

# Phenolic profile, antioxidant and anticandidal activities of *Inula oculus-christi* L. from Turkey

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Received: 21 February 2022 / Revised: 22 April 2022 / Accepted: 23 April 2022

**ABSTRACT:** The study aimed to determine the total phenol content of the extracts prepared with different solvents (70% methanol, ethyl acetate, 5% infusion) from the aerial parts of the *I. oculus-christi*, as well as to evaluate the antioxidant capacity and antimicrobial activity of the plant and to determine the main components responsible for the effect. The total phenolic content of the extracts prepared from the aerial parts of *I. oculus-christi* was calculated in gallic acid equivalents and the total phenolic content of the species was found to be in the range of 23.8-75.3 mg GAE/g. The highest total phenol content calculated as gallic acid equivalent was detected in the 5% infusion sample. The antioxidant activity of *I. oculus-christi* was also tested using DPPH<sup>•</sup> free radical scavenging in vitro assay (IC<sub>50</sub>: 0.092-1.03 mg/mL). The 5% infusion of the plant was shown to be the most effective extract in the antioxidant activity test performed using the DPPH<sup>•</sup> technique. The findings revealed that there is a correlation between total phenol content and antioxidant activity, as numerous research as suggested. By using the microdilution method, extracts from the aerial parts of *I. oculus-christi* were tested against *Candida* strains, and the MIC value for *C. utilis* was found to be 0.03 mg/mL in 5% infusion and ethyl acetate extracts. In terms of evaluating the biological effects obtained, the chemical composition of extracts was analyzed by HPLC-MS/MS, and the main components (5-caffeoylquinic acid, 1,5-dicaffeoylquinic acid, luteolin/kaempferol, apigenin, and rutin) were determined.

**KEYWORDS:** Anticandidal; DPPH; HPLC-MS/MS; *Inula oculus-christi*; phenolics.

## 1. INTRODUCTION

The Asteraceae family is a very large taxon that includes many genera with high medical and economic value and is widely used among the people. Among the various active substance groups isolated from the members of the family; essential oils, monoterpenes, sesquiterpenes, sesquiterpene lactones, and other sesquiterpene derivatives, diterpenes, triterpenes, flavonoids, phenolic acids, steroids, benzofurans, glycolipids, polyacetylenes, and amino acid derivatives can be counted [1,2,3,4].

*Inula* L. genus, especially in the Mediterranean Region; It has been naturalized in Europe, Asia, and Africa; It includes species showing a wide range of habitats from dry, rocky, mountainous areas to moist, shady, low-lying areas [5,6,7,8]. It has been widely used for many years in traditional medicine as a folk remedy and in modern medicine due to its various pharmacological activities. Various biological activity studies have been carried out on *Inula* species and in these studies; Antifungal, cardioprotective, antianginal, antidiabetic, and cytotoxic effects of these species have been reported due to the compounds they contain. Studies on species with high medicinal value, especially *I. helenium*, *I. racemosa*, *I. viscosa*, *I. britannica*, and *I. japonica*, have increased considerably in recent years. In previous studies, it has been noted that *I. helenium* was used as an expectorant, flowers of *I. britannica* and *I. japonica* were used as diaphoretic and bactericidal, as well as in the treatment of bacterial/viral infections and inflammations in addition to tumor treatment. In addition, it has also been reported that *I. viscosa* has antispasmodic, sedative, expectorant, antiseptic, wound healing, antirheumatic, antihemorrhoidal, antipyretic, antidiabetic, and anthelmintic effects [7,9,10]. In a recent study, *Inula* species grown in Turkey could potentially be used by diabetic patients due to their antidiabetic and antioxidant activities, and in addition, the antiprotozoal activity of *I. montbretiana* extract was reported in another study [11, 12].

**How to cite this article:** Saltan N, Köse YB, Göger F, İşcan G. Phenolic Profile, Antioxidant and Anticandidal Activities of *Inula oculus-christi* L. from Turkey. J Res Pharm. 2022; 26(4): 799-808

*Inula oculus-christi* L., belonging to the Asteraceae family is commonly known as “Sümenit, Şekerliot, Yolotu, İsağözü, Andız, Pire otu, Yünlüpirekran” in Turkey [13,14,15]. It is native to Iran, the Caucasus, Turkey, eastern Central Europe, Austria, and the Balkan Peninsula.

Because of their chemical composition, many phenolic chemicals, which are mostly found in plants, have antioxidant and free radical scavenging activities. These phenolic chemicals can be extracted from medicinal plants in a variety of ways, including Soxhlet, maceration, heat reflux, and microwave-assisted extraction. Antioxidants are critical sources in the body to eliminate free radicals and play an important role in maintaining our health. In recent years, the search for natural antioxidants, which are considered as alternative sources for antioxidant synthesis, has gained importance [16]. It has long been known that most of phenolics are important antioxidant substances derived from natural plants. Despite several investigations on various *Inula* species, little is known about the antioxidant capabilities and phenolic component composition of *I. oculus-christi*. To the best of our knowledge, hispidulin in *I. oculus-christi* from Serbia [17], a DPPH scavenging activity of aqueous extracts of the species from Turkey [8], and total phenolic and flavonoid contents of the methanol extract besides the DPPH and ABTS scavenging activity of extract of the species from Bulgaria [18], have been reported. Phytochemical studies have aimed to both confirm traditional applications and reveal pharmacological evaluations, as well as finding active ingredients. Ivanova et al. used DPPH and ABTS tests to assess chloroform and methanol extracts of *I. britannica* flower heads and leaves [19]. The methanol extracts were possessed the highest antioxidant activity and maximal total phenolic and flavonoid contents. Bucchini et al. (2015), determined that the methanol and hexane extracts of *I. crithmoides* had the strongest DPPH-radical scavenging activity with IC<sub>50</sub> values of 0.59 and 0.57mg DW/mL, respectively [20]. As a result, antioxidant capabilities, total phenolic content, and some phenolic compounds that may be responsible for the biological effect of *I. oculus-christi* extracts were determined in this study. We investigated ethyl acetate, aqueous methanol, and 5% infusion extracts in this study, as previous studies have shown that the choice of solvent has a significant effect on the concentration of antioxidants [21,22].

Further in vitro antimicrobial effects of all extracts of *I. oculus-christi* were evaluated against *Candida* strains. Despite a rich tradition of usage of medicinal plants for the treatment of various diseases in many parts of Turkey, reports on their antimicrobial activities are meager. Gökbulut et al. (2013) investigated the antibacterial activity of methanol extracts prepared from plant parts against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Candida tropicalis* in a recent paper [23]. The antimicrobial activity of the aqueous extract of *I. oculus-christi* was determined by Berk et al. [8]. They have observed no activity against *S. typhi*, *P. aeruginosa*, *E. coli*, *P. vulgaris* and *C. albicans*. By Omezzine et al. (2011) leaf and flower extracts (hexane, chloroform, and methanol) of 3 species of *Inula* were evaluated for their antifungal activity against two *Trichoderma* species and three formae specialties of *Fusarium oxysporum* [24]. There is a demand for novel, safer, and more effective antimicrobial agents in general, and medicinal plants might be considered a viable and alternative source of bioactive compounds [25].

Accordingly, the number of studies on *Inula* species spreading in the world due to their ethnopharmacological uses is increasing. Although *Inula* species grow naturally in a wide habitat in our country; chemical studies on plants are limited. Among the plants included in the genus *Inula*, the existence of species that have never been studied or have been studied in limited numbers attracts the attention of researchers in this direction. In addition to two studies on *I. oculus-christi*, which grows naturally in Turkey, published in our country, there are a small number of studies on antioxidant, antimicrobial, protective effect from DNA damage, and amibicidal activity; the number of international publications on this plant is also very limited [8,26,27].

In this study, it was aimed to determine the antioxidant and antimicrobial activity of the *I. oculus-christi*, as well as the total amount of phenols and active compounds. In the light of the data obtained from these studies, it is aimed to contribute to science in terms of human health.

## 2. RESULTS

The antioxidant capacity of *I. oculus-christi* was assessed using DPPH radical scavenging tests. The inhibition of the extracts against DPPH at a concentration of 1 mg/mL is given in Table 1. All of the extracts indicated antioxidant activity in varying doses (0.092-1.03 mg/mL) based on the IC<sub>50</sub> values obtained by this approach. The radical scavenging activity of the extracts was not effective as a positive control gallic acid (IC<sub>50</sub>: 0.018 mg/mL). As shown in Table 1, 5% infusion extract from aerial parts showed the highest radical scavenging activity (IC<sub>50</sub>: =0.092 ± 0.008 mg/mL), followed by aqueous methanol while ethyl acetate extract was lower than the others. DPPH radicals scavenging activity of *I. oculus-christi* extracts decreased in the following order: I > M > E > (Table 1). Total phenolic amounts were determined as gallic acid equivalent (mg/g extract). The content of total phenolics in extracts varied from 23.8 to 75.3 mg of gallic acid equivalent per gram

dried extracts (Table 1). As can be seen from Table 1, the highest amount of phenolics was detected in 5 % infusion extract of *I. oculus-christi* (75.3 mg GAE/g ext.). In compared to the 5% infusion and methanol extracts, the ethyl acetate extract displayed low antioxidant activity and high IC<sub>50</sub> values. Parallel to this, the lowest total phenolic concentration was found in ethyl acetate extract, which also had the highest IC<sub>50</sub> value.

**Table 1.** Total phenolic content (TPC) and DPPH• radical scavenging activity of different extracts derived from *I. oculus-christi* aerial parts

| Extracts       | TPC [mg GAE/g extract] <sup>a</sup> | DPPH test IC <sub>50</sub> (mg/mL) |
|----------------|-------------------------------------|------------------------------------|
| Ethylacetate   | 23.8                                | 1.03 ±0.1                          |
| Methanol (70%) | 71.3                                | 0.14 ±0.009                        |
| 5% infusion    | 75.3                                | 0.092 ±0.008                       |

<sup>a</sup> mg GAE/g extract: Total phenols expressed as gallic acid equivalents milligrams of gallic acid per gram (dry weight) of extract.

Measurements using HPLC-MS/MS were done in several extracts of the plant to determine some active components responsible for its antioxidant and antibacterial activities. It is known that the phenolic compounds contribute to the overall antioxidant potential of plants. The analyses of the extracts were carried out using HPLC-MS/MS systems and the results are presented in Table 2. In order to explain the chromatograms obtained as a result of the analysis, the code (Rt value), [M-H]<sup>-</sup> value, and fragments of each compound are given respectively. According to the identification of phenolic profile by HPLC-MS/MS, 5-caffeoylquinic acid, 1,5-dicaffeoylquinic acid, luteolin/ kaempferol, apigenin and rutin were determined (Fig. 1-2). These compounds were characterized according to their mass patterns and retention times by comparison with data from the background of different studies.

**Table 2.** HPLC-MS/MS results of *I. oculus-christi*

| Rt <sup>a</sup> | [M -H] <sup>-</sup> | Fragments      | Identified Compounds                     | References |
|-----------------|---------------------|----------------|--|------------|
| 13.3            | 609                 | 300            | rutin (M <sup>b</sup> , I <sup>c</sup> ) | [28]       |
| 17.3            | 353                 | 191, 179, 161  | 5-caffeoylquinic acid (M, I)             | [28]       |
| 20.6            | 515                 | 3353, 335, 191 | 1,5-dicaffeoylquinic acid (M, I)         | [29]       |
| 20.9            | 285                 | 151, 133       | luteolin/ kaempferol (M)                 | [28]       |
| 23.7            | 269                 | 151, 117       | apigenin (M)                             | [28]       |

<sup>a</sup> Rt: Retention time; [M - H]<sup>-</sup>: molecular ion.

<sup>b</sup> M: Methanol extract

<sup>c</sup> I: Infusion

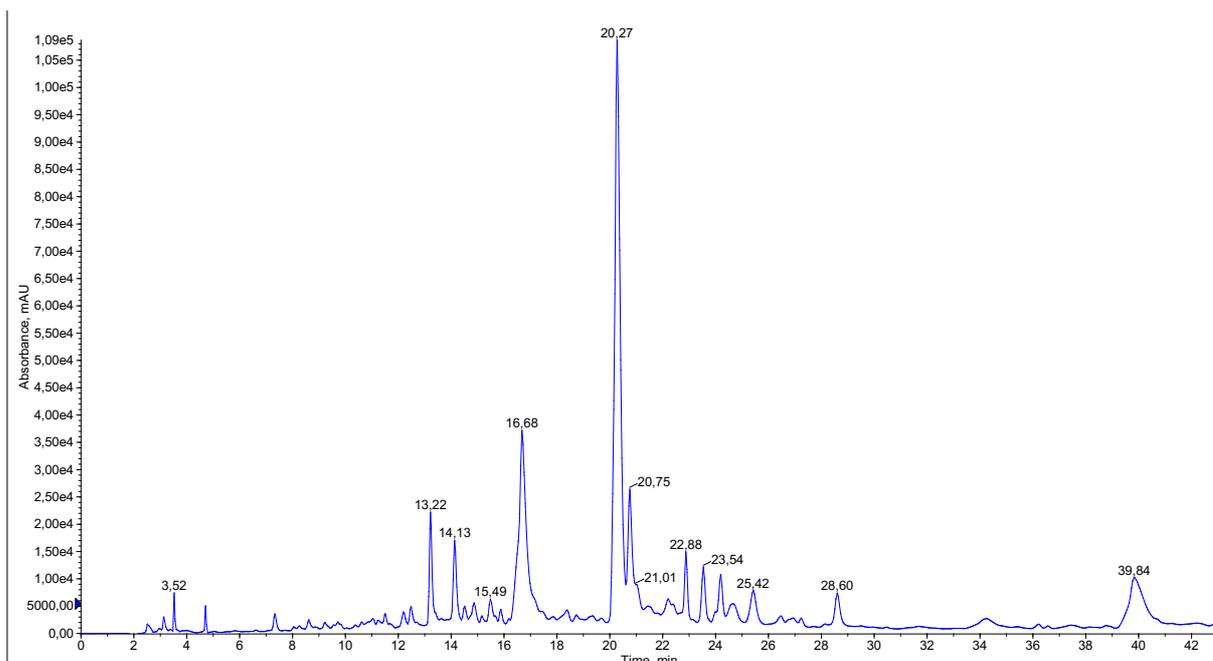


Figure 1. HPLC-MS/MS chromatogram of 70% MeOH extract of *I. oculus-christi*.

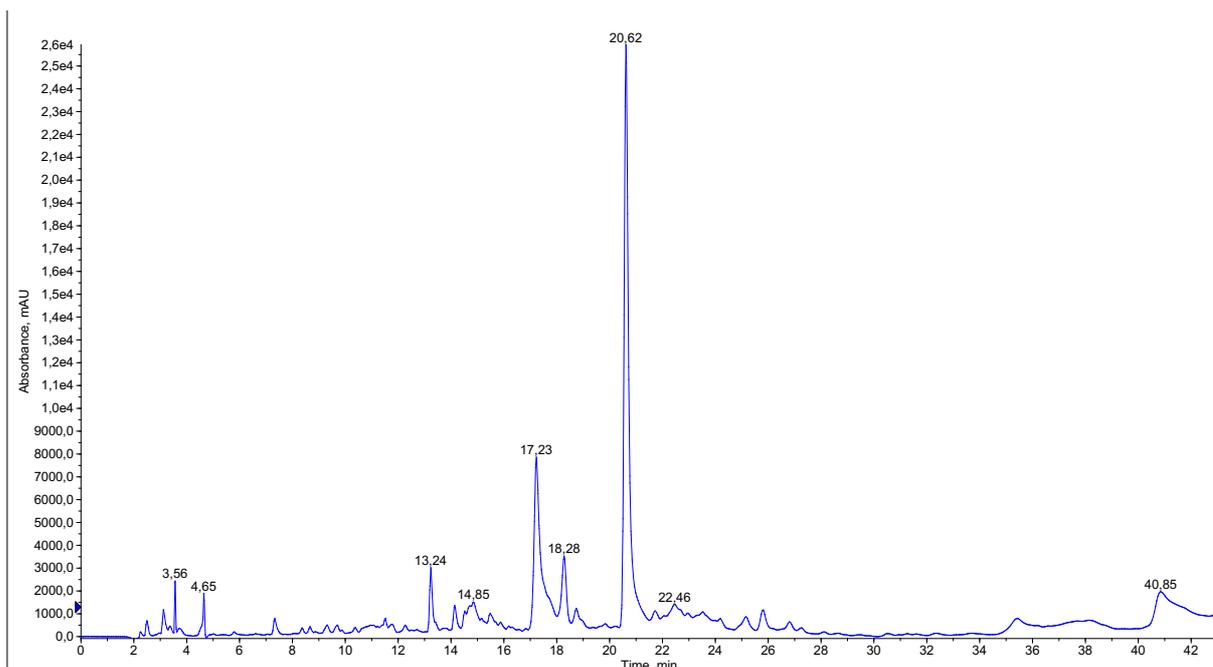


Figure 2. HPLC-MS/MS chromatogram of 5% infusion of *I. oculus-christi*.

*Candida* strains were also tested for anticandidal activity in vitro using microdilution techniques in this investigation. The anticandidal activity of *I. oculus christi* is summarized in Table 3. Anticandidal activity was found in all plant extracts, with MIC values ranging from 31 to 1000 g/mL (Table 3). While the MIC was found at 39 µg/mL against *C. utilis*, *C. krusei* for the extract of ethyl acetate, was found 78 µg/mL against *C. albicans*. The extract of 5% infusion had the lowest MIC value of all the extracts, at 31 g/mL against *C. albicans*. The most sensitive strain was *C. utilis*, according to our findings.

**Table 3.** Anticandidal MIC results ( $\mu\text{g/mL}$ ) of the extracts of the *I. oculus-christi*

| Microorganisms                     | E   | I    | M    | Ketoconazole | Amphotericin-B |
|------------------------------------|-----|------|------|--------------|----------------|
| <i>Candida albicans</i> ATCC 10231 | 78  | 125  | 125  | 0.03         | 0.12           |
| <i>C. utilis</i> NRRL Y-900        | 39  | 31   | 62   | 0.06         | 0.12           |
| <i>C. albicans</i> ATCC 90028      | 156 | 1000 | 1000 | 0.03         | 0.12           |
| <i>C. tropicalis</i> ATCC 750      | 156 | 250  | 500  | 0.03         | 0.25           |
| <i>C. parapsilosis</i> ATCC 22019* | 156 | 250  | 500  | 0.06         | 0.06           |
| <i>C. krusei</i> ATCC 6258*        | 39  | 62   | 125  | 0.5          | 0.5            |

\* Quality control strains recommended by CLSI protocol

E: ethyl acetate;  
M: Methanol extract;  
I: Infusion

### 3. DISCUSSION

The goal of the study was to investigate the phenolic components in *I. oculus-christi* extracts, as well as its antioxidant and anticandidal properties. The antioxidant capacity of plant extracts containing polar compounds was determined using a simple, repeatable approach called the DPPH test. Many papers about the antioxidant benefits of various extracts and chemicals derived from many *Inula* species can be found in the literature. Berk *et al.* (2011), reported that the free radical scavenging capacity of the *I. oculus christi* extract was determined as 53.34% at 0.8 mg/ml concentration value. Also, the amounts of phenolic compounds determined were  $33.17 \pm 0.34 \mu\text{g/mg}$  [8]. In another research, the highest radical scavenging activity was found in a methanol extract of *I. oculus-christi* flowers, followed by a methanol extract of leaves, while chloroform extract of leaves was nearly inert [18]. *Inula britannica* var. *chinensis* were prepared with different solvents and it was reported that the water fraction showed the strongest antioxidant activity (DPPH  $\text{IC}_{50}$  20.7  $\mu\text{g/mL}$ ; ABTS  $\text{IC}_{50}$  39.4  $\mu\text{g/mL}$ ) [30]. The total phenolic content of methanol, ethanol, water, and ethyl acetate extracts obtained by the Folin-Ciocalteu technique from the aerial parts of *I. helenium* species growing in Turkey ranged from 4.18 to 102.91 mg gallic acid equivalent/g dry extract [31]. In another study, it was reported that the total amount of phenol in the chloroform and ethyl acetate extracts of *I. helenium* root was  $5.06 \pm 0.06$  (mg gallic acid  $\text{g}^{-1}$ ) and  $42.2 \pm 0.07$  (mg gallic acid  $\text{g}^{-1}$ ), respectively [32]. Chahmi *et al.* assessed the antioxidant activity, total phenolic content, and flavonoid content of ethanol and ethyl acetate extracts of aerial parts of *I. viscosa* collected from three Moroccan regions, finding that the ethanol extract from Sefrou had the highest antioxidant capacity ( $274.39 \pm 6.94$  mg gallic acid equivalent/g dry extract), probably due to its high polyphenol concentration. [33]. In another study conducted in Morocco (2020), extracts were prepared from leaves and flower buds of *I. viscosa* by maceration with methanol or water, hot extraction and Soxhlet ethanol extraction [34]. The antioxidant capacity of *I. viscosa* leaf and flower bud extracts was assessed in vitro using the DPPH test, and the extracts were found to have a considerable effect ( $\text{IC}_{50}$   $54.24 \pm 0.21$  g/mL and  $39.77 \pm 0.23$  g/mL, respectively) [34]. According to Gökbulut *et al.*, the total phenolic content of extracts prepared from *I. viscosa*, *I. helenium* ssp. *turcoracemosa* and *I. montbretiana* collected from various parts of Anatolia ranged from  $21.1 \pm 0.8$  to  $190.9 \pm 6.1$  mg GAE/g [23]. Water extract of *I. viscosa* flowers ( $\text{IC}_{50}$ :  $0.28 \pm 0.03$  mg/mL) and methanol extract of *I. helenium* flowers ( $\text{IC}_{50}$ :  $0.14 \pm 0.06$  mg/mL) were also shown to have the highest antioxidant activities based on  $\text{IC}_{50}$  values obtained using the DPPH method. According to  $\text{IC}_{50}$  values obtained from the DPPH method, almost all ethyl acetate extracts had low antioxidant activity with high  $\text{IC}_{50}$  values, whereas water extract of *I. viscosa* flowers ( $\text{IC}_{50}$ :  $0.28 \pm 0.03$  mg/mL) and methanol extract of *I. helenium* flowers ( $\text{IC}_{50}$ :  $0.14 \pm 0.06$  mg/mL) has been reported to have the highest antioxidant activities [23]. In a study to determine the total phenolic contents, antioxidant and antimicrobial activities, and phenolic composition of *I. peacockiana* and *I. thapsoides* ssp. *thapsoides*, it was reported that ethyl acetate extracts of plants exhibited weak antioxidant activity with higher  $\text{IC}_{50}$  values compared to water and methanol extracts [35]. In our investigation, the antioxidant tests revealed

that *I. oculus-christi* extracts, such as from other *Inula* species, had considerable radical scavenging activity in the DPPH test. Furthermore, our findings are consistent with those of the authors, who found that antioxidant activity is related to phenolic chemicals.

The determination of antioxidant compounds such as rutin, apigenin, and luteolin in *I. oculus-christi* gives information about the compounds responsible for the effect in this plant. Several flavanones and flavonols (7-O-methylaromadendrin, sakuranetin, quercitrin, isorhamnetin, luteolin, hispidulin, and nepetin) have been isolated from *Inula* by different researchers [36,37,38,39,40] and some very interesting biological features antitumor, antibacterial, and anti-inflammatory activities have also been reported [39,41,42,43]. In a study by Gökbulut et al., three *Inula* species were researched for their phenolic composition and *I. viscosa* (L.) Aiton root extract was found to be rich in chlorogenic acid, while *I. helenium* flower extract contained caffeic acid and *I. montbretiana* flower extract contained high amounts of luteolin [23]. The plant kingdom, including *Inula* species, is rich in mono- and dicaffeoylquinic acid derivatives. Chlorogenic acid and 3,5-dicaffeoylquinic acid have been detected previously in *I. viscosa*, *I. britannica*, *I. helenium*, *I. cappa*, etc. [5,6]. Thirteen substances (nepetin, 3,3'-di-O-methylquercetin, hispidulin, and 3-O-methylquercetin, etc) were identified from *I. viscosa* in a study [39]. According to a study using HPLC and GC-MS techniques to obtain preliminary information about the composition of the extracts prepared with chloroform and ethyl acetate from the roots of *I. helenium*, phenolic acids (caffeic, chlorogenic, dicaffeoylquinic, hydroxybenzoic), terpenes (alantolactone) and different flavonoids (epicatechin, catechingallate, ferulic acid-4-O-glucoside, dihydroquercetin-pentosylrutinoside, kaempferol-7-O-dipentoside, quercetin-3-O- $\beta$ -glucopyranoside) of the extracts were investigated [32].

When compared to published data, the phenolic components and concentrations in our study were found to be similar, but there were minor variances due to different extraction processes and plant factors (plant origin, harvesting time, plant maturation, habitat). There have been some reports for antimicrobial activities belong to *Inula* genus. Berk et al. (2011) discovered that an aqueous extract of *I. oculus christi* had no effect on *C. albicans*. [8]. However, Gökbulut et al. (2013) examined at the antibacterial activity of three *Inula* species and showed that the yeast strain *C. tropicalis* was more sensitive to all of the extracts they examined. [23]. The antimicrobial efficacy of Soxhlet ethanol extracts against ATCC and food isolates strains was examined in a study by Mohti et al., and it was shown that the leaf extract had the best activity, with *C. albicans* being the most sensitive strain (MIC= 125  $\mu$ g/mL) [34]. Furthermore, Oskay and Sarı (2007) investigated the antimicrobial activities of ethanol extracts of 19 plants used in traditional medicine in Turkey against 14 pathogenic bacteria species and *C. albicans*, and found that the extract prepared from the leaves of *I. viscosa* had anticandidal activity [41].

Our findings support this and show that all *I. oculus-christi* extracts are more effective against *C. utilis* and *C. krusei* than the other *Candida* strains. Based on the findings of the investigations, the extracts are regarded to be a viable alternative for the prevention of *Candida* infections and candidal resistance. Additionally, in a study, the amoebicide activity of aqueous extracts (ranging from 1.0 to 32.0 mg/ml) of *I. oculus-christi* was assessed *in vitro* and the extracts were found to have a substantial amoebicide effect on trophozoites and cysts [26].

As a result, when prior studies are reviewed, the majority of them focus on different species of *Inula*, but there are just a few activities and analytic investigations on *I. oculus-christi* that we are aware of. It is thought that the reasons such as the screening of phenolic compounds of different extracts of this species and the determination that the extracts contain antioxidant and antimicrobial compounds, as well as the fact that *I. oculus christi* is a species used among the public, increase the importance of the study. By evaluating the total phenolic content, antioxidant and anticandidal activities, and main components of the extracts prepared from *I. oculus-christi*, it was determined that the extracts exhibited antioxidant and anticandidal activities at various doses in general, and results supporting the use among the public were observed.

Furthermore, while the findings suggest that the extracts of the studied plant may be anticandidal agents and might be utilized to treat a variety of yeast-related illnesses, more research is needed to determine *in vivo* efficacy.

#### 4. CONCLUSION

Medicinal plants have been employed by humans since the beginning of time as a rich source of bioactive components for medication research and manufacturing. Plants in the genus *Inula* are also thought to be sources of structurally diverse compounds with antioxidant and antimicrobial activity, which can act through different mechanisms in the fight against diseases and are useful for the development of new and effective drugs at lower doses.

According to the findings of this study, it can be said that the extracts prepared from the aerial parts of *I. oculus-christi* contain phytochemicals with antioxidant properties. Considering the total amount of phenol

contained in the infusion of the plant and the results of its antioxidant effect, results supporting its use among the public were observed. The results think that the preparations produced from this herbal raw material can be used as effective preventive tools and valuable additional drugs in the treatment of diseases caused by oxidative stress.

## 5. MATERIALS AND METHODS

### 5.1. Plant Material

Wild growing *I. oculus-christi* was collected in full flowering stage in July 2018 from Muğla, Turkey (C2 Muğla, Fethiye, Girdev Yaylası, 1960 m, 02.07.2018, K 36 44' 26", D 29 38' 34"). A voucher specimen (ESSE 15515) was deposited in the Herbarium of the Anadolu University Faculty of Pharmacy.

### 5.2. Extraction and Isolation

The dried and powdered aerial parts of *I. oculus-christi* were macerated with 70% methanol (M) and ethyl acetate (E) at 25°C for 24 hours. After evaporation of methanol and ethyl acetate, the aqueous portion was removed in the lyophilizer and the resulting dry extract was used in all experiments. Since the traditional use is in the form of tea, 5 g of the above-ground part of the plant was brewed with 100 mL of water (at 80 °C), allowed to rest for 10 minutes, and the 5% infusion (I) form was then lyophilized.

### 5.3. Microbial Strains

The anticandidal activities of the extract were tested against a panel of *Candida* strains, including: *Candida albicans* ATCC 10231, *C. utilis* NRRL Y-900, *C. albicans* ATCC 90028, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258.

### 5.4. Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of the strains were determined using broth microdilution procedures [44]. The extracts of *I. oculus-christi* was examined against a panel of *Candida* strains using the microdilution method according to CLSI M27-A2 reference protocol. Amphotericin-B (Sigma-Aldrich) and Ketoconazole (Sigma-Aldrich) were used as standard antifungals.

### 5.5. Total Phenol

The total phenolic content of *I. oculus-christi* extracts was evaluated using the Folin-Ciocalteu reagent and determined according to method of Gao et al. [45].

### 5.6. 1,1-Diphenyl-2-picrylhydrazyl (DPPH\*) radical scavenging activity

The DPPH radical scavenging ability of each extract was calculated by the protocol applied by Goger et al. [46].

### 5.7. Phenolic Compound Determination

The phenolic components of each extract were determined according to the method used by Goger et al. [46].

**Acknowledgements:** The authors are grateful to Scientific Research Projects of Anadolu University (Project No. 1805S204) for financial support.

**Author contributions:** Concept – Y.B.K.; Design – N.S., Y.B.K., F.G.; Supervision – Y.B.K.; Resources – N.S., Y.B.K., F.G., G.İ.; Materials – N.S., Y.B.K., F.G., G.İ.; Data Collection and/or Processing – N.S., Y.B.K., F.G., G.İ.; Analysis and/or Interpretation – N.S., F.G., G.İ.; Literature Search – N.S., F.G.; Writing – N.S., Y.B.K.; Critical Reviews – N.S., Y.B.K., F.G., G.İ.

The authors declared no conflict of interest.

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