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Phytochemical profile, antioxidant and antimicrobial assessments of *Cupressus semervirens* L. extracts

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Abstract: *Cupressus sempervirens* has a prominent role in the medicinal and pharmaceutical fields owing to the utility of this plant for the cure of many diseases in traditional medicine, as well as it comprises active chemical components. Accordingly, this study intended to inspect the chemical profile, antioxidant, and antimicrobial features of *C. sempervirens* (L.) extracts. Water and ethanol extracts of the dried aerial parts of *C. sempervirens* were prepared. The active secondary ingredients (phenolic, flavonoid, and tannin contents) were quantitatively estimated during four seasons. The active components in the ethanol extract were found to be higher than those of the water extract. The extracts acquired from plants gathered thru summer in July conveyed higher content of secondary metabolites followed by spring while the lowest content was in winter. The water extract exhibited more potent antioxidant activity in terms of DPPH radical scavenging assay comparative to the result of ascorbic acid. The antimicrobial activity revealed a broad antimicrobial spectrum for ethanol extract against *Staphylococcus aureus*, *Staphylococcus epidermidis, Enterobacter cloacae, Salmonella typhi, Bacillus cereus, Pseudomonas aeruginosa*, and *E. coli* although the extract prepared from water shown no activity against any of the tested species. The anti-oxidant and anti-microbial activities of the scrutinized extracts of *C. sempervirens* are ascribed to their amplitude with the therapeutically active metabolites.

keywords: *Cupressus sempervirens*, Antioxidant, Antimicrobial, DPPH, active constituents.

1.Introduction

Plants have been used in folklore medicine as pharmaceuticals and are thought to provide nutritional powers for people (Ekor, 2014). [1]. *Cupressus sempervirens* L. is used in folklore medications in the Mediterranean region. The medication was applied topically to treat bronchitis, coughs, and head colds [2]. The plant included alkaloids 0.7%, flavonoids 0.22%, tannin 0.31%, saponins 1.9%, and phenols 0.067%, according to the early phytochemical study. According to the plant's location and variety, the essential and volatile oils appeared to vary [3-4].

It originated in the Mediterranean area. The plant is widely distributed in North Africa, Asia (Iran, Palestine, Jordan, Lebanon, Syria, Iraq, and Turkey), Southern Europe (Greece and Italy), and North America [5-8]. The *Cupressus sempervirens* aerial parts extracts in methanol, ethanol, and ethyl acetate showed antibacterial efficacy against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumonia*, and *S. typhimurium* in a dose-dependent manner in addition to possessing antiviral activity [9-11].

The progress of antibiotic-resistant microbes *as Staphylococcus aureus* reduce the amount of antibiotics utilized for the cure of clinical infections and this has inspired the pursuit for innovative naturally antimicrobial drugs [12]. To our attentiveness, there have been few conveys on the potential activity of *C. sempervirens*. Therefore, the objectives of the present study to appraise the phytochemical profile, and biological features such as

antioxidant, and antimicrobial aptitude of polar extracts of this plant.

2. Materials and methods

2.1. Plant material

The aerial parts of *Cupressus sempervirens* were gathered from the Mansoura University garden during different periods covering the four seasons of the year (October 2021, January 2022, April 2022, and July 2022). The samples' botanic characteristics were isolated and authenticated according to Bolous (1999) [13]. Half a kilo of aerial parts of plant material gathered in each season was oven-dried at 45 ^{o}C and grinded into fine powder.

2.2. Preparation of the plant extracts

Extraction of the target species was carried out using two different extraction solvents including water and ethanol. 20 grams of the plant were extracted by shaking for two hours at 200 rpm using 200 ml of ethanol while water extract was prepared by shaking 10 grams of dried plant with water at 70^{o}C for 20 minutes. Subsequently, the extracts were filtered and evaporated to dryness using a rotary evaporator.

2.3. Determination of the active secondary metabolites

2.3.1. Total phenolics

The phenolic content of the studied extracts was measured using the Folin-Ciocalteu assay described by Lin and Tang (2007) [14] and determined as milligram gallic acid equivalent/gram dried extract.

2.3.2. Total flavonoids

The flavonoid content of the studied extracts was measured using an aluminum chloride assay described by Chang *et al*. (2002) [15] and determined as milligram catechin equivalent/gram dried extract.

2.3.3. Total tannins

Tannin content was determined by the Vanillin hydrochloride method of Sadasivam, & Maickam (1996) [17] and determined as tannic acid equivalent/ gram dried extract).

2.4. Evaluation of antioxidant activity

DPPH assay

The efficacy of the studied extracts against DPPH free radicals was determined according to the method of Kitts *et al* (2000) [18]. The antioxidant activity was calculated as the number of antioxidants necessary to decrease the initial DPPH$^{\cdot}$ concentration by 50% ($IC_{50\%}$). Ascorbic acid was used as the standard reference compound.

2.5. Screening of the antimicrobial activity

Well diffusion assay

The antimicrobial activity of the plant extracts was estimated using a filter paper disc assay [19].

Tested organisms

Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter cloacae, Salmonella typhi, Bacillus cereus, Pseudomonas aeruginosa, E. coli, and Candida albicans EMCC number-105.

3. Results and Discussions

The previous studies reported the ability of *Cupressus sempervirens* reported reasonable content of phenolics, flavonoids, tannins, and terpenoids. Such compounds from *C. sempervirens* were found to possess potent bioactivities and to be affordable sources for developing new drugs [20]. For example, phenolics are well known for their ability to express many biological properties such as antimicrobial, anti-inflammatory, and anti-carcinogenic. Flavonoids are helpful in preventing oxidative cell stress in addition to their anti-cancer and antimicrobial activity [21 - 23].

The phytochemical screening of water and ethanol extracts of *C. sempervirens* revealed many active metabolites including tannins, phenolics, and flavonoids. These active compounds were quantitatively analyzed during different seasons. The seasonal changes might contribute as a stress factor that leads to variation in the secondary metabolites content of *C. sempervirensis* such as phenolics, flavonoids, alkaloids, saponins, tannins, and many other compounds that have protective and therapeutic effects. The results obtained are summarized in (Table 1).

Ethanol extract expressed higher total phenolics and tannins content than water while water was higher than ethanol in flavonoid content. Regarding water extract, the phenolic content during different periods of the year expressed as mg gallic acid equivalent per gram

dried extract could be descending ordered as July 2022 (182.34), October 2021(170.86), April 2022 (161.26) and January 2022 (158.55), the flavonoids content during different periods of the year expressed as mg catechine acid equivalent per gram dried extract could be descending ordered as July 2022 (88.16), October 2021(85.76), April 2022 (75.90) and January 2022 (72.78) and tannins content during different periods of the year expressed as mg tannic acid equivalent per gram dried extract could be descending ordered as July 2022 (11.22), October 2021(8.87), April 2022 (7.68) and January 2022 (7.17), respectively.

Table (1): Total phenolics, total flavonoids, and total tannins content of *C. sempervirens* during different seasons.

Samples	Period of collection	Phenolics Content	Flavonoids Content	Tannins Content
Water	October 2021	170.86	85.77	8.88
	January 2022	158.55	72.78	7.17
	April 2022	161.26	75.90	7.68
	July 2022	182.34	88.16	11.22
Ethanol	October 2021	206.29	36.33	12.82
	January 2022	180.22	28.83	9.56
	April 2022	191.08	31.03	10.92
	July 2022	226.11	41.55	15.02

Phenolics Content “mg gallic acid/1 gm dry extract”

Flavonoids Content “mg catechine/1 gm dry extract”

Tannins Content “mg tannic acid/1 gm dry extract

Regarding ethanol extract, the phenolic content during different periods of the year expressed as mg gallic acid equivalent per gram dried extract could be descending ordered as July 2022 (226.11), October 2021(206.29), April 2022 (191.08) and January 2022 (180.22), the flavonoids content during different periods of the year expressed as mg catechine acid equivalent per gram dried extract could be descending ordered as July 2022 (41.55), October 2021(36.33), April 2022 (31.03) and January 2022 (28.83), the tannin content during different periods of the year expressed as mg catechine acid equivalent per gram dried extract could be descending ordered as July 2022 (15.02), October 2021(12.82), April 2022 (10.92) and January 2022 (9.56), respectively.

phenolics and flavonoids present in the prepared extracts in variable quantities are well known for their potential as antioxidant components [24-26] and antioxidant activity could be attributed to them.

The antioxidant activity of the extracts prepared from *C. sempervirens* is reported in Table 2. The antioxidant potential of *C. sempervirens* extracts was estimated by DPPH radical assay. The antioxidants scavenging activities for the free radical DPPH coincide with their hydrogen-donating capabilities [27]. Ascorbic acid was used as the standard compound (Table 2). The strongest activity was detected in water extract which was higher in its antioxidant activity than the ethanol. This antioxidant activity might be attributed to the presence of many active metabolites including phenolics, flavonoids, and tannins reported in Table (1).

There were several studies have been done for screening the antimicrobial potential of *C. sempervirens* where the plant showed antimicrobial potential against *Bacillus subtilis, Streptococcus pneumoniae* while essential oils against *Streptococcus pneumonia, E. coli*, *Bacillus subtilis*, *Aspergillus fumigatus*, and *Candida albicans* [28-29]. It has been reported in the literature that the major groups responsible for the antimicrobial activity of plant extracts are phenolics, flavonoids, terpenoids, essential oils, alkaloids, lectins, and polypeptides [30].

Microbial susceptibility test (well diffusion assay) was used to study the effect of *C. sempervirens* extracts against several pathogenic species of antibiotic-resistant bacteria including Three-Gram positive species (*Staphylococcus aureus, Bacillus cereus,* and *Staphylococcus epidermidis*), Four-Gram negative species (*Escherichia coli, Enterobacter cloacae, Salmonella typhi,* and *Pseudomonas aeruginosa*) and one pathogenic fungus (*Candida albican*)

Table (2): Antioxidant activity of the extracts prepared from *C. sempervirens*.

Sample	Concentrations (mg/ml)	% Remaining DPPH	% Scavenging activity	IC_{50} (mg/ml)
Water extract	0.078	14.48	85.52	0.034
	0.039	44.78	55.22	
	0.02	78.36	21.64	
	0.01	94.93	5.07	
	0.005	98.21	1.79	
Ethanol (80%) extract	0.154	13.88	86.12	0.067
	0.077	49.7	50.3	
	0.039	75.97	24.03	
	0.019	87.76	12.24	
Ascorbic acid	0.062	15.267	85.19	0.0222
	0.031	39.084	62.07	
	0.016	61.069	40.74	
	0.008	74.809	27.41	

Table (3): Antimicrobial activity of *C. sempervirens* ethanol and water extracts.

Pathogenic strains	Water	Ethanol
Staphylococcus aureus	-	21
Enterobacter colacae	-	13
Salmonella typhi	-	15
Bacillus cereus	-	17
Staphylococcus epidermidis	-	17
Pseudomonas aeruginosa	-	12
E. coli	-	12
Candida albicans	-	-

Values indicate zone of inhibition in mm and include well diameter (6 mm); "-": no inhibition.

The obtained result indicated that *C. sempervirens ethanol* extract possesses a wide spectrum of activity against most of the tested pathogenic microorganisms while water extract exhibited no activity against any of the tested strains as illustrated in Table (3).

4. Conclusion

The obtained results illustrated that *C. sempervirens* can be seen as a potential source of beneficial drugs. The obtained results could be considered as a reference to the antioxidant and antimicrobial activity of *C. sempervirens* with biologically active constituents. The plant possesses high medicinal importance as shown by its crude extracts' activity against various microorganisms. Thus, a scientific foundation to use this plant in medicine could be profound for improving the user's healthcare. Further studies are needed to be held for the isolation, identification, characterization, and structure elucidation of these active constituents.

Competing interests

We affirm no competing interests.

Authors' contributions

The authors all gathered the plant samples in all seasons, provided chemical and laboratory materials, conducted the biological and chemical tests, and then wrote, and interpreted the results.

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