Characterization of the Chemical Components of the Extracted Carduus Pycnocephalus L, and Assessment of Its Potential Novel Antioxidant, Antibacterial, and Anticancer Activities

Luay El Hayyany
Mansoura University Faculty of Science
El-Sayed El-Halawany
Mansoura University Faculty of Science
Hoda Soliman
Mansoura University Faculty of Science
Yasser El-Amier (yasran@mans.edu.eg)
Mansoura University Faculty of Science https://orcid.org/0000-0001-8920-8278

Research Article

Keywords: Carduus pycnocephalus, GC/MS Chromatography, DPPH Antioxidant, Cytotoxic and Antibacterial Activities

Posted Date: November 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1019997/v1

Abstract

Carduus pycnocephalus L, which is related to the Astraceae family, was well-known as a privileged medicinal plant that has innumerable respected biological potency. The current research aims to identify the chemical constitutes of the essential oils of the extracted *C. pycnocephalus* by Gas Chromatography-Mass Spectroscopic analysis (GC-MS) and to assess the biological profiles of the plant and its botanical ingredients as a precise antioxidant, and anticancer, as well as, antimicrobial agents. The extraction process of the medicinal plant by methanol provided a possibility to extract and identify the polar chemical constitutes that have the most effective categories of components. The DPPH antioxidant potency of the botanical ingredients of the plant indicated that the flower extract is the most potent with IC_{50} = 30.69 mg/L followed by leaves (IC_{50}= 32.78 mg/L), stem (IC_{50}= 41.31 mg/L), and root (IC_{50}= 46.84 mg/L). The antibacterial activities of the root, stem, leaf, and flower extracts of *C. pycnocephalus* exhibited remarkable potency to kill or inhibit the growth of the bacterial species. Leaf, and flower extracts revealed the most potent activities than the antibiotic standards against *E. coli*, *S. typhimurium*, and *B. cereus* species with inhibition zones ranged from 20-26 mm. Also, the extracted *C. pycnocephalus* revealed a moderate cytotoxic effect against hepatocellular carcinoma (HepG2) tumor cell line using MTT assay with IC_{50}= 46.2 µg/mL. The experimental interpretations inveterate the potential of *C. pycnocephalus* extract indicated its biological impacts as antioxidant, antibacterial, and moderate cytotoxic agents that provided the ease of using it in cancer therapy.

Introduction

The *Carduus* genus is related to Astraceae family, which included roughly 100 species worldwide according to Chaudhary (2000) that are extensively distributed from place to place in the Mediterranean. Only three species of *Carduus* genus were recorded in the flora of Egypt, *C. pycnocephalus, C. getulus* and *C. argentatus*. The latter is a spiny annual herb growing wildly in the coastal desert of Egypt. The stem usually branches and has spiny wings, the plant reaches up to 60 cm with the taproot, which gives greyish-tomentose on the lower surface, with spinose lobes, and its flowering time extends from March to May (Boulos 1983). Flu, stomach ache, and rheumatism of such human diseases were treated by *Carduus* genus in Chinese folk medicine (Esmaeili et al. 2005). In addition, the genus of *Carduus* revealed many biological characteristics, for example, antibacterial, antiviral, anticancer, antispasmodic, anti-inflammatory, and liver tonicity activities (Al-Shammari et al. 2015; Esmaeili et al. 2005; Jordon-Thaden & Louda 2003; Orhan et al. 2009). *Rhinocyllus conicus* (Coleoptera: Curculionidae), as a type of *Carduus* species (Asteraceae) that grown in Argentina, was applied as a control for thistles (de Briano et al. 2013). Many types of research since antiquity and in recent times have focused on the study of *Carduus* species such as the study of the reproductive system (Olivieri et al. 1983), ecology (Doing et al. 1969), the accumulation of heavy metals in plants growing in contaminated soils (Brunetti et al. 2009), recovery of the colonization of the rootstock of mycorrhizal fungi (Jaunatre et al., 2016), and comparative studies electrophoresis (Olivieri 1985).

The phytochemical analysis of several types of plants related to this *Carduus* genus indicated the characterization of diverse classes of chemical constitutes such as flavonoids, and Coumarin (Jordon-Thaden & Louda, 2003), as well as earlier reports, reported the identification of other classes of secondary metabolites such as lignans, alkaloids, sterols, and triterpenes. In this due course, *C. pycnocephalus* was well-known in the literature as an anti-inflammatory, antispasmodic, and hypotensive agent, in which the isolated components

from the different parts of this plant were characterized by spectroscopic analyses (Al-Shammari et al. 2015). The literature survey in earlier reports has also identified the isolation of many other classes from *C. pycnocephalus* such as essential oils (Esmaeili et al. 2005), sterols, and triterpenes (Gallo & Sarachine 2009). Al-Shammari et al. (2015), have characterized ten compounds from the aerial parts of this plant that were related to flavonoid class, and anthraquinone, anthraquinone-linked carbohydrate, steroids, and steroids-linked carbohydrate derivatives as subclasses including kaempferol-7-methoxy-3-*O*-*α*-L-rhamnoside, diosmetin-7-*O*-*β*-D-xylosyl-*β*-D-gluco-pyranoside, diosmetin-7-*O*-*α*-L-arabinosyl-*β*-D-gluco-pyranoside, and kaempferol-3-*O*-*α*-L-rhamnosyl-α-L-rhamnoside along with the structures that were depicted in Figure 1.

Esmaeili et al. (2005), have reported the analysis of the essential oils isolated from *C. pycnocephalus* that were grown in Iran by Gas Chromatography-Mass Spectroscopic analysis (GC-MS) with the identification of twenty-nine components demonstrating hexadecanoic acid (23.3%) as the main constituent. Marengo et al. (2017), have reported the characterization of four *Carduus* species, namely (*C. argyroa* Biv., *C. nutans* subsp. *macrocephalus* (Desf.) Nyman, *C. pycnocephalus*, *C. cephalanthus* Viv) that grown in the region of the Mediterranean by phytochemical and biomolecular analyses. The current research aimed to investigate the chemical constitutes of the essential oils of the extracted *C. pycnocephalus* by Gas Chromatography-Mass Spectroscopic analysis (GC-MS), and to utilize the extracted sample to explore its biological aptitude as cytotoxic, and antimicrobial potency, as well as, the antioxidant characters of the extracted plant parts.

Materials And Methods

Plant material and extraction process

The plant materials of *C. pycnocephalus* were collected from the sandy habitat near Gamasa City, northern Mediterranean coast, Egypt (31°30'22.24"N 31°22'4.70"E). The plant materials were cleaned and divided into small pieces. A weighed 20 gm of the previously prepared plant materials were placed in a conical flask (250 mL) contained 150 mL methanol and extracted by a shaker after two hours at room temperature. The extract was concentrated to a fixed volume after the complete extraction process. The produced methanol extract was filtered using qualitative Whatman filter paper no. 1 (125 mm, Cat No 1001 125, Germany) and stored at 4 ^{o}C (Abd-ElGawad et al. 2020; Souza et al. 2018).

Gas Chromatography-mass Spectroscopy (Gc-ms) Analysis

The chemical constitutes of the plant were characterized by implementing the plant extract on Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The temperature of the column oven was firstly held at 50 ^{o}C, raised subsequently by a rate of 5 ^{o}C per minute to reach 250 ^{o}C, and hold for 2 min, and then the temperature was raised to the final temperature (300 ^{o}C) by 30 ^{o}C per minute and hold for 2 min. the injector and MS transfer line temperature were kept at 270, and 260 ^{o}C, respectively. Helium (He) was used as a carrier inert gas at a constant flow rate of 1 mL/min. The solvent was released after 4 min and the diluted samples of 1 µl were injected directly using Autosampler AS1300 coupled with GC in the split mode. EI mass spectroscopy was collected at 70 EV ionization voltage over a range of 50-500 for *m/z* in packed scan mode. The temperature of the ion source was fixed at 200 ^{o}C. The chemical components of the individual extracted plant materials were

interpreted by a comparison of their mass spectral data with those of WILEY 09, and NIST 14 mass spectroscopic database.

Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH•), ascorbic acid, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), RPMI-1640 medium, and DMSO were purchased from Sigma Aldrich (St. Louis, USA). Fetal bovine serum (FBS; Gibco Life Technologies, Paisley, UK). Cephradin, Tetracycline, Azithromycin, and Ampicillin "antibiotics" were purchased from Merck (Darmstadt, Germany). Nutrient Agar (Contained Peptone, HM Peptone B #, Yeast extract, Sodium chloride, Agar, and Beef extract) were purchased from Himedia Laboratories Pvt. Ltd. LBS Marg, Mumbai-400086, India.

Instruments

The horizontal water bath shaker (Memmert WB14, Schwabach, Germany), for the plant extraction process. GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA), and UV/Vis spectrophotometer apparatus for colorimetric measurements (Spekol 11 spectrophotometer, analytic Jena AG, Jena, Germany). For antimicrobial tests: Laminar (HPH12, 1/PE AC, 50 Hz, 1P20, SN. 40328380) (Heraeus, Kendro, Langenselbold, Germany), and Incubator (Type: FRIOCELL 111, Medcenter Einrichtungen GmbH, D-82166, Grafelfing, Germany).

Potential Biological Characteristics

Antioxidant DPPH assay

The extracts of root, stem, leaves, and flower of *C. pycnocephalus* plant were *in vitro* screened as antioxidant agents by a colorimetric DPPH free radical assay. The method depended on measuring the intensity in the violet color of the DPPH solution. The assay was applied exactly as applied by the procedure reported by Kitts et al. (2000). In this course, different concentrations of each sample were prepared in serial dilutions (5-50 mg/L) in six testing tubes by mixing with methanol. 0.135 mM of DPPH• solution was prepared and subsequently add to each tube of the serial dilution of the investigated samples of the plant species. The tubes were next incubated in dark at room temperature for approximately 30 min. The intensity in the violet color was measured on Spectrophotometric apparatus at a maximum wavelength of 517 nm. The linear regression analysis was applied to calculate the inhibitive concentrations of each tested sample by plotting the exponential curve (Parejo et al. 2000) expressing the concentrations of the investigated samples against the percent of the remaining DPPH• radical. The percent of inhibitions were calculated by applying equation (1):

% Inhibition = [(A control − A sample)/A control] × 100 Eq. (1)
(A control) expressed the absorbance of the control sample.

(A sample) expressed the absorbance of each sample concentration.

Procedure Of Anticancer Activity

Tumor cell lines

HePG-2 (Hepatocellular carcinoma) was selected as a human tumor cell line was purchased from ATCC *via* a holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Preparation of MTT solution

The MTT solution was prepared by mixing a solution of MTT in water (10 mg/mL), ethanol (20 mg/mL), and buffered salt solutions and media (5 mg/mL). The mixture was mixed by vortex or sonication, then filtered, and stored at -20°C.

Protocol of MTT assay (Zhu et al. 2017)

The cytotoxic activity of the investigated sample *C. pycnocephalus* was evaluated by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay utilizing an altered process termed by Terblanche et al. (2017). For the determination of the IC_{50} of each particular drug, the adherent HepG2 cells were seeded onto 96-well plates at an initial density (3×10^3 cells/well suspended in 100 µL of complete medium). The crude plant extracts were prepared and used to stimulate the cells using five concentrations (31.3, 62.5, 125, 500, and 1000 µg/mL) in culture media. The experimental techniques were performed in duplicates, then plates were incubated for 24 hours in a 5% CO_2 at 37 ºC for settle down and adherence. Next, a serial dilution of each prepared concentration of the drug was applied onto the cells for 48 hours after adherence. The medium was removed by aspiration and a weighed MTT (0.5 mg/mL) was dissolved in a culture fresh medium and practically applied onto cells and the plates were incubated at 37 ºC and 5% CO_2 for 4 hours. Ultimately SDS (100 µL) was added into each well. The Reduction at cell growth was measured at ($\lambda_{max.}$ = 570 nm) (BioTek, Elx800, US) and the results were expressed as a percentage of control. The IC_{50} values of the drug, articulated the concentration that affects roughly 50% death of tumor cells, were estimated through a straight linear regression, type sigmoidal, analyzed using Origin 8.0® software (OriginLab Corporation). The IC_{50} values were calculated by the fit line *i.e.* Y= a*X + b, in which IC_{50}= (0.57-b)/a. The percentage of inhibition in cell growth was calculated from the following equation (Eq. 2), in which A is the absorbance of each control, and the tested sample:

$$\%\text{Inhibition} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100 \text{Eq}\,(2)$$

The relative cell viability percentage was subsequently calculated from the following equation (Eq. 3), in which A expressed the control, and sample absorbance at $\lambda_{max.}$ = 570 nm.

$$\%\text{Cellviability} = \frac{\text{Atreatedsamples} - \text{Ablank}}{\text{Acontrol} - \text{Ablank}} \times 100 \text{Eq}\,(3)$$

Assessment Of The Antibacterial Activity

Microbial Tendency

The antimicrobial aptitudes of *C. pycnocephalus* extract were estimated using the agar well diffusion assay (Magaldi et al. 2004; Valgas et al. 2007). Cephradin, Tetracycline, Azithromycin, and Ampicillin were used as

standard antibiotics for comparison scales.

Bacterial species

The microbial species were purchased from the Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. **Gram-negative bacteria**: *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhimurium* (ATCC 25566), *Staphylococcus epidermidis* (ATCC 12228). **Gram-positive bacteria**: *Bacillus cereus* (EMCC number 1080), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus haemolyticus* (ATCC 29970), *Staphylococcus xylosus* (NCCP 10937), and *Klebsella pneumonius* (ATCC 10031).

Preparation of the culture media

28.0 grams of the nutrient agar were placed in a 2 L conical flask and suspended in 1 L distilled water. The conical flask contents were heated to boiling point to a completely dissolved medium. The mixture was sterilized by autoclave at 15 lbs pressure, 121 ^{o}C for 15 minutes. The medium was left to cool to 45-50 ^{o}C. The medium can be enriched with 5-10% blood or biological fluids. The mixture was well-shaken and poured sterile Petri plates.

Microbial Susceptibility Testing

The antimicrobial activity of the investigated plant extract was estimated by filter paper disc technique (Murray et al. 1995; Kim et al. 2019) using inoculums containing 10^6 bacterial cells / mL to spread on nutrient agar plates. Sterilized filter paper discs (Whatman no.1, 6 mm in diameter) were saturated with the extracted sample and another set of filter paper discs were soaked in methanol and served as controls. The discs were placed on the surface of agar plates seeded with the test bacterial species. The plates were incubated at 37 ^{o}C. The inhibition zone diameters (mm) were measured after 18-24 hours (Sardari et al. 1998).

Results And Discussion

In this research, we aim to explore the reactive chemical components of the essential oil of the methanol extracted *C. pycnocephalus* that are considered as the major factors affected on the biological aptitudes such as antioxidant, cytotoxic, and antibacterial activities. Each type of biological character is affected by a significant class of compounds that control its biological behavior *i.e*. the phenolic contents are crucial for potent antioxidant characters.

Gas-chromatography Mass Spectroscopy “gc-ms”

The components of the essential oil of the extracted *C. pycnocephalus* were elucidated by Gas-Chromatography Mass Spectroscopy “GC-MS” (*c.f*. Fig. 2 & Table 1). The results revealed that the extract of *C. pycnocephalus* includes about mainly six characterized components. In general, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one located the major component with 57.43% composition which was identified after 9.11 min. Accordingly, other constitutes were characterized with high composition % such as (*E*)-4-(((2-methoxyoctadec-4-en-1-yl)oxy)methyl)-2,2-dimethyl-1,3-dioxolane (11.53%), methyl (*E*)-octadec-11-enoate (10.98%), and ethyl *iso*-allocholate (11.89%), which were identified after 4.92, 29.18, and 35.71 minutes. Additionally, 2-(hept-6-yn-1-yl)malonic acid (3.53%), and methyl 11-((2*R*,3*S*)-3-pentyloxiran-2-yl)undecanoate

(4.64%) were recorded after 4.15, and 25.89 minutes of retention time. The compounds were classified under several types of naturally occurring components, in which the class of ester of fatty acid has the majority of these components. Therefore, the esters of fatty acids includes (*E*)-4-(((2-methoxyoctadec-4-en-1-yl)oxy)methyl)-2,2-dimethyl-1,3-dioxolane, methyl 11-((2*R*,3*S*)-3-pentyl-oxiran-2-yl)undecanoate, and methyl (*E*)-octadec-11-enoate. Another class of compounds was identified as hydrocarbons including two components namely, 2-(hept-6-yn-1-yl)malonic acid, and 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one. Ethyl *iso*-allocholate followed the steroid class was found with 11.89% of composition (*c.f*. Supplementary file).

Alternatively, Al-Shammari et al. (2012), have interpreted the chemical constitutes isolated from the essential oil of *C. pycnocephalus* L. that grown in Saudi Arabia as basically nineteen components. Thus, the interpreted GC/MS of the plant extract indicated tetradecanoic acid (2.74%), hexadecanoic acid ethyl ester (4.81%), hexadecanoic acid (39.62%), phytol isomer (6.31%), linolenic acid ethyl ester (4.84%), 9,12-linoleic acid (19.46%), 1,2-benzendicarboxylic acid *iso*-octyl ester (7.11%), and heptacosane (2.34%). Therefore, the major component of this plant was related to hexadecanoic acid (39.62%), which followed the fatty acid class of compounds. On the other hand, the GC-MS spectroscopic analysis of *C. pycnocephalus* that grown in Iran revealed that the essential oil of this extract contains twenty-nine components such as hexadecanoic acid (23.3%), dibutyl, 1,2-benzene dicarboxylate (8.2%), 6,10,14-trimethyl-2-pentadecanone (7.4%), 1-pentyl octylbenzene (3.7%), and tetradecanoic acid (4.3%). The major component was found to be hexadecanoic acid (23.3%), which is related to the fatty acid class. The established components are terpenes with low composition percentages, fatty acids, and their esters, hydrocarbons, and alkylbenzenes.

Additionally, twenty components of the esters of fatty acids isolated from *C. pycnocephalus* that grown in Saudi Arabia including 1,2-benzene dicarboxylic acid, dimethyl ester (31.08%), azelaic acid dimethyl ester (7.6%), and palmitic acid methyl ester (20.08%) as the major constitutes of this plant extract. Other researches have been reported the composition of the components of the volatile oils of *C. pycnocephalus* grown in Iran (Esmaeili et al. 2005), and the identification of the terpenes, and flavone glycoside of *C. pycnocephalus* (Al-Shammari et al. 2015).

Table 1
The interpreted components of the essential oil of the extracted *C. pycnocephalus.*

Entry	Chemical name	Classification	Retention time (RT, min)	Molecular Weight	Molecular formula	Composition %
1	2-(Hept-6-yn-1-yl)malonic acid	Hydrocarbon "dicarboxylic acid"	4.15	198.22	$C_{10}H_{14}O_4$	3.53
2	(*E*)-4-(((2-Methoxyoctadec-4-en-1-yl)oxy)methyl)-2,2-dimethyl-1,3-dioxolane	Ester of fatty acid	4.92	412.66	$C_{25}H_{48}O_4$	11.53
3	3,5-Dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	Oxygenated hydrocarbon	9.11	144.13	$C_6H_8O_4$	57.43
4	Methyl 11-((2*R*,3*S*)-3-pentyloxiran-2-yl)undecanoate	Ester of fatty acid	25.89	312.49	$C_{19}H_{36}O_3$	4.64
5	Methyl (*E*)-octadec-11-enoate	Ester of fatty acid	29.18	296.50	$C_{19}H_{36}O_2$	10.98
6	Ethyl iso-allocholate	Steroid	35.71	436.63	$C_{26}H_{44}O_5$	11.89
						= 100.0

The Biological Characteristics Of The Plant Extracts

Once upon a time, medicinal plant extracts have been applied in the treatment of various diseases, where the discovery of medicines lies in the presence of their active essential components in the contents of the plant extract, which led to an advance in pharmacology. The *C. pycnocephalus* plant has been widely used in traditional medicine for the treatment of many diseases.

Antioxidant Activity - Dpph Assay

As commonly reported, the antioxidant activities of *C. pycnocephalus* extracts were evaluated by DPPH• (2,2'-diphenyl-1-picrylhydrazyl radical) colorimetric assay as the most commonly used assay for the extracted plants. The procedure is usually applied for the hydrophilic antioxidant constitutes, while their application for evaluating the antioxidant capacity of the hydrophobic components is limited. In this method, the reactive components that provided potent radical scavenging activities should contribute with a weak A-H bonding, which will increase the possibility for stabilizing or trapping the free radicals of DPPH• at a maximum wavelength 517 nm resulting in a discoloration of the DPPH molecule. The violet color of the DPPH radical will change subsequently to colorless by increasing the concentration of the scrutinized samples. The importance of antioxidants arises from the ability of these components to inhibit the oxidation of lipids. The process followed by scavenging or trapping the free radicals of DPPH, and hence determining the radical scavenging activity, as well as the assay in another route is an indication for reducing the capacity of the antioxidants in their reactions with DPPH radical.

The inexpensive reagents applied to enable to run of the investigated sample by this proficient colorimetric assay (Blois 1958; Bondet et al. 1997; Brand-Williams et al. 1995). The mechanism of DPPH assay is postulated to underwent by a mean of a HAT (Litwinienko & Ingold 2005), SET (Fotiet al. 2004; Huang et al. 2005), or mixed (Schaich et al. 2015) mechanisms as rendering to the succeeding reaction sequences:

DPPH·(violet) + XOH → DPPHH (colorless) + $XO^{\bullet}$ HAT Eq. (1)

DPPH·(violet) + XOH → DPPH⁻ (colorless) + $XO^{\bullet+}$ SET Eq. (2)

The phytochemical constitutes (Kozyra et al. 2019) such as phenolic, and flavonoid contents are proficient components that act as strong scavengers for the DPPH radical increasing the antioxidant potential of these samples to protect the lipoperoxy, and lipid peroxidative from oxidation progression. The plausible mechanism of action was controlled by several beneficial effects. Thus, the presence of antioxidant agents enables the scavenging of the free radicals of DPPH depending on the nature of the source of the free radical components, and the concentration of the inspected sample. Therefore, by increasing the sample concentration, the percent of radical scavenging activity will increase resulting in a release in the color intensity of the DPPH (Siripatrawan & Vitchayakitti 2016).

In this work, the antioxidant properties of root, stem, leaves, and flower extracts of *C. pycnocephalus* were *in vitro* evaluated by DPPH assay. The samples were tested for six diversified concentrations (5, 10, 20, 30, 40, and 50 mg/L). The results in Table 2 verified that flower extract is the most potent antioxidant agent with IC_{50} = 30.69 mg/L relative to the results of ascorbic acid (IC_{50} = 13.30 mg/L). The leaves extract of *C. pycnocephalus* located the second-order of radical scavenging capacity with IC_{50} = 32.78 mg/L, succeeding by the stem extract (IC_{50} = 41.31 mg/L), and root extract (IC_{50} = 46.84 mg/L). Table 2 consistently also presented the radical scavenging activity (%) of the extracted root, stem, leaves, and flower of *C. pycnocephalus* plant.

Figure 3 indicated the plotted percentages of radical scavenging activity against the various concentrations of each tested sample of *C. pycnocephalus* extract. A linear correlation between the scavenging activity % and their applicable concentrations (5-50 mg/L). The radical scavenging activity percent increased by increasing the sample concentration in a proportional relationship. Besides, the stem extract has the most potent scavenging activity for DPPH radicals at the lower concentration (5 mg/L) with % scavenging radical activity at 17.14±1.01%, however, the root extract located the second-order of activity with % scavenging radical activity at 12.58 ± 0.74% at the same concentration.

Table 2
The antioxidant results of the extracted root, stem, leaves, and flower of *C. pycnocephalus* plant.

Sample	Conc. (mg/L)	R_1 [a]	R_2 [b]	Mean value	Radical Scavenging Activity (%) [c]	IC_{50} (mg/L) [d]
Root	5	0.644	0.662	0.653	12.58 ± 0.74	46.84
	10	0.553	0.531	0.542	27.44±1.61	
	20	0.508	0.53	0.519	30.52±1.80	
	30	0.457	0.475	0.466	37.62±2.21	
	40	0.415	0.41	0.4125	44.78±2.63	
	50	0.375	0.353	0.364	51.27±3.02	
Stem	5	0.623	0.615	0.619	17.14±1.01	41.31
	10	0.575	0.564	0.5695	23.76±1.40	
	20	0.465	0.457	0.461	38.29±2.25	
	30	0.427	0.419	0.423	43.37±2.55	
	40	0.391	0.397	0.394	47.26±2.78	
	50	0.338	0.331	0.3345	55.22±3.25	
Leaves	5	0.666	0.673	0.6695	10.37±0.61	32.78
	10	0.595	0.611	0.603	19.28±1.13	
	20	0.439	0.431	0.435	41.77±2.46	
	30	0.394	0.371	0.3825	48.80±2.87	
	40	0.341	0.337	0.339	54.62±3.21	
	50	0.213	0.208	0.2105	71.82±4.22	
Flower	5	0.676	0.688	0.682	8.70±0.51	30.69
	10	0.579	0.591	0.585	21.69±1.28	
	20	0.411	0.401	0.406	45.65±2.69	
	30	0.324	0.327	0.3255	56.43±3.32	
	40	0.292	0.285	0.2885	61.38±3.61	
	50	0.233	0.238	0.2355	68.47±4.03	

[a], [b]: R_1, and R_2 referred to the values of the first and second read for the absorbance of the samples at different concentrations; [c]: RSA (%) indicated the Radical Scavenging Activity (%); [d]: IC_{50} referred to the inhibitive concentrations of the tested samples in mg/L.

At the concentration of 10 mg/L, the root extract was found with the most potent antioxidant activity (% RSA= 27.44±1.61%), and the stem extract has the second-order of antioxidant capacity (% RSA= 23.76±1.40%). The concentration of 20 mg/L of the investigated sample is more applicable for the comparison of the antioxidant scavenging radical activities for all the tested samples relative to the results of ascorbic acid. Thus, the flower extract revealed the potent antioxidant capacity of the other plant extracts with % RSA at 45.65±2.69%, this result is comparable with that of ascorbic acid (% RSA= 64.97%). Leaves extract situated the second-order of antioxidant potency (% RSA= 41.77±2.46%), then stem extract (% RSA= 38.29±2.25%), and finally the root extract (% RSA= 30.52±1.80%) at a concentration of 20 mg/L. In the same sequence, flower extract is the most potent antioxidant agent at the concentrations of 30, and 40 mg/L with % scavenging radical activity at 56.43±3.32, and 61.38±3.61%, respectively. The order of activity of the other plant constitutes is followed the extract of leaves (% RSA= 48.80±2.87 & 54.62±3.21%), stems (% RSA= 43.37±2.55 & 47.26±2.78%), and to end with the root (% RSA= 37.62±2.21 & 44.78±2.63%). Unexpectedly, at the higher concentration (50 mg/L), we noticed that leaves extract has the most potent antioxidant activity with % scavenging radical activity at 71.82±4.22% than the flower extract with % scavenging radical activity at 68.47±4.03%. The behavior of stem and root extracts at the concentration of 50 mg/L followed the same sequence of the lower concentration (20, 30, and 40 mg/L) with % scavenging radical activity at 55.22±3.25%, and 51.27±3.02% (Table 2). The values of inhibitive concentrations "IC_{50}" is the half-maximal inhibitory concentration expressed the potential concentration of the tested sample that achieves scavenging for the radicals of DPPH by 50%. The values of IC_{50} were calculated from the exponential curve (Parejo et al. 2000) plotting the sample concentrations against the remaining percent of $DPPH^{\bullet}$ radical applying linear regression analysis.

The relationship between the IC_{50} values and the free radical scavenging activity percentages are inversely proportional. As specified from the results of IC_{50} values in Table 2, and Fig. 4, the flower extract is the most potent antioxidant agent with IC_{50} = 30.69 mg/L, relative to the IC_{50} value of ascorbic acid (IC_{50}= 13.30 mg/L). The order of antioxidant capacity of the other plant materials is found in the following order: leaves (IC_{50}= 32.78 mg/L) > stem (IC_{50}= 41.31 mg/L) > root (IC_{50}= 46.84 mg/L). The significant antioxidant potency of the samples depended in this scale of comparison on the nature of the chemical components contained in the distinct extract as the presence of electron sources such as reactive oxygen or nitrogen sorts (Tiwari et al. 2013; Singh et al. 2021) such as hydroxyl, phenoxyl, alkyl peroxyl, linoleic acid, peroxyl, and glutamyl radicals that can stabilize or trap the DPPH free radical improve the antioxidant aptitude (Kwak et al. 2009; Mishra 2016).

Potential Antibacterial Activity

The antibacterial activity of the extracted botanical ingredients of *C. pycnocephalus* from methanol was assessed by a disc diffusion technique as an *in vitro* antimicrobial susceptibility testing. The tested samples were prepared in a concentration of 10 mg/L from the root, stem, leaf, and flower extracts. The results are shown in Table 3, and Figure 5 revealed that the four samples are potent antibacterial agents against *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *B. cereus* bacterial species. Particularly, potent antibacterial activities were recorded for leaf, and flower extracts against *E. coli* species with inhibition zones equivalent to that of antibiotic standards (20 mm). The root and stem extracts revealed remarkable antibacterial activities against *P. aeruginosa* species with inhibition zones 22, and 20 mm, respectively, with high potency than the antibiotic

standard “Azithromycin” (13 mm), along with good activities of the leaf, and flower extracts with inhibition zones at 10 mm.

By studying the results of the antibacterial activities of samples as inhibitors of bacterial growth, we found that the four samples had distinct activities in the process of inhibiting the growth of bacterial species of the type *S. typhimurium* by 14, 13, 26, and 25 mm, respectively, relative to the results of the antibiotic “Tetracycline” (10 mm). Medium to high activities of the four samples was observed against *S. epidermidis* bacterial strains compared to the results of the four antibiotics, while the highest result of inhibiting the growth of those bacterial species was for the flower extract with an inhibition zone at 15 mm. Looking at the results of the four samples as components of *C. pycnocephalus*, we found that the activity of methanolic extracts against Gram-positive bacterial species is not good compared to their results with Gram-negative bacteria. This does not indicate the lack of quality of those extracts to inhibit the growth of Gram-positive bacteria, as some extracts have higher results than antibiotics such as extracts of leaves and flowers towards inhibiting the growth of bacterial species of type *B. cereus* by an amount of 23 and 25 mm, respectively, and these results are higher than that obtained by all the antibiotics (5-20 mm) (Table 3).

Table 3
The antimicrobial activities are represented by the inhibition zone diameter (mm) of the methanol extract of *C. pycnocephalus* and standard antibiotics.

Microbes	Inhibition zone in mm							
	C. pycnocephalus (10 mg L^{-1})				Standard antibiotic (10 mg L^{-1})			
	Root	Stem	Leaf	Flower	Cephradin	Tetracycline	Azithromycin	Ampicillin
	Gram-negative bacteria							
E. coli	13	10	20	20	15	20	20	20
P. aeruginosa	22	20	10	10	0	0	13	0
S. typhimurium	14	13	26	25	0	10	0	0
S. epidermidis	10	5	5	15	10	20	23	10
	Gram-positive bacteria							
B. cereus	11	13	23	25	20	10	20	5
S. aureus	12	10	10	14	20	20	20	30
S. haemolyticus	0	0	5	5	25	23	23	20
S. xylosus	0	0	0	5	20	20	20	25
K. pneumonius	10	10	13	13	10	20	13	5
$LSD_{0.05}$ [a]	1.61	1.32	1.47	1.50	1.71	1.84	1.41	2.31

[a] $LSD_{0.05}$ expressed the calculated least of the smallest significance between two means as each test was run on those two means (calculated by Factorial ANOVA).

Among the good results are also a high activity of inhibiting the growth of *S. aureus* of the flower methanolic extract by an amount of 14 mm and the good activity of leaf and flower extracts as inhibitors of *K. pneumonius* growth with an amount of 13 mm compared to the highest inhibitory activity of the antibiotic "Tetracycline" with an inhibition efficiency of 20 mm. The results also demonstrated that the lowest efficiency to inhibit the growth of bacterial microorganisms was found against *S. haemolyticus* and *S. xylosus* using any of the four extracts with inhibition efficiency from inactive to 5 mm (Table 3).

The mechanism of action for the bacterial infections (Sawa, et al. 2019; Bascones-Martínez et al. 2009) is controlled by six main factors: (1) the interface with the bacterial cell wall (inhibit cell wall synthesis) (Mohammad et al. 2017), (2) depolarize the cell membrane (Higgins et al. 2005), (3) inhibition of the protein synthesis (De Vriese et al. 2006), (4) inhibition of nucleic acid synthesis (Srivastava et al. 2011), and (5) metabolic pathway inhibition (Li et al. 2019). Additionally, five basic mechanisms of action for antibiotics

(Etebu & Arikekpar 2016) are known including the inhibition of cell wall synthesis, protein synthesis (translation), nucleic acid synthesis, cell membranes alteration, and antimetabolite activity. The action of the tested samples as antibacterial agents is to continually disrupt and prevent the growth of bacterial species. The interpretation of these results is consistent with previous results in deducing the efficacy of the plant extract as an antimicrobial agent (El-Shahaby et al. 2013).

Cytotoxic Activity

The cytotoxic activity of *C. pycnocephalus* methanol extract was assessed using 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay under distinct conditions. The method is identical to a cell number growth curve. The MTT reagent as a reliable indicator is so sensitive to the light, this engaged the run of the experiments in the dark. Hepatocellular carcinoma (HepG2) tumor cell line was selected to assess the anticancer potency of the investigated extracted medicinal plant. The method was applied for determining the cell metabolic activity based on the aptitude of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes to reduce the MTT tetrazolium dye to its formazan “insoluble” that has a purple color. The number of viable cells should increase with growth, decrease with cytotoxic treatments, and remain the same (or plateau) with cytostatic treatments. The IC_{50} values expressed the concentration that represented 50% of the inhibition of cell growth was calculated by applying the curves obtained from plotted the percentages of cell survival versus drug concentration (μM). Thus, the potency of cytotoxicity will rise by the decrease in the extract concentration and IC_{50} values. The MTT solution may be affected by the results of cytotoxicity, so we have run a control sample “blank” that was a few "empty" wells containing MTT solution without any of the cell lines. The control sample is a benefit for calculating the cell viability percent, as it produces 100% viability of healthy cells. The experiments were run using five concentrations of each plant extract (31.3, 62.5, 125, 500, and 1000 μg/mL) prepared in a serial dilution (Table 4).

Table 4
Cytotoxic results of the extracted *C. pycnocephalus* against HepG2 tumor cell line.

Samples	Conc. (μg/mL)	R_1 [a]	R_2 [a]	IC_{50} (μg/mL) [b]
C. pycnocephalus extract	1000	0.714	0.755	46.2
	500	1.4	1.32	
	125	2.4	1.7	
	62.5	1.6	1.7	
	31.3	1.8	1.8	
	0	1.3	1.3	

[a] R_1, R_2 are the absorbance read of the extracts at diverse concentrations.

[b] IC_{50} values are the inhibitive concentration expressed sample concentration that contributes roughly 50% of the death of cancer cells.

Table 4 signified the results of the cytotoxic effects specified by the inhibitive concentration values of the extract of *C. pycnocephalus* on HepG2 tumor cell line. The results of cytotoxicity revealed that the extract of *C. pycnocephalus* has a potent cytotoxic effect on HePG-2 cell line with an IC_{50} value at 46.2 µg/mL. It is worth mentioning that the mechanism of cytotoxicity of the extracted samples as cytotoxic agents on HepG2 tumor cell line is commonly dependent on the structural nature of the components of each extract, and the nature of the cancer cell line. Additionally, the nature of the particles of the extracted plants such as surface morphology, size, and aggregation of the particles might control the results of cytotoxicity. The efficiency of the plant extract as an efficient anti-cancer agent for tumor cell growth depended on several factors as previously noted, including the nature of the chemical components of this extract, the type of cancer cell, and the concentration of the extracted plant used in this assess (Khacha-Ananda et al. 2013).

EC_{50} of C. pycnocephalus **extract**

The dose-response relationship of the assessed *C. pycnocephalus* extract is plotted in Figure 6. The dose-response curve in Fig. 6a was normalized in the X-axis direction by its EC_{50} value (Fig. 6b). The value of EC_{50} of the methanol extract of *C. pycnocephalus* was initially calculated by plotting the sample absorbance against the log of doses at different concentrations of the serial dilution (Fig. 6). The low concentrations of the extract are not enough to produce a response, while the high doses produce a maximal response, and the vertical point of the curve resembles an EC_{50} value. The data analysis specified that the higher concentration (dose = 1000 µg/mL) as calculated for EC_{50} value (2.82 µg/mL) has a cytotoxic effect on HepG2 cell lines.

Conclusion

C. pycnocephalus plant is related to Asteraceae family displayed a wide spectrum of biological activities (Rustaiyan & Faridchehr 2021; Conforti et al. 2008). In this work, we used the extract of *C. pycnocephalus* as a medicinal plant or any of its plant parts such as the stem, leaves, flowers, and roots to identify the chemical components that were isolated from the essential oils of the methanolic extract to study the effect of these components on the different biological results. We found that the four parts of the plant have high antioxidant capacities compared to the results of ascorbic acid as a result of these extracts containing a high percentage of phenolic components. Higher than antibiotics. Through that study, we discovered the efficiency of the plant extract as a cytotoxic agent with a moderate potency against the growth of hepatocellular carcinoma (HepG2), which indicated that the drug will not affect the growth of normal cells. The results also specified that the extracts of different parts of the plant, especially the that of leaves and flowers, have high and distinctive anti-bacterial activities to kill or inhibit the growth of the bacterial species such as *E. coli*, *S. typhimurium*, and *B. cereus* strains with higher efficiency than antibiotics.

Declarations

The authors declare no conflict of interest.

References

1. Al-Shammari LA, Hassan WHB, Al-Youssef HM (2012) Chemical composition and antimicrobial activity of the essential oil and lipid content of *Carduus pycnocephalus* L. growing in Saudi Arabia. J Chem Pharm Res 4(2):1281–1287
2. Al-Shammari LA, Hassan WHB, Al-Youssef HM (2015) Phytochemical and biological studies of *Carduus pycnocephalus* L. J Saudi Chem Soc 19(4):410–416. https://doi.org/10.1016/j.jscs.2012.05.002
3. Al-Shammari LA, Abdallah RH, Al-Youssef HM, Hassan WHB (2015) New pentacyclic triterpene ester and flavone glycoside from the biologically active extract of *Carduus pycnocephalus* L. J Pharmacogn Phytotherapy 7:45–55
4. Bascones-Martínez A, Muñoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Trapero J (2009) Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. Med Oral Patol Oral Cir Bucal 14:680–5
5. Blois M (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200
6. Bondet V, Brand-Williams W, Berset C (1997) Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT-Food Sci Technol 30(6):609–615
7. Boulos L (1983) Medicinal Plants of North Africa - Medicinal plants of the world 3. Reference Publications, Inc., 218 St. Clair River Drive, Box 344. Algonac, Michigan 48001, pp. 286
8. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28(1):25–30
9. Brunetti G, Soler-Rovira P, Farrag K et al (2009) Tolerance and accumulation of heavy metals by wild plant species grown in contaminated soils in Apulia region, Southern Italy. Plant Soil 318:285–298. https://doi.org/10.1007/s11104-008-9838-3
10. Chaudhary SA (2000) Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and Water, National Herbarium, National Agriculture and Water Research Center, Riyadh, KSA, vol II, part 3, pp 117–202
11. Conforti F, Ioele G, Statti GA, Marrelli M, Ragno G, Menichini F (2008) Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. Food Chem Toxicol 46(10):3325–3332. https://doi.org/10.1016/j.fct.2008.08.004
12. de Briano AEE, Acciaresi HA, Briano JA (2013) Establishment, dispersal, and prevalence of *Rhinocyllus conicus* (Coleoptera: Curculionidae), a biological control agent of thistles, *Carduus* species (Asteraceae), in Argentina, with experimental information on its damage. Biol Control 67(2):186–193. https://doi.org/10.1016/j.biocontrol.2013.07.009
13. De Vriese AS, Van Coster R, Smet J, Seneca S, Lovering A, Van Haute LL et al (2006) Linezolid-induced inhibition of mitochondrial protein synthesis. Clin Infect Dis 42(8):1111–1117
14. Doing H, Biddiscombe EF, Knedlhans S (1969) Ecology and distribution of the *carduus nutans* group (nodding thistles) in Australia. Plant Ecol 17:313–351. https://doi.org/10.1007/BF01965915
15. El-Shahaby O, El-Zayat M, Salih E, El-Sherbiny IM, Reicha FM (2013) Evaluation of Antimicrobial Activity of Water Infusion Plant-Mediated Silver Nanoparticles. J Nanomed Nanotechnol 4:178. doi:10.4172/2157-7439.1000178
16. Abd-ElGawad AM, El-Amier YA, Assaeed AM, Al-Rowaily SL (2020) Interspecific variations in the habitats of *Reichardia tingitana* (L.) Roth leading to changes in its bioactive constituents and allelopathic activity. Saudi J Biol Sci 27:489–499. https://doi.org/10.1016/j.sjbs.2019.11.01

17. Esmaeili A, Rustaiyan A, Nadimi M, Masoudi S, Tadayon F, Sedaghat S, Ebrahimpur N, Hajyzadeh E (2005) Volatile Constituents of Centaurea depressa M.B. and Carduus pycnocephalus L. Two Compositae Herbs Growing Wild in Iran. J Essent Oil Res 17(5):539–541. https://doi.org/10.1080/10412905.2005.9698988
18. Etebu E, Arikekpar I (2016) Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Int J Appl Microbiol Biotechnol Res 4:90–101
19. Foti MC, Daquino C, Geraci C (2004) Electron transfer reaction of cinnamic acids and their methyl esters with the DPPH(*) radical in alcoholic solutions. J Org Chem 69(7):2309–2314. doi.: 10.1021/jo035758q
20. Gallo MBC, Sarachine MJ (2009) Biological activities of lupeol. Int J Biomed Pharm Sci 3:46–66
21. Higgins DL, Chang R, Debabov DV, Leung J, Wu T, Krause KM, Sandvik E, Hubbard JM, Kaniga K, Schmidt DE Jr, Gao Q (2005) Telavancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 49(3):1127–1134
22. Huang D, Ou B, Prior RL (2005) The chemistry behind antioxidant capacity assay. J Agric Food Chem 53(6):1841–1856. doi.: 10.1021/jf030723c
23. Jaunatre R, Fonvieille N, Spiegelberger T et al (2016) Recovery of arbuscular mycorrhizal fungi root colonization after severe anthropogenic disturbance: Four species assessed in old-growth Mediterranean grassland. Folia Geobot 51:319–332. https://doi.org/10.1007/s12224-016-9254-z
24. Jordon-Thaden FE, Louda SM (2003) Chemistry of Cirsium and Carduus: a role in ecological risk assessment for biological control of weeds. Biochem Syst Ecol 31:1353–1396
25. Khacha-Ananda S, Tragoolpua K, Chantawannakul P, Tragoolpua Y (2013) Antioxidant and anti-cancer cell proliferation activity of propolis extracts from two extraction methods. Asian Pac J Cancer Prev 14(11):6991–6995
26. Kim E, Kim H-J, Yang S-M, Kim C-G, Choo D-W, Kim H-Y (2019) Rapid Identification of Staphylococcus Species Isolated from Food Samples by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. J Microbiol Biotechnol 29:548–557. https://doi.org/10.4014/jmb.1901.01046
27. Kitts D, Wijewickreme A, Hu C (2000) Antioxidant properties of a North American ginseng extract. Mol Cell Biochem 203:1–10
28. Kozyra M, Komsta Ł, Wojtanowski K (2019) Analysis of phenolic compounds and antioxidant activity of methanolic extracts from inflorescences of *Carduus sp*. Phytochem Lett 31:256–262. https://doi.org/10.1016/j.phytol.2019.04.012
29. Kwak SY, Seo HS, Lee YS (2009) Synergistic antioxidative activities of hydroxycinnamoyl-peptides. Journal of Peptide Science: an official publication of the European Peptide Society 15(10):634–641
30. Li Y, Jia A, Wang Y, Dong L, Wang Y, He Y et al (2019) Immune effects of glycolysis or oxidative phosphorylation metabolic pathway in protecting against bacterial infection. J Cell Physiol 234(11):20298–20309
31. Litwinienko G, Ingold KU (2005) Abnormal solvent effects on hydrogen atom abstraction. 3. Novel kinetics in sequential proton loss electron transfer chemistry. J Org Chem 70(22):8982–8990. doi.: 10.1021/jo051477p
32. Magaldi S, Mata-Essayag S, de Capriles CH, Perez C, Colella MT, Olaizola C, Ontiveros Y (2004) Well diffusion for antifungal susceptibility testing. Int J Infect Dis 8(1):39–45. doi: 10.1016/j.ijid.2003.03.002

33. Marengo A, Maxia A, Sanna C, Bertea CM, Bicchi C, Ballero M, Cagliero C, Rubiolo P (2017) Characterization of four wild edible Carduus species from the Mediterranean region *via* phytochemical and biomolecular analyses. Food Res Int 100(Part 1):822–831. https://doi.org/10.1016/j.foodres.2017.07.071
34. Mishra SS (2016) Detection and Analysis of Spin Trapped Radical Adducts by Using α-Phenyl-tert-Butyl Nitrone (PBN) Derivatives and GC/MS. University of Salford (United Kingdom)
35. Mohammad H, Younis W, Chen L, Peters CE, Pogliano J, Pogliano K et al (2017) Phenylthiazole antibacterial agents targeting cell wall synthesis exhibit potent activity *in vitro* and *in vivo* against vancomycin-resistant Enterococci. J Med Chem 60(6):2425–2438
36. Murray PR, Baroon EJ, Pfaller MA, Tenover FC, Yolke RH (1995) Manual of Clinical Microbiology, 6th edn. American Society for Microbiology, Washington DC, p 1482
37. Olivieri I (1985) Comparative electrophoretic studies of *Carduus pycnocephalus* L., c. tenuiflorus curt. (asteraceae), and their hybrids. Am J Bot 72(5):715–718. https://doi.org/10.1002/j.1537-2197.1985.tb08330.x
38. Olivieri I, Swan M, Gouyon PH (1983) Reproductive system and colonizing strategy of two species of *Carduus* (compositae). Oecologia 60:114–117. https://doi.org/10.1007/BF00379329
39. Orhan I, Orhan D, Ozcelik B (2009) Antiviral activity and cytotoxicity of the lipophilic extracts of various edible plants and their fatty acids. Food Chem 115:701
40. Parejo I, Codina C, Petrakis C, Kefalas P (2000) Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. J Pharmacol Toxicol Methods 44:507–512
41. Rustaiyan A, Faridchehr A (2021) Constituents and biological activities of selected genera of the Iranian Asteraceae family. J Herb Med 25:100405. https://doi.org/10.1016/j.hermed.2020.100405
42. Sardari S, Amin G, Micetich RG, Daneshtalab M (1998) Phytopharmaceuticals. Part 1: Antifungal activity of selected Iranian and Canadian plants. Pharm Biol 36(3):180–188
43. Sawa T, Kinoshita M, Inoue K, Ohara J, Moriyama K (2019) Immunoglobulin for treating bacterial infections: One more mechanism of action. Antibodies 8(4):52
44. Schaich KM, Tian X, Xie J (2015) Reprint of “Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays”. J Funct Foods 18(Part B) 782–796. https://doi.org/10.1016/j.jff.2015.05.024
45. Singh AK, Kumar S, Sinha T (2021) Antioxidants in Plant–Microbe Interaction. Antioxidants in Plant-Microbe Interaction. Springer, Singapore, pp 3–20
46. Siripatrawan U, Vitchayakitti W (2016) Improving functional properties of chitosan films as active food packaging by incorporating with propolis. Food Hydrocolloids 61:695–702
47. Souza MM, Silva BD, Costa CS, Badiale-Furlong E (2018) Free phenolic compounds extraction from Brazilian halophytes, soybean and rice bran by ultrasound-assisted and orbital shaker methods. An Acad Bras Cienc 90:3363–3372
48. Srivastava A, Talaue M, Liu S, Degen D, Ebright RY, Sineva E et al (2011) New target for inhibition of bacterial RNA polymerase: “switch region”. Curr Opin Microbiol 14(5):532–543

49. Terblanche U, SSemakalu CC, Mtunzi F, Pillay M (2017) Screening of variables influencing extraction yield of cotyledon orbiculata: 2^3 full factorial design. Int J Pharmacogn Phytochem Res 9:303–312
50. Tiwari BK, Brunton NP, Brennan C (eds) (2013) Handbook of plant food phytochemicals: sources, stability and extraction. John Wiley & Sons
51. Valgas C, de Souza SM, Smânia EFA, Smânia A Jr (2007) Screening methods to determine antibacterial activity of natural products. Braz J Microbiol 38:369–380. https://doi.org/10.1590/S1517-83822007000200034
52. Zhu Q, Jiang L, Wang X (2017) The expression of Duffy antigen receptor for chemokines by epithelial ovarian cancer decreases growth potential. Oncol Lett 13(6):4302–4306. doi: 10.3892/ol.2017.5954

Figures

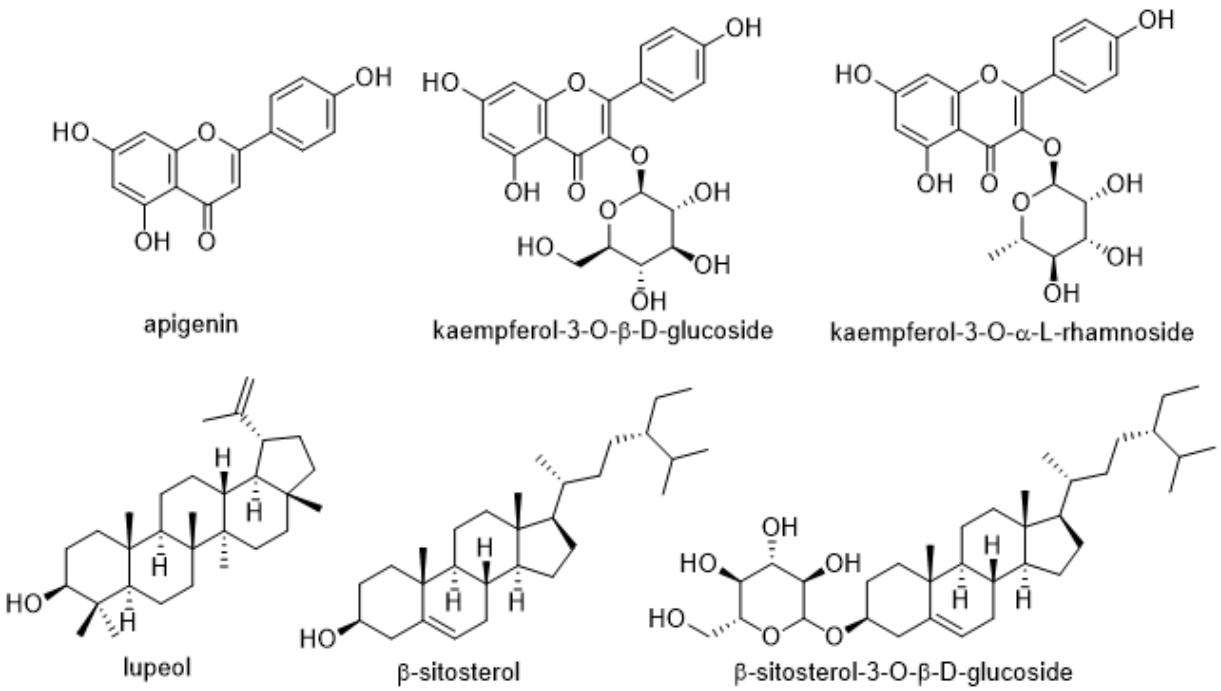

Figure 1

The effective chemical constitutes that were isolated from C. pycnocephalus.

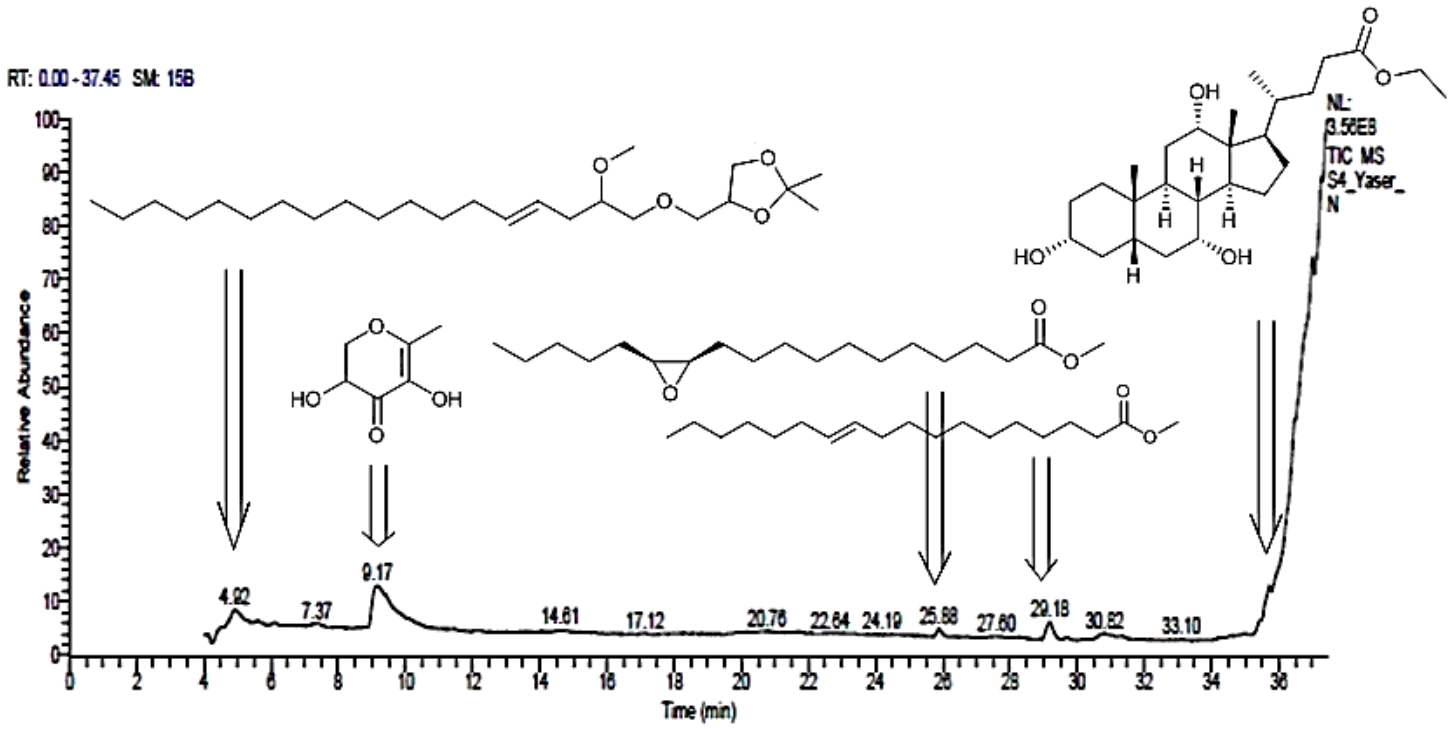

Figure 2

Chromatogram and structures of basic components of the essential oil of the extracted C. pycnocephalus by GC-MS.

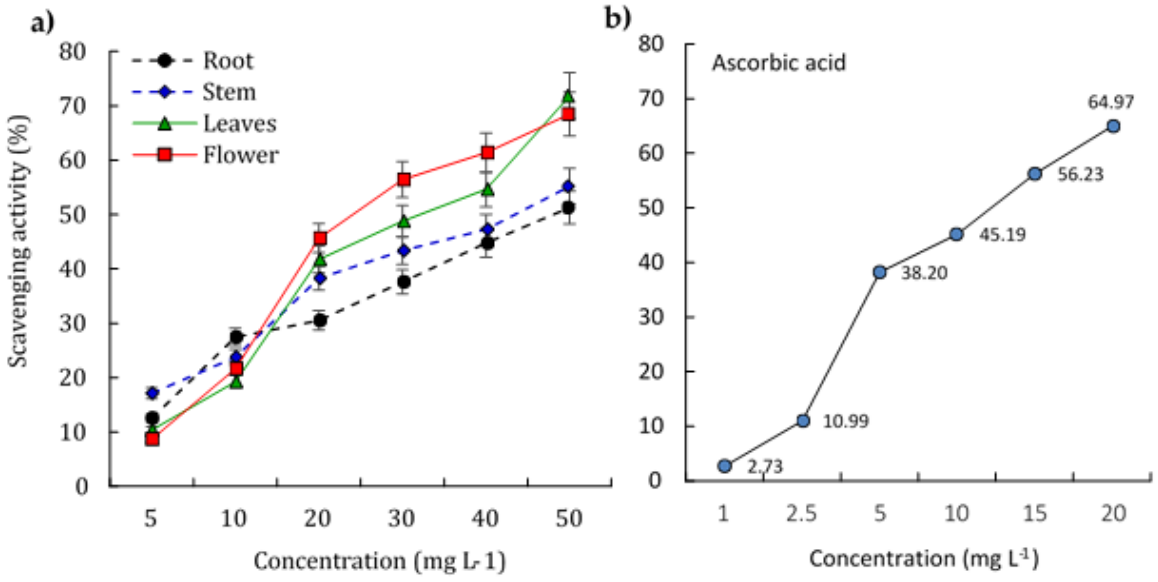

Figure 3

(a) Radical scavenging activity % plotted against the various concentrations of each species of C. pycnocephalus extract. (b) The plotted radical scavenging activity % against the various concentrations of ascorbic acid.

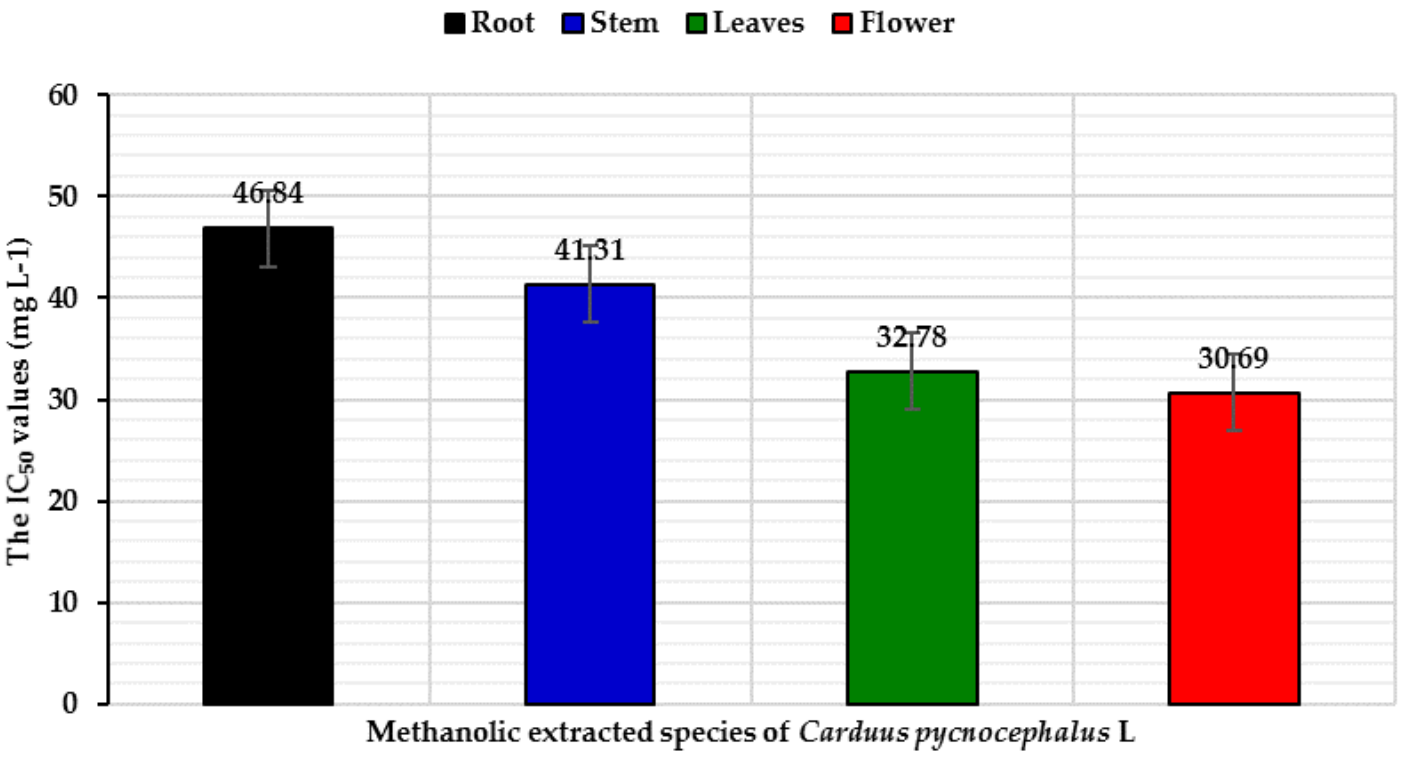

Figure 4

Comparison of the IC50 values of the investigated samples as antioxidant agents.

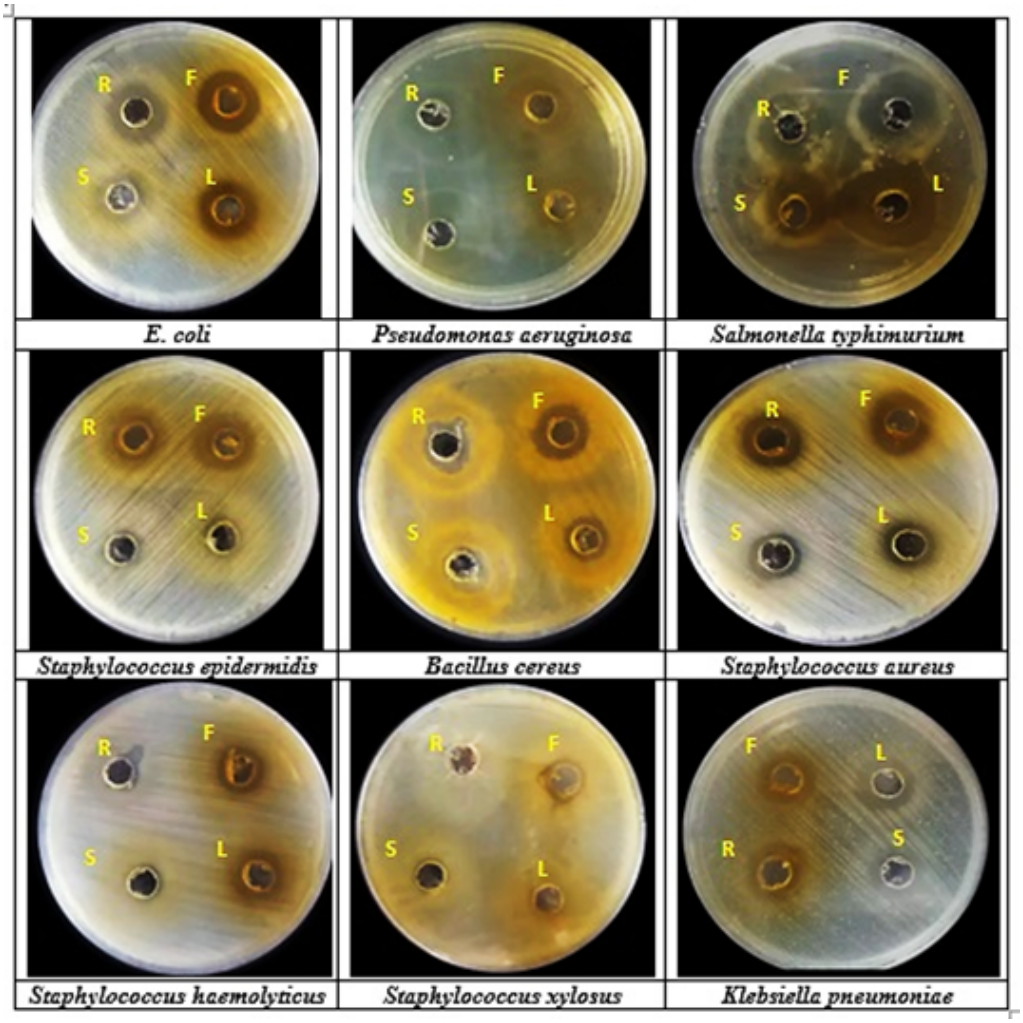

Figure 5

The inhibition zones were raised from the effects of the extracted root, stem, leaf, and flower of C. pycnocephalus on the plates seeded with different bacterial species by disc diffusion technique. R≡ Root extract; S ≡ stem extract; L ≡ Leaf extract; F ≡ Flower extract.

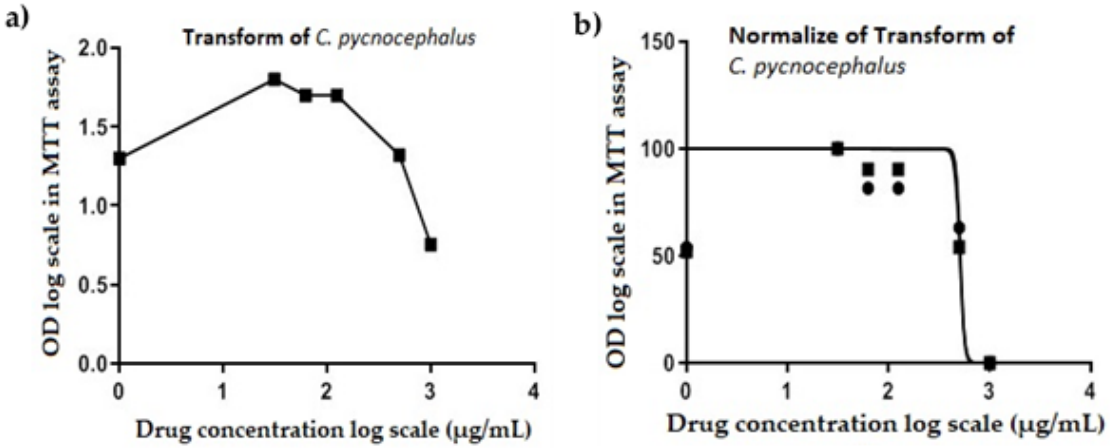

Figure 6

Dose response curve. (a) Transform of C. pycnocephalus extract. (b) Normalization of transform of C. pycnocephalus extract. (OD) expressed the measured absorbance value at a definite concentration of the investigated extracted sample.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx
- SupplementaryData.docx