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Phytochemical Analysis and Antioxidant Activities of Different Extracts of *Silene Vulgaris (Moench) Garcke* Roots and Leaves Grown Wild in the Algerian West

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Abstract

Silene vulgaris (Moench) Garcke is among many plants that people worldwide use for its roots and leaves as a traditional medication. The target of the current research was to analyse the phytochemical contents of several extracts derived from the roots and leaves of *S. vulgaris* and assess their antioxidant properties. Colorimetric phytochemical assays conducted on *S.vulgaris* have verified the availability of diverse secondary compounds such as saponins, alkaloids, flavonoids, and polyphenols. The crude extract obtained by decocting the butanolic solution (Dec-nBA) of both roots and leaves exhibited the highest concentration of secondary metabolites, including polyphenols, flavonoids, and flavonol. The assessment of the plant's antioxidant properties was successfully conducted using the 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay, the ferric reducing antioxidant power (FRAP) assay, and the total antioxidant capacity (TAC) assay. The DPPH antioxidant potential of *S. vulgaris* extracts indicates that Dec-nBA extract from the leaf is remarkably more powerful than the other extracts, as evidenced by its half-maximal inhibitory concentration (IC_{50}) value of 320±0.1 ug/ml. Furthermore, the FRAP assay turned out to show that the same extract provided the greatest performance, exhibiting an IC_{50} value of 151.98±0.3 ug/ml. Overall, the leafy parts of *S. vulgaris* exhibited significantly greater antioxidant activity compared to the roots.

Keywords: *Silene vulgaris*, traditional medication, phytochemical content, antioxidant activity, secondary metabolites

1. Introduction

Medicinal plants have therapeutic advantages due to their antioxidant characteristics [1], which aid in safeguarding against several diseases, notably cancer, Alzheimer, and diabetes [2-4]. Additionally, they serve as a significant reservoir of antimicrobial substances in the pharmaceutical business [5]. Phytopathogenic diseases are highly detrimental illnesses that represent significant economic burdens on human societies [6]. The use of artificial antibiotics in managing contagious diseases may have been beneficial but also raises the risk of acquiring antibiotic resistance, leading to decreased effectiveness [7]. Moreover, synthetic antibiotics can pose a risk due to their adverse side effects in some cases. Hence, some people are inclined to choose natural therapies owing to their abundant natural resources and the comparatively lower incidence of adverse effects compared to manufactured medications [8]. The Silene genus, belonging to the Caryophyllaceae family, consists of about 700 species classified under 39 sections [9]. These species include biennials, annuals, and perennials and are predominantly located in America and moderate parts of the Northern Hemisphere in Eurasia and on the African continent [10, 11]. S.vulgaris is a species belonging to this family that can be found in several regions, including central Asia, the Mediterranean,

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Iraq, Italy, Iran, Turkey, Spain, Russia, and England [12–14]. In traditional healing practices, people used it to cure a variety of disorders. In Algeria, people refer to it by various vernacular names, primarily Tighighet and Tighecht. It is particularly familiar to kids in farming regions who enjoy playing with the inflated calyxes. Furthermore, many people use the roots for their diuretic properties, treatment of uterine problems, and elimination of intestinal worms [15–17]. The plant is a popular substitute for soap in laundry because its roots contain a significant amount of saponins, a compound known for its detergent qualities [18]. Several medical conditions, such as digestive issues and respiratory problems, have also responded to its use [19]. In addition, traditional cuisine in Algeria, Italy, Spain, Turkey, and Austria highly values young shoots [19–23].

Researchers have not yet assessed the phytochemical and antioxidant properties of the S. vulgaris plant found in the western region of Algeria. From this point on, we present a study about the phytochemical profile of S. vulgaris roots and leaves using a variety of solvents and extraction techniques. The antioxidant activity was evaluated by applying various techniques, including the DPPH assay, the FRAP assay, and the TAC assay

2. Materials and methods

2.1. Plant material

A weight of 5 kg of both aerial and soil parts of *S. vulgaris* (Fig. 1) were collected from the area of Ain Dehab in Tiaret city (located in the west of Algeria, semi-arid climate, latitude: 34.8440, longitude: 1.5500, altitude: 1084 meters) in November 2021 (roots) and May 2022 (leaves).

Dr.Achraf Khaldi (Teacher in the Department of Biology at Bechar University, Algeria) performed the ethnobotanical authentication and a voucher specimen was deposited at the herbarium of the laboratory under the code number EHB-SV05-07. In addition, the plant was molecularly identified using ITS primers and the sequence was uploaded to the Genbank database (accession number: PP316332.1).The plant parts were properly washed and subsequently dehydrated in a dry and ventilated place in the dark. Afterward, a crusher was used to grind them into a finer powder, which was stored at a very low temperature until further use.

Figure 1: The roots (A) and the aerial parts (B) of Silene vulgaris (Moench) Garcke

2.2. Extraction

Preparing plant extracts involves decoction and maceration-filtration processes. The methanol extraction process was performed twice (2×100 ml) on 10 grams of powdered dried parts of the plant. The residual marc of methanol was dried and then partitioned three times with N-butanol (3×100 ml) [24]. All the solvents evaporated using a rotary evaporator and vacuum (Hei-VAP value). Ten grams of the plant's powdered parts were combined with 100 ml of distilled water to make the aqueous decocted extract. For half an hour, the ingredients were heated in the reflux assembly until boiling, then filtered and concentrated to dryness. With the same proportion, the macerated extract was left in the agitator at 200 rpm. Following 24 hours, the mixture underwent filtration and further evaporation until complete dryness was achieved. The weight of all the extracts was measured to determine the yield and subsequently stored away from the light at 4 °C for further use [25].

2.3. Qualitative phytochemical screening

Phytochemical tests aid in the qualitative identification of the primary chemical groups found in the plant. Decoction and maceration were used as extraction methods. By using three different solvents-water, methanol, and n-butanol-it was possible to extract different types of molecules. We analysed this plant's phytochemical content using standard qualitative methods. Some critical methods used were alkaloids with Dragendorff reagent, sterols and terpenes with sulfuric acid and anhydride acetic acid, flavonoids with metallic magnesium, and hydrochloric acid [26, 27].

Furthermore, the foam index was determined by extracting 1 gram of plant material with 100 milliliters of distilled water using a reflux assembly for 30 minutes. After filtering the resulting aqueous solution, a range of dilutions from 1 to 10 were prepared in test tubes. Subsequently, each tube was vigorously agitated for a period of 15 seconds and then left untouched for 15 minutes to allow the foam to settle. The height of the foam was subsequently measured. We take the number of tube where the height of foam is above or equal to at least 1 cm and then determine the foam index according to the following formula:

$I\% = 1000/A$

"A" represents the volume, measured in milliliters, of the decoction used to prepare the dilution in the tube where foaming reaches a height of 1 cm [28].

2.4. Quantitative Phytochemical Screening

2.4.1. Total polyphenolic content

By using the Folin-Ciocalteu (FC) reagent, the polyphenol content has been quantified [29]. 1 ml of FC reagent that had been diluted tenfold was combined with 200ul of extract solution (1 mg/ml) to

create the reaction combination. Sodium carbonate (Na_2CO_3) , at a concentration of 7.5%, was added in 0.8 ml after 5 minutes. After 30 minutes of darkness, the absorbance reactions were quantified at a wavelength of 765 nm using a UV/Vis spectrophotometer (model 6715, Jenway) with methanol serving as a blank and a standard of gallic acid developed in various concentrations (20–180 ug/mL). The results were calculated using the calibration curve equation (Y=0.0045x+0, 2498; R^2=0.997) expressed in mg of GAE/g dry matter.

2.4.2. Total flavonoid content

The aluminium trichloride method [30] determined the total flavonoid concentration in *S. vulgaris*. A 200-ul extract solution (1 mg/ml) was added to 150-ul of sodium nitrite ($NaNO_2$). After 5 minutes, 150 ul of trichlorure of aluminium ($AlCl_3$) was added. Following a 6-minute incubation period at ambient temperature, 1 ml of sodium hydroxide (NaOH) solution (1M) was added to the mixture. Distilled water served as a reference sample, while quercetin was employed as a standard prepared at different concentrations (20–200 ug/ml). The absorbance was determined at 440 nm. Using the calibration curve calculation (Y = 0.0042x + 0, 0475; R^2 = 0.997), the total flavonoid content was determined and expressed in mg of QE/g of dry matter.

2.4.3. Total flavonol content

The aluminium trichloride method determined the total flavonol content using quercetin as a standard [31]. A volume of 500 ul extract solution (10 mg/ml) was added to 500 ul of $AlCl_3$ 2% dissolved in methanol. After adding 1.5 ml of sodium acetate ($C_2H_3NaO_2$) at 50 mg/ml to the combination, it was then incubated for two and a half hours in the dark. As a standard, a quercetin solution was prepared with different concentrations (10–100 ug/ml), and methanol was served as a blank. The absorbance was measured at a wavelength of 440 nm, and the results were obtained by applying the calibration curve equation (Y=0.0181x-0.0306; R^2 =0.997) expressed in mg of QE/g of dry matter.

2.5. Antioxidant Activity

2.5.1. Determination of DPPH free radical scavenging activity

The in vitro antioxidant properties of *S. vulgaris* extracts were assessed using the DPPH-free radical scavenging test method [32]. A stock solution was prepared in small glass vials by dissolving 4 mg of DPPH (0.004%) in 100 ml of methanol. We added 100 ul of each concentration of extract solution (15–1000 ug/ml) to 1900 ul of DPPH solution. Following a half hour of dark incubation, the disappearance of the DPPH reagent was read spectrophotometrically at a wavelength of 517 nm, and due to DPPH's light sensitivity, optimization experiments on the mixture's absorbance stability show that readings need to be performed immediately. For comparative analysis, we prepared a standard ascorbic acid solution with different concentrations (15–1000 ug/ml) under the same conditions. Pure methanol was used as the reaction blank. We performed the experiment in triplicate. The formula determined the DPPH's scavenging inhibitory effect:

Percentage Inhibition % = [(Absorbance of the control - Absorbance of the sample)/Absorbance of the control] x 100.

The IC_{50} is the dose of antioxidants that reduces the initial DPPH concentration by 50% [33].

2.5.2. Ferric-reducing antioxidant power (FRAP) assay

The antioxidant power was measured via the method stated in [34]. The technique relies on converting the colorless Fe^{3+} tripyridyltriazine (TPTZ) complex into the Fe^{2+}-TPTZ complex by reduction. The outcome of this chemical reaction yields a vivid blue color. 0.4 ml of samples made at various concentrations were combined with 1 ml of potassium ferricyanide K_3 [Fe (CN) $_6$] freshly prepared reagent at 1%, then vortex well. Following a 20-minute period of incubation at a temperature of 50 °C in a water bath, 1.2 ml of trichloroacetic acid ($C_2HCl_3O_2$) was introduced into the mix, which was then centrifuged for 10 minutes at 3000 rpm. The supernatant was combined with distilled water and 0.2 ml of 0.1% ferric chloride ($FeCl_3$), freshly prepared. At 700 nm, the absorbance was evaluated against a blank. As a reference standard, ascorbic acid was prepared at different concentrations. The linear calibration curve (y = 0.0089x + 0.0205; R^2 = 0.9984) was applied to assess the extract's antioxidant activity.

2.5.3. Total antioxidant capacity

The phosphomolybdenum approach was used to calculate the total antioxidant capacity, following the procedures provided by [32]. The test tubes containing 0.1 mL of each sample solution were filled with 1.0 mL of the reagent solution (containing 0.6 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After securing the tubes tightly, they were incubated for ninety minutes at 95 °C. After the sample reached room temperature, we measured the absorbance at 695 nm. We generated the blank solution by mixing 1 ml of the reagent solution with the proper volume of the sample's solvent. After that, the solution was incubated similarly to the other samples. As a positive control, ascorbic acid was subjected to the same conditions.

3. Results

3.1. Qualitative Phytochemical Screening

The preliminary phytochemical screening of bioactive compounds in root extracts of *S. vulgaris* showed the presence of coumarins, flavonoids, terpenoids, sterols, fatty acids, reducing agents,

alkaloids, and triterpenes, as well as a strong presence of saponosids in remarkable quantity (Table 1).
In addition to the previously mentioned substances, the tests conducted on leaf extracts revealed the presence of tannins and anthocyanins, which were absent in the roots, as shown in Table 1. Nevertheless, both parts did not include emodols, quinones, or volatile oils.

Table 1: Qualitative phytochemical screening of *S.vulgaris* parts

Secondary metabolites	Results	
	Roots	Leaves
Flavonoids	+	+
Saponosids	+++ I %= 333,33	++ I %= 166.66
Tanins	-	+
Coumarins	+	+
Sterols	+	+
Emodols	-	-
Alkaloids	++	+
Terpenoids	+	+
Quinone	-	-
Fatty acids	+	+
reducing agents	+	+
Triterpenoid	+	+
Volatile oils	-	-
Anthocyanin	-	+

(+) Presence, (++) Medium presence, (+++) Strong presence, (-) Absence. I % means foam index percentage

3.2. Quantitative phytochemical screening

The therapeutic advantages of medicinal herbs are derived from the chemical composition of the plants. Examining the chemical composition of plants enhances our understanding of their possible medical uses. Plant cells utilize the basic metabolic processes to generate enormous secondary metabolites from plants [35, 36]. Phenolic substances, a type of secondary metabolite of plants, have been confirmed to have considerable anti-inflammatory, antioxidant, and anticancer properties [36, 37].
Table 2 displays the average yields and quantitative tests of S. vulgaris leaves and roots extracts. The current research revealed that the components of *S. vulgaris* leaves extract contain phenolic (62.21, 40.04, 33.63, and 41.32 mg GAE/g dry extract), flavonoids (15.55, 8.61, 12.43, and 5.34 mg QE/g dry extract), and flavonol (10.4, 5.38, 11.32, and 20.60 mg QE/g dry extract) for the Mac-H_2O, Dec-H_2O, Dec-MeOH, and Dec-nBA extracts, respectively. For the root extracts, they contain polyphenols (0.95, 2.54, 6.21, and 13.63 mg GAE/g dry extract), flavonoids (0.33, 1.91, 4.43, and 10.61 mg QE/g dry extract), and flavonol (0.24, 1.18, 3.68, and 4.68 mg QE/g dry extract) for the Mac-H_2O, Dec-H_2O, Dec-MeOH, and Dec-nBA extracts, respectively. The results observed can be attributed to environmental factors, taxonomic variances, plant maturity, genetic changes in organs, and the state of secondary metabolism in different conditions for development [38].

Table 2. The yields and quantitative phytochemical analysis of *S.vulgaris* parts

Extracts	Yields (w/w %)		Polyphenol content		Flavonoid content		Flavonol content	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
Mac-H_2O	27.33	13.65	0.95±0.01	62.21 ±0.76	0.33 ±0.49	15.55 ±0.72	0.24±0	10.4±0.58
Dec- H_2O	23	12.1	2.54±0.02	40.04 ±0.1	1.91 ±0.1	8.61 ±0.27	1.18±0.27	5.38±0
Dec-MeOH	13.6	18.1	6.21±0.3	33.63 ±0.31	4.43 ±0.51	12.43 ±1.42	3.68±0.27	11.32±0.21
Dec-nBA	2.3	1.4	13.63±0.84	41.32 ±0.21	10.61 ±2.06	25.34 ±0.69	4.68±2.09	20.60±0.24

Values was obtained by calculating the average of three experiments and data are presented as Mean ± SEM .Phenolic Content "mg gallic acid/ g dry extract", flavonoid Content "mg quercetin/ g dry extract", flavonol Content "mg quercetin/ g dry extract". Yield % Expressed as (g dry extract/g dry root/leaves) *100.

3.5 Antioxidant activity

3.5.1 DPPH radical-scavenging activity

The antioxidant activity of extracts of *S. vulgaris* parts was assessed by their capacity to scavenge DPPH-free radicals. The IC_{50} value represents the amount of antioxidants required to reduce the starting DPPH concentration by half. Better antioxidant potential correlates with a lower IC_{50} value [39]. Fig. 2 and Table 3 provide the DPPH radical scavenging activity of *S.vulgaris* extracts.

The results demonstrate that the leaves extracts, mainly the Dec-nBA extract with an IC_{50} of 320 ug/ml, have the greatest antioxidant activity compared to ascorbic acid (IC_{50} of 90 ug/ml) (Figure 5),, followed by Dec-MeOH, Dec-H_2O, and Mac-H_2O (599 ug/ml, 695 ug/ml, and 823 ug/ml), respectively. Furthermore, the root extracts show weak antioxidant activity compared to ascorbic acid and leaves extracts. The greatest root extract was Dec-nBA, followed by Dec-H_2O, Mac-H_2O, and Dec-MeOH (1320 ug/ml, 1870 ug/ml, 3080 ug/ml, and 3460 ug/ml), respectively.

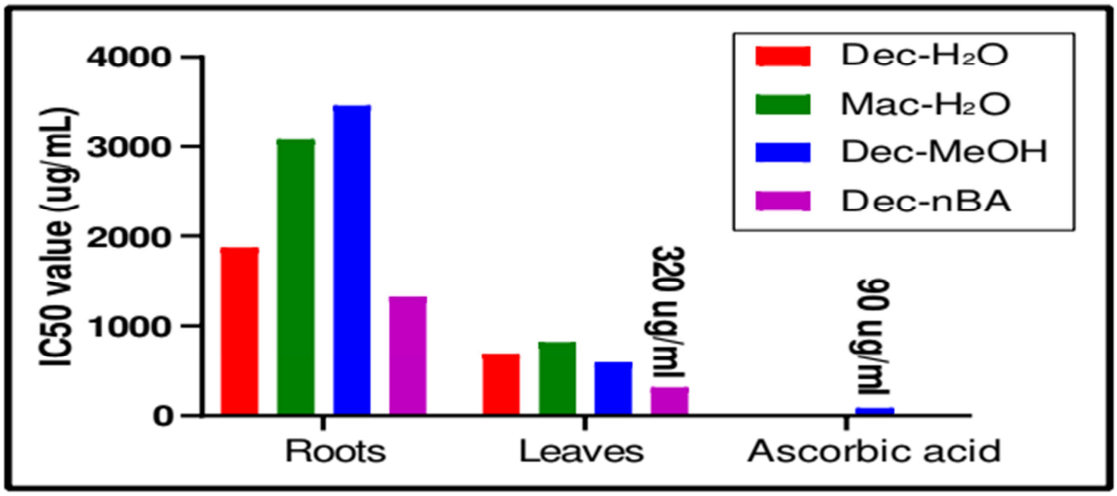

Figure 2: DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *S.Vulgaris* roots and leaf extracts

Table 3: Antioxidant activity (DPPH scavenging, FRAP assay, TAC assay) of the different extract of *S.vulgaris* parts

Extracts	DPPH test IC50		Fe2+ chelating activity IC50		Total antioxidant capacity IC50	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
Mac-H_2O	3080±0	823±0.061	>1000	446.82±0.2	830 ±0.01	1990 ±0.12
Dec- H_2O	1870±0.01	695±0.029	>1000	240.52±0.11	860 ± 0.02	1500 ±0.1
Dec-MeOH	3460±0.04	599 ±0.020	566.77±0.03	198.96± 0.08	1390 ±0.41	710 ±0.03
Dec-nBA	1320±0.034	320±0.1	259.51±0.04	151.98±0.3	ND	ND
Standard	AA		AA		AA	
	90±0.002		53.59±0.003		130 ±0.1	

Values was obtained by calculating the average of three experiments and data are presented as Mean ± SE. IC_{50}: the antioxidant concentration capable of diminishing 50% of the used DPPH radical. ND is referred to as the not detected value. AA: ascorbic acid.

3.5.2 FRAP assay

Ferric-reducing antioxidant power was detected in all the extracts of *S.vulgaris* roots (Fig. 3, Table 3): very weak in aqueous extracts (Mac-H_2O and Dec-H_2O>1000 ug/ml) compared to the reference compound (ascorbic acid: 53, 59 ug/ml) (Figure 5) and the organic solvent performed well (Dec-MeOH: 566.77 ug/ml, Dec-nBA: 259.51 ug/ml). The antioxidant activity of the leaves extracts was greater than that of the root extracts.

The IC_{50} of the aqueous extracts (Mac-H_2O and Dec-H_2O) were 446.82 ug/ml and 240.52 ug/ml, respectively. For organic solvents, the Dec-nBA extract was strong with an IC_{50} value of 151.98 ug/ml, followed by the Dec-MeOH extract with 198.96 ug/ml.

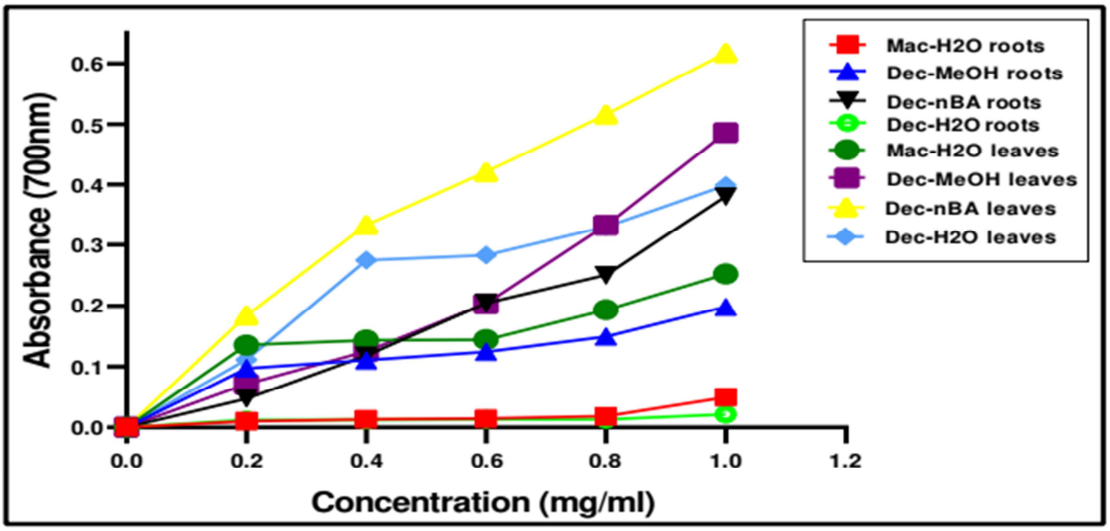

Figure 3: FRAP assay of *S.Vulgaris* roots and leaves extracts in different solvents

3.5.3 Total antioxidant capacity (TAC) assay

S.vulgaris extracts' total antioxidant capacity was determined by reducing molybdenum (VI) to molybdenum (V) using antioxidants. Subsequently, a phospho-molybdenum (V) compound is formed under acidic reaction conditions. The findings illustrated in Figure 4 and Table 3 demonstrate that the Dec-MeOH extract from the leaves (IC_{50} of 710 ug/ml) exhibited stronger antioxidant activity than all other extracts but was lower than that of ascorbic acid (130 ug/ml) (Figure 5). For the roots, the aqueous extracts (Mac-H_2O: 830 ug/ml and Dec-H_2O: 860 ug/ml) were much better than Dec-MeOH (1390 ug/ml).

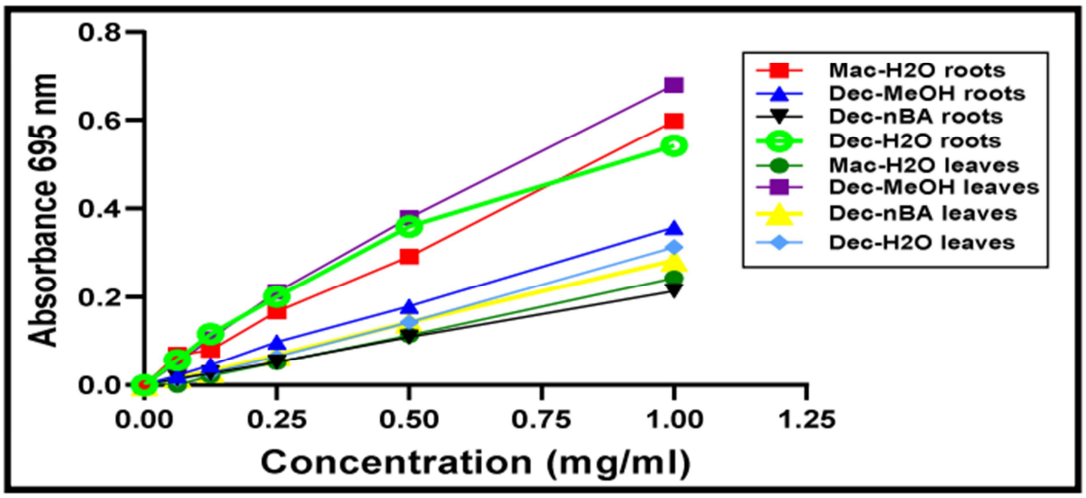

Figure 4: TAC assay of *S.Vulgaris* roots and leaves extracts

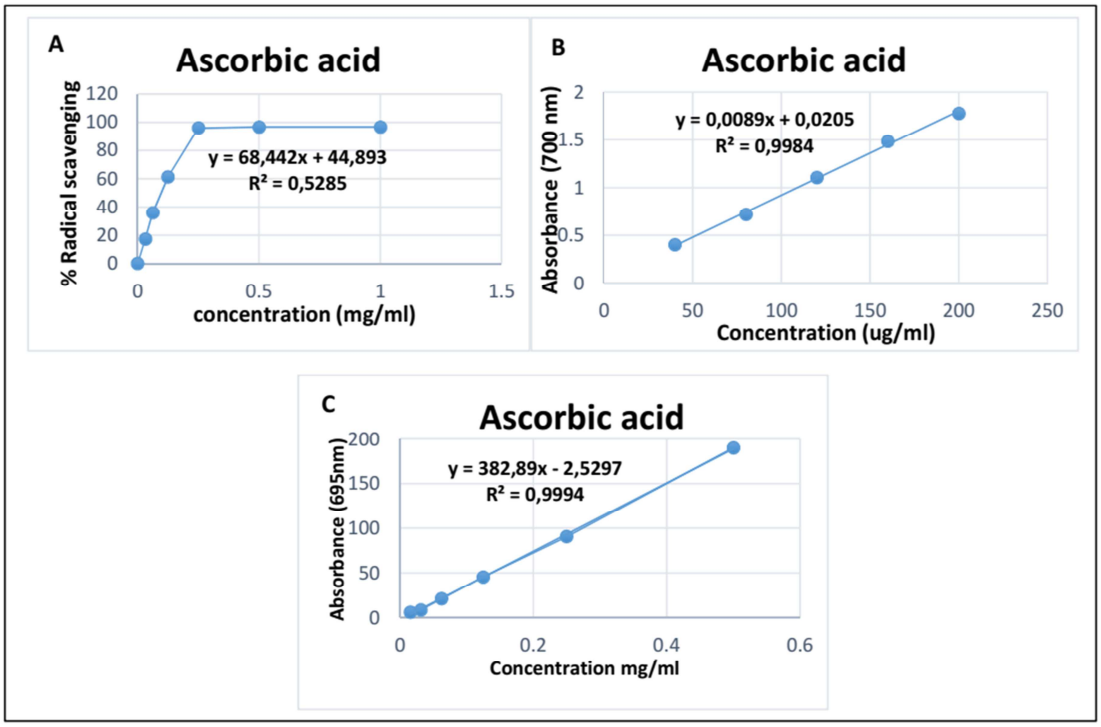

Figure 5: The calibration curves of the antioxidant activity of the used standard (Ascorbic acid), **A**: DPPH assay, **B**: FRAP assay, **C**: TAC assay

4. Discussion

Known by most as an emollient, *S.vulgaris* roots are frequently used in cleaning routines as disinfectant. Moreover, the young leaves can be consumed either in their raw form as a salad or cooked in a manner similar to spinach. The juice derived from *S.vulgaris* can be used for treating ophthalmia and serves as a universal antidote for poisoning. It is also used as a therapy for constipation and intestinal discomfort. Owing to these benefits, the current biological study was launched to evaluate the phytochemical and antioxidant characteristics of *S.vulgaris*, a native edible plant grown wild in Algeria. Using the calibration curve's regression equation, the extract's total polyphenol content was determined and represented as gallic acid equivalent. The total amount of polyphenols found in the leaves was 3.35± 0.12 (mg gallic acid/g of extract, as reported by Smahane et al, 2015 [40]. The current screening results conducted on the Mac-H_2O extract of the leaves were computed as 62.21 mg Gallic acid /g of extract, which justifies its large consumption and benefits. Polyphenolic substances are recognised for their efficacy as antioxidants in plants owing to the presence of hydroxyl groups, which effectively shield cells from the oxidative damage inflicted by free radicals [41- 42].

The flavonoid content recorded in the Dec-nBA extract was higher than in the Dec-MeOH extracts; this indicates that selecting the appropriate solvent is critical to extracting the most significant possible amount of secondary compounds with powerful antioxidant properties. The total flavonol content of the Dec-MeOH extract of the leaves is 15.32 mg expressed as quercetin equivalent in micrograms per g of extract. It is possible that *S.vulgaris* leaves help shield against oxidative stress-related illnesses, including cancer, heart disease, and neurological disorders, due to their antioxidant activity. IC_{50} value of the results of the DPPH assay can offer good insight into the antioxidant capacity of *S.vulgaris*. Compared to IC_{50} values reported in prior research, the IC_{50} value for the root Mac-H_2O, as determined by DPPH, was the same in root extract and higher in leaf extract, especially for the Dec-nBA extract with an IC_{50} of 320 ug/ml. Compared to earlier research findings, the IC_{50} value in the S. vulgaris extracts was investigated using the FRAP assay. The reducing capacity of a

molecule is often determined by the similarity in the inhibition pattern at different concentrations, which is influenced by the presence of reductants [43]. By giving up a hydrogen atom, these reductants can break up the chain of free radicals and act as antioxidants [43]. The only technique that can determine the precise concentration of antioxidants in a sample is the FRAP test. Indirect categorization is applied by the remaining assays as they measure the degree of inhibition of reactive substances (free radicals) produced in the reaction mixture. However, the TAC of the roots was considerably greater than the results of the DPPH results, indicating that more than two methods must be used to assess a plant's antioxidant activity.

The outcomes of these assays are heavily influenced by the specific kind of reactive species employed [35]. The key feature of this outcome is that multiple variables can affect antioxidant properties, like the part of the plant under investigation and its unique variables (such as the nature of substances and released chemical compounds), as well as the characteristics of solvents employed (such as kind and polarity) and the technique of extraction (in this case, decoction and maceration). Moreover, *S.vulgaris* extracts' antioxidant properties may be correlated with the sample's total polyphenol, flavonoid, and flavonol content.

5. Conclusion

S.vulgaris (Moench) garcke roots and leaves extracts were tested in this study to evaluate phytochemical and antioxidant properties in vitro. The findings reveal the detection of secondary metabolites in both plant parts, such as flavonoids, alkaloids, polyphenols, saponosids, sterols, terpenoids…etc. Furthermore, it turned out that the most effective solvent to extract antioxidants from leaves was n-butanol. These results justified the extensive consumption of *S.vulgaris* leaves by the local population to support their health care and energy, indicating we could use the potential effectiveness of the crude extracts as an antioxidant agent.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

"None"

7. References

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