokialakis N., et al.:Antileishmanial Activity of Natural Diterpenes from *Cistus* sp. And Semisynthetic Derivatives Thereof. Biol. Pharm. Bull. 29: 1775-1778; 2006.

Sayah K., et al.: Antioxidant Activity and Inhibitory Potential of *Cistus salviifolius* (L.) and *Cistus monspeliensis* (L.) Aerial Parts Extracts against Key Enzymes Linked to Hyperglycemia. BioMed Res. Int., Volume 2017, Article ID 2789482, 7 pages.

VIII. CISTUS POPULIFOLIUS EXTRACTS

The Cistaceae is a Mediterranean native family of almost 200 species of shrubs. Some of these plants including C. populifolius are widespread in the south-east of Iberian Peninsula, northwestern Africa, Greece and Portugal. *Cistus populifolius* shows one of the largest biomass productivity in the wilderness and natural parks of the south area of Spain.

VIII.1. Anti-bacterial effects of C. populifolius extracts

Study of Barrajón-Catalán et al. (2010).

The antibacterial activity of an aqueous *C. populifolius* extract was measured with Gram-positive (*Staphylococcus aureus*) and a Gram-negative (*Escherichia coli*) bacteria. The concentrations of the extract corresponding to 50% bacterial growth inhibition (MIC_{50}) were 0.12 mg of dry extract/ml for *E. coli* and 0.34 mg of dry extract/ml for *S. aureus*..

VIII.2. Anti-oxidant effects of C. populifolius extracts

Study of Barrajón-Catalán et al. (2010).

In this study, phenolic and tannin content of aqueous extracts of *C. populifolius* leaves were determined and their antioxidant and antimicrobial activity were studied by several in vitro assays. Their major compounds were identified and quantitated by high-performance liquid chromatography with diode array detection coupled to electrospray ion-trap mass spectrometry. *C. populifolius* extract showed significant antioxidant activity in electron transfer reaction based assays. The major compounds were identified as ellagitannins, especially punicalagins derivatives.

VIII.3. Anti-cancer effects of C. populifolius extracts

Study of Barrajón-Catalán et al. (2010)

The cytotoxic activity of an aqueous *C. populifolius* extract for several cancer cell lines, including pancreatic, colon and breast cancer cells was determined. The antitumor activity was measured on subconfluent (80–90% confluent) cells as the capacity of the extracts to inhibit cell proliferation.

Pancreatic cancer cells HS-766T and M186 were somehow resistant to the extracts, whereas pancreatic cancer cells M220 were highly sensitive, showing 50% cytotoxic concentration (CC50) values of 0.66 mg of dry extract/ml. Regarding breast cancer cells, MCF7/HER2 and JIMT-1 cells were the most sensitive to this Cistus extract showing CC50 values within the range of 0.5–1 mg of dry extract/ml for MCF7/HER2 and 1.6–2 mg of dry extract/ml for JIMT-1 cells. SKBr3 breast cancer cells and HT29 colon cancer cells were more resistant to this Cistus extract.

VIII.4. References

Barrajón-Catalán E., et al.: *Cistaceae* aqueous extracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. Food Chem. Toxicol. 48: 2273–2282; 2010.

IX. CISTUS SALVIIFOLIUS EXTRACTS

The genus *Cistus* L. (Cistaceae) comprises many medicinal plants, distributed primarily in the Mediterranean region. Among them, twelve species are members of Moroccan flora including *Cistus salviifloius*.

IX.1. Anti-oxidant effects of C. salvifolius extracts

Study of Sayah et al. (2017)

In this study, the antioxidant α-amylase and α-glucosidase enzyme inhibitory effects of aqueous and hydromethanolic extracts from the aerial parts of Moroccan *Cistus salviifolius* (CS) are demonstrated.

Antioxidant activity has been assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2‘-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals and ferric reducing/antioxidant power (FRAP) methods.

The α-amylase and α-glucosidase inhibitory activity has been assessed using an in vitro model.

The extract exhibited potent antioxidant activity in all used systems and possess strong inhibitory effect towards α-glucosidase (IC_{50}: 0.95to 14.58 μg/mL) and significant inhibitory potential against α-amylase (IC_{50}: 217.1 to 886.1 μg/mL).

The potential of the extracts to inhibit α-glucosidase enzyme and their significant inhibition of α-amylase indicate that CS extracts may be effective therapeutic agents for controlling hyperglycemia.

IX.2. Reference

Sayah K., et al.: Antioxidant Activity and Inhibitory Potential of *Cistus salviifolius* (L.) and *Cistus monspeliensis* (L.) Aerial Parts Extracts against Key Enzymes Linked to Hyperglycemia. BioMed Res. Int., Volume 2017, Article ID 2789482, 7 pages.