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Microscopical, macroscopical studies, and GC/MS analysis of leaves and seeds hexane extract of Iraqi wild *Amaranthus viridis* L

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ABSTRACT

Medicinal herbs are the largest source of chemical compounds on the planet and are utilized as drugs of traditional medicine. *Amaranthus viridis* L, a herbaceous plant from Amaranthaceae family, has sparked a lot of interest in the field of folk medicine due to its diverse complex chemical content. *Amaranthus viridis* is known to include many of active phytochemicals e.g., tannins, resins, alkaloids and terpenes. However there is none or very little pharmacognostical reports about the macroscopical and microscopical study of plant parts. **Aim**: investigate the morphological characteristics of stems, leaves, and seeds, besides investigation of leaves under microscope to seek for the type of stomata and trichomes. In addition, the active ingredients in the leaves and seeds extract of Iraqi grown plant were estimated using gas chromatography/mass spectroscopy (GC/MS) analysis. Furthermore, this study aimed to extract the flavonoids from dried leaves and seeds using the hot extraction method, and these compounds were to be estimated using chromatographic methods such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). **Methods:** *Amaranthus viridis* L was collected from Al-Musayyib region, Iraq. The microscopical study was performed on fresh leaves and stems to assess the kinds of stomata and trichome. The plant seeds and leaves were extracted with n-hexane using the hot method, and the extract was analyzed using GC/MS analysis. For the extraction of the flavonoids, the defatted materials were re-extracted with ethanol 75% and partitioned with ethyl acetate, and the ethyl acetate layer investigated for the presence of flavonoids using both TLC and HPLC analysis. **Results**: the microscopical study exhibited that the plant has anomocytic type stomata with un-branched unicellular trichome. Eight compounds (5-methyl, 1-Undecane,2-Undecanone,6,10-dimethyl-hexahydropseudoionone,2-Pentadecanone, 6,10,14-Trimethyl-hexahydrofuransyl acetone, n-Hexadecanoic acid, Palmitaldehyde,diisopentyl acetal, Oleic acid , Pentafluoropropanoic acid, octadecyl ester , and Hexadecanoic acid ,2,3-dihydropropyl ester) were identified by the GC/MS analysis. Among these compounds two were identified to be pharmacologically active n-Hexadecanoic acid (palmitic acid) and oleic acid (omega-9-fatty acid). The most abundant compound in the hexane extract was n-Hexadecanoic acid with percent area of 28.36%. Both TLC and HPLC detected the existence of epicatechin in the ethyl acetate fraction of plant extract. **Conclusion:** The presence of many bioactive compounds in the *Amaranthus viridis* L explains the use of whole plant to eradicate different ailments traditionally. However, identification and isolation of individual phytochemicals and studying of their biological activities will definitely provide rich results. This is the first report about the microscopical characteristic of the Iraqi grown plant which considered as diagnostic parameters of this genus. In addition, the current study showed the existence of epicatechin in *A.viridis* L plant and the presence of such an important flavonoid with many important pharmacological activities is a very important discovery especially in the Iraqi grown plant and it needs further investigations and quantitative analysis.

Keywords: Amaranthus GC/MS, Amaranthus TLC, Amaranthus epicatechin, Amaranthus flavonoids, Amaranthus HPLC, Amaranthus ethyl acetate extract, Amaranthus microscopical study

INTRODUCTION

Medicinal herbs are the largest source of chemical compounds on the planet and are utilized as drugs of traditional medicine, contemporary medicines, nutritional supplements, folk remedies, pharmaceutical intermediates and chemical building block for synthesized medications. Hence plants are known as the chemical factories due to the presence of abundant phytochemicals which are the chemicals or bioactive elements present naturally in any organ of the plant such as bark, leaves, flowers, roots, fruits, and seeds (1). *Amaranthus viridis* L, a herbaceous plant from Amaranthaceae family, has sparked a lot of interest in the field of folk medicine due to its diverse complex chemical content. This plant is known to include a diversity of bioactive phytochemicals that take part to its medicinal usefulness. The plant is grown in Latin America, Asia, and Africa. It is one of the most frequent weeds in tropical, subtropical climates and warmer rejoins (2). *Amaranthus viridis* is known to contain many of active phytochemicals e.g., tannins, resins, alkaloids and terpenes. Leaves methanol extract was discovered to contain two significant active flavonoids rutin and quercetin (3). It also included spinosterol (24-ethyl-22-dehydrolathosterol) as major component along with 24-methyl-22-dehydrolathosterol, 24-methyllathosterol 24- ethyllathosterol, 24-ethyl cholesterol and 24-ethyl-22-dehydrocholesterol as lesser compounds in sterol part (4). *A.viridis* L has many of traditional applications, the leaves, seeds, and roots were used in the treatment of many conditions including diuretic, analgesic, antipyretic, antiulcer, antidiabetic, anti-cholesterolemic, and laxative effect. Good 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect has been achieved in the methanolic and aqueous methanolic leaves and seeds extract as revealed by IC_{50} (14.25 to 83.43 µg/ml). Furthermore, *A.viridis* L was traditionally employed as vermicides. The methanolic extracts of the plant was studied for anthelmintic activity using earthworms and the results revealed that it caused paralysis and worms death due to the presence of polyphenolic compounds (5). Microscopical techniques can be applied to solve taxonomic problems in the plant field. Using of classical microscopic and taxonomic tools can be helpful in the accurate identification of the plant genera. Amaranthus genus has many of taxonomical complexities and identification difficulties because of big morphological diversities so the internal structures and morphological features can be useful in solving the taxonomic problems. However there is none or very little pharmacognostical reports about the macroscopical and microscopical studies of plant parts so the current research focus on the investigation of the morphological characteristics of leaves, stems, and seeds and investigation of mature leaves under microscope to seek for the type of stomata and trichomes. In addition to the analysis of the phytochemical content of leaves and seeds hexane extract of Iraqi grown plant using gas chromatography/ mass spectroscopy (GC/MS) and extraction of the flavonoids from the dried leaves and seeds using hot extraction method and identify the active ingredients founded in the extract using both thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Materials and Methods

Amaranthus viridis L was collected in Septemper-2023 from Al-Musayyib region, Iraq. *A.viridis* L was recognized and certified at the department of pharmacognocy/College of pharmacy/Mustansiriyah University. The sources of materials and instruments used were as follows: Chloroform (BHD, England), Ethanol HPLC-grade (Alpha Chemika, India), FeCl3 (BHD,England) , H2So4 (India), Iodine (Alpha chemical, India), NaOH (China), n-hexane (Romil, UK), Potassium iodide (Alpha chemical, India), epicatechin (hyperchim, China), toluene (Alpha chemical, India), acetic acid (Alpha chemical, India), HPLC grade acetonitrile (Alpha chemical, India), formic acid (Alpha chemical, India), rotary evaporator (Bochi-Rota), HPLC (Shimadzu, Jaban), GC/MS (Shimadzu, Jaban), electronic balance (OHAUS, US), and electronic microscope (Olympus, Japan). Furthermore the class wares used in the work were: Beaker (1000-500-100-25 ml), spatula, cylinder, separatory funnel, soxhlet 250 ml (round flask, extracting chamber, and condenser), watch glass, and pipette (10 ml).

Extraction of the phytochemicals

For the preliminary phytochemical screening, the plant was shade dried, then the leaves, and seeds were cleaned with water, allowed to dry in shade, and pulverized together to powder by an electric blender. After that, 10 g of this powder was heated separately with 100 milliliters ethanol and water. After that, the extracts were undergoing filtering by Whatman filter paper and used in the preliminary tests. For GC/MS analysis, the collected plant seeds and leaves have been washed properly and allowed to air-drying for two weeks at room temperature. An electric blender was then used to gather the plant materials into fine powder which was stored in clean container until required for use. Hot extraction method was used to extract the plant phytochemicals. Powdered plant material (25 g) was extracted with n-hexane (250 ml) using soxhlet apparatus for 3 hours. This extract was undergoing filtering, and the volume was reduced by the rotary evaporator. The obtained extract was collected, weight and stored. For the extraction of flavonoids, the defatted material was re-extracted with 75% ethanol (250 ml) using a soxhlet apparatus for 9 hours. Then, the ethanolic extract was undergoing filtering, and the volume was reduced to the fourth by the rotary evaporator. The obtained extracts were collected, weight and stored. About 5 milliliters of distilled water was added to the concentrated ethanolic extract and partitioned with ethyl acetate (15 mlx3) using the separatory funnel. The top layer (ethyl acetate fraction) was collected in a beaker and left to dry at room temperature, then it was weight and sealed tightly with a para film and stored in the refrigerator at 4 C^0 (6).The percentage yields of the plant extract (n-hexane, ethanol, and ethyl acetate) were measured as follows:

$(W_2\text{-}W_1/W_0)$x 100

Were W_2 is the weight of extract and container, W_1 is the weight of container alone, and W_0 is the weight of dried initial plant sample.

Preliminary phytochemical screening of *Amaranthus viridis L* (6)

Test for tannins (Ferric chloride test)

Aqueous extract (2ml) was mixed with one to two drops of 10% $FeCL_3$. The presence of tannins is indicated when colors like green-blackish or blackish-blue are seen.

Test for saponins (Froth test)

Aqueous plant extract 5 milliliters was combined with same quantity of distilled water and shaken thoroughly for 15 second. The formation of froth of about 1cm in height which is stable for 15 min reveals that saponins are present

Test for flavonoids (Alkaline reagent test)

Ethanolic extract (3ml) was mixed with 1 ml (10%) NaOH, the existence of flavonoids was indicated by the formation of distinct yellow color.

Test for terpenoids (Salkowski's test)

Ethanolic plant extract (5 ml) was combined with chloroform (2 ml). This step was followed by the careful addition of concentrated H_2SO_4 (3 ml). The reddish brown color precipitate implies a positive result.

Test for alkaloids (Wagner's reagent test)

One to two drops of Wagner's reagent were mixed with 2 milliliters of the ethanol plant extract; the observation of a reddish-brown precipitate implies a positive result.

Pharmacognostical study

1-Macroscopical study

Fresh leaves, flowers, seeds and stems of *A.viridis* L were studied for morphological characteristics such as shape, color, and size.

2-Microscopical study

The microscopical study was performed on fresh leaves and stems. To identify the type of stomata and trichome, a cut of epidermal layer from a fresh leaf was examined on a slide and the method was repeated on ten slides to ensure the kind of stomata in the tested plant. Chloral hydrate was applied for two to three times and discarded in order to remove the color of the plant. The slide was then heated to give a clear section and examined. The same process was repeated with stems, and the photographs containing various cell components were captured using a digital camera (7).

Gas chromatography-mass spectrometry (GC/MS) analysis of the hexane extract

Plant hexane extract was estimated by combined gas chromatography system and mass spectrophotometer (Shimadzo 2010, Japan). An HP-5MS fused silica column (5% phenyl methyl silicon, 30 m x 250 µm, film thickness 0.25 µm) and an MSD transfer line were included with this system. The ionization energy of 70 eV was used in the electron ionization system (EI). The carrier gas was helium which was employed at a flow rate of 1 ml/min. A split mode injector was used to inject 10 µl of sample. With a total elusion time of 30 minutes, the oven temperature was intended to begin at 80 °C for two minutes, increase to 280 °C at a rate of 10 °C per minute, and then hold for five minutes. The temperature of the injection and the interface was 280 °C, while the ion source was 200°C. The peak area, molecular ion peak, base peak, and retention time (RT) of each compound were detected. The compounds were identified from the spectral data based on the available mass spectra records in the National Institute of Standards and Technology (NIST) library (8).

Detection of flavonoids in the ethyl acetate part of *Amaranthus viridis* L by analytical thin layer chromatography (TLC)

Flavonoids were detected using a ready-made TLC aluminium sheet (20x20 cm) pre-coated with silica gel 60F254. On little pieces of TLC plate, a small amount of the sample was applied along with epicatechin standard with the help of the capillary tube, and then the plates were run in three solvent systems as follows:

S1: Toluene:ethyl acetate:formic acid (3.5:1.2:0.6)(9)

S2: Chloroform:ethyl acetate (5:2)(10)

S3: Chloroform:methanol (9:1)(11)

After the development of the solvents, the plates were taken from the developing jars, air-dried, and investigated under long-waved UV light at (365nm) for the existence of the flavonoids by comparing the colors and the R_f values with the standard.

Detection of flavonoids in the ethyl acetate part of Amaranthus viridis L by high performance liquid chromatography (HPLC)

The ethyl acetate part of the plant was analyzed by a Shimadzu LC-20AD liquid chromatography system. This system was equipped with a DGU-20A5 degasser and SPD-20A UV-

visible detector. The column was reversed-phase (RP-C18) with a 250 mm x 4.6 mm internal diameter (id) and a particle size of 5 µm. The mobile phase was isocratic acetonitrile: methanol: ethyl acetate: glacial acetic acid (60:5:30), the standard used was Epicatechin at a concentration of 1mg /ml using ethanol (HPLC- grade) as a solvent. The injection volume was 20 µl at the flow rate of 0.7 ml/min, the wavelength was 280 nm, and the column temperature was 25 ^{O}C. The stored plant ethyl acetate fraction was re-dissolved with ethanol (HPLC –grade) to prepare a concentration of 1mg/ml. The concentration of epicatechin was calculated as follows:

(Area of sample/area of standard) x concentration of standard (12).

Results and Discussion

The preliminary tests are essential in predicting different types of phytochemicals that are exist in the plant extract. The preliminary tests results of *A.viridis* L extract and the percentage yield of plant extract are summarized in table 1 and 2. The findings in table (1) demonstrated that the Iraqi grown plant contained a diversity of phytochemicals including, flavonoids, terpenes, saponins, and alkaloids. These phytochemicals are critical in the biological activities of *A. viridis* L. plant, and these findings were agreed with reported literature in which positive results for flavonoids, tannins, alkaloids and terpenes tests were obtained (13-15) . Plant materials continue to be a vital resource to fight against serious illness in the world. The traditional medical approaches, particularly the application of herbs, continue to play a crucial role in delivering essential medical care. Certain medical active components in these herbs have specific physiological impact on human body are the source of these herbs medicinal benefit. Alkaloids, tannins, flavonoids, and phenols are the most important bioactive components in the medicinal plant (16). Infections have grown exponentially within the recent years and antibiotics resistance has become a major treatment issue (17) . Natural substances founded in plants might provide a fresh supply of antimicrobial agents with potentially unique modes of action. They are useful in curing many infections while avoiding several negative effects that are commonly linked with synthetic agents (18) .

Table (1): Phytochemical screening of Iraqi *Amaranthus viridis L*.

Test	Positive Indicator	Result
Saponins test	Foam observed 1cm in high	+ ve
Tannins test	Dark green	+ ve
Flavonoids test	Yellow color	+ ve
Alkaloids test	Reddish- brown precipitate	+ ve
Terpenes test	Reddish- brown color	+ ve

Table (2): Percentage yield of plant extract

Plant extract	Weight of extract (gram)	Percentage yield
Hexane extract	3	12%
Ethanol extract	2.75	11%
Ethyl acetate fraction	0.75	3%

There for, screening of these plants is very crucial in verifying their applications in traditional medicine and identifying the active chemicals by separation and characterization. The systemic screening of these compounds may lead to discovering of novel drugs (19). Phytochemicals screened in the present study include tannins, saponins, flavonoids, alkaloids, and terpenoids. The phytoactivity and pharmacological effects of some of these phytochemicals have been proved by different authors. Tannins was discovered to exert several medicinal effects including anti-inflammatory, antioxidant, analgesic, and wound healing properties (20,21) . Saponins are known to possess bitter taste, and are foamy in nature. They have reported to possess antibacterial properties (22) . Dietary flavonoids have been found to protect against coronary heart disease .In addition, alkaloids possess reductive effect towards fever and headache.

The macroscopical examination revealed that *A.viridis* is an annual herbaceous plant with long petioles and distinctive venation. It is a durable, straight, branching plant that may reach a height of 10-100 cm tall. It grows on heavy, sandy loam soils, including muddy soil after the water recedes throughout the growing season, Figure (1). **Stems**: the stems are green, erect, 60-80 cm glabrous to pubescent. It is fairly thin and densely branched. **Leaves** are oval with broad base, simple and alternating with a long petiole. They have mid to light green in color but the lower side is reddish purple, they are heavily veined, measuring up to 5 centimeters long and 3 centimeters wide. The apex of the leaf blade is notched and terminates in a short tip. **Flowers** are small, and range in hue from green to reddish in color. They are arranged in little compact balls spread along thin spikes at the base of the leaves and the top of the stalks. **Seeds** are lenticular, smooth and glossy with a diameter of 1-1.25 mm and a hue ranging from dark brown to black. Figure (2) shows the examined parts of the plant. A close-up view of the abaxial surface of the leaf's epidermal peel, revealing the stomatal guard cells (GC) and the epidermis' subsidiary cells (SC). The stomata are anomocytic in type, with guard cells surrounded by cells that are identical in size, shape, and organization to the rest of the epidermis cells. In addition, unicellular unbranched trichome was also found, figure (3), the types of stomata and trichome were same in all the tested ten slides ensuring that the plant has one type of stomata and trichome. Amaranthus genus is taxonomically complicated due to its high morphological variation which may result in nomenclatural confusions, misapplication of names, and misidentification. Anatomical studies of this genus are still insufficient. The microscopical structures are parameters that are unique to the plant and are necessary for its standardization. There for, the main object of the study was to seek if the features of mature leaves and stems under microscope are helpful in the taxonomy of Amaranthus. The findings of the current study could, therefore, provide the basis for correct plant identification. The macro- and micro- morphological features of this plant were described in the current study can help in distinguishing it from other members of the genera.

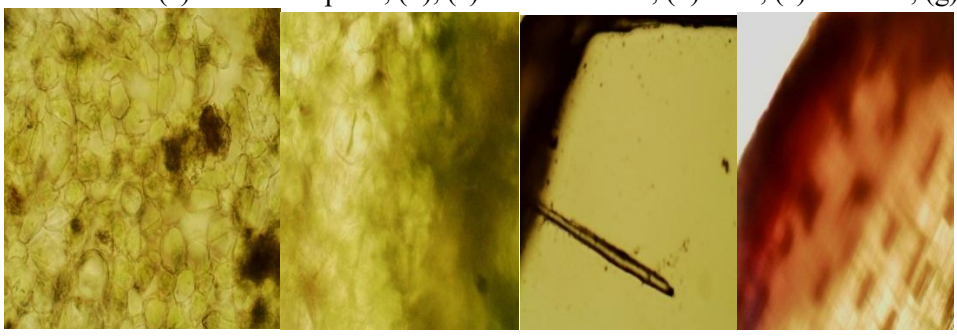
Figure (1): *Amaranthus viridis* L

(a) (b) (c)

(d) (e)

(g)

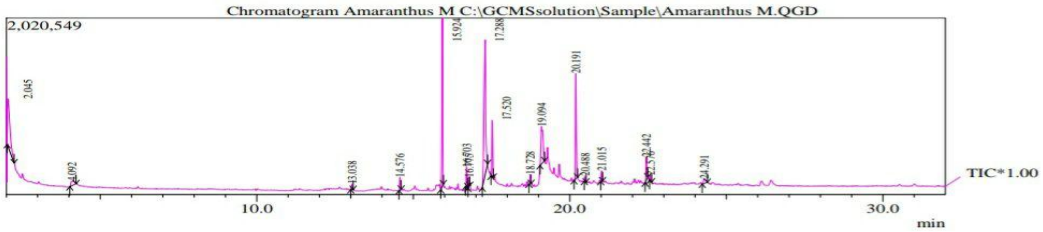
Figure (2): morphological examination of *Amaranthus viridis* L
(a): branch of plant, (b), (c): stem and leaf, (d): leaf, (e): flowers, (g): seeds



(a) (b) (c) (d)

Figure (3): microscopical examination of *Amaranthus viridis* L.
(a): Anomocytic stomata X40 (b): Anomocytic stomata X10 (c): Trichomes X10
(d) Transverse section of stem shows xylem and phloem.

Several peaks were found in the hexane extract of *A.viridis* L by GC/MS analysis (figure 4); eight of them were identified by comparing the retention time (RT), base peak, molecular ion peak, and the mass fragmentation pattern to those of the realized compounds listed in the NIST library, as illustrated in table (3). Figures (5, 6, 7,8,9,10,11, and 12) show the mass fragmentation of 5-methyl, 1-Undecane,2-Undecanone,6,10-dimethyl-hexahydropseudoionone,2 Pentadecanone 6,10,14-Trimethyl-hexahydrofuransyl acetone, n-Hexadecanoic acid, Palmitaldehyde,diisopentyl acetal, Oleic acid , Pentafluoropropanoic acid, octadecyl ester , and Hexadecanoic acid ,2,3-dihydropropyl ester, respectively. The GC/MS chromatogram revealed that the most predominant compound in the hexane extract of the plant was n-Hexadecanoic acid with percent area of 28.36% followed by 2-Undecanone,6,10-dimethyl-hexahydropseudoionone (16.54%), Pentafluoropropanoic acid, octadecyl ester (11.29%), oleic acid (9.99%), Hexadecanoic acid ,2,3-dihydropropyl ester (2.84%), Palmitaldehyde,diisopentyl acetal (1.42%), and 5-methyl, 1-Undecane (1.30%). The hexane extract of *A.viridis* leaves and seeds was analyzed by GC-MS spectrometry, resulting in spectra with discrete peaks. Each component in the extract was separated according to its boiling point, with peaks indicated in the order of their retention times. Eight compounds (5-methyl, 1-Undecane,2-Undecanone,6,10-dimethyl-hexahydropseudoionone,2-Pentadecanone, 6,10,14-Trimethyl-hexahydrofuransyl acetone, n-Hexadecanoic acid, Palmitaldehyde,diisopentyl acetal, Oleic acid , Pentafluoropropanoic acid, octadecyl ester , and Hexadecanoic acid ,2,3-dihydropropyl ester) were identified using the fragmentation pattern obtained from the mass spectrometry. Furthermore, the identities of these compounds were confirmed by comparison their retention time (RT), molecular ion peak, and fragmentation pattern with the database of the GC-MS library. Among these compounds two were identified to be pharmacologically active; n-Hexadecanoic acid (palmitic acid) and oleic acid (omega-9-fatty acid). n-Hxadecanoic acid is a saturated fatty acid and several fatty acids possess antibacterial and antifungal activity, as well as they can modulate immune response. n-Hexadecanoic acid has anti-inflammatory effect since it demonstrated significant inhibitory activity in the enzyme kinetics study of phospholipase A_2 (PLA_2)(23) . n-Hexadecanoic acid also shows an antibacterial properities against both *E.coli* and *S.aureus* (24) . This compound was found to be the most predominant one in the plant extract according to the GC/MS analysis and this may be responsible for the medicinal activities of this plant. The second important compound identified in the hexane extract of *A.viridis* plant was oleic acid which is mono-unsaturated omega-9-fatty acid. Oleic acid (cis-9-octadecenoic acid) is the most prevalent naturally occurring fatty acid and it makes up 55-80% of olive oil. It is also derived from vegetable and is regarded as one of the alternative better source of fat in diet which can replace the animal saturated fat. Researches showed that the lower occurrence of coronary heart disease (CHD) is associated with a higher dietary intake of monounsaturated fatty acids (MUFA), specifically oleic acid. To get this advantage, the same amount of saturated fat must be replaced with olive oil. Oleic acid can exert beneficial effects by several mechanisms, since it can modulate the amounts of lipids and lipoprotein in the plasma, inhibit coagulation, improve homeostasis of glucose, and reduce the fasting inflammatory and oxidative states. Recently, studies revealed that metabolism of fats after meal have a crucial effect in the pathogenesis and progression of CHD. The presence of such an important monounsaturated fatty acid in *A.viridis* extract making it an important vegetable source of healthy fat and this wild plant should be exploited for this purpose.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	2.045	2.025	2.250	2972735	14.35	540303	7.14	5.57	MI	
2	4.092	4.025	4.225	380728	1.84	50142	0.66	7.22	MI	
3	13.038	13.000	13.075	132970	0.64	61829	0.82	2.15	MI	
4	14.576	14.542	14.608	268723	1.30	147059	1.94	1.81	MI	
5	15.924	15.875	15.958	3427009	16.54	1925729	25.45	1.78	MI	
6	16.703	16.658	16.733	401346	1.94	227233	3.00	1.77	MI	
7	16.775	16.742	16.800	193293	0.93	109911	1.45	1.73	MI	
8	17.288	17.200	17.367	5874415	28.36	1547594	20.45	3.79	MI	
9	17.520	17.475	17.550	1148931	5.55	647097	8.55	1.78	MI	
10	18.728	18.692	18.758	192094	0.93	107704	1.42	1.78	MI	
11	19.094	19.042	19.192	2069975	9.99	428554	5.66	4.81	MI	
12	20.191	20.133	20.242	2339469	11.29	1203081	15.90	1.94	MI	
13	20.488	20.458	20.517	109329	0.53	63124	0.83	1.73	MI	
14	21.015	20.983	21.042	213414	1.03	119509	1.58	1.78	MI	
15	22.442	22.400	22.483	588032	2.84	261345	3.45	2.22	MI	
16	22.576	22.542	22.608	123512	0.60	72343	0.96	1.74	MI	
17	24.291	24.225	24.392	279533	1.35	55459	0.73	5.00	MI	
				20715508	100.00	7568016	100.00			

Figure (4): GC/MS chromatogram of hexane leaves and seeds extract of *Amaranthus viridis* L plant

Table (3): The phytochemical constituents identified in *A.viridis* L hexane leaves and seeds extract using GC-MS analysis with their retention time (RT), base peak, molecular ion peak (g/mole), similarity index (SI), molecular formula, and area %.

No	compound	RT(min)	Base peak	Molecular ion peak (g/mole)	SI	Molecular formula	Area %
1-	5-methyl, 1-Undecane	14.575	56	168	91	$C_{12}H_{24}$	1.30
2-	2-Undecanone,6,10-dimethyl-hexahydropseudoionone	15.925	43	198	87	$C_{13}H_{26}O$	16.54
3-	2-Pentadecanone,6,10,1,4-Trimethyl-hexahydrofuransyl acetone	16.700	58.05	268	85	$C_{18}H_{36}O$	1.94
4-	n-Hexadecanoic acid	17.292	43	256	86	$C_{16}H_{32}O_2$	28.36
5-	Palmitaldehyde,diisopentyl acetal	18.725	71.10	398	84	$C_{26}H_{54}O_2$	1.42
6-	Oleic acid	19.092	55.10	282	85	$C_{18}H_{34}O_2$	9.99
7-	Pentafluoropropanoic acid, octadecyl ester	20.192	43	416	85	$C_{21}H_{37}F_5O_2$	11.29
8-	Hexadecanoic acid ,2,3-dihydropropyl ester	22.442	43	330	80	$C_{19}H_{38}O_4$	2.84

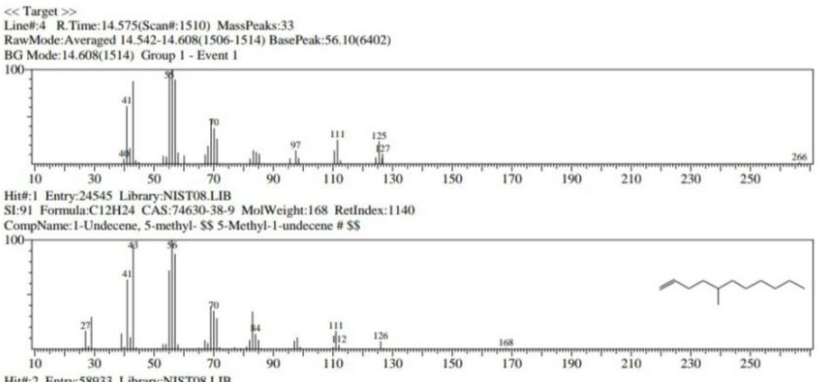


Figure (5): mass fragmentation of 5-methyl, 1-Undecane

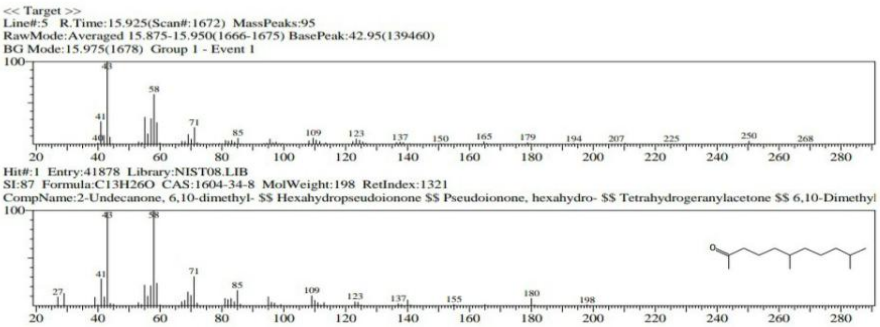


Figure (6): mass fragmentation of 2-Undecanone,6,10-dimethyl-hexahydropseudoionone

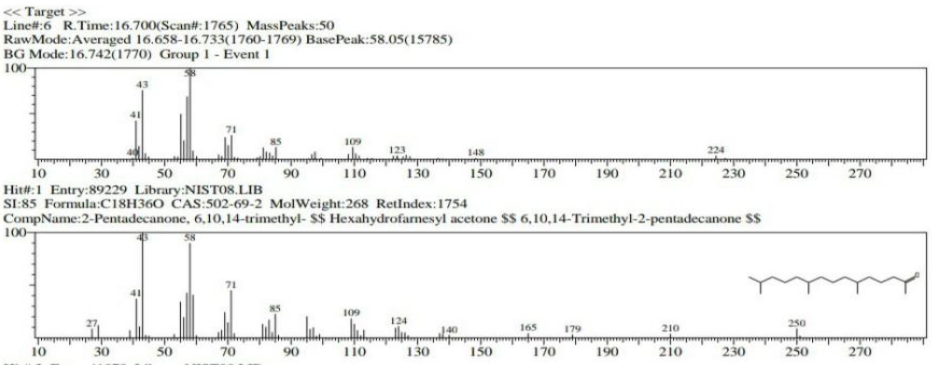


Figure (7): mass fragmentation of 2-Pentadecanone,6,10,14-Trimethyl-hexahydrofuransyl acetone

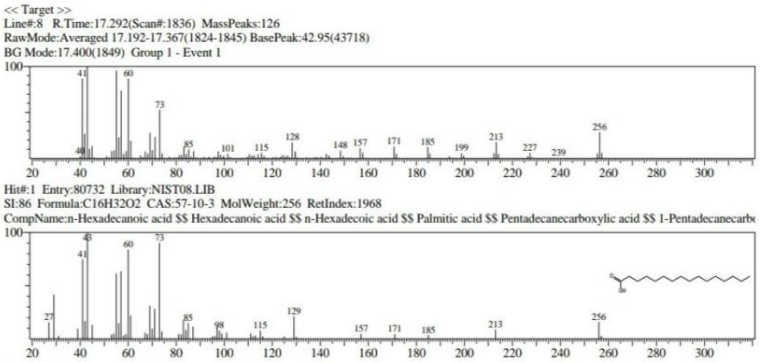


Figure (8): mass fragmentation of n-Hexadecanoic acid

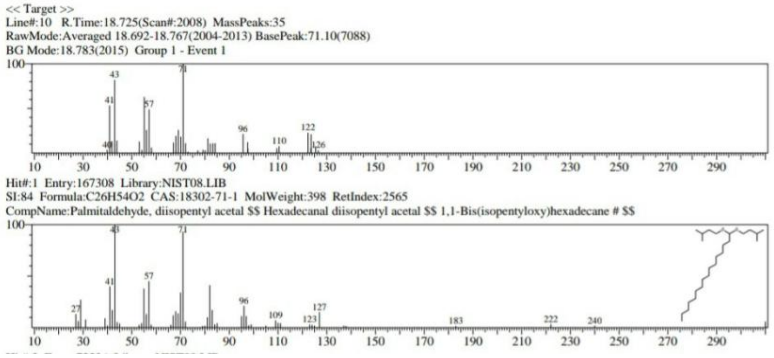


Figure (9): mass fragmentation of Palmitaldehyde,diisopentyl acetal

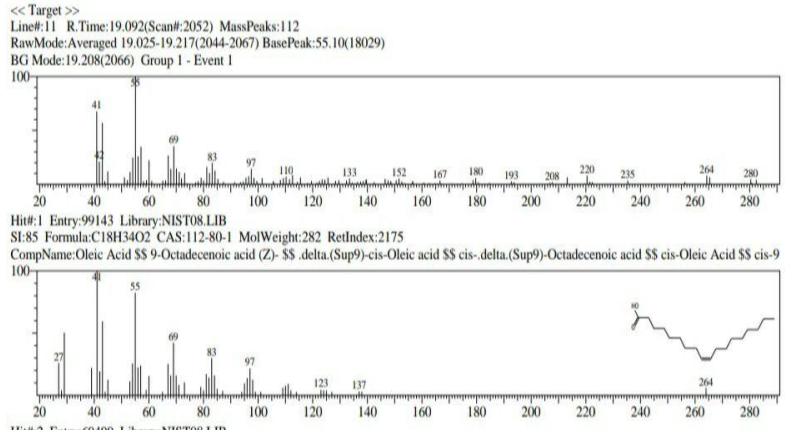


Figure (10): mass fragmentation of Oleic acid

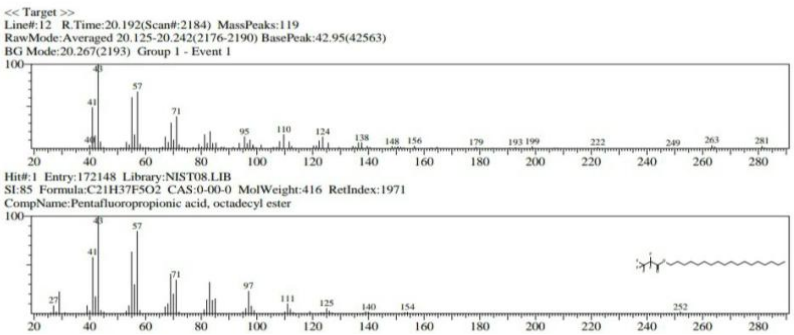


Figure (11): mass fragmentation of Pentafluoropropanoic acid, octadecyl ester

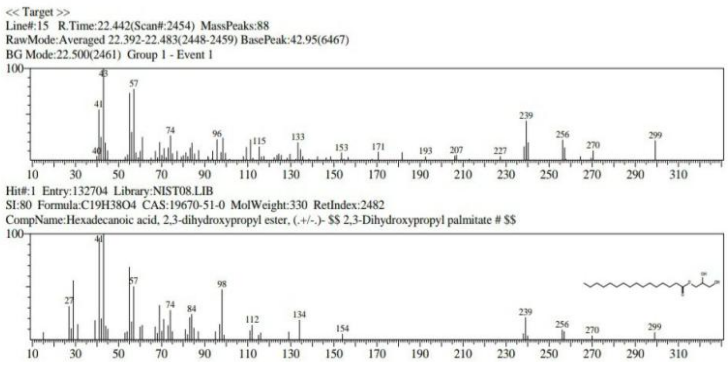


Figure (12): mass fragmentation of Hexadecanoic acid,2,3-dihydropropyl ester

The existence of flavonoids in the ethyl acetate part of plant sample was detected by analytical TLC in three mobile phase systems along with the use of epicatechin as reference standards. Epicatechin was observed as a fluorescent blue spot at long wave UV light (365 nm), figure (13). The color and the Rf value of the sample was detected and compared to the reference standard, table (4). This test was conducted to detect the existence of epicatechin in the plant sample. The retention time (RT) of the sample was compared to the reference standard. The HPLC chromatogram of epicatechin standard revealed the appearance of a single peak at a RT of 5.746 min with percent area of 100%, figure (14) while the HPLC chromatogram of the sample showed the appearance of several peaks, among them epicatechin separated at RT of 5.880 min with percent areas of 1.204%, figure (15). The concentration of epicatechin was 0.177 mg/ 1ml plant extract which was obtained from 0.75 gram plant ethyl acetate fraction.

Polyphenol are part of large family of naturally produced substances that includes a lot of other types of phytochemicals incorporating flavonoids. Anthocyanin, flavanols, flavonones, and flavones are the several categories that make up flavonoids. These compounds are called polyphenols because of the existence of many phenolic groups in their chemical structure. Consequently, polyphenols are compounds that share structural features including phenolic and aromatic ring. Polyphenols are most abundantly founded in fruits, vegetables, cereals, and beverages. Fruits such as apples, grapes, pears, cherries, and berries contain about two to three hundreds mg of polyphenols per one hundred grams (25). (−)-Epicatechin is one of the most important flavonoids of flavanol type. It composed of two aromatic rings joined by an oxygenated heterocycle with a 4-hydroxyl group. Epicatechin found in many foods such as dark chocolate and green tea. It is usually used as support supplement because of its potential advantages for athletes performance. It may enhance blood flow to the muscle helping in the removing of waste, delivering of nutrient, recovering and growth of muscle. It also have many other health benefits including improving the cardiovascular health, reducing the inflammation, and antioxidant capacity. This is the first report proved the presence of epiccatechin in *A.viridis* L plant and the presence of such an important flavonoid with many important pharmacological activities is a very important discovery especially in the Iraqi grown plant remembering that this plant is wild so it represents a natural economically sources of important polyphenolics compounds including epicatechin and it needs further investigations and quantitative analysis.

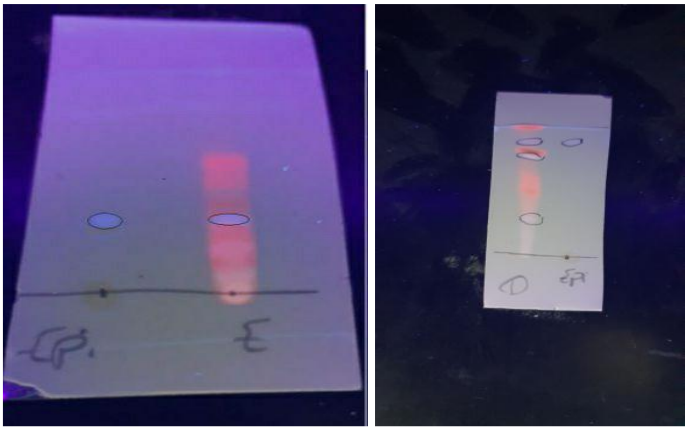


Figure (13): Analytical TLC under UV light (365 nm) using the solvent systems (S2 to the left, S3 to the right) for the detection of flavonoids in the ethyl acetate part . EP= Epicatechin.

Table (4): The Rf values of epicatechin in the ethylacetate part *Amaranthus viridis* L extract in three mobile phase systems as compared with the standards**.**

Solvent system	R_f of epicatechin (standard)	R_f of epicatechin (extract)
S_1	0.27	0.27
S_2	0.5	0.5
S_3	0.59	0.57

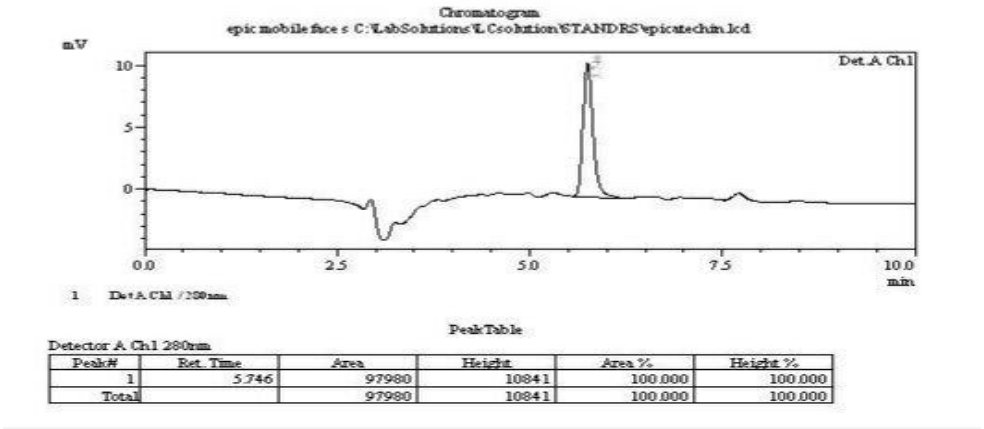
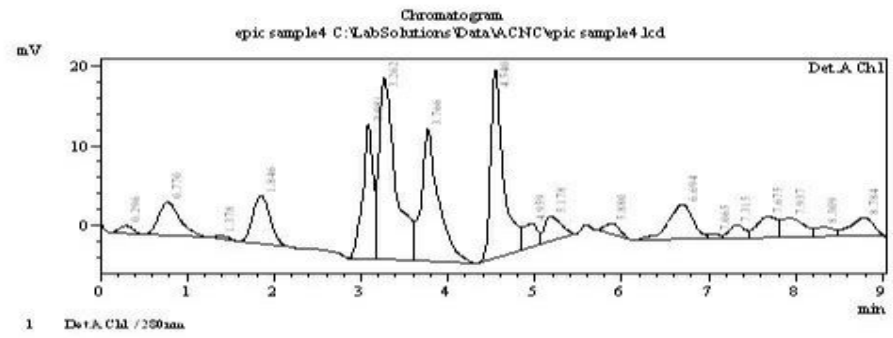


Figure (14): HPLC chromatogram of epicatechin standard



PeakTable

Detector A Ch1 280nm

PeakN	Ret. Time	Area	Height	Area %	Height %
1	0.296	10445	952	0.723	0.835
2	0.770	71742	4224	4.969	3.704
3	1.378	4139	401	0.287	0.351
4	1.846	84679	6054	5.865	5.309
5	3.081	140179	16882	9.709	14.804
6	3.262	307960	22863	21.329	20.049

PeakN	Ret. Time	Area	Height	Area %	Height %
7	3.766	231173	16580	16.011	14.539
8	4.540	241596	23585	16.733	20.682
9	4.959	32688	2897	2.264	2.540
10	5.178	40754	3123	2.823	2.739
11	5.880	17389	1401	1.204	1.229
12	6.694	88804	4396	6.151	3.855
13	7.065	5502	608	0.381	0.533
14	7.315	21546	1709	1.492	1.498
15	7.675	44122	2620	3.056	2.298
16	7.937	42401	2353	2.937	2.063
17	8.309	18018	1145	1.248	1.004
18	8.784	40714	2244	2.820	1.968
Total		1443848	114037	100.000	100.000

Figure (15): HPLC chromatogram of ethyl acetate fraction of *A.viridis* L leaves and seeds extract

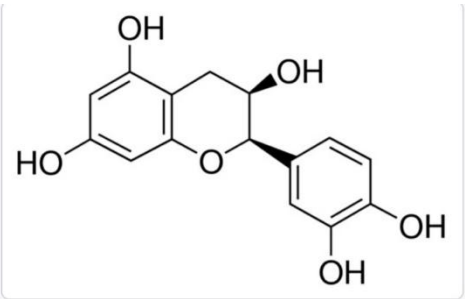


Figure (16): Epicatechin

Conclusion

The presence of many bioactive compounds in the *Amaranthus viridis* L explains the use of whole plant to eradicate different ailments traditionally. However, identification and isolation of individual phytochemical and studying of their biological activities will definitely provide rich results. This is the first report about the microscopical characteristic of the Iraqi grown plant which considered as diagnostic parameters of this genus. Epicatechin which is and important polyphenolic compound was also determined in the plant for the first time. The presence of such an important flavonoid with many important pharmacological activities is a very important discovery especially in the Iraqi grown plant and it needs further investigations and quantitative analysis.

REFERENCES

[1] Gaibimei, P., O. Yousuf, A. Singh and N. M. Devi, 2018. A study on phytochemical screening of Celosia argentea var. cristata inflorescence extract. The Pharma Innovation Journal, 7, 284-287.

[2] Kumar, B., K. Lakshman, V. N. Swamy, P. A. Kumar, D. S. Shekar, B. Manoj and G. Vishwantha, 2011. Hepatoprotective and antioxidant activities of Amaranthus viridis Linn.

[3] Kumar, B. A., K. Lakshman, K. Jayaveera, N. V. Krishna, M. Manjunath and M. Suresh, 2009. Estimation of rutin and quercetin in Amaranthus viridis Linn by HPLC. *Asian J. Exp. Sci,* 23**,** 51-54.

[4] Kumar, B. S. A., K. Lakshman, K. K. N. Jayaveera, D. S. Shekar, C. S. V. Muragan and Manoj, 2009. Antinociceptive and antipyretic activities of Amaranthus viridis Linn in different experimental models. *Avicenna journal of medical biotechnology,* 1**,** 167.

[5] Kumar, A., K. Lakshman, K. Jayaveera, R. Nandeesh, B. Manoj and D. Ranganayakulu, 2010. Comparative in vitro anthelmintic activity of three plants from the Amaranthaceae family. Archives of Biological Sciences, 62, 185-189.

[6] Abubakar, A. R. and M. Haque, 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12, 1-10.

[7] Al-ani, W. M., R. Eldalawy and T. M. Jasiem, 2017. Pharmacognostic and Volatile Oils Content for Iraqi and Turkish Pinus halepensis. Int. J. Pharm. Sci. Rev. Res, 45.

[8] Vidhate, M., A. Ranade, B. Vidhate and P. Birajdar, 2015. Isolation, characterization and quantification of extracted D-pinitol from Bougainvillea Spectabilis stem park. World J Pharm Res, 4, 1669-1683.

[9] Abdul-Jalil, T. Z., K. Saour and A. Nasser, 2010. Phytochemical study of some flavonoids present in the fruits of two Ammi L. species wildly grown in Iraq. Iraqi J Pharm Sci, 19, 48-57.

[10] Mhamdi, B., F. Abbassi, A. Smaoui, C. Abdelly and B. Marzouk, 2016. Fatty acids, essential oil and phenolics composition of Silybum marianum seeds and their antioxidant activities. Pakistan journal of pharmaceutical sciences, 29.

[11] Pandey, K. B. and S. I. Rizvi, 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity, 2, 270-278.

[12] Lopes, G. C., A. Blainski, P. V. P. d. Santos, M. C. Diciaula and J. C. P. d. Mello, 2010. Development and validation of an HPLC method for the determination of epicatechin in Maytenus ilicifolia (Schrad.) Planch., Celastraceae. Revista Brasileira de Farmacognosia, 20, 789-795.

[13] Sivagnanam, S. and A. P. Chandra, 2016. Preliminary phytochemical analysis of Amarathus viridis. RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES, 7, 1-7.

[14] Srivastava, R., M. R. Abdulmalik, P. Mishra, A. S. Ibrahim and C. Rajak, 2018. Preliminary phytochemical investigation and TLC fingerprint profile of Amaranthus herbs with nutraceutical potential. The Pharma Innovation, 7, 224.

[15] Sunday, E. A., W. P. Gift and W. J. Boobondah, 2021. Phytochemistry and antioxidant activity of Amaranthus viridis L (Green leaf). World Journal of Advanced Research and Reviews, 12, 306-314.

[16] Edeoga, H. O., D. Okwu and B. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology, 4, 685-688.
[17] Majumdar, N. and N. C. Mandal, 2019. Screening of different botanicals extract on two polyphagous postharvest pathogens from mango and banana. Journal of Pharmacognosy and Phytochemistry, 8, 4253-4256.
[18] Iwu, M. W., A. R. Duncan and C. O. Okunji, 1999. New antimicrobials of plant origin. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA, 457, 462.
[19] Nitta, T., T. Arai, H. Takamatsu, Y. Inatomi, H. Murata, M. Iinuma, T. Tanaka, T. Ito, F. Asai and I. Ibrahim, 2002. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. Journal of Health Science, 48, 273-276.
[20] Okwu, D. and M. Okwu, 2004. Chemical composition of Spondias mombin Linn plant parts.
[21] Ayinde, B. A., E. Omogbai and F. C. Amaechina, 2007. Pharmacognosy and hypotensive evaluation of Ficus exasperata Vahl (Moraceae) leaf. Acta Pol Pharm, 64, 543-546.
[22] Wadood, A., M. Ghufran, S. B. Jamal, M. Naeem, A. Khan and R. Ghaffar, 2013. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem anal biochem, 2, 1-4.
[23] Aparna, V., K. V. Dileep, P. K. Mandal, P. Karthe, C. Sadasivan and M. Haridas, 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical biology & drug design, 80, 434-439.
[24] Auttajinda, N., P. Raviyan, W. Klangpetch, W. Jirarattanarangsri and S. Osiriphun, 2023. Antibacterial Activities of Palmitic and Lauric Acids from Palm Kernel Oil for the Development of Food-Grade Disinfectants, 2, 1-15.
[25] Pandey, J., T. Bastola, J. Tripathi, M. Tripathi, R. K. Rokaya, B. Dhakal, R. DC, R. Bhandari and A. Poudel, 2020. Estimation of total quercetin and rutin content in Malus domestica of Nepalese origin by HPLC method and determination of their antioxidative activity. Journal of Food Quality, 2020, 8853426.

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