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Phytochemical Screening and Antioxidant Potential of *Lotus corniculatus* and *Amaranthus viridis*

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THE QUALITIES of plant secondary metabolites as a novel source of natural antioxidants have recently become the focus of research in this field. The current study's objectives were to assess the phytochemical profile, find any natural medicinal compounds present, and determine the antioxidant capacity of two Egyptian toxic plants, *Amaranthus viridis* (Amaranthaceae) and *Lotus corniculatus* (Fabaceae) These plants are widespread in Egypt's polluted lands and have not attracted much attention. According to the data, the amounts of linoleic acid, oleic acid, behenic acid, TSFA (total saturated fatty acids), and TUSFA (total unsaturated fatty acids) in the seeds of *Lotus corniculatus* were 40.13, 28.88, 0.49, 22.14, and 83.62 %, respectively. Additionally, the amounts of linoleic acid, palmitic acid, lignoceric acid, TSFA, and TUSFA in *Amaranthus viridis* were 45.2, 23.15, 0.13, 19.99, and 75.18 %, respectively. Protein, tanines, HCN, saponins, glycosides, nitrate, essential elements, polyphenolic substances (flavonoids and phenolics), amino acid composition, and antioxidant potential, $ABTS^{\bullet+}$[2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], [$DPPH^{\bullet}$(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and ferric-reducing antioxidant power (FRAP)] in both plants were quantitatively measured. Both plants include naturally occurring antioxidant chemicals that are clearly capable of removing reactive oxygen species, which then significantly improves the cellular equilibrium (the oxidant/antioxidant process) and protects typical physiological conditions. As a result, the two plants may be employed as potential sources of compounds with antioxidant characteristics in the cosmetics, pharmaceutics, and food industries.

Keywords: Antioxidant capacity, Egyptian poisonous plants, Photochemical profile, Polyphenols, Phenolic compounds.

Introduction

In developed nations, where the use of herbal remedies is prevalent for people's fundamental healthcare requirements, there is an increasing interest among the scientific community in studying chemicals with plant origins (Moussa & Hassen, 2018; Yadav, 2018). A wide variety of toxic plants produce compounds that may cause clinical symptoms in humans, some of which have caused severe poisonings. A few plants give rise to serious poisoning after ingestion of even a limited amount of plant material (Frohne et al., 2004). Four poisonous weeds (*Datura stramonium, Chenopodium murales, Lotus corniculatus,* and *Amaranthus viridis)* were collected from the Nile Delta coast of Damietta Province (Mamdouh et al., 2020a& b; Moussa et al., 2022; Mohesien et al., 2023). *Lotus corniculatus* (Fabaceae) is a wild plant that grows in Egypt's Mediterranean region,

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along the banks of the Nile, on cultivated land, and in marshes (Boulos, 2004). Proanthocyanidins, tanines, flavonoids, oleanolic acid, and saponins were shown to be present in these species and have potential uses as contraceptives, treatments for peptic ulcers, sexually transmitted diseases, and treatment and as antioxidants and anticancer agents (El Mousallami et al., 2002; Khalighi-Sigaroodi et al., 2012). *Amaranthus viridis* L. is a perennial herb in the Amaranthaceae family, a decoction of the entire plant is used as antihyperlipidemic, antidiabetic, and antioxidant (Ashok et al., 2011), antiproliferative and antifungal lectin (Kaur et al., 2006), constipation and urination-related irritation are both treated with the root juice (Manandhar, 2002). Amaranthus species is a blooming plant that has historically been used for both food and medicine (Bokaeian et al., 2013). The Amaranthus and Lotus genera are weeds that have taken over the New Damietta Botanical Garden (Serag et al., 2020). Furthermore, ruminants that ingest Lotus spp. do not experience meteorism, a nutritional condition, because the condensed tanines precipitate the proteins and prevent foam from forming in the rumen (Ramírez-Restrepo et al., 2004; Acuńa et al., 2008). Condensed tanines have been shown in studies to be an additional option to chemical anthelmintics for the management of gastro-intestinal parasitic nematodes (Niezen et al., 1995). Alkaloids, tanines, saponins, and cardiac glycosides were discovered to have both pharmacological and physiological activity in their crude yield (Pietta, 2000), in an effort to stop damaging free radicals, such as ROS, from oxidizing cells (Choi et al., 2007). Organic molecules known as "secondary metabolites" are predominantly produced by plants as byproducts of their metabolism. They have important pharmacological and biological characteristics and are therefore employed in medicine (Wink, 2015).

In this study, the classes of secondary metabolites taken into account included saponins, polyphenols (flavonoids and phenolics), and tanines. Saponins are glycosidic substances with the ability to interact with many elements of cell membranes, including cholesterol and phospholipids, due to their amphipathic character. According to reports, saponins are powerful *in vitro* antioxidants (Gülçin et al., 2004). So, based on the increased antioxidant activity, we expected that the crude extract with a higher amount of these secondary metabolites would have considerable free radical quenching characteristics (ABTS, DPPH, and FRAP). The antioxidant properties of polyphenols are well recognized (Scalbert et al., 2005). Their capacity to establish hydrophobic and ionic interactions with proteins is what gives them their bioactivity (Wink, 2015). In vitro, flavonoids have antioxidant effects (Pietta, 2000). Tanines as proton donors have the antioxidant property of scavenging free radicals (Amarowicz, 2007; Nsimba et al., 2008). Amaranth seeds have recently been shown to have a variety of phytonutrients that play a key role in inhibiting oxidative chain reactions as well as free radical interactions with tissue and membranes (Nsimba et al., 2008). Antioxidant properties of polyphenols are well recognized (Scalbert et al., 2005); their bioactivity comes from their ability to form hydrophobic and ionic bonds with proteins (Wink, 2015), In vitro, flavonoids have antioxidant effects (Pietta, 2000; Moussa & Mohamed, 2016). Free radical scavenging is one of the antioxidant characteristics of tanines (proton donors) (Amarowicz, 2007). Amaranth seeds have recently been shown to have a variety of phytonutrients that play a key role in inhibiting oxidative chain reactions as well as free radical interactions with tissue and membranes (Nsimba et al., 2008; Moussa et al., 2023). *Amaranthus viridis* and *Lotus corniculatus* were chosen for the study because of their potential as natural medicines and to be evaluated for their phytochemical content and antioxidant capability.

Materials and Methods

Study area

Damietta Province is located in the downstream part of the Damietta branch of the River Nile at 31° 25' 10'' north to 31° 48' 54'' east N-32° 00' longitude to the north east of the Nile Delta region of Egypt. The coast of Damietta Governate extends from El-Deeba village (about 20 km from Port-said) to Gamasa at west along the Mediterranean Sea for about 42km. This province is bounded by Lake Manzala at the east, Mediterranean Sea from the north and El-Dakahlia Governate from the west and the south. The total average area of Damietta Province is about 1029 Km^2 and the total agricultural area is about 48675 hectares (Mamdouh et al., 2020b; Moussa et al., 2022).

Collection and identification of plant materials

Two poisonous plants, *Amaranthus viridis* (Amaranthaceae) and *Lotus corniculatus* (Fabaceae) were collected from 0.5m × 0.5m area during summer of 2020 across the Nile Delta coast

from Damietta Province. Different healthy parts of leaves, stem, and roots of *Lotus corniculatus* and *Amaranthus viridis* were washed under tap water followed by distilled water and air-dried at room temperature in the shade and then ground to a coarse powder by mechanical mills (Moussa et al., 2022).

Preparation of crude extracts

The dried powder (100g) was extracted with 1000mL of ethyl alcohol as the polar solvent at -4°C. After 72h, the resulting extracts were filtered through Whatman™ no. 1, and the solvent filtrates were concentrated on rotary evaporator under reduced pressure and a BenchTop Lyophilizer. The extracts were stored at -4°C until further utilization in the designated experiment (Sima et al., 2018; Ezekwe et al., 2021).

Metabolomic profiling and an antioxidant assay of Lotus corniculatus and Amaranthus viridis

Flavonoids and phenolic fractions of polyphenols were assessed using HPLC (Waters, USA) by the techniques of Abdel-Farid (2020) and Rodriguez-Bernaldo et al. (2010). Fatty acids were identified by Christie (1990). Amino acids Profiling by the technique of Adeyeye & Afolabi (2004). Evaluation of condensed tanines (proanthocyanidins) by the methodology of Morel et al. (2014). The method described in Ebrahimzadeh & Niknam (1998) was used to identify saponins. The crude protein content was calculated by the method of Bradford (1976). Glycosides were determined by the method of Tyler (1994). The nitrate assay was determined by the method of Lufei & Yong (2017). The hydrogen cyanide (HCN) content was estimated by the method of Pushpa et al. (2019). Minerals analysis were investigated by the method of Kocak et al. (2011).

Antioxidant capacity

Antioxidant potential, $ABTS^{\bullet+}$[2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], [$DPPH^{\bullet}$(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and ferric-reducing antioxidant power (FRAP)] in both plants were established.

$DPPH^{\bullet}$(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The method described by Sayed et al. (2018) and Chu et al. (2000) was applied to assess the $DPPH^{\bullet}$(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the ethanolic extracts of *Lotus corniculatus* and *Amaranthus viridis*. Briefly, 1mL of the ethanolic extract of *Lotus corniculatus* and *Amaranthus viridis* was added to 0.5mL of a methanolic $DPPH^{\bullet}$ solution (100µM) at different concentrations (25, 50, 75, and 100µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30min. The absorbance was measured at 515nm. using a Unicum UV-300 UV/Vis spectrophotometer. Butylated hydroxytoluene (BHT) was used as a positive control, while the negative control only contained $DPPH^{\bullet}$ and methanol. The data was expressed as a percentage of $DPPH^{\bullet}$ radical scavenging activity and calculated as follows:

$DPPH^{\bullet}$ radical scavenging activity (inhibition %)= (Ac-As/Ac) X 100

where AC is the absorbance of the control reaction, and AS is the absorbance of the seaweed extract. The results were expressed as IC50 [the concentration (µg/ml) of the ethanolic extract of *Lotus corniculatus* and *Amaranthus viridis* that scavenges 50% of $DPPH^{\bullet}$ radicals]. All determinations were performed in triplicate. The lower the EC50, the higher the antiradical efficiency.

ABTS•+[2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity

The experiment was performed according to Arnao et al. (2001). In brief, ABTS radical cations ($ABTS^{\bullet+}$) were generated by reacting to the strong oxidizing agent potassium persulfate (2.6mM) with ABTS salt (7.4mM). The reaction was prepared by mixing equal volumes (5mL) of the two reagents and then kept in the dark for 12–16h at room temperature. After that, the solution was diluted by adding 60mL of methanol to 1mL of $ABTS^{\bullet+}$ radical cations to obtain an absorbance value of 1.1±0.02 at 734nm using Unicum UV-300UV/Vis spectrophotometer. The ethanolic extracts of *Lotus corniculatus* and *Amaranthus viridis* (150µL) were allowed to react with 2850µL of the freshly prepared $ABTS^{\bullet+}$ radicals at different concentrations (25, 50, 75, and 100µg/mL) for 2h in the dark and at room temperature. The absorbance was measured at 734nm. Trolox was used as a positive control. All determinations were performed in triplicate. Data was expressed as $ABTS^{\bullet+}$ radical scavenging activity % and calculated as follows:

$ABTS^{\bullet+}$ radical scavenging activity (inhibition %)= (Ac-As/Ac) X 100

where AC is the absorbance of the control, and AS is the absorbance of the ethanolic extract of *Lotus corniculatus* and *Amaranthus viridis*. The results were expressed as IC50 [the concentration (µg/ml) of the ethanolic extract of *Lotus corniculatus* and *Amaranthus viridis* that inhibits 50% of $ABTS^{\cdot+}$ radicals].

Ferric reducing antioxidant power (FRAP) assay

The total reducing capacity of the ethanolic extracts of *Lotus corniculatus* and *Amaranthus viridis* depends on its ability to reduce Fe^{+3} to Fe^{+2} according to the method of Oyaizu (1986). The reducing power of the ethanolic extract of *Lotus corniculatus* and *Amaranthus viridis* was determined following the method of (Kuda et al., 2005). 1mL of the ethanolic extract of *Lotus corniculatus* and *Amaranthus viridis* at different concentrations (25, 50, 75, and 100µg/mL) was thoroughly mixed with 2.5mL of 50mM phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide. After the incubation of the mixture at 50°C for 20 min, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000×g for 10min. The upper layer (1.25mL) was mixed with 1.25mL of deionized H_2O_2 and 0.25 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700nm using a Unicum UV-300 UV/Vis spectrophotometer. Higher absorbance indicates a higher reducing power. The assay was carried out in triplicate. Butylated hydroxytoluene was used as a positive control, and the results were expressed as EC50.

Statistical analyses

Statistical analysis was performed with SPSS Version 17 statistic software package. Data were expressed as means ± standard error (SE) as described by Richard & Gouri (2019).

Results

Fatty acids

Information about the fatty acid content of seeds (g/100g DW) in *Lotus corniculatus* and *Amaranthus viridis* is listed in Table 1. There are eleven fatty acids in each of the two plants. In *L. corniculatus,* the maximum value was 40.13% for linoleic acid, followed by 28.88% for oleic acid, and the minimum value was behenic acid at 0.49% as shown in Table 1. Also, our data in Table 1 showed that the TSFA concentrations of the *L. corniculatus* seed were 22.14% and the TUSFA content was 83.62%. However, the data in Table 1 showed that in *Amaranthus viridis*, the maximum value was 45.2% for linoleic acid, followed by 23.15% for palmitic acid, and the minimum value was 0.13% for lignoceric acid. Our results in Table 1 show that the TSFA concentrations of *Amaranthus viridis* were 19.99% and the TUSFA concentration was 75.18%.

TABLE 1. Fatty acid composition of seeds (g/100g DW) in *Lotus corniculatus* and *Amaranthus viridis*

Fatty acid components	*Lotus corniculatus*	*Amaranthus viridis*
14:0	0.82 ± 0.06	0.25±0.02
16:0	19.98±1.79	23.15±2.01
16:1Δ9	0.75 ±0.04	0.20±0.02
18:0	2.81±0.23	2.92±0.13
18:1Δ9	28.88 ±3.18	24.7±1.98
18:2Δ9,12	40.13±4.81	45.22±2.73
18:3Δ9,12, 15	0.98±0.07	0.92±0.05
20:0	2.05±0.21	0.72±0.06
20:1	3.06±0.27	0.22±0.02
22:0	0.49±0.06	0.18±0.04
24:0	0.92±0.08	0.13±0.05
TSFA	22.14±2.77	19.99±2.39
TUSFA	83.62±8.22	75.18±9.36

14:0: myristic acid, 16:0: palmitic acid, 16:1Δ9: palmitoleic acid, 18:0: stearic acid, 18:1Δ9: oleic acid, 18:2Δ9,12: linoleic acid, 18:3Δ9,12,15: linolenic acid, 20:0: arachidic acid, 20:1: gadoleic acid, 22:0: behenic acid, 24:0: lignoceric acid, TSFA: Total saturated fatty acid, TUSFA: Total unsaturated fatty acid. Values are mean±SE of samples in triplicate.

Chemical constituents (protein, tanines, HCN, saponins, glycosides, and nitrate)

The data for quantitative estimation of the percentages of crude chemical constituents (protein, tanines, HCN, saponins, glycosides, and nitrate) in *L. corniculatus* and *A. viridis* are summarized in Table 2. In *Lotus corniculatus*, the protein, HCN, glycosides concentrations are respectively being higher than those in *Amaranthus viridis*. However, tanines, saponins, nitrate contents respectively in *Amaranthus viridis* are higher than *Lotus corniculatus*. Protein content is higher in the seeds of the two plants than in the stems and leaves. Tanines, HCN, saponins, glycosides, and nitrate contents, on the other hand, are higher in the stems and leaves of the two plants than in their seed.

Profile of amino acids

Findings regarding the amino acid composition of *A. viridis* and *L. corniculatus* aerial parts are listed in Table 3. There are typically 24 standard amino acids found as constituents of proteins. *Lotus corniculatus* had eleven amino acids, and *A. viridis* had thirteen amino acids. However, some of the amino acids are not detected in either *L. corniculatus* or *A. viridis* (Table 3). *Lotus corniculatus* had a maximum value of tryptophan of 8.45%, followed by arginine at 4.38%, proline at 4.28, alanine at 3.12%, and lysine at 3.08%. Our data showed that *Amaranth* grains have a high content of tryptophan (4.13%) and lysine (3.81%).

Phenolic compounds

The data for phenolic compounds in aerial parts of *L. corniculatus* and *A. viridis* were summarized in Table 4 and Figs. 1, 2. *Lotus corniculatus* contains eleven fractions, but *A. viridis* contains ten fractions. The major phenolics of the two weeds, *L. corniculatus*, and *A. viridis*, were hydrocarbon compounds: pyrogallol (1.32 and 0.86mg/100g DW), caffeine (1.01 and 1.12mg/100g DW), and benzoic acid (0.43 and 0.87mg/100g DW), respectively. The hydroxycinnamic acid derivative, *P*-coumaric (0.87mg/100g DW), was identified only in *A. viridis*. Meanwhile, coumarin (0.21mg/100g DW), catechol (0.09mg/100 g DW), and catechin (1.02mg/100g DW) were identified only in *L. corniculatus*. The other phenolic acids showed small amounts in the two plants.

Flavonoids compounds

The flavonoid compounds of *L. corniculatus* and *A. viridis* were summarized in Table 5 and Figs. 3, 4. *Lotus corniculatus* contains twelve fractions, but *A. viridis* contains eleven fractions. The data show that the maximum value in *L. corniculatus* was quercetrin at 3.98mg/100g DW and the minimum value was hesperidin at 0.28mg/100g DW. However, in *A. viridis*, naringenin had the maximum value of 3.32mg/100g DW and kaempferol-3-2-*p*-comaroyl had the lowest value of 0.10mg/100g DW.

TABLE 2. Quantitative study of the phytochemical components of *Amaranthus viridis* and *Lotus corniculatus*

Parameters	*Lotus corniculatus*		*Amaranthus viridis*	
	Seed	**Stem + leaves**	**Seed**	**Stem + leaves**
Protein (g/100g DW)	40.01±2.41	29.18±2.33	28.13±1.69	17.76±1.43
Tanines (g/100g DW)	0.89±0.06	1.73±0.10	2.63±0.10	3.78±0.26
HCN (mg/100g DW)	8.98±0.89	28.23±2.54	7.15±0.43	12.23±0.74
Saponins (g/100g DW)	23.35±1.87	65.34±5.87	30.82±1.85	61.22±4.89
Glycosides (g/100g DW)	36.0 ± 0.94	71.3±0.82	29.0 ± 0.87	64.3±0.92
Nitrate (mg/100g DW)	8.99±0.54	35.34±2.83	12.67±0.53	31.45±1.88

Values are mean±SE of samples in triplicate.

TABLE 3. Amino acid composition in seeds (g/100g DW) of *Lotus corniculatus* and *Amaranthus viridis*

Amino acids	*Lotus corniculatus*	*Amaranthus viridis*
Alanine	3.12±0.09	2.12±0.06
Arginine	4.38±0.08	2.01±0.08
Asparagine	-	-
Aspartic acid	-	-
Cysteine	0.28±0.01	0.76±0.04
Glutamic acid	-	-
Glutamine	-	-
Glycine	0.98±0.07	2.87±0.14
Histidine	-	0.56±0.03
Isoleucine	2.18±0.17	0.76±0.04
Leucine	1.12±0.03	0.97±0.06
Lysine	3.08±0.12	3.81±0.03
Methionine	-	0.19±0.01
Phenylalanine	-	0.86±0.04
Proline	4.28±0.29	3.11±0.25
Serine	2.11±0.10	-
Threonine	-	0.88±0.06
Tryptophan	8.45±0.04	4.13±0.17
Tyrosine	-	-
Valine	2.05±0.16	-

Values are mean±SE of samples in triplicate.

TABLE 4. Phenolic compounds (mg/100g DW) in aerial parts (stem+leaves) of *Lotus corniculatus* and *Amaranthus viridis* using HPLC

Phenolic compounds	*Lotus corniculatus*	*Amaranthus viridis*
Gallic acid	0.28±0.01	0.56±0.01
Catechol	0.09±0.01	-
Caffeine	1.01±0.02	1.12±0.03
P-Coumaric acid	-	0.87±0.02
α-Coumaric acid	0.10±0.01	0.31±0.01
4-Aminobenzoic acid	-	0.19±0.001
Coumarin	0.21±0.01	-
Chlorogenic acid	0.19±0.01	1.23±0.02
Benzoic acid	0.43±0.02	0.87±0.03
Catechin	1.02±0.03	-
Vanillic acid	0.16±0.02	1.01±0.02
Caffeic acid	0.33±0.01	0.54±0.02
Pyrogallol	1.23±0.02	0.86±0.01

Values are mean±SE of samples in triplicate.

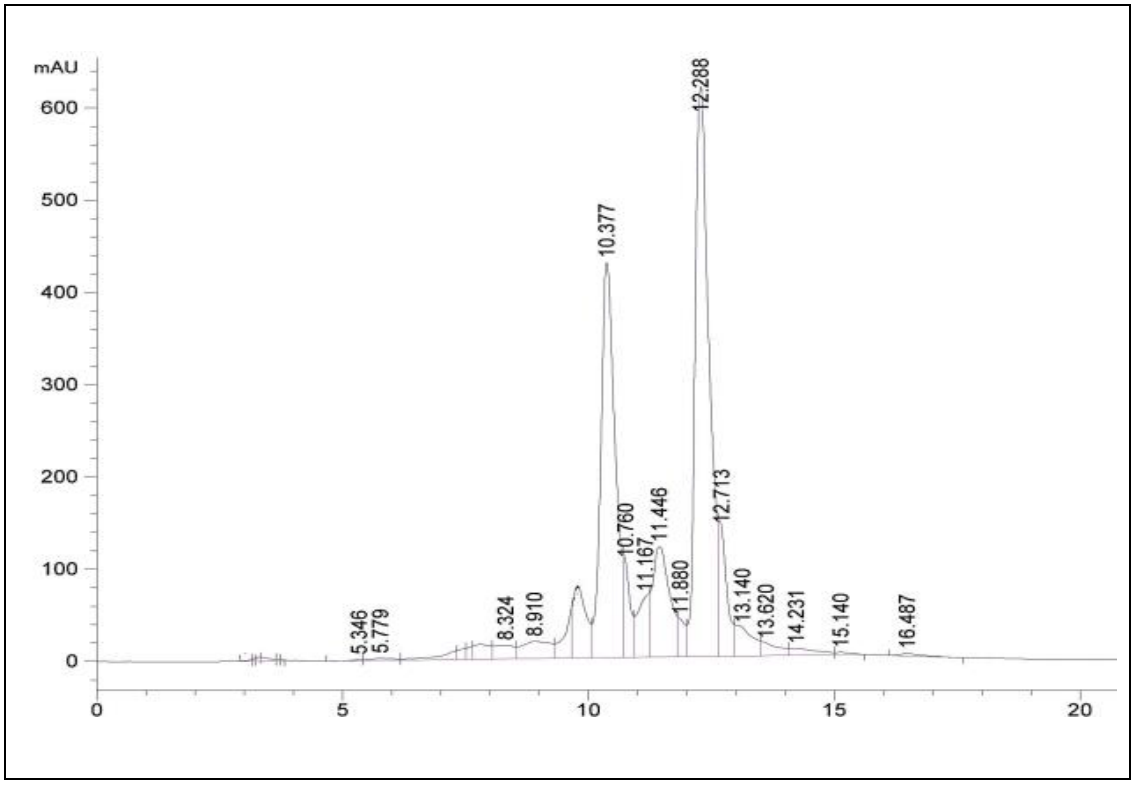

Fig. 1. Phenolic compounds in *Lotus corniculatus* by HPLC spectra

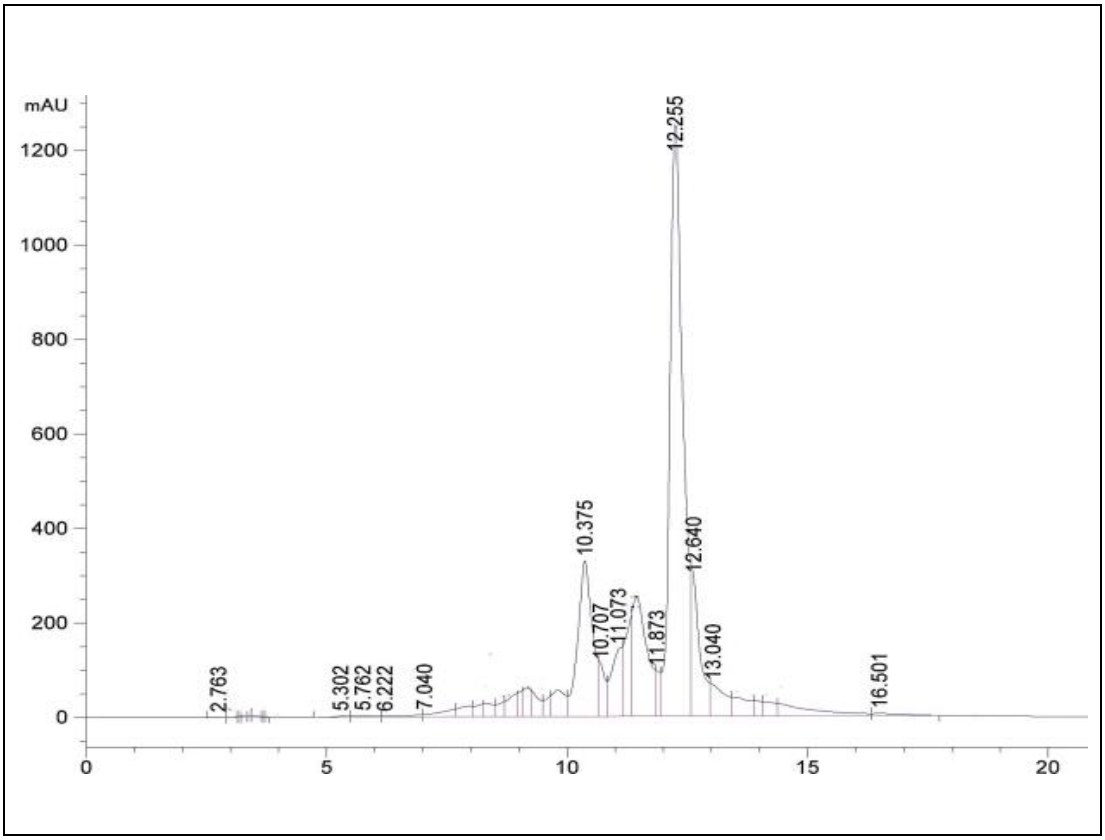

Fig. 2. HPLC spectra of flavonoid compounds in *Amaranthus viridis*

TABLE 5. Flavonoids compounds (mg/100g DW) in aerial parts (stem+leaves) of *Lotus corniculatus* and *Amaranthus viridis* using HPLC

Flavonoids compounds	*Lotus corniculatus*	*Amaranthus viridis*
Kaempferol	0.23±0.01	0.45±0.01
Quercetin	2.12±0.06	1.88±0.17
Hesperetin	0.16±0.01	-
Rutin	2.76±0.07	3.01±0.12
Quercitrin	3.98±0.16	2.45±0.15
Naringenin	2.14±0.17	3.32±0.18
Apigenin	0.67±0.02	0.45±0.01
Hesperidin	0.28±0.01	0.51±0.03
Rosmarinic	-	0.88±0.05
Naringin	2.08±0.08	1.75±0.15
Luteolin-7-O-glucoside	1.22±0.12	2.33±0.17
Kaempferol-3-2-p-comaroyl	0.19±0.02	0.10±0.01

Values are mean±SE of samples in triplicate.

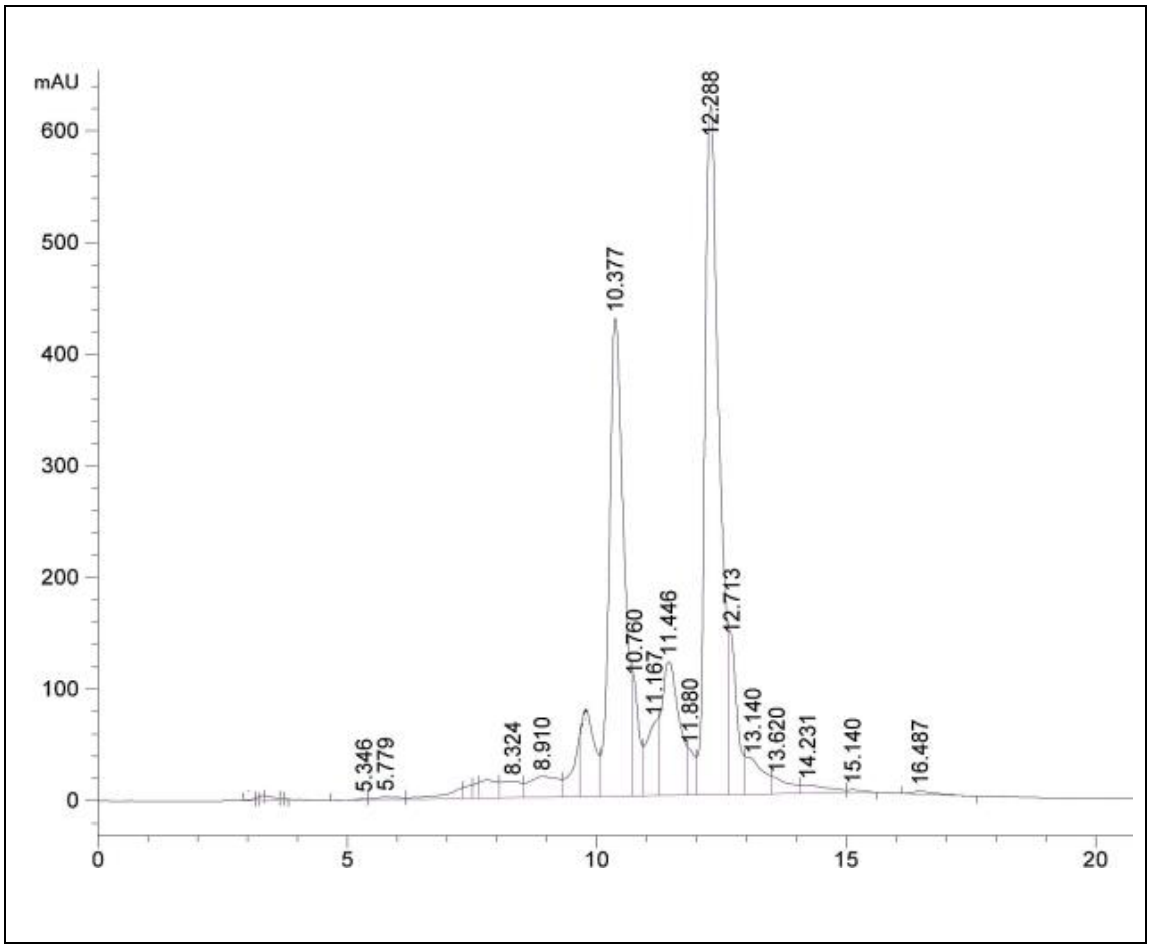

Fig. 3. HPLC spectra of flavonoid compounds in *Lotus corniculatus*

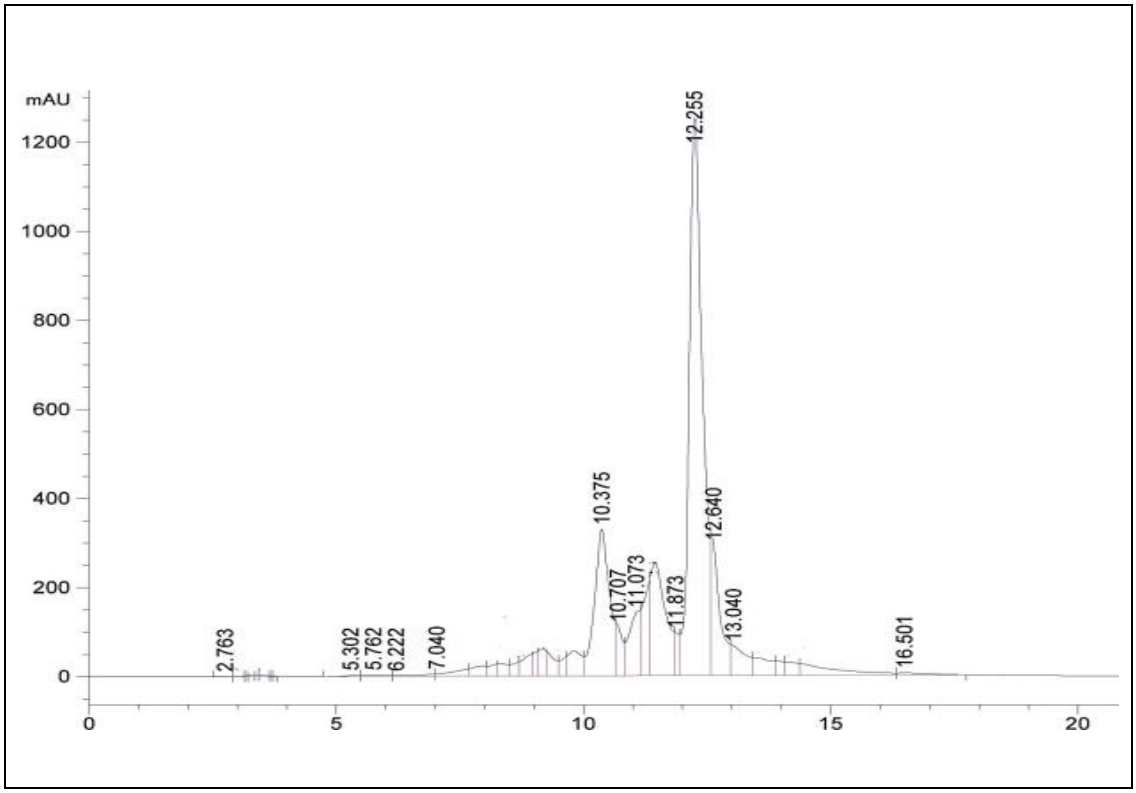

Fig. 4. HPLC spectra of flavonoid compounds in *Amaranthus viridis*

Mineral contents

The data for the mineral composition of roots (mg/100g DW) in *L. corniculatus* and *A. viridis* are listed in Table 6. The two weeds have eleven minerals (calcium, copper, magnesium, manganese, phosphorus, potassium, sodium, zinc, selenium, and nitrogen). The two weeds have a high level of calcium, magnesium, phosphorus, potassium, selenium, and nitrogen.

Antioxidant capacity

Antioxidant capacity ABTS$^{\bullet+}$[2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)], [DPPH$^{\bullet}$(2,2-diphenyl-1-picrylhydrazyl), radical scavenging activity, and ferric-reducing antioxidant power (FRAP)], in *L. corniculatus* and *A. viridis* plants were determined (Table 7). The DPPH and FRAP levels were higher in *Lotus corniculatus* than in *Amaranthus viridis*. On the contrary, the ABTS level was higher in *Amaranthus viridis* than in *Lotus corniculatus*.

Discussion

The fatty acid content of seeds in *Lotus corniculatus* and *Amaranthus viridis* is consistent with the information provided by Bakoglu et al. (2009) and Kocak et al. (2011). Also, the TSFA and TUSFA content are consistent with the data of Kocak et al. (2011), who investigated that TSFA content of the *L. corniculatus* seed was found between 15.06 and 25.96% and TUSFA contents was found between 72.28 and 85.06%. The elevated TUSFA contents in *L. corniculatus* seed have nutritional importance. The principal constituents of Fabaceae seed oils are linoleic-oleic type fatty acids, and their saturated and unsaturated fatty acid levels are strongly related to one another (Kocak et al., 2011). Additionally, in *Amaranthus viridis*, the levels of linoleic acid, palmitic acid, and lignoceric acid agree with the data from the literature (Jahaniaval et al., 2000; Hlinková et al., 2013). The concentration of TSFA and the TUSFA of *Amaranthus viridis* agree with the findings of Soyibjon et al. (2018). Amaranth oil is a popular antioxidant since it contains a lot of unsaturated fatty acids (Soriano-García et al., 2018). Seed fatty acids are used for animal feed because of their excellent nutritional value (Jahaniaval et al., 2000; Bagci, 2006; Bakoglu et al., 2009; Kocak et al., 2011; Hlinková et al., 2013; Soyibjon et al., 2018; Soriano-García et al., 2018).

TABLE 6. Mineral contents of roots (mg/100g DW) in *Lotus corniculatus* and *Amaranthus viridis*

Minerals	*Lotus corniculatus*	*Amaranthus viridis*
Calcium	210±18.9	165±13.2
Copper	0.43±0.02	0.61±0.03
Iron	7.21±0.25	3.89±0.05
Magnesium	213±15.88	302±20.17
Manganese	6.72±0.33	28.93±2.99
Phosphorus	872±67.23	783±98.09
Potassium	912±91.45	946±96.34
Sodium	10.12±0.60	8.7±0.52
Zinc	9.77±0.45	13.9±0.69
Selenium	28.15±1.71	36.14±2.17
Nitrogen	977±91.45	788±85.33

Values are mean±SE of samples in triplicate.

TABLE 7. Antioxidant capacity ABTS$^{\cdot+}$[2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)], [DPPH$^{\cdot}$(2,2-diphenyl-1-picrylhydrazyl), radical scavenging activity, and ferric-reducing antioxidant power (FRAP)], of *Lotus corniculatus* and *Amaranthus viridis*

Parameters	*Lotus corniculatus*	*Amaranthus viridis*
ABTS (mg VCE/g DW)	122±7.6	185±14.6
DPPH (mg VCE/g DW)	156±5.9	123±8.9
FRAP (mM Fe^{2+} equivalent/g DW)	3.9±0.09	1.5±0.04

(VCE) mg vitamin C equivalent. Values are mean±SE of samples in triplicate.

Aman et al. (2015) reported that the protein content is 37.93g/100g DW in *L. corniculatus*, which is in accordance with our results (28.13g/100g DW). Bagci (2006) investigated that the tanines were 1.63 % and the protein was 34% in *L. corniculatus*, which was similar to our results of 1.73% for tanines and 29.18% for protein. Previous phytochemical studies on the aerial parts of the genus Lotus revealed the existence of condensed tanines, which decreased meteorism in ruminants (Acuña et al., 2008; Naumann et al., 2013). The outcomes showed that the preliminary phytochemical screening was successful. Important bioactive substances such as phenolic, flavonoids, saponins, and tanines were found in *L. corniculatus*. Our findings are consistent with the information from the literature (Acuña et al., 2008; Moro et al., 2010; Naumann et al., 2013; Girardi et al., 2014). Our findings suggested that *L. corniculatus* contained significant levels of condensed tanines, and protein, identical with those mentioned by Pedro et al. (2005), Abdulrazak et al. (2006), Bakoglu et al. (2009), and Kocak et al. (2011). Saud et al. (2013) investigated the phytochemical information of *Amaranthus viridis* and established that saponins (53-32%), tanines (6.07-5.96%), proteins (16.76-24.51%) are consistent with our results. Proteins, tanines, and saponins show medicinal activity as well as exhibiting physiological activity (Saud et al., 2013). Umar et al. (2011) found that the tanine content in *A. viridis* leaves is high (> 7.5%). Tanines are astringent polyphenolic macromolecules and complex phenolic substances (water-soluble) that attach to and precipitate proteins, serve a role in protection against predators (pesticides), and may aid in controlling plant growth (USEA, 2016). Umar et al. (2011) reported that the HCN concentration in *A. viridis* was 13.07mg/100g DW, which is consistent

with our data (12.23mg/100g DW). The only plants that are regarded as harmful (dangerous) are those that have more than 200mg/100g DW of HCN (Betancur-Ancona et al., 2008). This shows that the aerial parts (seeds, stems and leaves) of *A. viridis* and *L. corniculatus* as far as HCN is concerned, are safe for eating. Umar et al. (2011) showed that the nitrate concentration was 25.35 mg/100 g DW, which is in accordance with our data (31.45mg/100g DW). This value was less than the daily maximum of 3.7mg/kg of body weight, which is equal to 220mg/60kg per person (Hassan & Umar, 2004). The existence of active components such as saponins, carotenoids, phenolic acids, amino acids, betaine, vitamins, glycosides, steroids, alkaloids, minerals, flavonoids, lipids, terpenoids, and tanines, has been confirmed by phytochemical composition in aerial sections of several *Amaranthus* spp. (Kavita & Puneet, 2017).

The findings presented here show that *A. viridis* and *L. corniculatus* have enormous potential as food sources, particularly given their protein and amino acid compositions, and can be used to alleviate amino acid and other nutrient shortages that are common in developing countries (Abdel-Ghani et al., 2001; Pedro et al., 2005; USEA, 2016; Kavita & Puneet, 2017). The advantage of *Amaranth* grains over traditional cereals is that they have a relatively high protein content and a structure rich in important amino acids. The high content of tryptophan and lysine in *Amaranth* grains is equivalent to proteins derived from animals, and their outcomes are in line with the information of Pisarikova et al. (2005). Amaranth grain's employment as a replacement for meat and bone meals is predetermined due to its comparatively high amount of necessary amino acids (Umar et al., 2011).

The phenolic compounds in aerial parts of *L. corniculatus*, and *A. viridis* were is consistent with the data from the literature (Pisarikova et al., 2005; Moro et al., 2010; Umar et al., 2011; Girardi et al., 2014; Ekeke et al., 2019). The results showed that, according to the preliminary phytochemical screening of *L. corniculatus* and *A. viridis*, essential bioactive molecules were present, such as phenolics, flavonoids, saponins, and tanines. The information from the literature is congruent with our findings (Acuña et al., 2008; Moro et al., 2010; Girardi et al., 2014).

The flavonoid compounds of *L. corniculatus* and *A. viridis* were in accordance with the data from the literature (Abdel-Ghani et al., 2001; Reynaud & Lussignol, 2005; Abdel-Kader et al., 2007; Barba de la Rosa et al., 2009; Umar et al., 2011; Venskutonis & Kraujalis, 2013; Rijke et al., 2015; Soriano-García et al., 2018; Ekeke et al., 2019; Garcı´a-Caldero´n et al., 2020). Other studies have also demonstrated that *Lotus corniculatus* has high agronomic value due to its rich flavonoid content in aerial parts (Reynaud & Lussignol, 2005). The analysis of *A. viridis* leaves revealed that they have significant potential as food sources, particularly when considering their protein and amino acid profiles. As a result, they can be utilized to reduce the shortages of amino acids and other nutrients that are common in many developing nations (USEA, 2016). Besides, *Lotus* species contain flavonoids of the flavonol type, like kaempferol and quercetin (Reynaud & Lussignol, 2005), vitexin, and rutin (Moro et al., 2010). The most prevalent components of the aerial sections of *Lotus* species are flavones and flavonols (Abdel-Ghani et al., 2001; El Mousallami et al., 2002; Abdel-Kader et al., 2007).

In *L. corniculatus* and *A. viridis*, there are eleven elements (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, selenium, and nitrogen). with different concentrations that are of important value for human health (Moussa, 2006, 2008, 2009; Bakoglu et al., 2009; USEA, 2016; Li et al., 2019; Mohamed et al., 2021).

The high antioxidant capacity of both plants is very important for human health (Gabriela & Raquel, 2013). The DPPH and FRAP levels were higher in *Lotus corniculatus* than in *Amaranthus viridis*. On the contrary, the ABTS level was higher in *Amaranthus viridis* than in *Lotus corniculatus*. The ability of the extracts to operate as reducing agents is based on their redox properties; this ability is typically linked to the presence of reductants, which disrupt the free radical chain by giving an atom of hydrogen or inhibiting the development of peroxide to produce antioxidant action (Kumaran, 2006).

Conclusions

Overall, the research found that *Lotus corniculatus* and *Amaranthus viridis* extracts have promising

natural antioxidant properties that can be used in culinary, medical, and agricultural applications. As a result of the study, it was determined that *Lotus corniculatus* and *Amaranthus viridis* had a significant amount of valuable ingredients in their crude yield, comprising minerals (Iron, Phosphorus, Sodium, Manganese, Potassium, Calcium, Copper, Magnesium, Zinc, Selenium, and Nitrogen), saponins, and cardiac glycosides that are both physiologically active and beneficial to health (exhibiting medicinal activity). In both plants having a strong antioxidant capacity $ABTS^{\cdot+}$[2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)], [$DPPH^{\cdot}$(2,2-diphenyl-1-picrylhydrazyl), radical scavenging activity, and ferric-reducing antioxidant power (FRAP)]. Naturally occurring antioxidant substances in both plants have the ability to scavenge ROS, improving cellular equilibrium (the antioxidant/oxidant status) and preserving physiologically normal conditions. Therefore, *Lotus corniculatus* and *Amaranthus viridis* plants may serve as a natural source of important compounds having antioxidant characteristics in the cosmetics, food, and pharmaceutical industries. Also, *Lotus corniculatus* and *Amaranthus viridis* preferred an arid environment and can be considered a new source of several different metabolites that can contribute to drug improvement. In addition, the study provides insight into unusual strategies to increase the efficiency of wild plants for accumulation of phytochemicals.

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الفحص الكيميائي الضوئي وقدرة مضادات الأكسدة في اللوتس كورنيكولاتوس و أمارانثوس فيريديس

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حديثا، تركزت الأنشطة البحثية في دراسة المركبات الأيضية الثانوية النباتية على خصائصها كمصدر جديد لمضادات الأكسدة الطبيعية. الهدف من الدراسة الحالية هو تقييم الفحص الكيميائي للنباتين ، للكشف عن وجود عوامل علاجية طبيعية ومضادات الأكسدة المحتملة لنباتين سامين فى مصر اللوتس كورنيكولاتوس و أمارانثوس فيريديس لم تكتسب هذه النباتات أهمية كبيرة وهي شائعة الانتشار في الأراضي المصرية الملوثة. الأحماض الدهنية للبذور في نبات اللوتس 40.13، 28.88، 0.49، 22.14 و 83.62٪ لحمض اللينوليك وحمض الأوليك وحمض البوهينيك وإجمالي الأحماض الدهنية المشبعة وإجمالي الأحماض الدهنية غير المشبعة على التوالى. أيضًا، في أمارانثوس فيريديس 45.2، 23.15، 0.13، 19.99 و 75.18٪ لحمض اللينوليك، حمض البالمتيك، حمض اللجنوسريك، الأحماض الدهنية المشبعة والأحماض الدهنية غير المشبعة، على التوالي. تم تقدير بيانات التقدير الكمي للمكونات الكيميائية الخام (البروتين ، التانين ، سيانيد الهيدروجين ، الصابونين، الجليكوزيدات والنترات). أيضًا ، يوجد عشرين حمضًا أمينيًا قياسيًا بشكل شائع كمكونات للبروتينات. يحتوي اللوتس كورنيكولاتوس على أحد عشر حمضًا أمينيًا وأمارانثوس فيريديس يحتوي على ثلاثة عشر حمضًا أمينيًا. ومع ذلك ، لم يتم الكشف عن بعض الأحماض الأمينية سواء في اللوتس كورنيكولاتوس وأمارانثوس فيريديس. المركبات الفينولية هي أحد عشر وعشرة كسورًا في اللوتس كورنيكولاتوس وأمارانثوس فيريديس على التوالي. ومع ذلك، فإن مركبات الفلافونويد هي اثني عشر وأحد عشر في اللوتس كورنيكولاتوس و أمارانثوس فيريديس على التوالي. يوجد في كلا النباتين أحد عشر عنصرًا ذات قيمة مهمة لصحة الإنسان (الكالسيوم والنحاس والحديد والمغنيسيوم والمنجنيز والفوسفور والبوتاسيوم والصوديوم والزنك والسيلينيوم والنيتروجين). تم تقدير قدرة مضادات الأكسدة (ABTS, DPPH, and FRAP) لما لها من أهميه كبيرة فى الحماية من الشوارد الحرة في كلا النباتين اللوتس كورنيكولاتوس و أمارانثوس فيريديس .

لذلك، يمكن استخدام نباتات اللوتس كورنيكولاتوس و أمارانثوس فيريديس كمصدر قوى متاح للمنتجات ذات الخصائص المضادة للأكسدة في مستحضرات التجميل والصيدلة وصناعة الأغذية.