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TOTAL PHENOLIC AND FLAVONOID CONTENTS, ANTIMICROBIAL AND ANTIOXIDANT POTENTIALS OF CAMPANULA STRIGOSA BANKS & SOL

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ABSTRACT

Humans find several uses for plants. Not only is it good for you from a nutritional standpoint, but it has also been used to cure a wide range of illnesses. In this research, we analysed *Campanula strigosa* Banks & Sol. for its antioxidant and antibacterial properties. Total phenolic and flavonoid concentrations were also calculated. For this purpose, a soxhlet apparatus was used to extract ethanol and methanol from the plant's above-ground portions. The effectiveness of antioxidants was measured using Rel Assay kits. The agar dilution technique was used to test for antimicrobial activity against various bacterial and fungus species. Folin-Ciocalteu reagent was used to quantify total phenolic content. An aluminium chloride assay determined the total flavonoid concentration. Studies found that the plant extract had a TAS value of 4.974±0.259 mmol/L, a TOS value of 12.437±0.150 µmol/L, and an OSI value of 0.251±0.014. The plant extract was shown to be effective against bacterial strains at concentrations between 100 and 400 µg/mL, and against fungal strains at concentrations between 50 and 100 µg/mL. Ethanol extract also had a greater total phenolic concentration and total flavonoid content. *C. strigosa* was shown to have antibacterial and antioxidant properties in this study.

Keywords: Antioxidant, Bellflower, Complemantary medicine, Little bell, medicinal plants

INTRODUCTION

Natural remedies including mushrooms, plants, and animals are employed in the context of alternative medicine. (Korkmaz et al., 2018). Vitamins, minerals, and other nutrients are plentiful in plant foods. They are not only useful for human health because of their nutritional value, but also because of the secondary metabolites they generate. (**Mohammed *et al.* 2021a**). Plants have been shown to provide several health benefits, including those related to cancer prevention, infection prevention, DNA protection, allergy relief, cell growth inhibition, inflammation reduction, and liver protection (**Miastkowska and Sikora, 2018; Mohammed *et al.* 2020a; Maseehullah *et al.* 2021; Osanloo et *al.* 2022; Venmathi Maran *et al.* 2022; Uysal *et al.* 2023**). Determining the biological functions of plants is, thus, crucial. Anti-oxidant and antimicrobial properties of *C. strigosa* were investigated here. Total phenolic and flavonoid concentrations were also calculated for the plant.
More than 500 species and subspecies of bellflowers are included in the genus *Campanula* (Campanulaceae). There are both annuals and perennials in this genus. It is the temperate zones of the northern hemisphere and the Mediterranean and Caucasus with the highest diversity. It is generally grown as an ornamental plant (**Tsiftsoglou *et al.* 2022**).

MATERIALS AND METHODS

Gaziantep was the site of the gathering of plant specimens. (Turkey). The aerial parts were dried in a cool, dry place with plenty of shade. Plant samples were dried, then pulverised into powder. Then, after soaking the plant sample at 50 °C for around 6 hours, 30 g of it was extracted with 250 mL of ethanol. The same method was applied for the methanol extract.. In a rotary evaporator, the solvents were evaporated off of the resulting extracts.

Antioxidant tests

The sample of plant material was analysed for its status of antioxidants and oxidants with Rel Assay Diagnostics' TAS and TOS kits. Calibrators for TAS and TOS testing included trolox and hydrogen peroxide. TAS values were expressed as mmol Trolox equiv./L and TOS values as µmol H_2O_2 equiv./L (**Erel, 2004; Erel, 2005**). The OSI was calculated by dividing the TAS by the TOS (**Sevindik, 2019**).

Antimicrobial activity tests

The agar dilution technique was used to evaluate the plant sample for its antimicrobial activity against a panel of reference bacteria and fungal strains. The plant extract was diluted to amounts ranging from 12.5 to 8.0 µg/mL. To dilute, we used distilled water. Gram positives and negatives included *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, and it was cultured in Muller Hinton Broth medium. The fungi were cultivated in RPMI 1640 Broth medium, and the strains utilised were *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, and *Candida glabrata* ATCC 90030. The minimum inhibitory concentrations (MIC) of an extract were calculated. (**Bauer *et al.* 1966; Hindler *et al.* 1992; Matuschek *et al.* 2014**).

Total Phenolic and Flavonoid Tests

The plant extract was dissolved in distilled water to make a 1 mL stock solution, and then 1 mL of Folin-Ciocalteu reagent (1:9, v/v) was added and the mixture was vortexed. The resultant solution was diluted to 0.75 mL with 1% Na_2CO_3 and let to incubation for 2 hours. Then, a reading was taken at 760 nm. Based on the gallic acid standard solution calibration curve (**Yumrutaş *et al.* 2009**), total phenolic content was calculated and reported as mg.GAE g^{-1}.

Aluminum chloride analysis was used to calculate the total flavonoid content of the plant extract. (**Chang *et al.* 2002**). The mixture included 0.1 mL of 10% $Al(NO_3)_3$, 0.1 mL of 1 M NH_4CH_3COO, 4.3 mL of methanol, 0.5 mL of Quercetin, and 0.5 mL of plant extract. It was then incubated for 40 minutes and absorbance was measured at 415 nm. The amount of flavonoids was expressed as mg.QE g^{-1}.

RESULT AND DISCUSSION

Antioxidant activity

The metabolic processes of all living creatures result in the production of reactive oxygen species (ROS), also known as free radicals. (**Baba *et al.* 2020**). At low concentrations, it has no ill effects, but at higher concentrations, it may cause serious damage to cells. The antioxidant defence system helps mitigate these negative consequences. (**Bal *et al.* 2017**). When antioxidant defence mechanisms are overwhelmed, oxidant chemicals become unchecked, leading to oxidative stress. Cancer, Parkinson's, cardiovascular problems, neurological illnesses, depression, and Multiple Sclerosis are only some of the major diseases that have been linked to oxidative stress in humans (**Gürgen *et al.* 2020; Eraslan *et al.* 2021**). Antioxidant supplements are useful in preventing or alleviating illnesses caused by oxidative stress. (**Bal *et al.* 2019**). Plants are significant here because they provide additional antioxidants from a natural source. Antioxidant status of *C. strigosa* was evaluated in this research. In addition, oxidant status were measured to calculate the oxidative stress index. Table 1 displays the findings.

Table 1 Antioxidant and oxidant status of *Campanula strigosa*

	TAS (mmol/L)	TOS (µmol/L)	OSI
Campanula strigosa	4.974±0.259	12.437±0.150	0.251±0.014

Values are presented as mean±S.D

TAS, TOS, and OSI values of *C. strigosa* have not been determined in the literature. It was detected for the first time in our study. Numerous *Campanula* species, each harvested in their own unique way, have been reported to have antioxidant potentials in the scientific literature (Table 2).

Table 2 Antioxidant activity of Some *Campanula* species in world

Species	Method	Extract	Country	References
Campanula alata Desf.	DPPH radical scavenging assay	n-butanol	Algeria	**Touafek *et al.* 2011**
C. alliariifolia Willd.	DPPH radical scavenging assay, Reducing power	Methanol	Turkey	**Dumlu *et al.* 2008**
C. camptoclada Boiss.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. cretica (A.DC.) D.Dietr.	DPPH radical scavenging assay	Hexane, Dichloromethane, Methanol, hydromethanolic	Greece	**Dimitriadis *et al.* 2022**
C. cymbalaria Sm.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. damascena Labill.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. erinus L.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. hierosolymitana Boiss.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. kotschyana A.DC.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. latifolia L.	DPPH radical scavenging assay	Ethanol	Iran	**Moosavi *et al.* 2018**
C. latifolia L. subsp. latifolia (Current name: Campanula latifolia L.)	Phosphomolibdenum reducing, Ferric reducing, DPPH radical scavenging assay	Water, Methanol, Acetonitrilen, Hexane	Turkey	**Korkmaz *et al.* 2020**
C. lyrata Lam.	Metal chelating, DPPH radical scavenging assay, ABTS radical cation	Ethanol	Turkey	**Taskin and Bitis, 2016**
C. lyrata subsp. lyrata (Current name: Campanula lyrata Lam.)	Metal chelating, DPPH radical scavenging assay, ABTS radical cation, β-Carotene / linoleic acid	Water, Methanol	Turkey	**Ayaz, 2021**
C. macrostachya Waldst. & Kit. ex Willd.	DPPH radical scavenging assay, ABTS radical cation, Ferrous ion chelating	Water, Methanol, Ethanol	Turkey	**Sarikurkcu *et al.* 2021**
C. medium L.	DPPH radical scavenging assay	Essantial oil	Saudi Arabia	**Assiri *et al.* 2014**
C. peregrina L.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. phrygia Jaub. & Spach	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. rapunculus L.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. retrorsa Labill.	DPPH radical scavenging assay	Methanol, Water	Lebanon, Palestine	**Jaradat and Abualhasan, 2015; Alhage *et al.* 2018**
C. sidoniensis Boiss. & Blanche	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. stellaris Boiss.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. strigosa Banks & Sol.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. stricta L.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. sulphurea Boiss.	DPPH radical scavenging assay	Methanol	Palestine	**Jaradat and Abualhasan, 2015**
C. takesimana (Current name: Campanula punctata Lam.)	ABTS radical scavenging, Reducing power	n-butanol, ethyl acetat, Ethanol, Chloroform, water	Japan	**Kim *et al.* 2012**

When Table 2 is examined, it is seen that *Campanula* species have antioxidant potentials. In our study, antioxidant potential of *C. strigosa* was determined by using Rel Assay kits. TAS, TOS and OSI values of different plant species have been reported with this method in the literature. In these studies, TAS, TOS and OSI values of *Mentha longifolia* ssp. *longifolia* (TAS: 3.628, TOS: 4.046, OSI: 0.112), *Allium calocephalum* (TAS: 5.853, TOS: 16.288, OSI: 0.278), *Scorzonera papposa* (TAS: 5.314, TOS: 24.199, OSI: 0.456), *Rumex scutatus* (TAS: 8.656, TOS: 4.951, OSI: 0.057), *Glycyrrhiza glabra* (TAS: 8.770, TOS: 14.590, OSI:

0.167), *Galium aparine* (TAS: 5.147, TOS: 18.679, OSI: 0.346), and *Alcea kurdica* (TAS: 3.298, TOS: 8.312, OSI: 0.252) have been reported (**Sevindik *et al.* 2017; Mohammed *et al.* 2019; Mohammed *et al.* 2020b; Korkmaz *et al.* 2021; Mohammed *et al.* 2021b; Mohammed *et al.* 2022; Unal *et al.* 2022**). Compared to these studies, TAS value of *Campanula strigosa* was higher than *M. longifolia* ssp. *longifolia* and *A. kurdica*, and lower than *A. calocephalum, S. papposa, R. scutatus, G. glabra* and *G. aparine*. The TAS value is an indicator of the whole of the antioxidant compounds found in natural products. A high TAS value indicates that the natural product has a high antioxidant potential (**Krupodorova and Sevindik, 2020**). In our study, it is seen that *C. strigosa* has an overall lower TAS value compared to the studies reported in the literature. However, it was determined that *C. strigosa* has antioxidant potential. TOS value is an indicator of all endogenous oxidant compounds produced in natural products (**Krupodorova and Sevindik, 2020**). The TOS value of *C. strigosa* used in our study was determined to be higher than *M. longifolia* ssp. *longifolia*, *R. scutatus* and *A. kurdica*, and lower than *A. calocephalum, S. papposa, G. glabra* and *G. aparine*. In this context, it was observed that the oxidant values of *C. strigosa* were at normal levels. OSI value shows how much oxidant compounds are suppressed by antioxidant compounds (**Krupodorova and Sevindik, 2020**). The OSI value of *C. strigosa* used in our study was determined by *M. longifolia* ssp. *longifolia*, *R. scutatus* and *G. glabra*, and lower than *A. calocephalum, S. papposa, G. aparine* and *A. kurdica*. In this context, it is thought that *C. strigosa* can be used as a natural antioxidant source.

Antimicrobial Activity

The prevalence of microbial illnesses continues to rise. For several reasons, including the development of antibiotic-resistant microbes and the potential negative effects of synthetic medications, scientists are looking to natural items for novel antimicrobial solutions. (**Selamoglu *et al.* 2020; Vaou *et al.* 2022; Sevindik *et al.* 2023**). Plants created secondary metabolites to protect themselves from the wide variety of microbes they came into contact with in the wild **(Mohammed *et al.* 2023**). Because of these characteristics, plants may be used as antibiotics. In this context, ethanol and methanol extracts of *C. strigosa* were used in our study and antimicrobial activity was determined against standard bacterial and fungal strains. The obtained results are shown in Table 3.

Table 3 MIC values of *Campanula strigosa* against Bacterial and Fungal strains

	A	B	C	D	E	F	G	H	J
Ethanol	200	200	200	100	100	200	50	50	50
Methanol	200	400	400	100	100	400	100	100	100

*(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei*
*400, 200, 100 and 50 µg/mL extract concentrations

A study on the antimicrobial activity of *C. strigosa* was found in the literature. Several *Campanula* species have been discovered to possess antimicrobial properties.In these studies, it was reported that volatile oil and aqueous extracts of *Campanula portenschlagiana* were effective against *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Penicillium* sp. and *Rhizopus stolonifer* at different concentrations (**Politeo *et al.* 2013**). *Staphylococcus aureus, Bacillus cereus, Enterococcus faecium, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanii*, and *Candida albicans* were all shown to be inhibited by methanol and water extracts of *Campanula retrorsa* at varying doses, according to another research. (**Alhage *et al.* 2018**). Our research demonstrated that the activity of *C. strigosa* ethanol extracts was often greater than that of metanol extracts. Extract concentrations between 100 and 400 µg/mL were effective against several bacterial strains. The extract's efficacy against fungal strains was determined to be at its peak between 50 and 100 µg/mL. It exhibited the highest activity against fungal strains. *E. coli* and *P. aeruginosa* were the two bacterial species it was most effective against. These findings suggest that *C. strigosa* may have antibacterial properties.

Total phenolic and flavonoid contents

Phenolic and flavonoid chemicals may be found in plants. Their medicinal value is an essential function of these substances. (**Iamkeng *et al.* 2022**). *C. strigosa* was extracted in both ethanol and methanol, and their total phenolic and flavonoid contents were analysed. Table 4 displays the findings collected.

Table 4 Total phenolic and flavonoid contents of *Campanula strigosa*

Plant extracts	TPC (mg/g)	TFC (mg/g)
Etanol	78.70±2.57	109.05±1.53
Metanol	56.28±2.72	108.07±3.48

Ethanol extract of *C. strigosa* employed in our research had a greater total phenolic and flavonoid content. The total phenolic content of *C. lyrata* subsp. *lyrata* methanol extracts has been reported in the literature to be 48.97 mg/g, while the total flavonoid content has been reported to be 67.02 mg/g. (**Ayaz, 2021**). Based on our findings, *C. strigosa* is a superior source of phenolic and flavonoid content. *C. strigosa* has been identified as a potential natural source due to its high phenolic and flavonoid content in this context.

CONCLUSION

The research here looked at the antimicrobial and antioxidant properties of aerial parts of *C. strigosa* plant.. Total phenolic and flavonoid concentrations were also calculated for the plant. Plant extracts were shown to have antioxidant and antimicrobial properties, as hypothesised by the study. The phenolic and flavonoid concentrations found in its structure further suggest it may be a naturally occurring source.

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