Phytochemical and Biological Evaluation of *Carduus getulus* Pomel (Asteraceae) Naturally Growing in Arid Ecosystem

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Abstract

One of the most important public health issues is antimicrobial resistance. *Carduus getulus* Pomel (family Asteraceae), known as hashroof by Egyptians, is wild annual plant. The present study aims to evaluate the antioxidant and antimicrobial activities of *C. getulus* collected from coastal desert. In the investigated plant, total phenolics, tannins, alkaloids, flavonoids, saponins, glucosides, and steroids were measured. The antioxidant activity was measured based on the reduction of DPPH.The IC_{50} values of the antioxidant inhibition of *C.getulus*was43.32 mg ml^{-1} compared to control. In this investigation, the methyl alcohol extract of *C. getulus* has a high broad spectrum (89 percent) of antibacterial activity against the microorganisms tested. The pathogen *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (17.22, 16.33 and 16.18 mm, respectively) were the most sensitive bacteria. In conclusion, *C. getulus* extracts can be employed as natural antioxidant and antibacterial agents in pharmacotherapy, according to this study.

Keywords: *Carduus getulus,* bioactive compounds, antioxidant, antimicrobial

1. Introduction

Bioactive chemicals are naturally occurring substances created by living organisms (Sánchez and Vázquez, 2017). They are bioactive substance produced by flora, microalgae, seaweeds, and invertebrates that are not required for existence but give evolutionary benefits to the organisms that yield them by acting as anti-diabetic, antioxidants, cytotoxic, anti-inflammatory, and other properties (Firn and Jones,2000) (Cotas et. al, 2020).

Traditional medicine has a wealth of ethnomedical information about the advantages of medicinal wild plants in the treatment of infectious illnesses, which is based mostly on medicinal plants (Iwu and Holzemer,2014)Khan et. al, 2021). Several African and Arabian countries have recognized the need to produce improved traditional medicines based on native plants that have historically been used to treat a variety of illnesses (Inngjerdingen et. al, 2004) (Van and

Prinsloo,2018).This necessitates a comprehensive investigation of the use of native plants in basic healthcare as well as antimicrobial investigation to verify the usage of such medicines in varied areas. Traditional medicinal knowledge is responsible for around 25% of all pharmaceutical products in the globe. There is a lot of interest in creating new types of therapeutic drugs that are more potent and have fewer adverse effects (Fauci et. al, 1998) (Palhares et. al, 2021).

Carduus is a genus in the Astraceae family with approximately 100 species worldwide (Chaudhary,2000). In the Mediterranean area, Asia, and the East African mountains, there are 91 species of the genus *Carduus* (Asteraceae family). In Egypt, three species may be found along the Mediterranean coast and in Sinai (Boulos et. al, 2002). The member of the genus *Carduus* are often used in Chinese traditional medicine to cure a variety of human ailments, including colds, stomachaches, and rheumatism (Esmaeili et. al, 2005). Species of *Carduus* possesses anti-inflammatory, anti-spasmodic, anti-cancer, anti-viral, and anti-bacterial activities, among other biological effects (Esmaeili et. al, 2005) (Orhan et. al, 2009) (Frydman, 1962).Phytochemical studies on several *Carduus* species revealed the presence of lignans(Fernández-Moreno et. al, 1991), flavonoids(F.E. Jordon-Thaden and S.M. Louda, 2003) (El-Lakany et. al, 1997) (Kaloshinaand Mazulin,1988), coumarin (F.E. Jordon-Thaden and S.M. Louda, 2003) (Cardona et. al, 1992), alkaloids (Frydman, 1962), sterols and triterpenes, among other chemical constituents(Abdel-Salam et. al, 1982) (Abdel-Salam et. al, 1983).

Concerning the *Carduus getulus*, known as hashroof by Egyptians, is an annual plant rising 10 to 30 cm tall with branched, spiny erected stem. Foliage leaves are lobed, spiny, sessile or short pedunculate and small pinkish flowers with little phytochemical and biological information. Antimicrobial screening and liver function for *C. getulus* have both been described as biological effects (Abdel-Salam et. al, 1982) (Abdel-Salam et. al, 1983).The purpose of this study is to identify the main constituents of the aerial portions of *C. getulus* growing in Egypt's coastal desert. In addition, antioxidant and antibacterial properties were investigated.

2. Materials and Methods

2.1. Plant Materials

During the month of March 2021, fresh and healthy *C. getulus* was taken in flowering stage from the Coastal Desert (northern sector of the Nile Delta, Egypt). According to Boulos (Boulos et. al, 2002) the plant species was recognized. It was dried at room temperature, then blended into a powder and stored in dark plastic containers until needed.

2.2. Phytochemical screening

Quantitative phytochemical analysis was performed on powdered samples of *C. getulus* aerial parts(Obadoni and Ochuko, 2002) (Evans,2002) (Sadasivam and Manickam,1992).

2.3. Extraction

With 500 mL of methanol solvent, the dehydrated plant powder (50 g) was extracted for 3 hours. Filtered and evaporated until absolutely dry, the extracts were used. The extract was produced as a stock solution of dimethyl sulfoxide (DMSO) and stored at -20 °C for future usage(Hassani-Mehraban, 2005).

2.4. Biological Activity

2.4.1. Determination of Antioxidant Activity

A stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was tested for antioxidant properties(Miguel,2010).At varied doses, two ml of 0.15 mM DPPH was added to two ml of plant extracts (4000, 2000, 1000, 500, and 250 ppm). 2 mL DPPH in 2 mL solvent was used as a control.For 30 minutes, the mixture was incubated at room temperature in the dark.The IC50 turned into anticipated visually after measuring the absorbance at 517 nm. The antioxidant pastime turned into calculated the usage of the technique below:

% Radical scavenging pastime = [1– (A plant sample/A control)] × 100

2.5.2. Antimicrobial Bioassay

The MeOH extract of *C. getulus* was evaluated against three-gram negative bacteria such as *Klebsiella pneumoniae* (ATCC10231), *Escherichia coli* (ATCC10436), and *Salmonella typhi* (ATCC25366), as well as four-gram positive bacteria such as *Staphylococcus aureus* (ATCC7539), *Bacillus subtilis* (DMS1278), *Staphylococcus pneumoniae* (VGM83160), and *Pseudomonas aeruginosa* (BAP12528) were used in the screening by filter paper discs assay(Cappuccino and Sherman,2008).At 37 degrees Celsius, all Petri plates were incubated for 24 hours. After incubation, the diameter of the inhibitory zone (cm) was measured to record the clear zone, and the results were compared to DMSO as a control. Standard antibiotic (cephradin, tetracycline, azithromycin and ampicillin) were utilized to compare the plant extracts that were examined.

2.6. Statistical Analysis

The average of all secondary chemicals, antioxidant, and antibacterial activity was calculated after triplicate testing. Using the CoStat software package, the data were submitted to a one-way ANOVA and Duncan's post hoc test (CoHort Software, Monterey, CA, USA).

3. Results and Discussion

3.1. Phytochemical analysis

Tannins, alkaloids, saponins, flavonoids, phenolics, and glycosides were found in the quantitative phytochemical analysis of C. getulus (Table 1). Tannins, saponins, and phenolics were found in abundance. These findings are consistent with those of (Abdel-Salam et. al,1982 and

1983), who reported comparable findings on *C. getulus* from Egypt. The phytochemical study of *C. getulus* corresponds with those described by (El-Amier et. al, 2014). when compared to other plant species in the same family of Egyptian flora. But it is higher than those reported by (El-Amier and Aisha, 2019) and (Abd-ElGawad et. al, 2020).

Phytochemical investigations on many *Carduus* species were carried out in the literature and demonstrated the existence of several chemical elements such as flavonoids (F.E. Jordon-Thaden et. al, 2003) (El-Lakany et. al, 1995) (El-Lakany et. al, 1997) (O.M. Abdallah et. al, 1989)(Calliste et. al, 2001).Coumarin(F.E. Jordon-Thaden and S.M. Louda, 2003)Cardona et. al, 1992). alkaloids(Frydman and Deulofeu, 1962)sterols and triterpenes(Abdel-Salam et. al, 1982 and 1983).

Table 1.Preliminary phytochemical investigation of *Carduus getulus*

Plant species	**Bioactive compounds (mg.g^{-1} dry weight)**						
	Tannins	**Saponins**	**Phenolics**	**Flavonoids**	**Alkaloids**	**Glycosides**	**Steroids**
P. tomentosa	10.39±0.02	4.45±0.04	8.94±0.04	3.24±0.02	3.81±0.03	2.68±0.02	1.67

3.2. Antioxidant Activity

Antioxidant activity is influenced by a variety of parameters, including lipid composition, antioxidant content, and plant type(Calliste et. al, 2001) (Khasawneh et. al, 2011).Plant extracts' radical scavenging ability is frequently measured using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).The MeOH extract of the aerial parts of *Carduus getulus* exhibited strong and significant antioxidant activity with an IC_{50} value of 43.32 mg ml^{-1} as comparable with ascorbic acid as a standard reference (Table 2). It is clear that raising the concentration of plant extract resulted in a constant rise in scavenging activity. The rise in MeOH extract was up to 50 mg ml^{-1}, with a scavenging activity of 54.07 percent.The antioxidant activity of *Carduus getulus* was lower than *Carduus nutans*, but greater than *C. nigrescens* and *C. nigrescens*, according to the findings of (Kozyra et. al, 2019) (Hammad et. al, 2013).

Many studies have shown that phenolic compounds have a strong antioxidant effectAbd-(ElGawad et. al, 2020) (El-Amier et. al, 2022). and that the antioxidant activity increases as the amount of phenolic compounds increases.Variations in the antioxidant activity are explained by changes in the phenolic content and other chemicals contained in the extracts(Abd-ElGawad et. al, 2021) (El-Amier et. al, 2021). These findings show that *Carduus getulus* extract might be a cost-effective source of natural antioxidants. The high flavonoid concentration of *Carduus getulus* may account for its antioxidant properties(F.E. Jordon-Thaden et. al, 2003) (Kaloshinaand Mazulin,1988).Coumarin(F.E. Jordon-Thaden and S.M. Louda, 2003) (Cardona et. al, 1992).

alkaloids (Frydman and Deulofeu, 1962). sterols, and triterpenes, among other chemical constituents(Abdel-Salam et. al, 1982 and 1983).The phenolic content of shoot extract is primarily responsible for its potent antioxidant activity, and a strong courting among overall phenolic content material and antioxidant pastime has been proven in lots of plant species(El-Amier et. al, 2019) (Abd-ElGawad et. al, 2021).

Table 2.*Carduus getulus* MeOH extract scavenging activity % of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and IC_{50} values.

Treatment	Concentration (mg ml^{-1})	Scavenging activity[1] (%)	IC_{50} (mg ml^{-1})	$LSD_{0.05}$
Carduusgetulus	5	12.24±0.72	43.32	1.26***
	10	18.50±1.09		
	20	29.09±1.53		
	30	40.43±2.13		
	40	47.22±2.49		
	50	54.07±3.21		
Ascorbic acid	1	3.78±0.92	12.97	1.97***
	2.5	12.04±0.84		
	5	39.25±1.68		
	10	46.24±2.34		
	15	57.28±2.90		
	20	66.02±3.67		

The values are the averages of three replicates with a standard error, IC_{50}: the sample concentration that is necessary to lower DPPH absorbance by 50%.

3.3. Antimicrobial assay

Antibiotic resistance among bacteria has prompted scientists to look for new strategies to inhibit bacterial development and illnesses(Johnson et. al, 2005) (Zaki et. al, 2017) (Fayed et. al, 2021). The plant metabolites were extracted using MeOH in this work, and the antibacterial activity of *Carduus getulus* was tested in vitro using the agar well diffusion technique. All pathogenic micro organism are inhibited via way of means of methyl alcohol at various rates, starting from 13.450.fifty four to 17.220.69 (Table 3).The methanol extract of *Carduus getulus* aerial parts was reported to be efficacious against the same tested bacterium strain by (Rahman et. al, 2011).(Joshi et. al, 2009) assessed the Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum, and Origanum majorana extracts in opposition to pathogenic bacteria, at the same time as (Iranbakhsh et. al, 2010). in Datura stramonium (E. coli, Staphylococcus aureus, and S. pneumoniae) in addition to Deverra tortuosa and observed comparable effects(Fayed et. al, 2021).

In this study, a methyl alcohol extract of *Carduus getulus* was shown to have a high broad spectrum (100 percent) of antibacterial activity against Gram positive and negative microorganisms (**Figure 1**). Plant extracts were more effective than antibiotics against the bacteria

in all of the examples previously described. The pathogen *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (17.22, 16.33 and 16.18 mm, respectively) were the bacteria with the highest sensitivity. In many plant species the diameter of the inhibition quarter changed into inspired through quite a few parameters, consisting of the ability of chemicals (blanketed with inside the extracts) to diffuse with inside the agar medium, the antibacterial hobby of dispersed compounds, and the boom and metabolic hobby of microorganisms with inside the medium (Bandeira et. al, 2006) (Sultan et. al, 2021). The sensitivity of any micro organism to plant extract established no courting with antibiotic susceptibility. The sensitivity to extract of microbial traces from a particular species touchy to sure medicines changed into virtually better than that of resistant species. The methanol plant extract has a variety of antibacterial hobby in opposition to the pathogenic micro organism that had been tested.

According to (Favel et. al, 1994), (El-Amier and Al-hadithy, 2020). phytochemical materials are identified to have antibacterial characteristics. Previous research have found out that Carduus species found out the presence of lignans, flavonoids, coumarin, alkaloids, sterols, and triterpenes (Fernández-Moreno et. al, 1991) (F.E. Jordon-Thaden and S.M. Louda, 2003) (Cardona et. al, 1992) (Frydman and Deulofeu, 1962) (Abdel-Salam et. al, 1982 and 1983).

Table 3. The inhibitory activity of *Carduus getulus*MeOH extract against the examined organisms, as measured by inhibition zone diameters (mm)

Test microorganisms	*Carduus getulus* extract	Standard antibiotic			
		Cephradin	Tetracycline	Azithromycin	Ampicillin
Gram negative bacteria					
Klebsiella pneumoniae	14.52±0.58	12.31±0.49	22.4±0.68	15.09±0.60	7.58±0.30
Escherichia coli	17.22±0.69	17.22±0.69	22.63±0.91	22.34±0.89	22.67±0.91
Salmonella typhi	13.45±0.54	0.0±0.0	12.44±0.52	0.0±0.0	0.0±0.0
Pseudomonas aeruginosa	16.33±0.65	0.0±0.0	0.0±0.0	15.21±0.61	0.0±0.0
Gram positive bacteria					
Bacillus subtilis	13.42±0.54	22.42±0.82	12.77±0.89	22.31±0.72	7.36±0.29
Staphylococcus aureus	16.18±0.65	22.61±0.90	22.63±0.65	22.11±0.88	32.11±1.28
Staphylococcus pneumoniae	14.34±0.57	12.52±0.50	22.18±0.51	25.04±1.0	12.19±0.49

Values are means of triplicate ± standard error

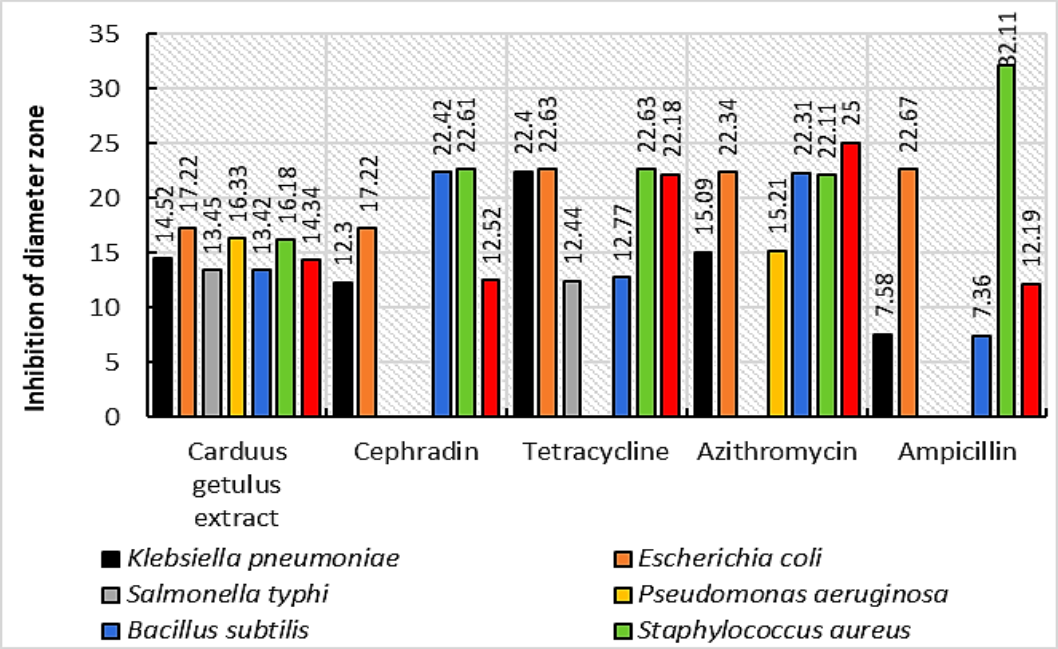

Figure 1. Antimicrobial activity of *Carduus getulus* extracts and conventional antibiotics

4. Conclusion

In conclusion, our look at showed that MeOH extract of Carduus getulus exhibited excessive wide spectrum (100%) towards examined bacteria. The maximum inclined microorganisms have been Escherichia coli, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.* According to this work, *Carduus getulus* extract may be used as herbal antibacterial dealers in pharmaceutical and meals protection systems. The MeOH extract of the aerial components of Carduus getulus exhibited robust and vast antioxidant pastime with an IC50 price of 43.32 mg ml-1 as similar with ascorbic acid as a well known reference. In addition, the organic pastime turned into correlated with the phenolic acids and flavonoids content.

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