Phytochemical and Antioxidant Screening of Some Folkloric Medicinal Plants from Bechar Region, Southwest Algeria

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Summary

This study focuses on the assessment of the phytochemical content and antioxidant potency of the crude hydromethanolic and aqueous extracts of nine folkloric medicinal plants from the Bechar region (Southwest Algeria). Phytochemical screening of the subjected extracts was done qualitatively and quantitatively using standard chemical tests. Antioxidant activity was performed according to various tests having different mechanisms. Among all extracts, the aqueous extract of *Rhus tripartita* DC. (276.221 ± 0.079 mg GAE g^{-1}) showed the highest phenolic content. The hydromethanolic extracts of *Periploca laevigata* Aiton showed the highest flavonoid content (646.531 ± 0.234 mg QE g^{-1}), whereas the hydromethanolic extracts of *Andropogon schoenanthus* Spreng. and *R. tripartita* exposed the highest polysaccharide content (356.609 ± 0.005 and 350.440 ± 0.049 mg GE g^{-1} respectively). The performed antioxidant activity proved that the hydromethanolic extracts had a strong radical scavenging ability comparing with the aqueous extracts. The hydromethanolic and aqueous extracts of *R. tripartita* exhibited the highest total antioxidant activity with a very low IC_{50} (15.838 and 19.539 mg mL^{-1} respectively). The hydromethanolic extracts of *R. tripartita* and *P. laevigata* had the highest ferric reducing antioxidant potency (624.194 ± 0.294 and 589.195 ± 0.054 AAEFRAP g^{-1} respectively). Hydromethanolic and aqueous extracts of *R. tripartita* had the highest total antioxidant capacity (361.507 ± 0.326 and 426.581 ± 0.1812 mg AAE g^{-1} respectively). The preliminary results obtained in this study serve as an important guide or tool in the selection of potential candidates for further pharmacological studies.

Key words

medicinal plants, phytochemical screening, polyphenolic content, total flavonoid content, total polysaccharides content, antioxidant potency

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Introduction

The reliance on traditional medicines in developing countries, especially among the most populated groups, to prevent and treat different illnesses is still alarming despite continuous advancements in modern medicines. This is often due to cultural beliefs, lower cost, availability, and effectiveness in treating various ailments (Makhuvele et al., 2020). Natural products derived from this kind of plant possess exceptional values and supply incredible sources of novel drugs and chemical entities for developing various drugs (Tabassum and Vidyasagar, 2017; Telichowska et al., 2020). Currently, natural products cover about 25-30% of available drugs for treating various diseases (Atanasov et al., 2015; Behera, 2018). In line with several studies, ethnomedicinal values of plants have been reported owing to the significant therapeutic advantages of phytochemicals, mainly flavonoids, phenols, tannins, and alkaloids. Thanks to their unique properties as pain relievers and effectiveness in treating different diseases (Karim et al., 2020; Khan et al., 2020). The southern region of Algeria is known for its rich endowment in different medicinal plants for the effective treatment of various ailments. However, the studies about their pharmacological potency are very scarce, thus there is a great need for a detailed study to provide sufficient information about the constituents for such pharmacological potency. As part of our group continuous efforts and investigations in the Bechar region (located in the southwest of Algeria) to pharmacologically evaluate folkloric medicinal plants, this study, therefore, focuses on the assessments of phytochemical contents and antioxidant potency of aqueous and hydromethanolic crude extracts of nine folkloric plants, which have been habitually used as traditional medicine by the people in this area. Since the study about the study region is scarce, the results obtained serve as important preliminary guide or tool in the future selection of potential candidate(s) for further pharmacological studies.

Materials and Methods

Collection and Extraction Procedure

The samples used for this study were collected from the nine medicinal plants between February and March 2015 from different places within the Bechar province located in the southwestern part of Algeria (Table 1). The exsiccates of the plants collected were deposited in the herbaria of the biology laboratory University, Bechar, Algeria, and identified by an expert, Mohamed Tahri. The sample (fresh plants) was then cut into pieces and dried in ambient shade before grinding. A 50 g sample was completely refluxed with distilled water for each plant material, followed by a separate admixture of 80% water-methanol for 3 h. The extracts obtained from this process were then filtered, evaporated and dried under reduced pressure using a rotatory vacuum evaporator. The dried extracts were weighed to determine the percentage of yield of the soluble constituents using the following formula:

$$\text{Yield (\%)} = (\text{Weight of dry extract})/(\text{Weight of extraction}) \times 100$$

Phytochemical Screening

A qualitative phytochemical screening was carried out on the extracts to detect the presence or the absence of secondary metabolites using standard procedures as described in the previous studies (Sofowora, 1993; Trease and Evans, 1989).

Determination of Total Phenol Content (TPC)

Total phenolic contents were determined by following Folin-Ciocalteu colorimetric method using gallic acid as standard (Singleton et al., 1999). Briefly, 0.2 mL of each extract were added to 0.8 mL of a Na_2CO_3 solution (75 mg mL^{-1} distilled water) and stirred for few minutes. Thereafter, a 1 mL of Folin-Ciocalteu solution (1/10 dilution) was added to the overall solution and incubated for 2 h in a dark room under room temperature. The absorbances were measured using a UV-visible spectrophotometer at a wavelength of 765 nm. The concentrations of the total phenolics were then estimated as mg of equivalent gallic acid using the equation obtained from the gallic acid calibration curve.

Determination of Total Flavonoid Content (TFC)

Total flavonoid contents were performed by following a colorimetric assay reported by (Kim et al., 2003) and quercetin as a standard. Briefly, 0.25 mL of each extract were added to 1.25 mL of ddH2O separately. This was followed by the subsequent addition of 75 µL of 5% $NaNO_2$ into the mixture and then allowed to stand for 5 minutes before adding a 150 µL of 10% $AlCl_3$. The mixture obtained was incubated at ambient temperature for another 5 min. Thereafter, 0.5 mL of 1 M NaOH was added, and the mixture was immediately diluted with the addition of 275 µL of ddH_2O. The absorbance of the melange was recorded at 510 nm. The concentrations of the total flavonoids were evaluated as mg of equivalent quercetin using the calibration curve equation for quercetin.

Determination of Total Polysaccharide Content

The total polysaccharide contents were determined with glucose as a standard using the Phenol-Sulfuric acid method (Dubois et al., 1956). Briefly, 2 mL of each extract were pipetted into a test tube, and 1 mL of 5% phenol solution was added. Then 5 mL of concentrated H_2SO_4 were added rapidly. The tubes were shaken and placed in a water bath at 30 °C before the readings were recorded. The absorbance of the characteristic yellow-orange color was recorded at 490 nm. The concentrations of the total polysaccharide were evaluated as mg of equivalent glucose using the calibration curve equation for glucose.

Evaluation of *in Vitro* Antioxidant Assay

In this study, the antioxidant potentials of the plant extracts were evaluated using three methods: DPPH, FRAP, and TAC as described below. Ascorbic acid, gallic acid, and quercetin were used as reference antioxidants.

Total Antioxidant Capacity (TAC)

The TAC of the plant extracts was obtained following a previous method by (Prieto et al., 1999). The extracts were prepared in their respective solvents (1mg/mL) and admixed with 1mL of the reagent solutions (0.6M H_2SO_4, 28mM Na_3PO_4, 4mM $(NH_4)_2MoO_4$ mixtures). The tubes were incubated at 95 °C for 90 min. The mixtures were then allowed to cool to room temperature, and the respective absorbances were recorded at 695 nm against a blank sample. The TAC was expressed as mg/gram of plant extracts in ascorbic acid equivalent.

Table 1. List of selected traditional medicinal plants

N°	Scientific name	Family	Vernacular name	Region of collect	Date of collect
1	*Andropogon nardus* L.	Poaceae	ليدخير	Bechar	February 2015
2	*Andropogon schoenanthus* L.	Poaceae	اللماد	Bechar	March 2015
3	*Globularia alypum* L.	Globulariaceae	تسلغا	Bechar	March 2015
4	*Hammada scoparia* Pomel. red	Chenopodiaceae	الرمث الأحمر	Bechar	March 2014
5	*Hammada scoparia* Pomel. green	Chenopodiaceae	الرمث الاخضر	Lahmer	March 2014
6	*Periploca laevigata* Ait.	Asclepiadaceae	الحلاب	Bechar	March 2015
7	*Rhus tripartita* R. Sch.	Anacardiaceae	الجداري	Bechar	February 2015
8	*Tamarix gallica* L.	Tamaricaceae	فرسيق	Bechar	March 2015
9	*Traganum nudatum* Del.	Chenopodiaceae	الضمران	Kenadsa	February 2015

Ferric Reducing Antioxidant Power (FRAP)

This study employed the method described by Yildirim et al. (2001) for determining the FRAP of the plant extracts. Here, the mixture involves mixing the extracts with 2.5 mL phosphate buffer (0.2M, pH 6.6) and 2.5 mL of 1% $C_6N_6FeK_3$, then followed by 30 min incubation at 50 °C. 2.5 mL of 10% trichloroacetic acid were further added to the as-obtained mixture and centrifuged for 10 min at 3000 rpm. Lastly, 2.5 mL of upper solution layer were mixed with 2.5 mL distilled water and 0.5 mL of 0.1% $FeCl_3$, and the corresponding absorbance was taken at 700 nm. The results obtained were expressed as mg/gram of the extract in ascorbic acid equivalent.

1, 1 Diphenyl-2-picrylhydrazyl Radical (DPPH) Scavenging Assay

The antioxidant activities of the extracts were determined qualitatively by TLC assay and Microtiter plate assay (Hsiao et al., 1996; Purushothaman et al., 2013). For the TLC assay, the aliquot (3 µL) of each extract and standard (Quercetin and Ascorbic acid) was carefully loaded onto a silica gel plate and allowed to dry. After 5 minutes, the TLC plate was sprayed with 0.2% DPPH in methanol. The DPPH discoloration is an indication of the scavenging potentials of the compounds tested.

For the microtiter plate assay, an aliquot (50 µL) of each extract and standard was taken separately in the microtiter plate. Methanolic DPPH (100 µL of 0.1%) was added over the samples and then incubated for 30 minutes under dark conditions. The samples were then observed for discoloration from purple to pale pink and yellow, indicating weak and strong positive, respectively.

Radical scavenging activity of the plant extracts was also determined quantitatively using the method of (Samarth et al., 2008) with ascorbic acid, gallic acid and quercetin as standards. Briefly, 1.9 mL of DPPH solution were added to 0.1 mL of various concentrations of each extract (0.01-0.5 mg/mL). Equal amounts of DPPH and CH_3OH were used as the control. The mixture was then shaken vigorously and allowed to stand in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm, and the percentage scavenging activity of each extract on DPPH radical was determined using the expression in Equation 1:

$$\text{Scavenging Activity (\%)} = (1\text{-Absorbance of the sample})/(\text{Absorbance of the control}) \times 100 \quad (1)$$

The scavenging activities of the DPPH radical of the extracts were expressed as IC_{50} values (IC_{50}), which can be defined as the effective concentrations of an extract required for 50% scavenging of DPPH radical. It was estimated from the plot of scavenging activity against the concentration of the sample.

Statistical Analysis

For statistical analysis, the measurements of all data recorded were obtained with three independent replicates to express the mean ± standard deviation (SD). One-way analysis of variance (SAS, 1990; ANOVA model) was conducted for comparing the means and testing the significant difference between the means obtained amongst the treatments at $P < 0.05$ significance level.

Results and Discussion

Sample Description

Nine plant species, namely, *Andropogon nardus* L., *Andropogon schoenanthus* L., *Globularia alypum* L., *Hammada scoparia* Pomel. red, *Hammada scoparia* Pomel. green, *Periploca laevigata* Ait., *Rhus tripartita* R. Sch., *Tamarix gallica* L., *Traganum nudatum* Del., were obtained from various regions of Bechar province. These plants were chosen based on a survey of the ethnopharmacological population with knowledge of their use in traditional medicine. Complete details of identified plants with botanical name, family, vernacular name, region and date of collection are summarized in Table 1. Among the chosen samples, three plants belong to the Chenopodiaceae family, two of them belong to the Poaceae family, while the rest belong to Anacardiaceae, Asclepiadaceae, Globulariaceae, and Tamaricaceae each.

Pre-Preparation of Plant Samples

Plant sample preparation is the initial stage in medicinal plant studies because it is the step for conserving the biomolecules present in the plants before extractions. Other plant material processing, including drying and grinding also influences the phytochemicals preservation in the final extract(s) (Abubakar and Haque, 2020; Azwanida, 2015). In most cases, the dried samples are preferred when considering the time required for designing the experiment. Between grinded and powdered samples, the lower particle sizes increase surface contact between the extraction solvents and samples. Furthermore, powdered samples usually contain smaller and more homogenized particles thereby offering better surface contacts with the extraction solvents (Azwanida, 2015; Deli et al., 2019).

Extraction Procedure

Extraction remains the major step to recover and isolate the phytochemicals from plant materials. The initial crude extracts of these processes contain a complex mixture of many plant metabolites. The efficiency of the extraction is largely affected by the chemical nature of phytochemicals, extraction method(s), particle sizes of the samples, solvents and other interference substances that may be present (Benzarti, 2016; Lourenço et al., 2019).

The present study used two solvents including water and 80% methanol for the extraction of plants under investigation. This is based on the understanding that water or a combination of water with alcohol are common solvents usually employed for extraction process for assessing the contents of the biological active compound(s) in the raw plant materials. However, vitamins, minerals and polyphenols were simply extracted using the polar solvents in order to enable the extraction with higher pharmacological activity (Telichowska et al., 2020).

The yield of each plant extract as presented in Table 2 was determined by weighing the dry matter obtained after the extraction and recovery of the solvents from the extracts. The extraction yields obtained for the aqueous extracts ranged from 2.44% to 41.64% and those of the hydromethanolic extracts from 5.25% to 31.42% (for *A. nardus* and *P. laevigata* respectively in both cases). By comparison, the extraction yields of the aqueous extracts (32.99, 34.40, 41.64, 25.12, 34.58 and 20.61%) were higher than those of hydromethanolic extracts (20.85, 19.59, 31.42, 21.37, 31.15 and 7.76%) for *H. scoparia* green, *H. scoparia* red, *P. laevigata*, *R. tripartita*, *T. gallica*, and *T. nudatum*, respectively) as shown in Table 2. It can also be noticed that the yield of the extracts of *A. schoenanthus* is much lower than *G. alypum* in both extracts, whereas the yield of *A. nardus* hydromethanolic extract (5.25%) is higher than that of its aqueous extract (2.44%). These results imply that using water as a solvent enhanced the extraction yields more than the methanol aqueous extract (80%), thus providing more future economic prospects than the methanol counterpart. It should however be noted that the extraction yields depend on the solvents with different polarities, extraction time, composition, pH and temperature of the samples. Previous studies have also shown that many phytochemicals might have been extracted and contributed to higher yield (Cho et al., 2020; Franco et al., 2008).

Table 2. Total extraction yield of selected plant species

	Yield (%)	
	A	HM
A. nardus	2.44	5.25
A. schoenanthus	4.34	5.75
G. alypum	20.01	19.57
H. scoparia green	32.99	20.85
H. scoparia red	34.40	19.59
P. laevigata	41.64	31.42
R. tripartita	25.12	21.37
T. gallica	34.58	31.15
T. nudatum	20.61	7.76

Note: A - Aqueous extracts, HM - Hydromethanolic extracts

Preliminary Phytochemical Analysis

Phytochemical compositions and the corresponding biological activities are crucial to grasp the therapeutic potential and feasibility of the medicinal herbs. Many studies suggest that the pharmacological activity of the medicinal plants is due to the presence of secondary metabolites (Behera, 2018; Fardiyah et al., 2020; Gorlenko et al., 2020). The aqueous and hydromethanolic extracts of the selected plant species were subjected to preliminary qualitative phytochemical screenings for the detection of major chemical groups which might be responsible for their medicinal attributes (Table 3).

The maximum number of phytochemicals was found in the aqueous and hydromethanolic extracts of *R. tripartita* followed by the aqueous extracts of *P. laevigata* and *T. gallica* (Fig. 1). As presented in Table 3, the phytochemical analysis of *A. nardus* and *A. schoenanthus* showed that all their extracts contained an abundance of alkaloids, carbohydrates, flavonoids, glycosides, phenols and quinones, while coumarins and resins were absent. Also, the results indicated that *G. alypum* extracts had almost all the tested phytoconstituents such as carbohydrates, flavonoids, phytosterols, quinones, terpenoids, tannins and phenols. The extracts of the two species of *H. scoparium* were very rich in many biomolecules like alkaloids, flavonoids, phenols, quinones, and tannins, whereas some phytoconstituents such as carbohydrates, coumarins, proteins, and resins were totally absent in all the tested extracts.

Moreover, the extracts of *P. laevigata* and *R. tripartita* are rich sources of all the tested compounds as alkaloids, carbohydrates, coumarins, flavonoids, glycosides, phytosterols, proteins, phenols, quinones, tannins, and terpenoids. Saponins were detected abundantly in the extracts of *R. tripartita* but poorly detected in the extracts of *P. laevigata*. However, the extracts of *T. gallica* were rich sources of coumarins, flavonoids, glycosides, phytosterols, proteins, phenols, and tannins, whereas alkaloids and resins were absent.

Table 3. Preliminary qualitative phytochemical analysis of the aqueous and hydromethanolic extracts of plant species

		A. nardus		*A. schoenanthus*		*G. alypum*		*H. scoparia* green		*H. scoparia* red		*P. laevigata*		*R. tripartita*		*T. gallica*		*T. nudatum*	
		A	HM	A	HM	A	HM	A	HM	A	HM	A	HM	A	HM	A	HM	A	HM
Alkaloids	*Mayer's Test*	+	++	++	+	+	-	+++	+++	+++	+++	++	++	+	+	-	-	+	+
	Wagner's Test	+	+	++	+	+	-	+++	+++	+++	+++	++	+++	++	+++	-	-	-	+
Carbohydrates	*Molisch test*	++	++	+	+	+	++	-	-	-	+	++	-	+++	++	+	-	++	-
	Fehling's test	+	+	-	++	++	++	-	-	-	-	+++	+++	+++	+++	++	-	-	++
	Benedict's test	-	-	-	+	++	++	-	-	-	-	+++	+++	+++	+++	+	-	-	-
Coumarins	*Sodium hydroxide test*	-	-	-	-	-	+	-	-	-	-	+++	+++	+++	++	+	++	-	+
Flavonoids	*Lead acetate Test*	++	++	+	+	+	-	+	+++	++	+++	+++	+	+++	++	++	++	+	+
	Shinoda test	-	+	+	+	+	-	+	+++	++	+++	+++	+	+++	++	++	++	+	+
	Ammoniac test	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++
	Zinc test	-	+	+	-	-	-	+	++	+	++	++	+	++	++	+	++	-	-
Anthocyanin	*Sodium hydroxide test*	++	-	-	-	-	-	+	-	+	-	+	-	-	++	-	-	-	-
Betacyanin		-	++	++	-	++	+++	-	-	-	+	+	-	+	-	++	++	+	++
Cyanidin aglycones	*Willstätter cyanidin test*	-	-	-	+++	+++	-	-	+++	-	-	-	-	+	+++	++	+	-	-
Leucoanthocyanins	*Isoamyl alcohol test*	-	+	-	-	+	++	-	++	-	++	+	-	-	-	-	-	-	-
Glycosides	*Modified Borntrager's Test*	++	+	+	+	+	+	++	++	++	+	++	+	++	+	++	+	+	+
Phytosterols	*Salkowski's Test*	+	-	-	+	+++	++	-	+++	-	+	+	++	++	+++	+	+++	-	-
	Libermann Burchard's Test	+	-	-	++	-	+++	+	++	+	++	++	++	+	++	+	++	-	+
Proteins	*Xanthoproteic test*	+	+	-	+	+	-	+	++	+	+	+	+	+	++	+++	+	-	-
	Biuret Test	-	-	-	-	-	-	-	+	-	-	-	+	+	+++	+	++	-	-
Phenols	*Ferric Chloride Test*	+	++	++	+++	++	+++	++	++	++	++	+++	+++	+++	-	+++	+++	+	+
Quinones	*Chlorhydric acid Test*	++	+++	+	+++	++	++	+++	+++	++	+++	+++	++	+++	++	+	+	++	+++
Saponins	*Froth Test*	-	+++	+++	+	+	+	+	+	+	+	-	-	+++	++	+++	-	+++	-
Tannins	*Ferric Chloride Test*	+	-	-	++	++	++	+	+++	+	+	+	++	+	+++	+	+	-	+
Terpenoids	*Gelatin test*	+	+	-	-	-	+	-	+	-	+	+++	+	+++	+++	+++	+++	-	+
Resins	*Sulfuric acid Test*	+	+	-	+	+++	+++	-	++	-	++	+	++	++	++	+	+	-	-
	Acetone-water test	-	-	+	-	+++	-	-	-	-	-	+++	-	-	-	-	-	-	-

Note: A: Aqueous extracts, HM: Hydromethanolic extracts; (-): Absence, (+): Poor, (++): Moderate, (+++): Abundant

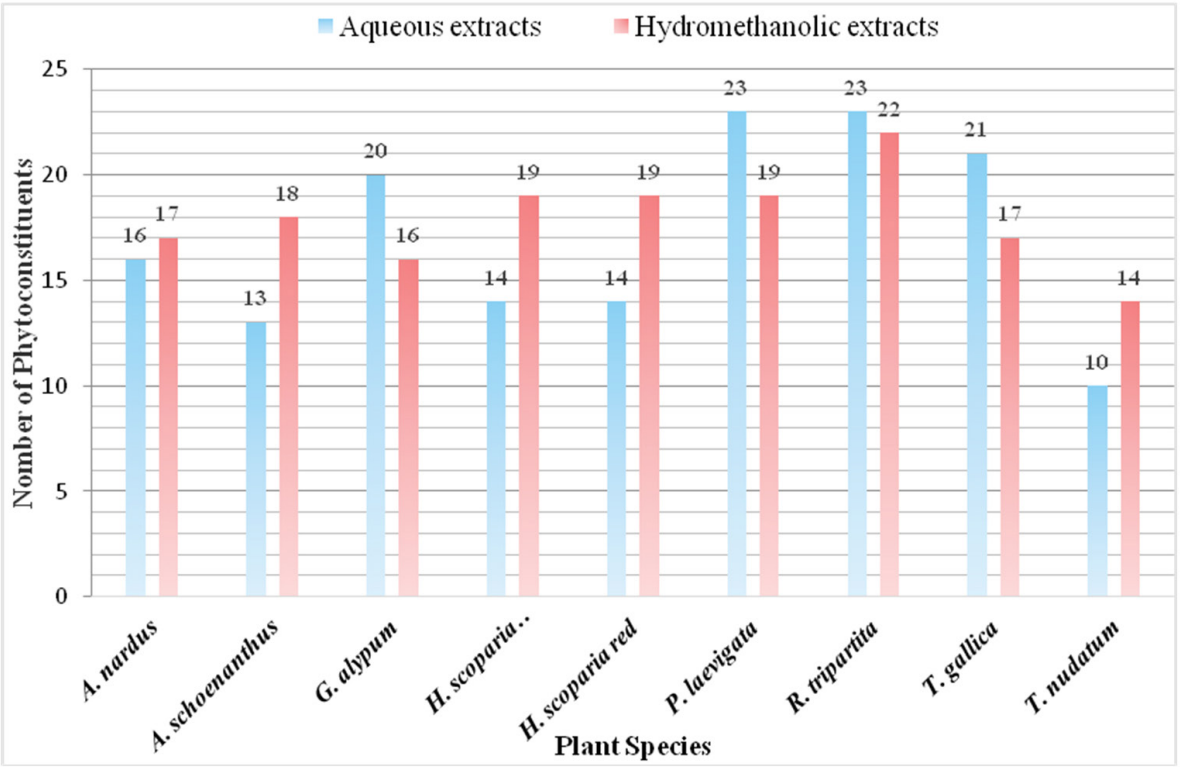

Figure 1. Number of phytochemicals in each extract

By comparison, *T. nudatum* was the poorest plant because almost all the phytoconstituents were poorly detected except for some flavonoids and quinones (Table 3).

Each living body, from one cell bacterium to million-cell plants processes different chemical compounds for their subsistence and survival. All the biological system compounds are often categorized into two broad arenas. One is primary metabolites (proteins, amino acids, lipids and carbohydrates), which are chemical substances needed for growth and development. Another is secondary metabolites, including alkaloids, phenols, flavonoids, quinones, coumarins, etc. These are groups of compounds apart from the primary metabolites and are believed to facilitate the increments of the overall ability of the plants for surviving and overcoming local or surrounding challenges so that they allow their interactions with the immediate surroundings (Erb and Kliebenstein, 2020; Harborne, 1993). These classes of phytochemicals have been observed to have broad range of biological activities such as antimicrobial, anti-inflammatory, anticancer, antioxidant, and antiplasmodial, etc. Therefore, these observations partially explain the traditional usages of the plants under study for treating various diseases, indicating that the investigated plants can be the sources of bioactive compounds against different illnesses (Le Anh Dao et al., 2020).

Total Phenol Content (TPC)

Plant-derived phenols are of great importance because of their exceptional potential antioxidant and antimicrobial properties (Karim et al., 2020; Kumar and Goel, 2019). Folin-Ciocalteu is a very sensitive reagent containing phosphomolybdate and phosphotungstate which by the reduction of phenols forms a blue complex in alkaline solution (Tabasum et al., 2016). Using the Folin-Ciocalteu method, the total phenol compounds of the present study were reported as mg gallic acid equivalents per gram (mg GAE g^{-1}) by reference to standard curve ($y = 8.2995x + 0.1085$, $R^2 = 0.9942$). Table 4 shows the results of different extracts under investigation indicating a wider range of variation in the total phenolic contents from 16.848 ± 0.002 to 276.221 ± 0.079 mg GAE g^{-1}. Total phenol contents of the hydromethanolic extracts varied from 76.732 ± 0.031 to 245.095 ± 0.037 mg GAE g^{-1}, while they ranged between 16.848 ± 0.002 and 276.221 ± 0.079 mg GAE g^{-1} in aqueous extracts of plant samples (Table 4).

As presented in Table 4, among all the extracts, aqueous extracts of *R. tripartita* (276.221 ± 0.079 mg GAE g^{-1}) and the hydromethanolic extracts of *P. laevigata* and *H. scoparia* red (245.095 ± 0.037 and 243.609 ± 0.231 mg GAE g^{-1} respectively) have the highest phenolic contents, followed by the aqueous extracts of *H. scoparia* red (222.523 ± 0.168 mg GAE g^{-1}) and the hydromethanolic extracts of *G. alypum* and *H. scoparia* green with 199.229 ± 0.180 and 190.674 ± 0.004 mg GAE g^{-1}, respectively. It should be noted that the recovery of phenolic compound(s) is generally determined by the type of solvents used, its polarity index, and the solubility of phenolic compounds in the extraction solvents. It has been observed that the solubility of polyphenols depends mainly on the presence and hydroxyl group positions, molecular sizes and lengths of hydrocarbon chain constituents (Karim et al., 2020; Lourenço et al., 2019).

Table 4. Phytochemical and Antioxidant Screening Results of the Investigated Plant Species

		TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	Total Polysaccharide Content (mg GE g^{-1})	TAC (mg AAE g^{-1})	FRAP (mg AAEFRAP g^{-1})	Inhibition Conc. IC_{50} (mg mL^{-1})
A. nardus	A	16.848 ± 0.561	5.531 ± 0.021	161.178 ± 1.084	3.497 ± 0.005	1.852 ± 0.006	251.466
	HM	76.732 ± 0.745	94.197 ± 0.110	235.460 ± 1.518	151.814 ± 0.102	84.322 ± 0.016	95.761
A. schoenanthus	A	54.562 ± 0.248	36.864 ± 0.052	167.432 ± 1.121	51.774 ± 0.073	4.272 ± 0.003	178.083
	HM	110.268 ± 1.024	127.197 ± 0.143	356.609 ± 2.225	49.238 ± 0.009	98.657 ± 0.027	90.985
G. alypum	A	144.045 ± 1.304	218.197 ± 0.234	145.327 ± 0.992	159.209 ± 0.048	40.760 ± 0.022	53.273
	HM	199.229 ± 1.762	494.197 ± 0.510	129.048 ± 0.897	218.895 ± 0.109	286.495 ± 0.031	38.287
H. scoparia green	A	145.009 ± 1.312	108.864 ± 0.124	315.055 ± 1.982	74.803 ± 0.259	38.340 ± 0.012	108.424
	HM	190.674 ± 1.691	179.197 ± 0.195	344.957 ± 2.157	146.849 ± 0.048	212.774 ± 0.020	82.051
H. scoparia red	A	222.523 ± 1.955	193.531 ± 0.209	190.479 ± 1.255	262.101 ± 0.150	68.499 ± 0.051	32.176
	HM	243.609 ± 2.130	371.197 ± 0.387	96.405 ± 0.706	289.673 ± 0.051	436.914 ± 0.153	29.123
P. laevigata	A	183.164 ± 1.629	310.864 ± 0.326	116.282 ± 0.822	146.532 ± 0.181	70.174 ± 0.038	107.724
	HM	245.095 ± 2.143	646.531 ± 0.662	167.860 ± 1.123	264.108 ± 0.170	589.195 ± 0.054	33.367
R. tripartita	A	276.221 ± 2.401	341.197 ± 0.357	226.293 ± 1.464	426.581 ± 0.181	176.473 ± 0.063	19.539
	HM	163.765 ± 1.468	510.531 ± 0.526	350.440 ± 2.189	361.507 ± 0.326	624.194 ± 0.294	15.838
T. gallica	A	124.887 ± 1.145	3.197 ± 0.019	336.989 ± 2.110	114.840 ± 0.306	0.549 ± 0.002	461.369
	HM	84.202 ± 0.807	83.531 ± 0.099	298.091 ± 1.883	295.166 ± 0.165	470.423 ± 0.141	19.203
T. nudatum	A	41.790 ± 0.455	61.531 ± 0.077	147.469 ± 1.004	17.230 ± 0.025	2.225 ± 0.001	354.937
	HM	90.608 ± 0.861	61.531 ± 0.077	137.016 ± 0.943	59.485 ± 0.033	135.145 ± 0.017	150.044

Note: A - Aqueous extracts, HM - Hydromethanolic extracts; TPC - Total Phenol Content; TFC - Total Flavonoid Content; TAC - Total Antioxidant Capacity; FRAP - Ferric Reducing Antioxidant Power

Total Flavonoid Content (TFC)

Different spectrophotometric techniques have been established for quantifying the flavonoid compounds over past years. These spectrophotometric methods are based on the formation of a compound or colored complex that is measured at a certain wavelength. The present study determined the TFC using aluminum chloride techniques and was reported as mg quercetin equivalent per gram (mg QE/g of dry extract), by reference to standard curve ($y = 1.398x + 0.022$, $R^2 = 0.989$).

The TFC of the hydromethanolic extracts was placed between 61.531 ± 0.014 and 646.531 ± 0.234 mg QE/g, whereas it ranged between 3.197 ± 0.004 and 341.197 ± 0.084 mg QE g^{-1} in water extracts as shown in Table 4. The hydromethanolic extracts of *P. laevigata*, *R. tripartita*, and *G. alypum* demonstrated the highest flavonoids contents (646.531 ± 0.234, 510.531 ± 0.023, and 494.197 ± 0.077 mg QE g^{-1} respectively), followed by the hydromethanolic extracts of *H. scoparia* red and *H. scoparia* green (371.197 ± 0.067 and 179.197 ± 0.008 mg QE g^{-1}) (Table 4).

Flavonoids are a particular group of phenolic compounds with a structural feature based on the carbon skeleton of the diphenylpropane. As indicated based on their *in vitro* and epidemiological evidence such as antioxidant, anticarcinogenic and cardioprotective activities, flavonoids are found to be beneficial for human health and are also known for their protecting ability against other non-transmissible chronic diseases (Khettaf et al., 2016; Kumar and Goel, 2019).

Total Polysaccharide Content

The polysaccharide is the higher molecular weight polymer consisting of a minimum of 10 monosaccharides which are mutually joined by glycosidic linkages. Due to the mixed complexity and combination of variety of monosaccharides, there are no direct measurements for polysaccharides. The phenol sulfuric acid method is a colorimetric method commonly used to assess the total carbohydrate contents of bacterial and plant polysaccharides (Wang et al., 2016).

The total polysaccharide contents of the plant extracts were estimated using a regression equation obtained from the calibration curve ($y = 5.8358x + 0.1439$, $R^2 = 0.991$).

Total polysaccharide contents in hydromethanolic extracts were placed between 96.405 ± 0.003 and 356.609 ± 0.005 mg GE g^{-1}, whereas they were between 116.282 ± 0.002 and 336.989 ± 0.043 mg GE g^{-1} in water extracts of plant samples (Table 4).

Among all the extracts, hydromethanolic extracts of *A. schoenanthus*, *R. tripartita*, and *H. scoparia* green had the highest polysaccharide contents (356.609 ± 0.005, 350.440 ± 0.049, and 344.957 ± 0.046 mg GE g^{-1} respectively), followed by the aqueous extracts of *T. gallica* and *H. scoparia* green (336.989 ± 0.043 and 315.055 ± 0.017 mg GE g^{-1}). Furthermore, the hydromethanolic extract of *H. scoparia* red and the aqueous extract of *P. laevigata* obtained the lowest polysaccharide contents (96.405 ± 0.003 and 116.282 ± 0.002 mg GE/g respectively) among all the tested extracts (Table 4).

Over the years, plants, microorganisms and animal polysaccharides have raised the attention of different researchers due to their numerous biological activities (Bose, 2016). Plant-based polysaccharide acts as antioxidant, antitumor, antiviral, immune-stimulating, and anticoagulant agents (Cho et al., 2020; Wang et al., 2016; Zhong et al., 2019).

In vitro Antioxidant Activity

Medicinal plants contain different ranges of free radical scavenging molecules, including phenolic and vitamins, nitrogen, terpenoids and other endogenous metabolites, which are enriched in antioxidant activities (Salehi et al., 2020; Shi et al., 2016). Epidemiological studies have revealed that a large number of these antioxidant compounds have to an extent antiatherosclerotic, antitumor, anti-inflammatory, antimutagenic, antiviral, anticarcinogenic and antibacterial activities (Le Anh Dao et al., 2020; Szerlauth et al., 2019). Therefore, to measure the efficiency of natural antioxidants, either as a pure compound or plant extract, many *in vitro* techniques such as DPPH Radical Scavenging Assay, FRAP, and TAC have been designed. These techniques are popular owing to their sensitivity and higher speed. However, due to the complex nature of phytochemicals, a reliable efficiency measurement require the use of more than one method to determine the antioxidant capacity of the plant materials (Essien et al., 2017).

In this study, *in vitro* antioxidant activities of the crude aqueous and hydromethanolic extracts of the investigated plants, compared to that ascorbic acid as a positive reference standard, were evaluated using three different assays: TAC, FRAP and DPPH Radical Scavenging Assay as discussed below:

Total Antioxidant Capacity (TAC)

The TAC is an efficient way of depicting the combined effects of flavonoids, phenolics and other reducing compounds in the plant extracts. It is expressed in terms of ascorbic acid equivalent (AAE) using the phosphomolybdenum method. As presented in Table 4 the TAC assay indicated a wide variation of total antioxidant capacity in the different extracts, ranging from 49.238 ± 0.009 to 361.507 ± 0.326 mg AAE/g in the hydromethanolic extracts. However, in the aqueous extracts of plant samples, it ranged from 3.497 ± 0.004 to 426.581 ± 0.1812 mg AAE g^{-1} (Table 4). By comparison, the aqueous and hydromethanolic extracts of *R. tripartita* (426.581 ± 0.1812 and 361.507 ± 0.326 mg AAE g^{-1}, respectively) and the hydromethanolic extracts of *T. gallica*, *H. scoparia* red, and *P. laevigata* (295.166 ± 0.165, 289.673 ± 0.051, and 264.108 ± 0.170 mg AAE g^{-1} respectively) had the highest TAC, followed by the aqueous extract of *H. scoparia* red and the hydromethanolic extract of *G. alypum* (262.101 ± 0.149 and 218.895 ± 0.109 mg AAE g^{-1}, respectively). Furthermore, the aqueous extracts of *A. nardus* and *T. nudatum* demonstrated the lowest antioxidant capacity (3.497 ± 0.004 and 17.230 ± 0.025 mg AAE g^{-1}, respectively) as shown in Table 4.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay may be assigned as an important index for the antioxidation effect of antioxidants (Rahman et al., 2015). In this study, the FRAP assay revealed that the existence of reductant in the antioxidant samples propelled the Fe^{3+} (ferricyanide complex) reduction into the Fe^{2+} (ferrous form), which enabled monitoring the reducing power of the samples by measuring the Prussian blue formation at 700 nm. The FRAP values were expressed as mg Ascorbic acid equivalent ferric reducing antioxidant potency per gram of plant extract (mg AAEFRAP g^{-1}).

The reductive potential of different plants exhibited a dose-dependent activity within concentration ranges of 84.322 ± 0.016 to 624.194 ± 0.294 mg mg AAEFRAP g^{-1} in the hydromethanolic extracts. On the other hand, the ranges of 0.549 ± 0.002 to 176.473 ± 0.063 0.079 mg mg AAEFRAP g^{-1} were observed in the aqueous extracts of plant samples (Table 4). Among all the extracts, the hydromethanolic extracts of *R. tripartita*, *P. laevigata*, *T. gallica*, and *H. scoparia* green demonstrated the highest reductive ability (624.194 ± 0.294, 589.195 ± 0.054, 470.423 ± 0.141 and 436.914 ± 0.153 mg mg AAEFRAP g^{-1} respectively), followed by the hydromethanolic extract of *G. alypum*, *H. scoparia* green and the aqueous extract of *R. tripartita* (286.495 ± 0.031, 212.774 ± 0.020 and 176.473 ± 0.063 mg mg AAEFRAP g^{-1} respectively). Furthermore, the aqueous extracts of *T. gallica*, *A. nardus*, *T. nudatum*, and *A. schoenanthus* showed the lowest reductive ability (0.549 ± 0.002, 1.852 ± 0.006, 2.225 ± 0.001, and 4.272 ± 0.003 mg AAEFRAP g^{-1} respectively). However, the hydromethanolic extracts of all the plant samples showed a very high ferric-reducing antioxidant potency compared with the aqueous extracts (Table 4).

DPPH Radical Scavenging Assay

DPPH Radical Scavenging Assay analysis remains one of the most accurate and best-known methods that is frequently employed in the evaluation of antioxidant activities (Raja et al., 2016). In this study, the TLC-DPPH and Microtiter plate screening methods were conducted to indicate the presence of antioxidant compounds in the investigated extracts. The degrees of activities of all the studied (tested) samples were qualitatively determined from the observation of a yellow colour intensity (Fig. 2). The species *G. alypum*, *H. scoparia* red, and *R. tripartita* showed the most prominent antioxidant activities. In addition, the hydromethanolic extracts of *T. gallica* and *P. laevigata* also demonstrated their abilities as good candidates for isolating the antioxidant compounds (Table 5).

Table 5. Qualitative DPPH assay on TLC of the studied plants

		A. nardus	*A. schoenanthus*	*G. alypum*	*H. scoparia* green	*H. scoparia* red	*P. laevigata*	*R. tripartita*	*T. gallica*	*T. nudatum*
TLC essay	A. extracts	+	+	+++	++	+++	++	+++	+	+
	HM. extracts	++	++	+++	++	+++	+++	+++	+++	+
Multiplate assay	A. extracts	+	+	+++	++	+++	++	+++	+	+
	HM. extracts	++	++	+++	++	+++	+++	+++	+++	+

Note: The degree of activity, determined qualitatively from observation of the yellow color intensity: weak (+), moderate (++) and strong (+++)

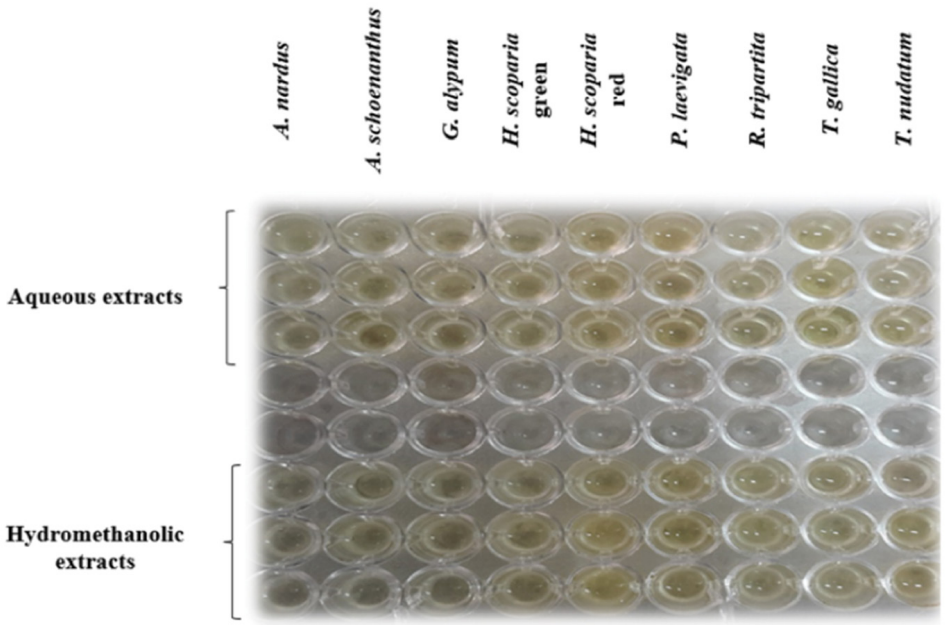

Figure 2. DPPH Microtiter plate screening methods

The DPPH method measured the "electron-donating" activities of other compounds in the mixture, thereby providing substantial evaluations of antioxidant activities due to scavenging free radicals. Any molecules with the potential of donating the electron or hydrogen into the mixture would react with the DPPH and bleach it. The DPPH was reduced from a purple-colored compound to a light-yellow compound by electrons from oxidant compounds (Ogbonnaya and Chinedum, 2013).

Quantitatively, the amount of sample needed to decrease the initial DPPH IC_{50} is a parameter commonly employed for measuring the antioxidant activities. The sample with the lowest IC_{50} value indicates the highest antioxidant activity (Gallego et al., 2017). As presented in Table 4, the hydromethanolic and aqueous extracts of *R. tripartita* exhibited the highest total antioxidant activity with the lowest IC_{50} (15.838 and 19.539 mg mL^{-1} respectively) followed by the hydromethanolic extract of *T. gallica* (19.203 mg/mL), hydromethanolic and aqueous extracts of *H. scoparia* red (29.123 and 32.176 mg mL^{-1} respectively), hydromethanolic extract of *P. laevigata* (33.367 mg mL^{-1}), hydromethanolic and aqueous extracts of *G. alypum* (38.287 and 53.273 mg mL^{-1} respectively). Furthermore, the aqueous extracts of *T. gallica* and *T. nudatum* obtained the lowest activity (461.368 and 354.937 mg mL^{-1}) among all the extracts, followed by the aqueous extracts of *A. nardus*, *A. schoenanthus* (251.466 and 178.083 mg mL^{-1} respectively), and the hydromethanolic extracts of *T. nudatum* (150.044 mg mL^{-1}). By comparing all the extracts in this study (Table 4), the hydromethanolic extracts were more effective than those of aqueous extracts for all plant samples.

Ascorbic acid, gallic acid and quercetin used in this study, are standard antioxidants and could be employed as good indicators for comparing scavenging activities between the extracts. None of the analyzed species displayed a higher result than the gallic and ascorbic acids (4.020 and 9.027 mg mL^{-1}, respectively. However, the standards used were pure and might possess higher antioxidant activities compared with the crude extracts. The activity of *R. tripartita* extracts and the hydromethanolic extract of *T. gallica* were high; not only compared with other plants but also it was elevated *vis-à-vis* the antioxidant standard "quercetin" (150.044 mg mL^{-1}) (Table 4).

Natural materials have recently been proven to be a highly promising source of antioxidants. A large number of bioactive constituents (such as polyphenols, flavonoids, polysaccharides, etc.,) have been derived from the natural materials and reported for possessing highly antioxidant abilities (Salehi et al., 2020; Szerlauth et al., 2019). The detection of antioxidant activities of the investigated plant species enhances remarkable importance as potential novel sources of natural drugs and nutritional supplements, which will need further investigations in the future.

Conclusion

This study has revealed that Bechar region (Southwest Algeria) comprises of underutilized (uncommon) plant species with numerous medicinal properties for identification of new compounds and candidate drugs. The phytochemical contents and the antioxidant potency of the novel crude aqueous and hydromethanolic extracts of nine Saharan medicinal plants were investigated. It was observed that *G. alypum*, *P. laevigata*, *H. scoparia* red, *T. gallica* and *R. tripartita* exhibited remarkable antioxidant activities, thereby demonstrating the potential sources of plant-based medicines for curing different ailments. The stronger antioxidant properties of the studied plants are well correlated with the presence of noticeable amounts of flavonoid and phenolic compounds thereby supporting their usages as traditional medicines. The highest flavonoid and phenolic contents were noticed in aqueous extract of *Rhus tripartita* (276.221 ± 0.079 mg GAE g^{-1}) and hydromethanolic extracts of *Periploca laevigata* (646.531 ± 0.234 mg QE g^{-1}), respectively. The highest polysaccharide contents were obtained in the hydromethanolic extracts of *Andropogon schoenanthus* and *R. tripartita* with 356.609 ± 0.005 and 350.440 ± 0.049 mg GE g^{-1}, respectively. Mush stronger antioxidant activity for radical scavenging ability was observed in hydromethanolic extracts than in aqueous extracts. The hydromethanolic and aqueous extracts of *R. tripartita* exhibited the highest TAC (361.507 ± 0.326 and 426.581 ± 0.1812 mg AAE g^{-1} respectively) with the lowest IC_{50} (15.838 and 19.539 mg mL^{-1} respectively) while the hydromethanolic extracts of *R. tripartita* and *P. laevigata* had the highest ferric reducing antioxidant potency (624.194 ± 0.294 and 589.195 ± 0.054 AAEFRAP g^{-1} respectively. Future investigation will include isolation and evaluation of the bioactive potential component(s) of the studied plants to establish the drug molecules that can further improve treatments of various diseases associated with the oxidative stress. In addition, further studies on the correlation of the antioxidant activities with the polyphenol contents are required for the clarification of the plants' phenolic actions *in-vivo*.

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