Assessment of the antioxidant and antimicrobial activities of *Caralluma deflersiana* growing in the South of Saudi Arabia

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Reactive oxygen species and oxidative stress are related to a large number of human degenerative diseases. Consequently, plants have been investigated across the world to exploit their potential antioxidant and antimicrobial activities. In the current study, *Caralluma deflersiana* is native to Saudi Arabia and was screened to assess its antioxidant and antimicrobial potential activities as well as the phenolic content/phytochemicals. Subsequently, the collected aerial parts were extracted by maceration with different solvents. The antioxidant activity was investigated using the total antioxidant capacity, diphenylpicryl hydrazine (DPPH) radical scavenging assay, ABTS radical scavenging assay, and Ferric reducing antioxidant power assay (FRAP). Regarding the antimicrobial activity, the Minimum Inhibitory Concentration (MIC) assays were used. The total phenolic content of *C. deflersiana* extracts was quantified using standard methods. As a result, the water extract of *C. deflersiana* displayed a strong antioxidant activity in all tested methods compared to other plant extracts. Moreover, it was also noted that water and methanolic extracts exhibited approximately similar bacterial and fungal growth inhibition. Additionally, the water extract of *C. deflersiana* also demonstrated the highest phenol content among other plant extracts, consistent with the higher antioxidant activity found in *C. deflersiana*. In conclusion, Caralluma species could be a promising source of antioxidant and antimicrobial agents.

**Key words:** *Caralluma deflersiana*, antioxidant, antimicrobial.

**INTRODUCTION**

Reactive oxygen species and oxidative stress are related to many human degenerative diseases, including cardiovascular diseases, cancer, inflammation, and diabetes (Waris and Ahsan, 2006, Liguori et al., 2018). The use of synthetic antioxidants compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are restricted due to their toxicity, instability, less efficacy and serious side effects (Sökmen et al., 2004). Infections caused by microorganisms are reported to possess drug resistance for the commonly used antimicrobials (Levy and Marshall, 2004). The improper use of antibiotics against pathogenic bacteria...
has resulted in antimicrobial resistance, which has become a major health issue worldwide (Murti and Radjasa, 2012). Therefore, to alleviate these problems, the search for new antimicrobial and antioxidant natural products continues to draw many researchers’ attention. Medicinal and aromatic plants constitute an alternative and new potential reservoir of new bioactive compounds (Hemaiswarya et al., 2008). Natural products are considered rich sources of phytochemical compounds with remarkable biological activities. Additionally, these phytochemicals are an important source with a variety of structural arrangements and properties (de Fátima et al., 2006; Calixto, 2019).

Saudi Arabia has a diversity of plant species that grow in harsh conditions, which makes it medicinally promising, such as Caralluma species (Osman et al., 2014). Caralluma genus is widely distributed in Saudi Arabia, which belongs to the Asclepiadaceae family, which comprised of 200 genera and 2500 species (Qiu et al., 1997; Sireesha et al., 2018). The Arabic and Indian traditional medicine had widely used Caralluma species for it is the treatment of fever, inflammation, snake and scorpion bites, diabetes, cancer, tuberculosis, skin rashes, scabies (De Leo et al., 2005; Oyama et al., 2007; Abdel-Sattar et al., 2009; Aruna et al., 2009; Touneki et al., 2019). Several reports stated that the Caralluma genus contains anti-inflammatory, antitumor (Zakaria et al., 2001), cytoprotective antiulcer activities (Zakaria et al., 2002). Other important pharmacological activities had been reported such as the antinoiceptive (Abdel-Sattar et al., 2007), the antioxidant, hypolipidemic (Tatiya et al., 2010), and the antidiabetic activities (Dra et al., 2019). Sparingly, pregnane glycosides, saponin, and flavonoids are the major phytochemical constituents isolated from this plant (Bauer et al, 1966). The presence of several important compounds, including pregnane glycosides (Abdel-Sattar et al., 2007), stigmasterol, and other constituents (Bader et al., 2003) in Caralluma species explains the range of biological activities (Dra et al., 2019).

Caralluma deflersiana is one of 14 species distributed in Saudi Arabia (Al-Massarani, 2011; Aati et al., 2019), which has no literature reports concerning the biological activities and phytochemical composition. Thus, the current work was undertaken to investigate the antioxidant and antimicrobial properties of C. deflersiana extract and fractions against a panel of pathogenic microorganisms. The study will be of value in highlighting that Caralluma species in Saudi Arabia are considered as a promising source for several compounds that can be used as antioxidants and antimicrobial agents.

MATERIALS AND METHODS

Plant material

The aerial part (stem) of C. deflersiana was collected from the south of KSA in March 2019. Four samples of the stems (100 g each) were dried to a constant temperature of 60°C for 24 h and filtered using a sterile filter paper. The extract obtained was stored at -20°C until use.

Extraction of C. deflersiana

The four samples of C. deflersiana were extracted by soaking in 500 ml of water, methanol, ethanol, and ethyl acetate with 24 h of agitation. Extracts were filtered with Whatman filter paper No.1, while solvents were evaporated by rotary evaporator at 40°C. Dry matter was collected, and yields were calculated. Dry materials were stored in Eppendorf tubes in no light condition with a temperature of 4°C for further analysis. Dry extracts were dissolved in the same solvents at the time of analysis.

Estimation of total phenol content

The total phenolic content of C. deflersiana was estimated by a modified Folin–Ciocalteu reagent (Al jawfi et al., 2013). Briefly, 1 ml of sample extract (0.1%, v/v), 0.5 ml Folin–Ciocalteu reagent (1:2, v/v), 2 ml of 5% sodium carbonate were mixed and allowed to stand at 30°C for 1 h. Absorbance was measured with a UV-VIS spectrophotometer at a wavelength of 765 nm. The total phenolic content of C. deflersiana extracts was expressed as Gallic acid equivalents per g dry weight (mg GAE/g).

Determination of antioxidant activity

Total antioxidant capacity

Antioxidant capacity of extracts (0.2-1 mg/ml) was estimated utilizing a slightly modified phosphomolybdenum method (Prieto et al., 1999). This method is based on the reduction of Mo (VI) to green Mo (V) in an acidic medium. Concisely, 0.1 ml of each plant extract (100 μg/ml) solution was mixed with 1 ml of reagent (0.6 M H2SO4, 28 mM Na2PO4, and 4 mM ammonium molybdate). The tubes were placed in a water bath at 95°C for 1.5 h after that, the mixture was cooled, and the absorbance was measured at 695 nm in contrast to a blank that contained 1 ml of reagent and an appropriate volume of solvent. Antioxidant capacity was calculated using an α-tocopherol standard curve and expressed as α-tocopherol equivalents (μg/g of extract).

2,2-diphenyl-1-picrylhydrazyl (DPPH)- radical scavenging assay

DPPH scavenging ability was evaluated as follows; a 1 ml of the DPPH, which as previously prepared in methanol (0.135 mM) combined with 1 ml of different concentrations of C. deflersiana extracts and standards (0.2-1 mg/ml). Mixtures were vortexed thoroughly and left in the dark at 28°C for 30 min (Wintola and Afolayan, 2011). Absorbance was then measured at 517 nm. DPPH scavenging ability was calculated by the following equation:

\[
\text{DPPH scavenging activity (%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%
\]

ABTS radical scavenging assay

A stock solution was prepared by mixing 1 ml of 7 mM ABTS solutions and 2.45 mM K2SO4 then left in the dark at 28°C for 12 min. A bluish-green solution was produced. The blend was then
diluted by adding 1 ml of the ABTS solution with methanol until an absorbance of 0.700 ± 0.01 at 734 nm was reached. 1 ml of extracts, standard (BHT, rutin), and blank (methanol) of different concentrations (0.2-1 mg/ml) were allowed to react with 1 ml of diluted ABTS solution. Absorbance was obtained at 734 nm after 7 min (Asghar et al., 2008). Inhibition percentage was then calculated by the following equation:

\[
\text{ABTS radical scavenging activity (\%)} = \left(\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}}\right) \times 100%
\]

**Ferric reducing antioxidant power assay (FRAP)**

A 1 ml of extract was dissolved in water, 2.5 ml of phosphate buffer (0.2 M, pH 6.6), and 2.5 ml of 1% potassium ferricyanide mixed, then it was placed at 50°C for 30 min. 2.5 ml of trichloroacetic acid (10%) was added, and the mixture was centrifuged for 10 min at 3000 rpm. 2.5 ml from the upper part of the supernatant was diluted with 2.5 ml of water and vortexed with 0.5 ml of 0.1% ferric chloride before measuring absorbance at 700 nm (Vijayalakshmi and Ruckmani, 2016). A reference solution was prepared as above but instead of extract, standard (BHT, rutin), and blank (methanol) were used. The absorbance of 0.700 ± 0.01 at 734 nm was reached. A reference solution was prepared as above but instead of extract, standard (BHT, rutin), and blank (methanol) were used. The absorbance of 0.700 ± 0.01 at 734 nm was reached. A reference solution was prepared as above but instead of extract, standard (BHT, rutin), and blank (methanol) were used. The absorbance of 0.700 ± 0.01 at 734 nm was reached.

**Determination of the antimicrobial activity**

**Test microorganisms**

Two gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) and two gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Proteus vulgaris* (ATCC 8427) and one fungal strain *Candida albicans* (ATCC 60193) were used in this investigation.

**Minimum inhibitory concentrations**

The MICs of *C. deflersiana* extracts were assessed using an improved micro-well dilution method (Mann and Markham, 1998; Sulaiman, 2013). To 96- sterile well plates, duplicate two-fold serial dilutions of each sample (100 µl/well) were made in the required broth media containing 5% (v/v) DMSO to achieve (2000 to 31.2 mg/ml) concentrations. The bacterial or fungal suspension (100 µl, 1106 CFU/ml) was then added. After that, the plates were incubated at 37°C for 24 h and 25°C and 72 h for bacterial and fungal strain, respectively. The MIC of *A. Judaica* and *A. Sieberi* methanol extracts were defined as the lowest concentration displaying no detectable bacterial or fungal growth. Gentamicin and nystatin were used as a positive control. For minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) estimation, 5 µl from the wells that exhibited no growth was transferred agar plates and further incubated for 24 or 72 h. MBC and MFC are the lowest concentrations that show evidence of no visible bacterial or fungal growth.

**Statistical analysis**

All the data were obtained from independent tests in triplicate (n = 3) and presented as means ± standard deviations. All data were analyzed with a one-way analysis of variance, followed by Tukey’s test. A p-value of ≤0.05 was considered to be statistically significant. The analyses were performed using SPSS software (V. 21).

**RESULTS**

**Total phenolic content**

The water extract of *C. deflersiana* exhibited the highest total phenolic content 35 mg of GAE/100 g powder weight followed by the methanolic extract with 33.1 mg/g of the total phenolic content of GAE/ g powder weight. On the other hand, the ethyl acetate extract exhibited the highest total phenolic content (27.1 mg/g); results are shown in Table 1.

The total phenolic content of *C. deflersiana* extracts varied by solvent (Table 1). Phenolic content among extracts was mostly significantly different (p ≤ 0.05). The methanol extract had significantly higher phenol content than ethanol and ethyl acetate extracts (p ≤ 0.05). The difference between ethanol and ethyl acetate extracts was not significant.

**Antioxidant activity**

**Total antioxidant capacity**

Total antioxidant capacity again showed the highest activity for the water extract, 1906 α-tocopherol equivalents µg/g dry weight) (p ≤ 0.05). Methanol, ethanol, and ethyl acetate extracts showed a decrease in the activity, 1865, 1497, and 1412 α-tocopherol equivalents µg/g dry weight, respectively (Figure 1). TAC of the methanolic extract was significantly higher than ethanol and ethyl acetate extracts (p ≤ 0.05).
**DPPH scavenging activity**

The DPPH free radical scavenging activity of *C. deflersiana* extracts and BHT showed a dose-dependent manner increment (Figure 2). The water extract showed the highest scavenging activity (91.73%), followed by BHT, methanol, ethanol, and ethyl acetate, with 88.18, 87.33, 75.74, and 75.10%, respectively, at extract concentrations of 1 mg/ml. The scavenging activity of water extract was significantly higher than that of BHT and other extracts (p ≤ 0.05). DPPH radical scavenging activity of BHT and the methanol extract was not significantly different, but they differed significantly from activities of ethanol and ethyl acetate extracts (p ≤ 0.05).

**ABTS radical scavenging activity**

The ABTS scavenging activity of extracts and standards were increased with increasing the concentrations, ranging between 93.8 and 65.79% at the highest concentration (1 mg/ml) (Figure 3). The highest percentage of inhibition was achieved by the water extract, and the lowest was the ethyl acetate extract. Scavenging activity of water and methanol extracts was significantly higher than BHT (p ≤ 0.05), while the BHT showed a significantly higher scavenging activity than ethanol and ethyl acetate extracts.

**Ferric reducing antioxidant power (FRAP) assay**

FRAP activity results showed a reduced power measured at 700 nm, which ranged from 1.38 to 1.94 at the highest concentration (1 mg/ml) (Figure 4). BHT had the highest reducing power, followed by water, methanol, ethanol, and ethyl acetate extracts. No significant differences in reducing power were found among the extracts.

**Antimicrobial activity**

MICs, MBCs, and MFCs of *C. deflersiana* extracts are
Figure 3. ABTS scavenging activity of *C. deflersiana* extracts. Data are means ± standard deviations from triplicate experiments. Different letters indicate significant differences at \( p \leq 0.05 \).

**Figure 4.** FRAP activity results for *C. deflersiana* extracts. Data are means ± standard deviations from triplicate experiments. Ferric reducing antioxidant power. Different letters indicate significant differences at \( p \leq 0.05 \).

displayed in Table 2. The *C. deflersiana* extracts expressed variable degrees of growth inhibition of the bacterial and the fungal strains with MIC-values ranging between (156.25 to 1250). The most active extracts were water and methanol extracts, and the most sensitive strain against *C. deflersiana* extract was the Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* (MIC: 156.25 mg/ml). MFC or MBC values were maintained about twofold higher than MIC’s (Table 2).

**DISCUSSION**

The medicinal and pharmacological actions of medicinal plants are often dependent on the presence of bioactive compounds (the secondary metabolites) (Heinrich et al., 2004; Calixto, 2019). The chemical composition can vary within the same species depending on the geographical location (Jaafari et al., 2007). As reported, many factors such as the climate, the soil, the plant material and the season in which the plants were collected, the method of preservation and extraction, and the genetic factors, could be responsible for the variation of the chemical compositions (Sivropoulou et al., 1997; Bakkali et al., 2008). Phenolic compounds are omnipresent plant metabolites and the largest group of compounds that contribute to the antioxidant properties; they may play a significant role in initiating harmful free radical actions (Wang et al., 2010).

*Caralluma* genus species are known for their abundance of phenolic compounds (Priya et al., 2012; Maheshu et al., 2014a; Devi and Dhamotharan, 2016). Total phenolic content of *C. adscendens* var. *Fimbriata* aerial parts were reported to be 21.0 ± 0.59, 18.8 ± 0.98, 14.9 ± 0.40 and 8.7 ± 0.63 mg GAE/g dry weight in methanol, water, ethyl acetate, and chloroform extracts, respectively (Maheshu et al., 2014a). Another study suggested that strong antioxidant activity of *Caralluma*
arabica correlated with phenolic content in different plant parts (Al-Attabi et al., 2015). Our data demonstrated that C. deflersiana is in agreement with the data mentioned above, it contains polyphenolic compounds that can play a significant role in the initiation of harmful free radical actions (Wang et al., 2010). On the other hand, regarding the antioxidant activity, it was stated that antioxidants had gained more popularity as health boosters in the treatment of many diseases, due to their beneficial effects (Unuofin and Lebelo, 2020). Several antioxidant phytochemicals that occur naturally in plant sources have been identified as free radical scavengers (Bauer et al., 1966). The antioxidant capacities of plant extracts cannot be measured using a single approach because of the dynamic existence of the various phytochemical groups present in plants. The FRAP, ABTS, DPPH, and total antioxidant capacity methods were used in the present work to determine antioxidant activities of C. deflersiana extracts.

The findings of the DPPH and FRAP assays for the four C. deflersiana extracts were demonstrated that water extract had conducted the strongest free radical scavenging behavior, possessing the highest amount of total phenolic moiety. On the other hand, the ethyl acetate extract had exhibited the lowest radical scavenging activity in both methods. Our results were in agreement with the data reported by Karthishwaran et al. (2018) who demonstrated that Caralluma flava extracts exhibited antioxidant activity as measured by DPPH assay. Another study also supported our results confirmed that Caralluma edulis extract showed strong scavenging activity (Ansari et al., 2005). Moreover, Maheshu et al. (2014a) indicated that C. adscendens var. Fimbriata methanol extracts have significantly higher FRAP activity than the other plant extracts. Likewise, the results of the ABTS radical scavenging method, as well as the total antioxidant capacity method, assured the earlier results, which showed that the water extract exhibited the strongest antioxidant activity amongst other plant fractions.

The total antioxidant capacity of all C. deflersiana extracts in the present study was higher than previous reports for C. adscendens var. fimbriata extracts (Maheshu et al., 2014a). Concomitantly, Marwah et al. (2007) reported that TAC in ethanol extracts from C. flava and C. quadrangula were 335 ± 0.5 and 899 ± 29.2 mg GAE/g, respectively, which considered lower than the current findings for C. deflersiana extracts.

Consistent with our results, various concentrations of C. flava extract and gallic acid were observed to scavenge ABTS radicals in a dose-dependent manner. The percentage of inhibition was reported to be 86 and 59% for the extract and gallic acid, respectively, at a concentration of 80 mg/ml (Pisoschi et al., 2016;
Karthishwaran et al., 2018). The ABTS free radical scavenging activity of C. deflersiana extracts was assumed to be higher than that of C. fimbriata crude extract, as stated by Devi and Dhamotharan (2016).

Concerning the antimicrobial activity of biosynthetically generated chemical compounds that could kill or usefully inhibit the metabolism of pathogenic microbes. These are referred to as antibiotics, which have been extensively studied in recent times in various higher plants. Nonetheless, we learn a bit about the antimicrobial activity of the genus Caralluma (Vajha et al., 2010). In the present study, antimicrobial activity was investigated against four bacterial strains (two grams +ve and two grams -ve). Our findings showed differences in C. deflersian antimicrobial activity. Various solvents were documented to be effective in extracting different phytoconstituents depending on their solubility or polarity in the solvent (Altemimi et al., 2017), which explains the dissimilarities in the antimicrobial activity of the extracts using different solvents (Yusha’u et al., 2008; Kawo et al., 2009; Altemimi et al., 2017).

Conclusion

The present study findings showed that stem extracts of C. deflersiana prepared by different solvents displayed various bioactive phytochemical constituents with high antioxidant activity. Antioxidant activity of extracts correlates with their phenolic content. Interestingly, the water extract contained the highest total polyphenol content and showed the highest overall antioxidant capacity. The high phytochemical constituent’s level, antioxidant and antimicrobial activities of C. deflersiana reflects its potential to be helpful in health maintenance and treatment of various diseases. C. deflersiana may possess a significant ability to counter oxidative stress and infections in humans and other animal systems. Thus, expanding its ethnomedical application is highly recommended. Further investigation should be conducted to isolate and identify specific antioxidant and antimicrobial components of C. deflersiana, both qualitatively and quantitatively, and assess the mechanisms of action underlying these activities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


