Acta Botanica Brasilica, 2024, 38: e20230204
doi: https://doi.org/10.1590/1677-941X-ABB-2023-0204

Original Article

Chemical constituents and biological activities of the aerial part of *Stipagrostis ciliata* (Desf.) De Winter, a perennial grass in North Africa

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ABSTRACT

The main objective of this research work was to investigate active constituents and potential biological activities of *Stipagrostis ciliata* aerial parts, which may re-explore this plant as an important medicinal plant rather than being used only forage for animals. The results indicated that the total phenolic contents of the methanol, hexane and acetate leaf extract vary from 34.45 and 263.16 mg gallic acid equivalent (EAG)/g of extract. The highest concentrations of phenolics were found in the methanol extract. The results were suggestive that methanol extract is very rich in antioxidant compounds. The highest antioxidant activity was obtained in methanol extract. *Stipagrostis ciliata* ethyl acetate extract has an IC_{50} from 0.3 µg/mL. The antibacterial activity of *S. ciliata* extracts against six bacterial strains showed varying degrees of inhibition on the tested strains. The methanol extract was found to be the most potent (8 to 20.41 mm) against most tested strains and *Escherichia coli* was the most vulnerable bacteria with a MIC value of 15 μgmL^{-1}. The Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC-ESI-MS/MS) analyses of methanol and ethyl acetate fractions allowed the identification of six phenolic acids and six flavonoids. The present study shows that *S. ciliata* could be regarded as a promising plant source of bioactive phenolic compounds with good antioxidant and antibacterial activities.

Keywords: Antibacterial activity, antioxidant activity, flavonoids phenolic acids

Introduction

Studies that address the bioactivity of plants still make an outstanding contribution to the field of medicine. Several plant species have been investigated for their medicinal properties and especially for their antioxidant features. Natural antioxidants extracted in their crude form involve numerous chemical constituents that are very effective in preventing the destructive processes triggered by oxidative stress (Zengin *et al.* 2011). Recent research studies on important bioactive compounds in various plants and food materials have whetted the interest of scientists. Plants are characterised by their ability to produce very diverse natural substances (Djeridane *et al.* 2006; Abinaya *et al.* 2013), mainly secondary metabolites. Plant antioxidants, which play a crucial role in converting free radicals to less reactive

Received July 24, 2023; Accepted February 2, 2024
Editor-in-Chief: Thaís Elias Almeida; Associate Editor: Bruno Garcia Ferreira

How to cite:
Fakhfakh LM, Dammak DF, Salah HB, Chaieb M. 2024. Chemical constituents and biological activities of the aerial part of *Stipagrostis ciliata* (Desf.) De Winter, a perennial grass in North Africa. Acta Botanica Brasilica 38: e20230204. doi: 10.1590/1677-941X-ABB-2023-0204

species, have health-promoting effects in terms of the prevention of degenerative diseases (Hanafey 2013). Indeed, in addition to the primary metabolites (carbohydrates, proteins and lipids that all living beings can synthesise and which are produced in high amounts by plants), there are also secondary metabolites that are uniquely generated by angiosperms.

Phenolic compounds derived from plant material correspond to one of the most important natural antioxidants that act as reducing agents and activators of antioxidative defence enzyme systems to suppress radical damage in biological systems (Anderson *et al.* 2001; Proestos *et al.* 2006). However, most plants' potential as sources for the production of new medicines remains largely undeciphered given the number of species of seed plants (gymnosperms and angiosperms) in the world, estimated at around 250,000. Indeed, only 6% of them have been tested for their biological activity, and 15% have been evaluated from a phytochemical aspect (Fabricant & Farnsworth 2010). In Africa, we are increasingly faced with the emergence of emerging diseases. Among the most prominent are diseases related to oxidative stress. Oxidative stress is involved in a myriad of diseases as either a triggering factor or an associated complication. Most diseases induced by oxidative stress appear with age because aging reduces antioxidant defences and increases mitochondrial-propagating radicals (Girodon *et al.* 1997). This important factor corresponds to the emergence of multifactorial diseases, such as diabetes, Alzheimer's disease and rheumatism. Given the diversity and severity of diseases induced by oxidative stress, several research teams have oriented their efforts toward the search for new powerful antioxidants that can fight against oxidative stress and its associated diseases. Flavonoids are one of the most outstanding groups of bioactive compounds in plants that exist in free glycine and glycoside forms, displaying a diverse structure and a broad range of biological activities (Plazonic *et al.* 2009). Phenylpropanoids sensu stricto is a derivative of shikimate and a phenolic derivative with a C_6-C_3 fragment in its structure, where C_6 corresponds to an aryl group. Flavonoids and phenolic acids play a significant protective role in carcinogenesis, inflammation, atherosclerosis and thrombosis and exhibit a high antioxidant capacity. Furthermore, flavonoids have been reported as aldose reductase inhibitors blocking the sorbitol pathway, which is linked to many problems associated with diabetes (Tapiero *et al.* 2002; Yonathan *et al.* 2006). Secondary metabolites as phenolic compounds, flavonoids and hydroxamic acids, commonly occur in both wild and cultivated Poaceae.

According to Soreng *et al.* (2015), Poaceae is one of the largest plant families, with around 12,000 species and roughly 800 genera. Some plants of the family Poaceae are used as medication for hypertension, anti-inflammatory, anthelmintic, diuretic and antioxidant effects (Moreira *et al.* 2010; Rathod *et al.* 2011). The main classes of bioactive compounds reported from the Poaceae family are steroids, terpenoids, flavonoids, fatty acids, hydroxycinnamic acids and alkaloids. Few studies showed the importance of the medicinal activities of Poaceae plants, including antibacterial (Singariya *et al.* 2012), antioxidant (Awaad *et al.* 2007), and cytotoxic activities (Hussein *et al.* 2015). Adom and Liu (2002) showed that some grass species exhibit powerful antioxidant properties and are effective in inflammation treatment. *Stipagrostis ciliata* (Desf.) De Winter, is a perennial grass and C_4 plant belonging to the Poaceae family and it invades arid tropical ecosystems (Hosny *et al.* 2009; Mnif *et al.* 2020). It is spread in the Mediterranean basin and the dry tropical African region (Danin & Orshan 1995). This species can tolerate arid bioclimate and wind erosion. Hence, *S. ciliata* is a useful grass species for fixing sand in shifting and semi-fixed sandy lands (Daur 2012). Ecologically, this species grows in sandy soil and can tolerate drought stress. It can be found in countries including Egypt, Zambia, South Africa, Israel, Namibia, Soudan, Libya and Mauritania. *Stipagrostis ciliata* provides excellent forage for animals. However, according to (Roger 2003), a review of the current literature revealed that nothing could be traced regarding phytochemical or biological studies on *S. ciliata*. This encourages us to investigate its active constituents and potential biological activities, which may re-explore this plant as an important medicinal plant rather than being used as a crop and animal food. The basic aim of this study was to analyse the flavonoid and total phenolic contents of the methanol and ethyl acetate extracts of *Stipagrostis ciliata* using high-performance liquid chromatography (HPLC) and LC-ESI-MS/MS techniques and to evaluate its antioxidant and antibacterial activities in an *in vitro* test system.

Material and methods

Plant material and extract preparation

Leaves of *Stipagrostsis ciliata* (Desf.) De Winter were collected at the flowering stage from their natural habitats in Bir Ali Ben Khélifa (34° 45'19 N, 10° 13' 18 E), west centre of Tunisia. The site is located 50 km away from Sfax city. The climate of this area is arid to Saharan, with a typical Mediterranean arid climate characterised by lower and irregular rainfall events and a dry summer period. The plant was identified by Prof. Mohamed Chaieb, a botanist at the Sciences Faculty of Sfax-Tunisia. A voucher specimen of *Stipagrostis ciliata* was (P-01-2014). Aerial parts of the plant were air-dried in the shade at room temperature over two weeks before extraction and deep analysis. The air-dried and powdered aerial part of *S. ciliata* was extracted by maceration with 80% aqueous ethanol for 24 hours three times at room temperature with regular stirring. The extracts were collected, filtered and concentrated. The dried crude extract was solubilised with distilled water for fractionation. The aqueous solution was partitioned with hexane, methanol and ethyl acetate.

Determination of Total Phenolic Contents (TPC) of *Stipagrostis ciliata*

The total phenolic content in different extracts was determined with the Folin–Ciocalteau reagent following Chen *et al.* (2007). A standard curve was plotted using gallic acid as the standard. Different concentrations of gallic acid were prepared in methanol, and their absorbances were recorded at 750 nm. The results were presented in mg gallic acid equivalents per gram of extract (mg GAE/g extract).

Determination of Total Flavonoid Contents (TFC)

The TFC in the extracts was specified using a method based on the formation of complex flavonoid aluminium, having the maximum absorbance at 430 nm[2]. Quercetin was used to generate the calibration curve. About 1 mL of diluted sample was mixed with 1 mL of 2% aluminium trichloride ($AlCl_3$) methanol solution. After 15 min of incubation, the absorbance of the reaction mixture was measured at 430 nm, and the TFC was presented in mg quercetin equivalents per gram of extract (mg QE/g extract).

Antioxidant activities

Ferric-reducing antioxidant power assay

The method of Yildirim *et al.* (2001) was used to assess the reducing power of 1 ml of different concentrations of each *Stipagrostis ciliata* sample (5, 10, 25, 50, 100 µg/mL). These concentrations were mixed with 2.5 ml of 1% potassium ferricyanide and 0.2 M sodium phosphate buffer and incubated in a water bath at 50 °C. Subsequently, 2.5 mL of trichloroacetic acid (10%) was added to the mixture and centrifuged at 650 × g. The absorbance of the mixture was measured at 700 nm. A standard curve was plotted using various concentrations of Ascorbic acid.

The 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging activity of different fractions of *Stipagrostis ciliata* was determined following the procedure described by Les *et al.* (2015). 1.5 mL of DPPH solution (4–10 M, in 95% ethanol) was added to 1.5 mL of each *S. ciliata* fraction at various concentrations (0.01–1 mg/mL). The final concentrations in the reaction mixture were 0.005–0.5 mg/mL. Each mixture was shaken and left in the dark for 30 min at room temperature. The percentage of inhibition (PI %) was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank. PI % was calculated using the following formula:

$$V(PI\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the blank, and A_{sample} is the absorbance of the test sample.

The calibration curve for the scavenging percentage against the extract concentration was plotted, and the IC_{50} value of each sample was calculated.

Determination of Total Antioxidant Capacity (TAC) assay

The total antioxidant capacity (TAC) of all *S. ciliata* fractions was spectrophotometrically assessed following Prieto *et al.* (1999). A volume of 0.1 mL of each *S. ciliata* fraction (1 mg/mL) was mixed with 1 mL of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate). The mixtures were incubated in a boiling water bath at 95 °C, for 90 min. After cooling the samples, absorbance was determined at 695 nm. Antioxidant capacity was expressed as the number of gram equivalents of α-tocophenol.

Chromatographic conditions and apparatus

HPLC procedure chromatographic separation was performed on an Aquasil C18 column (150 mm × 3 mm, 3 µm particle size). The solvents used in the chromatographic separation were (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol. The elution gradient established was 10–100% B, 0–45 min; 100% B, 45–55 min. The equilibration duration was 5 min between individual runs. The flow rate of the mobile phase was 0.4 mL/min, the injection volume was 5 µL, and the column temperature was guarded at 40 °C. Phenolics were characterised according to their retention times and UV and mass spectra compared with commercial standards when available. The quantification of phenolics was determined based on the DAD results using 280 nm.

The LC-ESI-MS/MS analysis was carried out using an LCMS-8030 triple quadrupole mass spectrometer equipped with electrospray ionisation. According to Ben Salah *et al.* (2019), the mass spectrometer was operated in negative ion mode with a nebulising gas flow of 1.5 L/min, a block source temperature of 400 °C, a dry gas flow rate of 12 L/min, a DL (dissolving line) temperature of 250 C, the negative ionisation mode source voltage of –4500 V, and a full scan spectrum from 50 to 2000 Da.

Antibacterial activity

Microbial Strains

The crude extract of *S. ciliata* was evaluated against a panel of microorganisms, namely six bacterial strains: Gram-negative: *Salmonella enterica* (CIP 8039), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC9027); Gram-positive: *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (LB 14110) and *Bacillus amyloliquefaciens* (FZB 425). All tested strains were obtained from the Microbiology Department, Faculty of Sciences (Sfax, Tunisia). Bacterial strains were cultivated in Mueller–Hinton agar (MHA) for 24 h at 37 °C.

Disk diffusion method

To assess antimicrobial activity, the disk diffusion method was used, following Nilsson (1978). Each extract was dissolved at 50 mg/ml in 100% dimethyl sulfoxide (DMSO) and sterilised by filtration through a 0.22-mm Nylon membrane filter. Culture suspensions (150 µL) of the investigated bacterial strains (10^6 colony-forming units (CFU mL^{-1}) were spread on the surface of MHA solid media plates. Then, wells of 6 mm in diameter were punched and loaded with 60 ml of each sample extract at 50 mg/ml. The same volume (60 ml) of DMSO (without extract) was used as a negative control. After being stored at 4 °C for 3 h, they were incubated at 37 °C for 24 h. Antibacterial activity was estimated by measuring the diameters of the inhibition zones against the test bacteria and comparing them to penicillin (10 µg per disk), which was considered the positive control. The accurate inhibition zone of any dimension surrounding the paper disk was measured using a sliding calliper. This test was conducted in triplicate. The zone of inhibition indicates the degree of sensitivity of bacteria to an extract, according to the following criteria (Barros *et al.* 2007).

Microdilution method

To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), we used the method of microdilution in broth described by Bassole *et al*. (2003). After the strains were cultured, the inocula were suspended in MHB to provide a final density of 10^6 CFU mL^{-1}. The different *S. ciliata* extracts were dissolved in 100% DMSO for a concentration of 100 mg/ml, and then a two-fold dilution series of these extracts was prepared in a 96-well microtiter plate. The DMSO solution was used as a blank control. Each well of the microplates included 40 ml of the growth medium, 10 ml of overnight bacterial culture at a density of 106 CFU/ml and 50 ml of the diluted sample extracts. The microplates were then incubated overnight at 37 °C. Bacterial activity in the test wells was detected by adding 40 ml of p-iodonitrotetrazolium violet (INT) aqueous solution as an indicator of microorganism growth. Penicillin was used as a standard reference against bacteria. In proportion to the ratios, the effect was bactericidal or bacteriostatic:

1 < MBC/MIC < 4: bactericidal action;
4 < MBC/MIC < 16: bacteriostatic action;

Statistical analysis

Each computation was carried out using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). The results were expressed as the means ± standard deviations of three replicates. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was performed to determine the differences between the means of various parameters.

Results and Discussion

Total flavonoid and phenolic contents

The total flavonoid compound (TFC) and total phenolic compound TPC of different leaf extracts from *S. ciliata* were specified. The percentage yields of *S. ciliata* extracts, as well as TPC and TFC, are depicted in Table 1. The extraction yield obtained with methanol (3.65%) was higher than that obtained with hexane (0.66%) and ethyl acetate (0.42%). In this context, Bouhadjera *et al.* (2005) showed that the yields of methanol extracts were more important than those of ethyl acetate for *Aristida pungens* L. The results revealed that there were significant differences ($P<0.05$) among yields of crude extracts. This is basically due to the affinity between the polarity of solvents and extracted compounds (Dash *et al.* 2005). Phenolic compounds (phenolic acids and flavonoids) have drawn considerable attention due to their potential antioxidant activity (Tungmunnithum *et al.* 2018). These compounds are known for their ability to scavenge free radicals and active oxygen species, such as singlet oxygen (Karker *et al.* 2016). The quantification of the TPC was carried out using a linear calibration curve produced by a calibrating solution (gallic acid) at various concentrations. Table 1 shows that the values of phenolic compounds ranged from 34.45±0.68 to 263.16±5.26 mg EAG/g of extract. The levels of phenolic compounds varied significantly ($P<0.05$) depending on the influence of solvent polarity (Karker *et al.* 2016). They were rich in all fractions, except hexane. The highest value of TPC was recorded in the methanol extract (263.16±5.26 mg EAG/g of extract), followed by ethyl acetate (70.93±1.41 mg EAG/g of extract) and hexane (34.45±0.68 mg EAG/g of extract) fractions. Within this framework, Hanafey (2013) highlighted that the extract of the aerial part of *Stipagrostis lannata* was not rich in TPC (84.21 mg EAG/g of extract). Iram *et al.* (2019) demonstrated that *Stipagrostis plumosa* hexane extract (29.5±1.3 and 29.2±3.9 mg QE/g) exhibited the highest flavonoid content. The TFC of each extract was then calculated from the calibration curve and expressed in equivalent milligrams of quercetin per g of extract. The density measurement was carried out at the 415 nm wavelength referring to Table 1. The values of TFC ranged from 7.56±0.03 to 107.21±0.53 mg QE/g of extract. However, the order of flavonoid content in the extracts was: Ethyl acetate > Methanol > Hexane. These results revealed that *S. ciliata* extracts may stand as a good source of natural phenolic compounds including flavonoids with relatively low polarity (e.g. methoxylated derivatives). In this context, Kalpna *et al.* (2011) asserted that phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods and beverages. Iram *et al.* (2019) clarified that the TPC of *Stipagrostis plumosa* ranged from 10.44±1.5 to 42.33±6.33 mg/g gallic acid equivalents of the dry fraction weight.

Table 1. Extraction yields (%), total phenolic contents (TPC) and total flavonoid contents (TFC) of aerial parts of *S. ciliata*.

Extract	Extraction yields (%)	TPC (mg GAE / g extract)	TFC (mg QE / g extract)	TFC/TPC ratio
Hexane	0.663[a]	34.45±0.68[a]	7.56±0.03[a]	0.21
Ethyl Acetate	0.422[b]	70.93±1.41[b]	107.21±0.53[b]	1.51
Methanol	3.655[c]	263.16±5.26[c]	14.7±0.07[c]	0.05

Values are means of 3 replicates ±SD. Within the same column, means followed by different letters are significantly different at $P < 0.05$. (GAE): Gallic Acid Equivalent; (QE): Quercetin Equivalent

DPPH free radical scavenging activities (%)

The DPPH scavenging method has been used to assess the antioxidant activity of compounds owing to simple rapid and sensitive procedures (Gonçalves *et al.* 2005). The DPPH assay has been extensively used to determine the antioxidant properties of multiple plant extracts (Baccouch *et al.* 2018). The DPPH radical scavenging activity and the extract concentration required to inhibit 50% of the initial DPPH free radicals (IC_{50}; µg/mL) are illustrated in Fig. 1. This activity increased by increasing the concentration of the sample extract. The DPPH assay rested on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolourised in the presence of antioxidants. The stable free radical DPPH has been widely used to test the free radical scavenging capacity of various antioxidants (Pavithra & Vadivukkarasi 2015). The DPPH scavenging activities of the *S. ciliata* extracts and standard antioxidants are displayed in Fig. 1. The highest antioxidant activity (IC_{50}=0.18 µg/mL) was mainly related to the methanol extract (Table 2). The order of DPPH scavenging activity of the extracts was: Methanol > Ethyl acetate > Hexane. The high scavenging activity of the DPPH radical with the lowest IC_{50} values of *S. ciliata* methanol extract was related to its high phenolic compound content. Sacan and Yanardag (2010) corroborated that this radical scavenging activity of extracts could be related to their phenolic compounds, thus contributing to their electron transfer/hydrogen donating ability. In the same context, Salaheldin *et al.* (2016) confirmed that ethyl acetate and *n*-butanol fractions of *Stipagrostis plumosa* display maximum antioxidant activity with IC_{50}=0.136±0.005 and 0.551±0.012 µg/mL, respectively. Accordingly, methanol was a suitable solvent for the extraction of polyphenolic compounds, such as flavonoids, from plant tissue. This refers to its ability to inhibit the action of polyphenol oxidase, triggering the oxidation of polyphenols and its ease of evaporation compared to water (Yao *et al.* 2004).

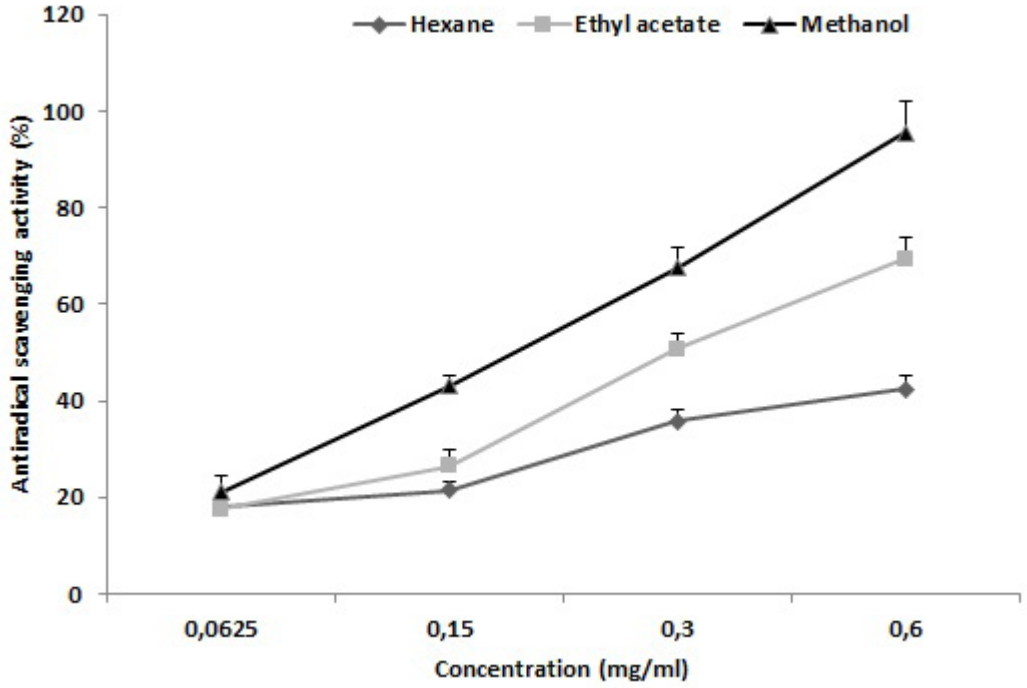

Figure 1. DPPH Radical Scavenging Assay of *Stipagrostis ciliata* extracts (Hexane, Ethyl Acetate and Methanol)

Table 2. Antioxidant activity of *S. ciliata* extracts.

Extracts	IC_{50} (µg/mL)
Hexane Extract	Not determined
Ethyl Acetate Extract	0.3 ±0.015[a]
Methanol Extract	0.18 ±0.009[b]

Values are means of 3 replicates ±SD.
a-b: averages with different letters in the same column are different (P <0.05)

Reducing power assay

Reducing power activity is often used to assess the ability of natural antioxidants to provide electrons (Dorman *et al.* 2003). The power of an extract is mainly related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow colour of the test solution changed to green and blue depending on the reducing power activity of the test specimen. As far as this work is concerned, reducing activity was determined based on the ability of extracts to reduce a Fe^{3+} ferricyanide complex to form a Fe^{2+} ferrous complex. The amount of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm (Ebrahimzadeh *et al.* 2010). The reducing power of various extracts of *S. ciliata* is shown in Fig. 2. Therefore, the reducing ability of all extracts increased when the concentration of the extracts increased. Among the reducing power tests, the methanol extract of *S. ciliata* displayed the highest reducing ability (P<0.05). This result indicates that methanol extract has a good ability to provide electrons with reactive free radicals, converting them into more stable products. This result can be related to the phenolic compounds that were quantified in the methanol extract. These compounds have a good ability to private electrons into reactive free radicals, converting them into more stable products and achieving a free radical chain reaction. These reducing properties are often attributed to the presence of reductones capable of exerting an antioxidant effect by breaking the three radicals' chains and generating a hydrogen atom. It has been reported that reductones respond to various precursors of peroxides and therefore prevent their generation (Kolsi *et al.* 2017).

Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity (TAC) of the *S. ciliata* extracts, expressed as the number of gram equivalents of α-tocophenol, is presented in Fig. 3. These results yielded that the ethyl acetate fraction had a higher (P<0.05) antioxidant capacity (726.83 mg of α-tocophenol /g of extract) than that of methanol (498.58 mg of α-tocophenol /g of extract) and hexane (397.76 mg of α-tocophenol/g of extract) extracts. The highest antioxidant capacities of methanol and ethyl acetate can be related to their high levels of TPC and TFC. This result is in good accordance with the phenolic content variation in all fractions. The high phenolic content of the methanol extract is suggestive of high antioxidant potentials since the phenolic constituents can react with active oxygen radicals, such as the hydroxyl radical (Hussain *et al.* 1987). State-of-the-art works have reported that there is a high correlation between antioxidant activity and phenolic content. Our results indicate that there is a high correlation between the total phenolic content and antioxidant activity observed with the methanol extract rather than with any other extract. Javanmardi *et al.* (2003) mentioned that the antioxidant activity of plant extracts is not confined to phenolic amounts. Activity may also result from the presence of such antioxidant compounds as carotenoid vitamins and others.

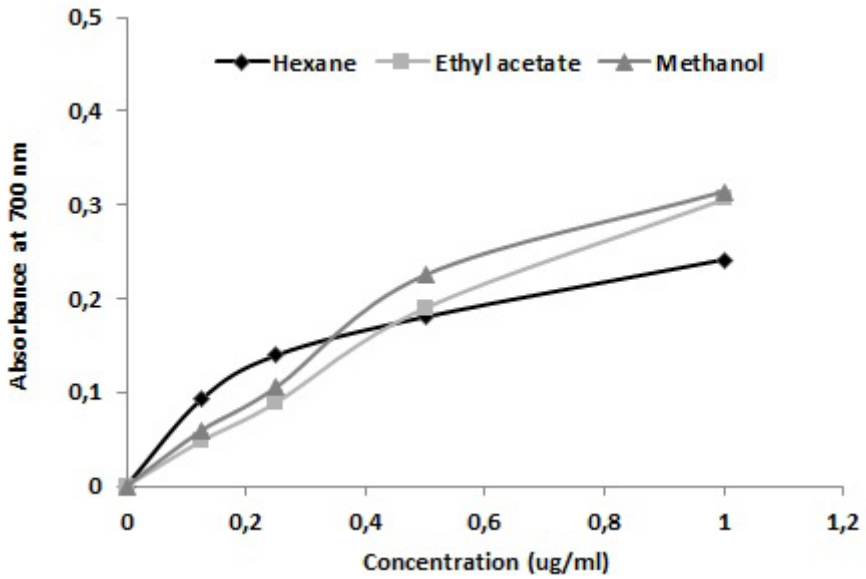

Figure 2. Reducing power of *Stipagrostis ciliata* extracts (Hexane, Ethyl Acetate and Methanol).

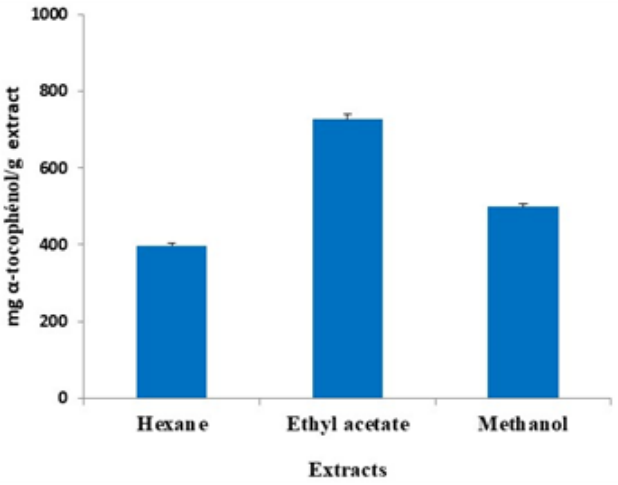

Figure 3. Total antioxidant capacity (TAC) of *Stipagrostis ciliata* extracts. Data are presented as the mean value ± (SD) (n = 3). Different letters are significantly different at P <0.01.

Antibacterial activity

The antibacterial activities of the extracts of *S. ciliata* were investigated against six pathogenic bacteria, three Gram-negative [(*Escherichia coli ATCC 8739, Salmonella enterica (CIP 8039*) and *Pseudomonas aeruginosa* (ATCC 9027)) and three Gram-positive bacteria [(*Staphylococcus aureus* (ATCC 6538), *Bacillus amyloliquefaciens* (FZB 425) and *Micrococcus luteus* (LB 14110)]. The antibacterial potential of the different extracts was assessed in terms of the inhibition zone of bacterial growth. The results of the antibacterial activities are outlined in Table 3. The standard antibiotic penicillin presented high antibacterial inhibition against all bacteria, with a diameter ranging from 8.44±0.21 to 15.20±0.10 mm. The growth inhibition zone measured for all extracts ranged from 6.23±0.20 to 20.41±0.32 mm for all sensitive bacteria. The highest activity (with an inhibition zone diameter of 20.41±0.32mm) was recorded for the methanol extract of *S. ciliata* against *Pseudomonas aeruginosa* (ATCC 9027), while the lowest activity (with an inhibition zone diameter of 6.23 mm) was recorded for the ethyl acetate extract against *Escherichia coli* (ATCC 8739). The methanol extract had the strongest antibacterial activity against *Pseudomonas aeruginosa* (20.41±0.32) and *Salmonella enterica* (18.29±0.4 mm) and was about three times higher than the positive control. However, the hexane extract displayed low antibacterial activity against all Gram-positive and Gram-negative bacteria, with diameters ranging from 6.23±0.2 to 10.23±0.4 mm.

The antibacterial activity of the methanol extract was assessed quantitatively using its MIC and MBC. The results of the MIC and MBC (Table 4) values varied depending on the bacterial species and were generally in accordance with those recorded for the inhibition zones (Table 3). The MBC values ranged between 150 and 1500 μgmL^{-1}, whereas the MIC values varied from 15 to 1500 $\mu g{\cdot}mL^{-1}$.The most vulnerable bacteria for the methanol extract were *Escherichia coli*, with an MIC value of 15 μgmL^{-1} (Table 4). According to the literature, an extract is bactericidal (B) when the ratio of MBC/MIC is lower than 4 and bacteriostatic (b) if the MBC/MIC is higher than 4 (Mhalla *et al.* 2017). The methanol extract had a bacteriostatic effect with an MBC/MIC ratio higher than 4 against all tested Gram-positive and Gram-negative bacteria. The biological activity of the methanol extract was linked to its chemical composition and the functional groups of the main compounds, such as flavonoids, in this work (quinic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, trans ferulic acid, rutin, quercetin (quercetin-3-O-rhamnoside), apigenin-7-O-glucoside, salviolinic acid, kampherol, apigenin, cirsilineol). Our results demonstrated that antibacterial activity depends on the content of phenolic components in the plant extracts. High amounts of phenolic groups in the aerial parts of *S. ciliata* imply that these components may be active compounds, which may be responsible for antibacterial activity. In this context, Shan *et al.* (2007) emphasised that polyphenolics, such as tannins and flavonoids, have high antibacterial activity.

Table 3. Antibacterial activity of *Stipagrostis ciliata* extracts using agar disk diffusion method.

Bacterial strains	Penicillin	Hexane	Ethyl Acetate	Methanol
	Gram -			
Escherichia coli (ATCC 8739)	14.33±0.20^{a}	6.23±0.20^{a}	10.5±0.30^{a}	12.35±0.40^{a}
Salmonella enterica (CIP 8039)	R	8±0.15^{a}	17.13±0.23^{b}	18.29±0.40^{b}
Pseudomonas aeruginosa (ATCC9027)	8.44±0.21^{b}	10.23±0.40^{b}	14.54±0.50^{c}	20.41±0.32^{c}
	Gram+			
Bacillus amyloliquefaciens (FZB)	12±0.15^{a}	9.45±0.20 ^{b}	10.23±0.14^{a}	12.65±0.30 ^{b}
Staphylococcus aureus (ATCC 6538)	15.2±0.10^{b}	6.25±0.23^{a}	14.25±0.15^{b}	8.35±0.30^{a}
Micrococcusluteus (LB 14110)	14.2±0.10^{b}	8.70±0.21 ^{c}	18.74±0.30^{c}	14.65±0.40^{c}

R: resistant. Inhibition zone in diameter (mm± SD) around the discs impregned with 100 µg per disc of each extract. ±: standard deviation of three replicates. $^{a-c}$: averages with different letters in the same column are different (P <0.05).

Phytochemical analyses of ethyl acetate and methanol fractions

The quantification analysis data of the identification constituents were performed using LC-ESI-MS/MS. The amounts of compounds detected in the samples are reported in Table 5. Our results revealed that quinic acid was the dominant component in methanol and ethyl acetate, with contents amounting to 1034.66 and 289.006 µg/g of dried material, respectively. However, the biological activities of the *S. ciliata* fractions could be related to high amounts of quinic acid. For both fractions, phenolic acids and flavonoids had lower content levels, which were less than 300 µg/g of dried material. p-Coumaric acid was the dominant compound in the methanol and ethyl acetate fractions, with 222.72 and 261.86 µg/g of dried material, respectively. Several works have confirmed the high phenolic compound content of *Stipagrostis* extract. However, Hussein *et al.* (2018) isolated ten compounds, including one new flavone glycoside, tricin 7-*O*-galactoside, three known flavones, three *C*-glycosyl flavones and three phenolic acids, from *S. plumosa* for the first time, although tricin was not found. The HPLC chromatograms (280 nm) of methanol and ethyl acetate fractions are portrayed in Fig. 4. Eighteen constituents, numbered 1–12, were detected and attributed to both phenolic acid and flavonoid groups. Fig. 4 and Table 5 summarise the identified peaks with retention time (T_R), Λ_{max} (nm), molecular formula, main fragments and pseudo-molecular ions.

Quinic Acid

Quinic acid exhibited characteristic UV spectra with an absorption maximum at Λ_{max} around 320–325 nm (Kumar 2017). Peak 1 was identified as quinic acid (T_R 6.2 min) compared to a standard, as well as its pseudomolecular ion $[M-H]^-$ at m/z 191 and its fragmentation pattern at m/z 173 (Gouveia & Castilho 2013).

Phenolic acid (salviolinic acid)

The MS spectra of compounds 2, 3, 4, 5 and 9 showed specific fragments corroborating the presence of phenolic acid in both extracts (Table 5). *Trans*-ferulic acid (Peak 5, T_R 42.2 min) was easily identified through its MS ions at 193$[M-H]^-$ m/z. Studying the ferulic acid fragmentation allowed us to identify that protonated ferulic acid produces three ionic fragments (m/z 178, 149 and 134) (Nayane Sinosakia *et al.* 2020). However, Peaks 2 (T_R 11.2 min), 3 (T_R 23.7 min) and 4 (T_R 33.3 min) were assigned to protocatechuic acid, syringic acid and *p*-coumaric acid, respectively, resting upon the UV spectra MS^2 fragmentations. The first fragments observed in p-coumaric were as follows: m/z 119, 125 and 135. Peak 9 was identified as salviolinic acid with MS ions at m/z 717 $[M-H]^-$.

Table 4. Determination of the minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of *Stipagrostis ciliata* leaf methanol extract by the microdilution method.

	Penicillin	Methanol extract			
Bacterial strains	MIC (μgmL^{-1})	MIC (μgmL^{-1})	MBC (μgmL^{-1})	R [a]	Inter. [b]
Escherichia coli (ATCC 8739)	10	15	150	10	b
Salmonella enterica (CIP 8039)	NA	150	1500	10	b
Pseudomonas aeruginosa (ATCC9027)	10	150	1500	10	b
Bacillus amyloliquefaciens (FZB)	10	1500	15000	10	b
Staphylococcus aureus (ATCC 6538)	10	150	1500	10	b
Micrococcus luteus (LB 14110)	10	1500	15000	10	b

[a] Ratios MBC/MIC for bacteria. [b] Interpretation: B: bactericidal, b: bacteriostatic, NA: not active

Table 5. Qualitative and quantitative phytochemical analyses of ethyl acetate and methanol extracts of *Stipagrostis ciliata*.

Peak	TR (min)	Λ_{max} (nm)	$[M-H]^-$ (m/z)	Main fragment ions MS^2 (m/z)	Molecular formula	Tentative identification	Content µg/g of dried material	
							Methanol extract	Ethyl acetate extract
1	6.2	321	191.0	173, 135	$C_7H_{12}O_6$	quinic acid	1304.660	289.006
2	11.2	294	153.0	109	$C_7H_6O_4$	Protocatechuic acid	43.117	42.684
3	23.7	280, 210	197.0	167, 153, 138	$C_7H_{12}O_6$	syringic acid	132.295	72.532
4	33.3	280, 334	163.0	119, 125, 135	$C_7H_{12}O_6$	*P*-coumaric acid	222.720	261.860
5	42.2	332, 234	193.0	177, 149, 163	$C_{10}H_9O_4$	*Trans* ferulic acid	141.864	137.746
6	59.0	355, 254	609.0	465, 303	$C_{27}H_{30}O_{16}$	Rutin	64.192	47.506
7	67.2	353	447.0	301	$C_{21}H_{20}O_{11}$	Quercetin-3-O-rhamnoside	45.867	46.302
8	68.7	334, 217	431.0	269	$C_{21}H_{20}O_{10}$	Apigenin-7-O-glucoside	1.128	0.142
9	73.6	280	717.0	339, 313, 179	$C_7H_{12}O_6$	Salviolinic acid	130.820	176.108
10	80.9	365, 264	285.0	269, 179	$C_{15}H_{10}O_6$	kaempherol	36.001	51.820
11	89.2	334, 268	269.0	179, 151, 119	$C_{15}H_{10}O_6$	Apigenin	0.765	6.746
12	99.7	340, 270	344.0	297, 255	$C_{18}H_{16}O_7$	Cirsilineol	2.549	16.725

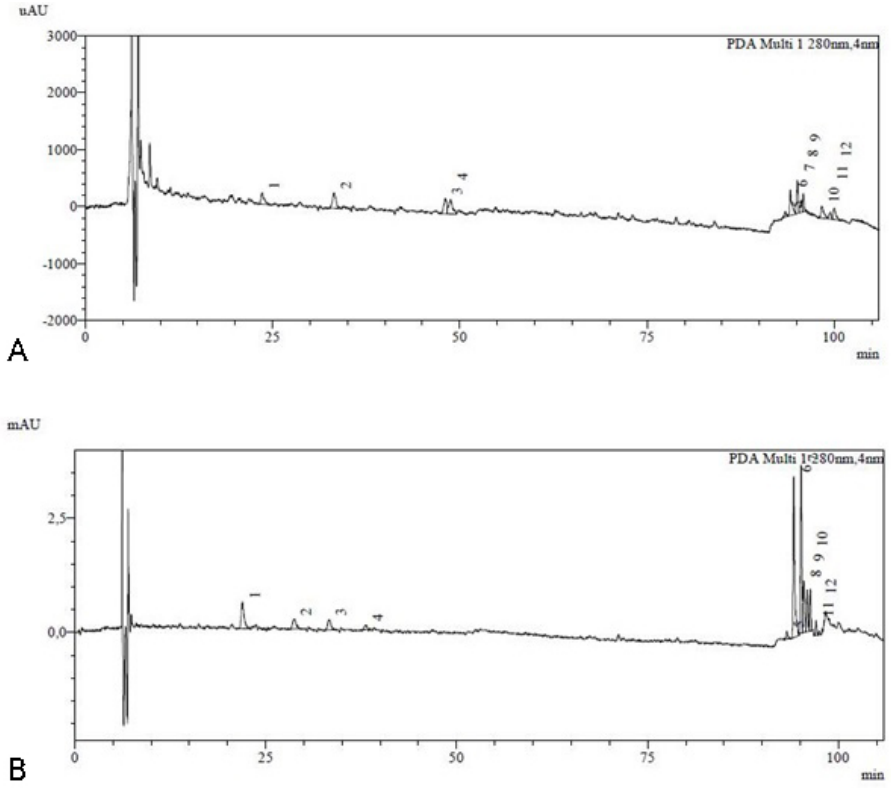

Figure 4. HPLC-based peak chromatogram at (280 nm) of components from methanol **(A)** and ethyl acetate **(B)** extracts of *Stipagrostis ciliata*

Flavonoids

In the methanol and ethyl acetate fractions, flavonoids were present with quercetin derivatives and kaempferol. Peak 7, having MS ions at m/z 447 $[M-H]^-$ and 301 [M-H rhamnosyl], was associated with quercetin (quercetin-3-O-rhamnoside) (Yao *et al.* 2013). Peak 6 was identified as a rutin. Indeed, it was revealed that a parent ion $[M-H]^-$ at m/z 609 released the MS^2 fragments at m/z 465. Peak 10 (T_R 80.9 min) was identified as kaempferol with pseudomolecular ions $[M-H]^-$ at m/z 285 and MS^2 ions at m/z 269 and 179 (Miceli *et al.* 2017). Three flavanones possessing similar UV spectra were identified in the methanol and ethyl acetate fractions. Compound 11 (T_R 89.2 min) was identified as an apigenin. Its MS ions occurred at m/z 269 $[M-H]^-$ and at 179, 151 and 119 (Gouveia & Castilho 2013). Compound 12 (T_R 99.7 min) was identified as cirsilineol. The literature data (Alexandru *et al.* 2013) proved that peak 8 (T_R 68.7 min) was identified as apigenin -7- O glucoside.

Conclusions

Based on these results, it is inferred that the *S. ciliata* methanol extract contains more antioxidant agents than the hexane and ethyl acetate extracts. The antioxidant capacity and antiradical activity of *S. ciliata* extracts against DPPH were correlated with the contents of total phenolics and flavonoids. Methanol and ethyl acetate extracts can be good candidates against infections triggered by Gram-negative bacteria [*Salmonella enterica* (CIP 8039), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027)]. However, antibacterial activity depends largely on the content of phenolic components in *S. ciliata* extracts. The quantitative analyses of methanol and ethyl acetate fractions yielded the identification of 12 compounds belonging to phenolic acids and flavonoids in which quinic acid was present in higher amounts. At this stage of analysis, *S. ciliata* can be regarded for the first time as a valuable and worthwhile extract that deserves further and deeper investigation as an effective source of natural antioxidants serving a wide variety of applications, notably in pharmaceutical manufacturing. Hence, the findings derived from the present study importantly highlighted the significance of this perennial grass as a reservoir of bioactive metabolites. Nevertheless, while this study preliminarily accumulated some important scientific data, further in vivo and clinical investigations could help to better understand the toxicity and safety profiles of this species before its applications in mainstream medicine. Indeed, this work could be promising in terms of paving the way for future research to explore and identify its antioxidant compounds and assess their antioxidant potential in an in vivo system.

Acknowledgment

Our deep and sincere gratitude goes first and foremost to the Arid Regions Institute of Medenine. We are deeply indebted to Mr Houcine Khattelli General Director of this institute, may his soul rest in peace, for his help. We also extend our honorable thanks to Mr Thalel Bouhamda, technician of the central laboratory for his implementation of HPLC and LC-ESI-MS/MS techniques.

Authors' Contributions

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication.

Lobna Mnif Fakhfakh and Doniez Frikha Dammak: concept and design and writing. Hichem Ben Salah: analysis. Mohamed Chaieb: revision of the manuscript.

Conflict of Interest

No potential conflict of interest was reported by the authors.

References

Abinaya G, Paulsmay S, Saradha M. 2013. Antioxidant potential of leaf, stem and root extracts of *Thalictrum javanicum blume*. International Journal of Biological & Pharmaceutical Research 4: 1217-1221.

Adom KK, Liu RH. 2002. Antioxidant activity of grains. Journal of Agricultural and Food Chemistry 450: 6182-6187.

Alexandru L, Pizzale L, Conte L, Barge A, Cravotto G. 2013. Microwave-assisted extraction of edible *Cicerbita alpina* shoots and its LC-MS phenolic profile. Journal of the Science of Food and Agriculture 93: 2676-2682.

Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. 2001. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Journal of Nutrition 131: 2837-2842.

Awaad AS, Mohamed NH, Maitland DJ, Soliman GA. 2007. Anti-ulcerogenic activity of extract and some isolated flavonoids from *Desmostachya bipinnata* (L.) Stapf. Records of Natural Products 2: 76-82.

Baccouch N, Ben Salah H, Belhaj S *et al*. 2018. Chemical characterization and biological activities of *Simmondsia chinensis* (Link) CK Schneid seeds oil. Molecular and Cellular Biology 64: 11.

Barros L, Calhelha RC, Vaz J, Ferreira A, Baptista IC, Estevinho P. 2007. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. European Food Research and Technology 225: 151-156.

Bassole IHN, Quattara AS, Nebie R, Quattara CAT, Kabore ZI, Traore SA. 2003. Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia mutiflora* from Burkina Faso. Phytochemistry 62: 209-212.

Ben Salah H, Smaoui S, Abdennabi R, Allouch N. 2019. LC-ESI-MS/MS Phenolic Profile of *Volutaria lippii* (L.) Cass. Extracts and Evaluation of Their In Vitro Antioxidant, Antiacetyl cholinesterase, Antidiabetic, and Antibacterial Activities. Evidence-Based Complementary and Alternative Medicine 2019: 9814537.

Belhaj D, Frikha D, Athmouni K, Jerbi B, Mohammad Boshir A, Bouallagui Z, Kallel M, Maalej S, Zhou J, Ayadi H. 2017. Box-Behnken design for extraction optimization of crude polysaccharides from Tunisian *Phormidium versicolor* cyanobacteria (NCC 466): Partial characterization, in vitro antioxidant and antimicrobial activities. International Journal of Biological Macromolecules 105 (2): 1501-1510.

Bouhadjera K, Bendahou M, Boufeldja T. 2005. Anti-microbial activity of extracts from algerian *Aristida pungens* L. Pakistan Journal of Biological Sciences 8: 206-210.

Chen HY, Lin YC, Hsieh CL. 2007. Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chemistry 104: 1418-1424.

Danin A, Orshan G. 1995. Circular arrangement of *Stipagrostis ciliata* clumps in the Neveg, Israel and near Gokaeb, Namibia. Journal of Arid Environment 30: 307-313.

Dash S, Nath LK, Bhise S, Bhuyan S. 2005. Antioxidant and antimicrobial activities of *Heracleum nepalense* D Don Root. The Tropical Journal of Pharmaceutical Research 4: 341-347.

Daur I. 2012. Plant flora in the rangeland of western Saudi Arabia. Pakistan Journal of Botany 44: 23-26.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. 2006. Antioxidant activity of some Algerian medicinal plants' extracts containing phenolic compounds. Food Chemistry 97: 654-660.

Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ. 2003. Characterization of the antioxidant properties of de-odorization aqueous extracts from selected Lamiaceae Herbs. Food Chemistry 83: 255-262.

Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. 2010. Antioxidant and free radical scavenging activity of *H. officinalis*, L. Var. angustifolius, V. odorata, B., hyrcana and *C. speciosum*. Pakistan Journal of Pharmaceutical Sciences 23: 29-34.

Fabricant DS, Farnsworth NR. 2010. The value of plants used in traditional medicine for drug discovery. Environnemental Health Perspectives 109: 69-75.

Girodon F, Lombard M, Galan P *et al*. 1997. Effect of micronutrient supplementation on infection in institutionalized elderly subjects: A controlled trial. Annals of Nutrition and Metabolism 41: 98-107.

Gonçalves C, Dinis T, Batista MT. 2005. Antioxidant properties of proanthocyanins of *Uncaria tomentosa* bark decoction: A mechanism for anti-inflammatory activity. Phytochemistry 66: 89-98.

Gouveia SC, Castilho PC. 2013. *Artemisia annua* L.: Essential oil and acetone extract composition and antioxidant capacity. Industrial Crops and Products 45 :170-181.

Hanafey FM. 2013. Assessment of total antioxidant capacity and antiradical scavenging activity of three Egyptian wild plants. Journal of Medical Sciences 13: 546-554.

Hosny AM, Morsy AA, Youssef A, Ahmed HA. 2009. Structure of the common plant population along Alamain-WadiEl-Natrun Desert Roat. Australian Journal of Basic and Applied Sciences 3: 177-193.

Hussain SR, Cillard J, Cillard P. 1987. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 26: 2489-2491.

Hussein IA, Mostafa EM, Ghoneim M, El-Hela A. 2015. Cytotoxic, antimicrobial and antiviral evaluation of the aerial parts of *Sorghum virgatum* Hack. Journal of Pharmaceutical Research 4: 17-20.

Hussein SR, Latif RRA, Marzouk MM, Elkhateeb A, Mohammed RS, Soliman AAF, Abdel-Hameed E-SS. 2018. Spectrometric analysis, phenolics isolation and cytotoxic activity of *Stipagrostsis plumosa* (Family Poaceae). Chemical Papers 72: 29-37.

Iram F, Sobia K, Tariq M. 2019. Microbiostatic, antioxidative and cytotoxic potentiation of some grasses of Bahawalpur. Journal of Traditional Chinese Medical Sciences 39: 482-491.

Javanmardi J, Stushnoff C, Locke E, Vivanco JM. 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chemistry 83: 547-550.

Kalpna DR, Mital K, Sumitra C. 2011. Vegetable and fruit peels as a novel source of antioxidants. Journal of Medicinal Plants Research 5: 63-71.

Karker M, Falleh H, Msaada K, Smaoui A, Abdelly C, Legault J, Ksouri R. 2016. Antioxidant, anti-inflammatory and anticancer activities of the medicinal halophyte *Reaumuria vermiculata*. EXCLI Journal 15: 297-307.

Kolsi RB, Salah HB, Saidi SA, Allouche N, Belghith H, Belghith K. 2017. Evaluation of nutritional value, characteristics, functional properties of *Cymodocea nodosa* and its benefits on health diseases. Lipids in Health and Disease 16: 238.

Kumar BR. 2017. Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). Journal of Pharmaceutical Analysis 7: 349-364.

Les F, Prieto JM, Arbon´es-Mainar JM, Valero MS, L´opezn V. 2015. Bioactive properties of commercialized pomegranate (*Punica granatum*) juice: Antioxidant, anti-proliferative and enzyme inhibiting activities. Food and Function 6: 2049-2057.

Mhalla D, Bouaziz A, Ennouri K *et al*. 2017. Antimicrobial activity and bioguided fractionation of *Rumex tingitanus* extracts for meat preservation. Meat Science 125: 22-29.b

Miceli N, Filocamo A, Ragusa S *et al.* 2017. Chemical characterization and biological activities of phenolic-rich fraction from Cauline leaves of *Isatis tinctoria* L. (*Brassicaceae*) growing in Sicily, Italy. Chemistry & Biodiversity 14: e1700073.

Mnif FL, Jeddi K, Anjum NA, Chaieb M. 2020. Plant traits and phenotypic variability effect on the phytomass production of *Stipagrostis ciliata* (Desf.) De Winter. Saudi Journal of Biological Sciences 27: 1553-1561.

Moreira FV, Bastos JF, Blank AF, Alves PB, Santos MR. 2010. Chemical composition and cardiovascular effects induced by the essential oil of *Cymbopogon citratus* DC. Stapf, *Poaceae*, in rats. Revista Brasileira de Farmacognosia 20: 904-909.

Nayane Sinosakia BM, Angélica Tonina PP, Marcos Ribeiro AS *et al.* 2020. Structural study of phenolic acids by triple quadrupole mass spectrometry with electrospray ionization in negative mode and H/D isotopic exchange. Journal of the Brazilian Chemical Society 31: 402-408.

Nilsson L. 1978. New rapid bioassay of gentamicin based on luciferase assay of extracellular ATP in bacterial cultures. Antimicrobial Agents and Chemotherapy 14: 812-816.

Pavithra K, Vadivukkarasi S. 2015. Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima* (Jacq.) Cogn. Food Science and Human Wellness 4: 42-46.

Plazonic A, Bucar F, Males Z, Mornar A, Nigovic B, Kujundzic N. 2009. Identification and quantification of flavonoids and phenolic acids in burr parsley (*Caucalis platycarpos* L.), using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. Molecules (Basel, Switzerland) 14: 2466-2490.

Prieto P, Pineda M, Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 269: 337-341.

Proestos C, Boziaris IS, Nychas GJE, Komaitus M. 2006. Analysis of flavonoids and phenolics acids in Graak aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chemistry 95: 664-671.

Rathod JD, Pathak NL, Patel RG, Jivani NP, Bhatt NM. 2011. Phytopharmacological properties of *Bambusaarundinacea* as a potential medicinal tree: An overview. Journal of Applied Pharmaceutical Science 1: 27-31.

Roger PE. 2003. *Stipagrostis ciliata* (Desf.) De Winter var. capensis (Trin. & Rupr.) De Winter. The Consilience Engine Ecoport RSA Country Programme. 2 p.

Sacan O, Yanardag R. 2010. Antioxidant and anti-acetylcholinesterase activities of chard (*Beta vulgaris* L. var. cicla). Food and Chemical Toxicology 48: 1275-1280.

Salaheldin Abdelkader MS, Orabi MMA, Shama SM, Assaf MH. 2016. Chemical constituents and biological activities of the aerial parts of *Stipagrostis plumosa* (L) Munro ex T. Anderson. Journal of Pharmaceutical Sciences and Research 8: 801-812.

Shan B, Cai YZ, Brooks JD, Corke H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. The International Journal of Food Microbiology 117: 112-119.

Singariya P, Kumar P, Mourya KK. 2012. Identification of steroid compound using preparative thin layer chromatography, GC-MS and antimicrobial and antioxidant properties of *Cenchrus setigerus* (Poaceae). International Journal of Pharmaceutical and Life Sciences 3: 1909-1916.

Soreng RJ, Peterson PM, Romschenko K *et al*. 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). Journal of Systematics and Evolution 53: 117-137.

Tapiero H, Tew KD, Ba GN, Mathé G. 2002. Polyphenols: Do they play a role in the prevention of human pathologies? Biomedicine & Pharmacotherapy 56: 200-207.

Tungmunnithum D, Thongboonyou A, Phoboon A, Yangsabai A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines (Basel) 5: 93.

Yao LH, Jiang YM, Datta N *et al.* 2004. HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. Food Chemistry 84: 253-263.

Yao XH, Zhang DY, Zu YG *et al.* 2013. Free radical scavenging capability, antioxidant activity and chemical constituents of *Pyrola incarnata* Fisch Leaves. Industrial Crops and Products 49: 247-255.

Yildirim A, Mavi A, Kara AA. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. Journal of Agriculture Food Chemistry 49: 4083-4089.

Yonathan MY, Asres K, Assefa A, Bucar F. 2006. In vivo anti-inflammatory and antinociceptive activities of *Chelianthes farinosa*. The Journal of Ethnopharmacology 108: 462-470.

Zengin G, Aktumsek A, Guler GO, Cakmak YS. 2011. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. Hayekiana wagenitz. Records of Natural Products 52: 123-132.