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**Original article**

# Effects of the stem extracts of *Cynara scolymus* L. on some microorganisms

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**Abstract**

In this study, the antimicrobial activity of *Cynara scolymus* L. stem, which are edible plants and sold at bazaars in Aydin vicinity, were tested against some microorganisms. Plants were dried and extracted with n-hexane, ethanol, n-propanol, acetone, methanol, chloroform and ethyl acetate. Antimicrobial activity of the extracts was determined by the disk diffusion method. Test microorganisms were 18 bacteria including, *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Proteus vulgaris* ATCC 33420, *Listeria monocytogenes* ATCC 19112, *Pseudomonas aeruginosa* ATCC 35032, *Streptococcus mutans*, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, and 4 yeasts including, *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida tropicalis*, *Candida glabrata*. Also different antibiotic discs were used for comparison of inhibition zones. Results show that the acetone extracts of *C. scolymus* stem inhibited the growth of fifteen microorganisms and the inhibition zones ranged between 8-14 mm. However, the ethanol and n-propanol extracts of *C. scolymus* stem inhibited the growth of thirteen microorganisms and the inhibition zones ranged between 9-15 mm. The inhibition zones of standard antibiotics used as positive control (chloramphenicol, gentamycin, tetracycline, erythromycin and ampicillin) were between 11-30 mm. against bacteria while the inhibition zones of standard antibiotic (Nystatin) were between 20-22 mm. against yeasts. While methanol, chloroform and ethyl acetate extract of *C. scolymus* stem has also showed moderate antimicrobial effect hexane extract of *C. scolymus* stem showed no effect against tested microorganisms.

**Key words:** *Cynara scolymus* L., antibacterial, anticandidal effect, agar disk diffusion method

## 1. Introduction

Antimicrobial agents are one of our most important tools fighting against infractions since their discovery (Nascimento *et al*., 2000). Multiple drug resistance has arisen because of the frequent use of antibiotics (Singh *et al*., 2017). This discovery of new antimicrobial agents is essential. However, these agents should be harmless to humans, can easily be produced and cost effective. Plants are rich in a wide assortment of auxiliary metabolites, for example, tannins, terpenoids, alkaloids, flavonoids, glycosides, and so on, which have been found *in vitro* to have antimicrobial properties (Akhtar *et al*., 2014; Babu *et al*., 2015; Mahendra *et al*., 2016; Torun *et al*., 2017; Biyik *et al*., 2017). This plant is well-known for its nutritional and curative properties due to some bioactive components that have antioxidant and antibiotic activities. In addition, it shows interesting tendency of protection against degenerative diseases such as cancer (Trichopoulos, 1995). In folk medicine, many parts of *C. scolymus* have been widely used as astringent, blood cleanser, cardiotonic, detoxifier, digestive stimulant, diuretic and hypoglycaemic, hypocho-lesterolemic as well as medicine for liver complaints (Lattanzio *et al*., 2009; Shalaby *et al*., 2016). The most active components found in artichoke species consists of flavones, their glycosides, coumarins, sterols caffeoylquinic acids and triterpenoid saponins (Willett *et al*., 1995; Martino *et al*., 1999; Acevedo, 1999; Visioli *et al*., 2004; Pinelli *et al*., 2007) which are associated with their relative activity against microorganisms. The mechanisms of phenolic toxicity against microorganism might be related to their reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987).

In this study, the antimicrobial effects of the stem extracts of *C. scolymus* were examined against some bacteria and yeasts.

## 2. Material and Methods

### 2.1 Plant material and preperation of extracts

*C. scolymus* was bought from bazaars in Aydin province in Turkey and was used stem of plant.

### 2.2 Preparation of plant extracts

Plant stems were washed with distilled water and reduced to powder with liquid nitrogen. Ten gram of this material was added to separately in 100 ml of n-hexane, ethanol, n-propanol, acetone,

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methanol, chloroform and ethyl acetate. Then the mixtures were agitated for a period of 72 h. They were filtered with Whatman No. 389 filter paper. Under aseptic conditions, the extracts were filtered through 0.45 µ-pore size diameter filters and stored at 4°C (Coban *et al*., 2017a).

### 2.3 Microorganisms and condition for cultivation

The eighteen bacteria and four yeasts species tested as *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Proteus vulgaris* ATCC 33420, *Listeria monocytogenes* ATCC 19112, *Pseudomonas aeruginosa* ATCC 35032, *Streptococcus mutans*, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida tropicalis*, *Candida glabrata*.

The bacteria and yeasts were cultured in Tryptic Soy Agar (Merck) at 30-37°C, Malt Extract Agar (Merck) at 27-30°C for 24 h, respectively (Coban *et al.*, 2017b).

**Table 1:** Antimicrobial effects of *C. scolymus* extracts and reference antibiotics

| Test microorganisms | Inhibition zones (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Extracts | | | | | | | Reference antibiotics | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | C 30 | CN 10 | TE 30 | E 15 | AMP 10 | NS 100 |
| *Escherichia coli* ATCC 35218 | - | 10 | - | 9 | - | - | - | 24 | 21 | 15 | 11 | - | NT |
| *Stapylococcus aureus* ATCC 25923 | - | 9 | - | - | - | - | - | 23 | 20 | 22 | 23 | 20 | NT |
| *Stapylococcus epidermidis* ATCC 12228 | - | - | - | - | - | - | - | 22 | 17 | 19 | 11 | 17 | NT |
| *Enterobacter aerogenes* ATCC 13048 | - | - | - | 9 | - | - | - | 19 | 20 | 14 | - | - | NT |
| *Salmonella typhimirium* ATCC 14028 | - | 9 | 9 | - | - | - | - | 17 | 16 | 15 | 8 | 8 | NT |
| *Klebsiella pneumoniae* ATCC 13882 | - | - | 9 | 9 | - | 9 | - | 21 | 19 | 20 | 14 | - | NT |
| *Mycobacterium smegmatis* ATCC 607 | - | 11 | 12 | - | - | - | - | 23 | 18 | 26 | 25 | 19 | NT |
| *Corynebacterium xerosis* ATCC 373 | - | - | 11 | - | - | - | - | 20 | 17 | 25 | 26 | 27 | NT |
| *Enterococcus faecalis* ATCC 29212 | - | 9 | 9 | 10 | - | - | - | 16 | 11 | 19 | - | 14 | NT |
| *Streptococcus pneumoniae* ATCC 27336 | - | - | - | - | - | - | - | 24 | 20 | 25 | 15 | 14 | NT |
| *Serratia marcescens* ATCC 13880 | - | 9 | 14 | 10 | - | 9 | - | 23 | 19 | 13 | - | 19 | NT |
| *Proteus vulgaris* ATCC 33420 | - | 9 | 9 | - | - | - | - | 17 | 24 | 17 | 20 | - | NT |
| *Listeria monocytogenes* ATCC 19112 | - | - | - | 9 | - | - | - | 19 | 14 | 12 | - | 12 | NT |
| *Pseudomonas aeruginosa* ATCC 35032 | - | 15 | 10 | 14 | - | 12 | 14 | 22 | 20 | 20 | 21 | - | NT |
| *Streptococcus mutans*** | - | - | - | 9 | - | 9 | - | 28 | 22 | 19 | - | - | NT |
| *Micrococcus luteus* ATCC 9341 | - | 9 | 11 | 10 | - | - | - | 25 | 15 | 26 | 30 | 28 | NT |
| *Bacillus cereus* ATCC 11778 | - | - | - | 11 | 9 | 10 | 9 | - | 23 | 24 | 25 | 26 | NT |
| *Bacillus subtilis* ATCC 6633 | - | 10 | 10 | 12 | - | 9 | - | 22 | 20 | 12 | 25 | - | NT |
| *Candida albicans* ATCC 10231 | - | 10 | 10 | 10 | - | - | - | NT | NT | NT | NT | NT | 22 |
| *Candida utilis* ATCC 9950 | - | 10 | 9 | 9 | - | 9 | - | NT | NT | NT | NT | NT | 21 |
| *Candida trophicalis** | - | - | - | 9 | - | - | - | NT | NT | NT | NT | NT | 20 |
| *Candida glabrata** | - | 9 | 10 | 8 | - | - | - | NT | NT | NT | NT | NT | 21 |

1: Hexane, 2: Ethanol, 3: n-Propanol, 4: Acetone, 5: Methanol, 6: Chloroform and 7: Ethyl Acetate

C30: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erytromycin (15mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid)

(-): Zone did not occur

NT: Not tested

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### 2.4 Antimicrobial assays

#### 2.4.1 Disc diffusion method

Screenings for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms (Collins *et al*., 1995; CLSI, 2015). The inoculum size of each group of bacteria and yeast were prepared by using a No. 0.5 McFarland tube to give a concentration of $1x10^8$ bacteria and $1x10^6$ yeasts per milliliter. In order to test the antimicrobial activity of plants, 20 ml of Mueller Hinton Agar (MHA) were poured in petridishes and kept to solidify at room temperature.

Then, it was inoculated with strains of bacteria and yeasts by taking 0.1 ml from cell culture media. Later on, a hole of 6 mm in diameter and depth were made on top with a sterile stick and was filled with 50 µl of plant extracts. Then, bacterial cultures were incubated at 30-37°C and yeast cultures were incubated at 27-30°C for 18-24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in millimeters. Discs of chloramphenicol (C30), gentamycin (CN10), tetracycline (TE30), erythromycin (E15), ampicillin (AM10), and nystatin (NS100) were used as positive controls (Coban *et al*., 2017c).

#### 2.4.2 Statistical analysis

Mean values and standard deviation calculations were made by SPSS v22 (Statistical Package for Social Sciences).

## 3. Results and Discussion

The extracts of the acetone and n-propanol of *C. scolymus* was found to be most effective against tested microorganisms (Table 1).

**Table 2:** Mean values of each extract with standart deviation

| Solvents | Mean | StdDev |
|---|---|---|
| n-propanol | 5,32 | 4,96 |
| Methanol | 1,96 | 5,4 |
| Acetone | 4,98 | 4,92 |
| n-hexane | 0 | 0 |
| Ethyl acetate | 3,54 | 3,85 |
| Ethanol | 5,23 | 5,63 |
| Chloroform | 3,04 | 4,60 |

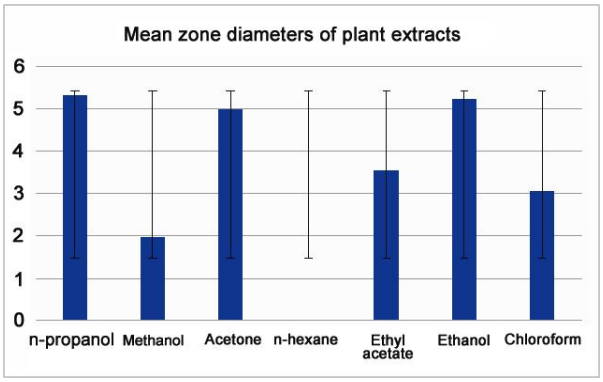


**Figure 1:** Zone range of each extract with standart deviation.

According to Table 1, ethanol extract of *C. scolymus* inhibited the growth of nine bacteria and three yeasts and the inhibition zones ranged between 9-15 mm. The n-propanol extract of *C. scolymus* inhibited the growth of ten bacteria and three yeasts and the inhibition zones ranged between 9-14 mm. The acetone extract of *C. scolymus* inhibited the growth of eleven bacteria and four yeasts and the inhibition zones ranged between 8-14 mm. The methanol extract of *C. scolymus* was only effective against *Bacillus cereus* ATCC 11778 (9 mm). The chloroform extract of *C. scolymus* inhibited the growth of six bacteria and one yeast and the inhibition zones ranged between 9-12 mm. The ethyl acetate extract of *C. scolymus* inhibited only two bacteria as *Pseudomonas aeruginosa* ATCC 35032 (14 mm) and *Bacillus cereus* ATCC 11778 (9 mm) but did not show any effect against yeasts. Besides this, n-hexane did not show any effect on tested microorganisms (Figures 2a,b).

While the n-propanol extract of *C. scolymus* showed high effect (14 mm) against *Serratia marcescens* ATCC 13880 the ethanol, acetone and ethyl acetate extracts of *C. scolymus* showed high effect against *Pseudomonas aeruginosa* ATCC 35032, respectively, 15 mm, 14 mm, 14 mm. However, the ethanol and n-propanol extracts of *C. scolymus* demostrated moderate effect (11-12 mm) against *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778 and *Bacillus subtilis* ATCC 6633.

The etanol, n-propanol, acetone, methanol, chloroform and ethyl acetate extracts of *C. scolymus* indicated slightly effect (9-10 mm) or did not show any effect against *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimirium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Enterococcus faecalis* ATCC 29212 *Proteus vulgaris* ATCC 33420, *Listeria monocytogenes* ATCC 19112, *Streptococcus mutans*, *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida trophicalis* and *Candida glabrata*.

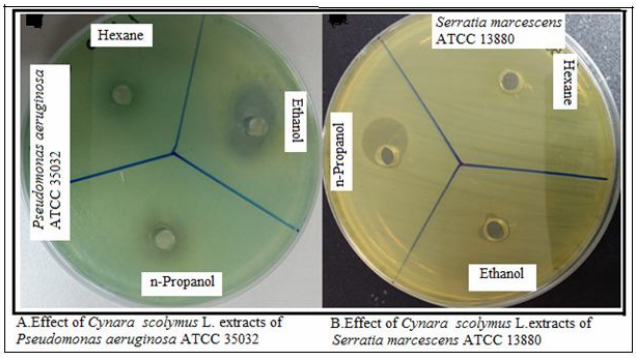


**Figure 2(a):** Effect of *C. scolymus* extracts of *Pseudomonas aeruginosa* ATCC 35032 . **(b).** Effect of *C. scolymus* extracts of *Serratia marcescens* ATCC 13880.

1: Hexane, 2: Ethanol, 3: n-Propanol

The extracts of the ethanol, n-propanol and acetone of *C. scolymus* stem showed high effect comparing to other solvents. While the methanol, chloroform and ethyl acetate extracts demostrated moderate effect, the extract of n-hexane showed no effect against tested bacteria and yeasts. Since water is one of the basic compounds

which has high polarity. Like water, all solvents except hexane are polar; it is easier for them to penatrate in the organisms and show their effect. The n-propanol and acetone brings out effective secondary metabolites like alkaloids, tannins, and flavonoids against microorganisms from *C. scolymus* stem while other solvents did not reveale secondary metabolites with antimicrobial effects.

According to these results, it may suggest that the methanol, n-propanol and acetone extracts of *C. scolymus* stem posses compounds with antimicrobial properties which can be used as antimicrobial agents.

Mossi and Echeverrigaray (1997) identified five different antimicrobial components from *C. scolymus* leaf extracts. Wang *et al*. (2003) analysed antioxidative phenolic compounds in artichoke. They used methanol and purified 7 phenolic compounds, 2 of which were unique to artichoke heads. Vamanu *et al*. (2011) studied antioxidant and antimicrobial effects of artichoke extracts. They found antimicrobial activity against 15 microorganisms. Sohaimy (2014) researched chemical composition, antioxidant and antimicrobial potential of artichoke. The methanol extract of *C. scolymus* is more effective and powerful in antioxidant and antimicrobial activity against 5 pathogenic bacterial strains. Ergezer *et al*. (2018) studied possible antioxidant and antimicrobial effects of *C. scolymus* extract in beef patties. They used *E.coli* and *L. monocytogenes* as test organisms and found 1000 ppm of artichoke extracts prevented the bacterial growth.

## 4. Conclusion

According to our findings, ethanol, n-propanol and acetone extracts of *C. scolymus* stem showed higher antimicrobial activity than other extracts against tested microorganisms. While methanol, chloroform and ethyl acetate extract of *C. scolymus* stem has also showed moderate antimicrobial effect of hexane extract of *C. scolymus* stem, but showed no effect against tested microorganisms. This could be the reasons of different uptake mechanisms of these chemicals. Solvents which have easy uptake in microorganisms, can be more effective. More research was needed to prove the reasons behind this and also the need to find new compounds from plants with antimicrobial effect.

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### Conflict of interest

We declare that we have no conflict of interest.

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