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Investigation of Antioxidant Activity (In Vitro) and Gas Chromatography-Mass Spectrometry Profiling of *Portulaca Oleracea* L Extract

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ABSTRACT

Background:Purslane was its common name for *Portulaca oleracea*. They're usually eaten as either a salads or fruit salad. Such herbs, that relate to a Portulacaceae family, are often used in African and Asian pharmacy for treat many diseases. Such plants are the main sources of natural antioxidants, essential amino acids, and sometimes even antimalarial drugs, according recent pharmacological research.**Methods:** Methanol had been used to extract the plant's vegetative components. The capacity of a species' methanolic extract to scavenge 2,2-diphenyl-1-picrylhydrazyl radical has been examined using gas chromatography-mass spectrometry (GC-MS).**Results:** The presence of 24 biologically active compounds was discovered by GC-MS analysis of P. oleracea extract. The extract bare antioxidant capacity.**Conclusion:**The study's findings may also be useful for future pharmacological and phytochemical studies to determine the species' medicinal benefits.

Keywords:*Portulaca 0leracea* L , 2,2-Diphenyl-1-picrylhydrazyl method, Gas chr0matography-mass spectr0metry analysis.

Introduction

Purslane, or Portulacaoleracea L., is a member of the Portulacaceae family. A herb may have occur in Asia and Africa [1]. In farming systems, it's also found to accumulate also as weeds [2,3]. Its acidic flavor makes it ideal for vegetables, appetizers, and pickles. It was previously thought to also be effective for the prevention for disorders of a stomach, heart, and abdomen, and also some cough, difficulty breathing, fatigue, fires, and nausea. It's often used as a painkiller, an anti - pyretic, an emollient, and an anti-inflammatory [4]. Purslane is still used in Folk remedies to prevent pathogens, stop bleeding, cooling blood, as well as prevent acne, cholera, but mostly hepatitis also with farm's plant propagation [5]. Purslane also has high concentration of β - carotene &vit C than most of the common nutritious crop plants, thus according phytochemical analysis. Furthermore, a herb is shown to be a storeroom for omega-3 fats like alpha-linolenic acid [6]. As a result, a crop could be considered a significant origin of antioxidants and vitamins in the diet[7]. Purslane is a widely spread plant that the Ministry Of Health identifies as one of the most useful medicinal vegetables [8]. Purslane has been shown to have antibacterial, analgesic, anti-inflammatory, muscular muscle relaxant, as well as incision properties in vivo experiments [9].Water or alcoholic extracts were shown to suppress cyclooxygenase and synthetic progesterone synthetase [10], toxic effects of doxorubicin in neuronal liver cells [11] and gentamicin nephrotoxicity [12], control the proliferation of cells [8], suppress respiratory inflammation induced via oxygen deprivation [13], as well as decreased blood sugar or blood lipids [13]. all biological causes for all these potential benefits were also unclear., However antioxidant capacity produced by the ingredients in this natural herb are believed to become a significant factor. Flavonoids, terpenoids, alkaloids, phenolic acids, saponins, vitamins, and minerals were available in purslane [14]. Other components contained in the plant include - carotene, glutathione, melatonin, and a high fatty acid content [15]. Any one of these substances joint venture to provide Free-radical chelating with antioxidant properties. Some purslane flavonoids (kaempferol and quercetin) and alkaloids (oleracein A, oleracein B, and oleracein E) have been shown to have even greater radical scavenging activity than Vit C as well as E [17]. Consequently,, numerous in vitro studies [17], as well as a growing number of in vivo studies [9], have supported the hypothesis that purslane constituents' beneficial effects are due to their free-radical scavenging action[9]

Methodology

Plant collection and identification:

This study's crop was collected from the a local medicinal shop. Dr. Neddaa Adnan (Crop Herbarium / Department of Biology / College of Science / University of Babylon) recognized the plant.The plant part used in this study was Portulaca oleracea L. the Arial parts of plant were grained into fine powder.

Preparation of plant extract:

The methanolic extract of porstulacaoleracea was prepared according [18]. In an average of 1 gm of powdered sample, a solution of methanol with distilled water in a ratio of 20percentage methanol: 80% distilled water (V/V) was being used to extract the powder. Blend 3 mL of the solution about 30 min at room temperature. A solution has been gauze filtered, and

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also the solution was filtered was stored in such a 45°C furnace. A methanolic extract were kept in the dark and kept at 4°C until they were needed.

Gas chromatography-mass spectrometry (GC-MS) analysis:
This test was carried out. in forensic laboratories in Babylon Governorate.

The methanolic extracts of portulaca oleracea was performed on gas chromatograph "Agilent 7890A" with mass spectrometer detector 5975C under computer control at 70 Ev . The capillary column was Hewlett Packard HP-5MS silica , 30 m in length, 250 m in internal diameter, but a film thickness of 0.25 m. Helium was used as carrier gas at a constant flow rate of 2 ml/min with an injection volume of 1 µl with an injector temperature of 250°C, after that the scanning was done for 18 min. The oven temperature was programmed from an initial temperature of 40°C for 2 min , then 12.5 °C/min to 250°C for 12.5 min. Full ion chromatography and mass chromatograms have been used to define the GCMS peaks.; A compounds were further identified using National Institute of Standards and Technology (NIST) 2008 mass spectral library [19].

DPPH radical scavenging activity test

The DPPH free radical scavenging behavior was used to assess antioxidant activity using the method of [20]. In this analysis, the standard was ascorbic acid. 50 µl of dilute extract solutions of various concentrations (200, 100, 50, 25, 12.5 µg/ml) have been captured, A 100 µl of each solvent was applied, and then 150 µl of DPPH, and then left to incubate at room temp for 30 minutes in a dark place. Each test tube received 3ml of each solvent, and the absorbance was measured at 520nm with the respective solvent as a blank. A control sample with the same volume was prepared, and ascorbic acid was used as a reference. The DPPH free radical scavenging percentage was determined using the following equation:

DPPH scavenging activity (%) = [Ac– As/ Ac] × 100

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Results

1.GC-MS analysis of methanolic extract of portulaca oleracea areal parts:
the data obtained from this test are presented in table 1 , table2 and figure 1 .

Table 1.class composition of purslane

Class of composition	*portulaca oleracea* shoot (%)	Number of Class of composition
Alkaloids	13.8%	6
Phenols	13.6%	5
Fatty acids	10.1%	6
Terpenes	9.8%	2
Saponins	1.4%	1
Sugar alcohols	1.1%	1
Carbohydrates	0.5%	1
Ethyl ester	0.5%	1
Amino acids	0.3%	1
Total compositions	**51.1%**	**24 phytochemical compounds**

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Table 2.phytochemicals of purslane identified by GC-MS.

Peak	Phytochemical compound	R.T (min)	Molecular Formula	Molecular weight (g/mol)	Peak area%	*Nature of compound	*biological activity
1.	d-Mannitol, 1-decylsulfonyl	3.1	$C_{16}H_{34}O_7S$	370.5	1.1	Sugar alcohol	Anti-cancer
2.	2,6,10-trimethyl,14-ethylene-14-pentadecne	3.2	$C_{20}H_{38}$	278	3.3	alkaloid	Anti-cancer
3.	gamma. Sitosterol	4	$C_{29}H_{50}O$	414.7	1.4	saponin	Anti-inflammatory
4.	2 Octen 1 ol, 3,7 dimethyl , isobutyrate	4.4	$C_{14}H_{26}O_2$	226.3	1.4	terpene	Cytotoxic activity
5.	Dodecane	5.6	$C_{12}H_{26}$	170.3	0.5	Carbohydrate	Anti-inflammatory
6.	Dodecanoic acid, 3-hydroxy-	5.9	$C_{12}H_{24}O_3$	216.3	0.7	Fatty acid	Anti-inflammatory
7.	dl-Allo-cystathionine	6.3	$C_7H_{14}N_2O_4S$	222.2	0.3	Amino acid	Anti-inflammatory
8.	Phenol, 4- propoxy-	7.3	C9H12O2	152.1	0.5	phenol	Anti-inflammatory
9.	Imidazole, 2 amino 5 [(2 carboxy) vinyl]	9.2	$C_6H_7N_3O_2$	153.4	2.3	alkaloid	anti-inflammatory
10.	Ethylbenzene	10.3	C_8H_{10}	106.1	0.5	Ethyl ester	Anti-oxidant
11.	Phenol, 2,6-dimethoxy-	10.8	$C_8H_{10}O_3$	154.1	1.2	phenol	Anti-inflammatory
12.	6,9,12,15 Docosatetraenoic acid, methyl ester	11.6	$C_{23}H_{38}O_2$	346.5	1.1	Fatty acid	Anti-cholesterol
13.	Octadecanoic acid, 2 (octadecyloxy) ethyl ester	12.3	$C_{38}H_{76}O_3$	581	1	Fatty acid	anti-cancer
14.	Vanillinalactoside	12.7	$C_{20}H_{28}O_{13}$	476.4	4.8	phenol	anti-oxidant
15.	1- (5- Methoxy- 4,4- di methyl- dihydro- furan- 2- ylidene)- propan- 2- one	12.8	$C_{10}H_{16}O_3$	184.2	1.6	phenol	anti-cancer
16.	Hexadecanoic acid, 2 hydroxy 1 (hydroxymethyl) ethyl ester	12.9	$C_{19}H_{38}O_4$	350.5	0.9	Fatty acid	anti-oxidant
17.	1 Benzoylamino 5 piperidinyl 1 phenylpentane	13	$C_{23}H_{30}N_2O$	350.5	2.7	alkaloid	anti-cancer
18.	Linoleic acid ethyl ester	13.2	$C_{20}H_{36}O_2$	308.5	6.3	Fatty acid	Hepatoprotective
19.	1 (5 Bicyclo[2.2.1] heptyl) ethylamine	13.5	$C_{23}H_{30}N_2O$	139	2.6	alkaloid	Anti-inflammatory
20.	3,3 Dimethoxy 2 butanone	14.4	$C_6H_{12}O$	132.16	5.5	phenol	Anti-cancer
21.	5alpha-Androstan-12-one, cyclic ethylene mercaptole	14.5	C21H34S2	350.5	8.4	terpene	Antioxidant, Anti-inflammatory and Cytotoxic activities

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22.	Papaveroline, 1,2,3,4 tetrahydro 3 O methyl	14.7	$C_{17}H_{19}NO_4$	270.5	1.2	alkaloid	Anti-inflammatory
23.	9,12 Octadecadienoic acid(Z,Z)-	14.7	$C_{18}H_{32}O_2$	280.4	1	Fatty acid	Hepatoprotective
24.	2 Pentanamine, N ethyl 4 methyl	14.8	$C_8H_{19}N$	129.2	1.7	alkaloid	Anti-inflammatory

* Nature of compounds ,activity from : Dr. Duke's Phytochemical and Ethnobotanical databases .
R.T: Retention time

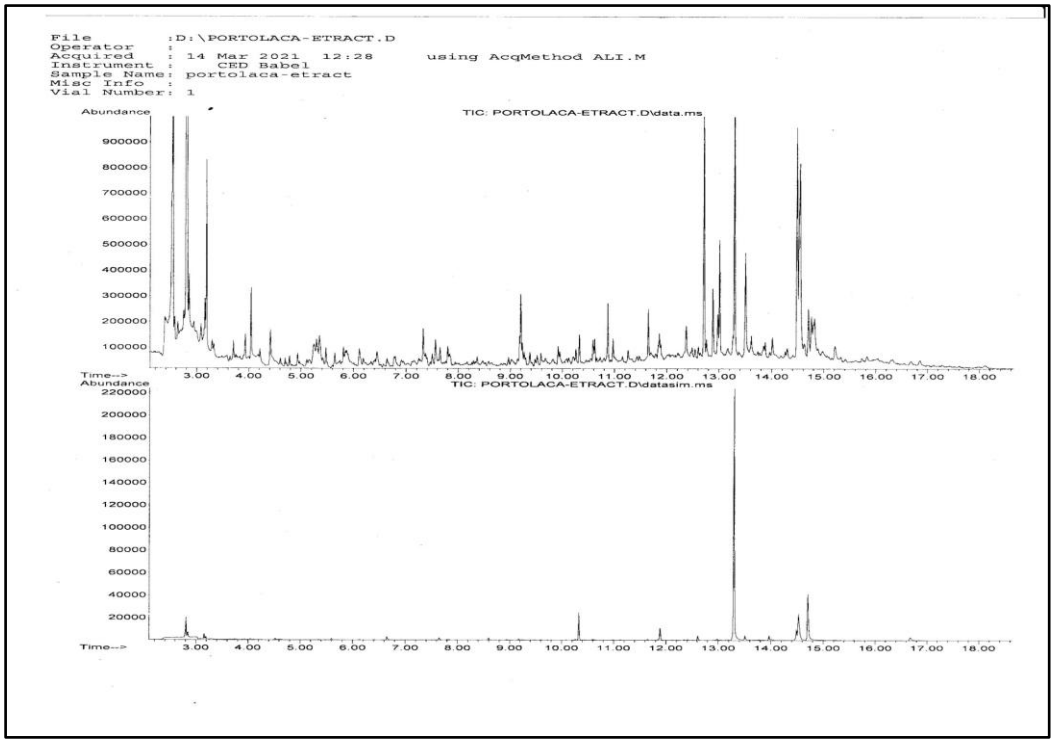

Figure 1.the result of GC-MS analysis of the compound of methanolic extract of portulaca oleracea , the main components were showed as the marked peak with retention time

2- DPPH radical scavenging activity test:

The results in table (3) and figure (2) showed comparison of the radical scavenging activity of Portulaca oleracea extract with ascorbic acidthat revealedthere was a significant decrease ($p<0.05$) in the radical scavenging activity of the extract at a concentration of 200µg/ml compared to ascorbic acid, and there were no significant differences ($p>0.05$) in the radical scavenging activity of the extract at concentrations of 100,50,25 and 12.5 µg/ml compared with the same concentrations of ascorbic acid.

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Table 3.percent of Scavenging activity in portulaca oleracea extract.

Concentration of extract (µg/ml)	Percentage scavenging of DPPH radical %		significance	P value
	Ascorbic acid	P. oleracea extract		
200	86.03±4.02	76.89±2.73	*	0.036
100	74.07±1.00	69.75±2.23	ns	0.616
50	57.60±2.20	55.63±0.85	ns	0.976
25	39.00±1.73	40.82±7.28	ns	0.983
12.5	22.90±1.86	18.02±6.78	ns	0.491

-($p<0.05$) significant differences
-($p>0.05$) non- significant difference

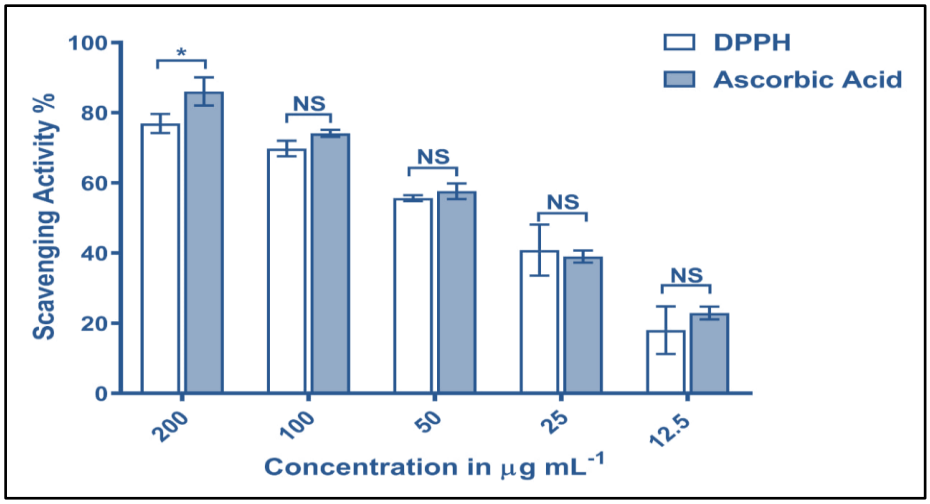

Figure 2. Percentage of inhibition of DPPH by 20% methanolic extract of P. oleracea as compared with standard ascorbic acid.

Discussion

1- GC-MS

twenty four biologically active compounds were identified in methanolic extract of portulaca oleracea. The GC-MS analysis of the compounds carried out in this extract was shown in table 2, class composition of the compounds carried out in this extract was shown in table 1. the GC-MS analysis also indicated the presence of a higher percentage of alkaloid compounds (13.8%) , followed by phenol compounds (13.6%) , fatty acids compounds and terpene compounds(9.8%) table 1 . The identified compounds with more percentage were, 5alpha-Androstan-12-one, cyclic ethylene mercaptole (8.4%) , Linoleic acid ethyl ester (6.3%) , Vanillinalactoside (4.8%) , 2,6,10-trimethyl,14-ethylene-14-pentadecne (3.3), 1 Benzoylamino 5 piperidinyl 1 phenylpentane (2.7%) it showed a wide range of potent bioactivity table 1, figure 1. These phytochemicals are responsible for various pharmacological actions like cytotoxic activity [21], Hepatoprotective activity [22] , anti-oxidant activity [21],anti-cancer activity [23] and anti-inflammatory activity[21]. among the twenty four compounds identified 9 showed anti-inflammatory, 6 showed anti-cancer, 4 showed anti-oxidant, 2 showed Hepatoprotective activity , 2 showed cytotoxic activity , 1 showed anti-cholesterol respectively.

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2- DPPH radical scavenging activity test

The DPPH assay measures the antioxidant reaction with the organic radical 2,2-diphenyl-1-picrylhydrazyl. DPPH is a stable free radical compounds and has an absorbance in its oxidized form around 515-520 nm[24].These results showed that the methanolic extract of portulaca oleracea shoot and leaves possessed antioxidant activity. The result may be due to the presence of high total fatty acid (a-linolenic acid and linoleic acid) and phenolic contents. The portulaca oleracea was had the special properties of suppression reactive oxygen species (ROS), free radicals, peroxides and various other harmful oxidizing agents. [25]. It inhibits xanthine oxidase, which is mainly involved in the production of reactive oxygen species. The first evidence for the antioxidant properties exhibited by portulaca oleracea is that it has the ability to degrade free radicals produced by various food radiations [25]It has been reported that portulaca oleracea protects DNA in vitro from stannous chloride-induced oxidative damage of ROS[20].

Conclusion

The existence of many useful compounds such as polyunsaturated fatty acids, alkaloids, and terpenoids was discovered using GC–MS analysis of methanolic extracts of purslane. Although the findings of the GC–MS study of purslane did not match those of previous studies, it is important to note that growth conditions and harvest time play a significant role in determining phytochemicals and their amounts [25]. According to the findings, the methanolic extracts of purslane have potent anti-oxidant activity, which may be attributable to the presence of specific phytocompounds. These phytocompounds have the potential to prevent the emergence of new free-radical species while also converting existing ones.

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