

Zaid M. Najah*, Najla A. Algaw

Chemistry Department, Science Faculty, Elmergib University, Alkoms, Libya

Article information	Abstract
Key words Cestus Parviflorus, GC analysis, Fatty acid esters, Bioactive compounds. Received 20/07/2024, Accepted 14/08/2024, Available online 24/08/2024,	Phytochemical screening of <i>Cestus Parviflorus</i> plant ethanolic extract was carried out revealing presence of most bioactive phytochemicals, including alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins, terpenes and quinones. For more depth analysis, GC-MS analysis was performed. Wide diverse of chemical classes was found in the ethanolic extract of the plant, long chain hydrocarbons, different types of terpenes, flavonoids, nitrogen and oxygen contained heterocyclic compounds and fatty acids and their esters. Five fatty acids were the most abundant in the ethanolic extract with 11.72% peak area, the acids are; Oleic acid (<i>Z</i>)-9-Octadecenoic acid, <i>trans</i> -13-Octadecenoic acid, <i>cis</i> -13-Octadecenoic acid and <i>cis</i> -Vaccenic acid. Anti oxidation activity of the plant powder also investigated, IC_{50} value of <i>C. parviflorus</i> plant was 3.95 µg/ml, lower than standard reagent ascorbic acid with 13.49 µg/ml and indicates that, <i>C. Parviflorus</i> is a strong and promising natural antioxidation agent.

I. Introduction

Cictus Parviflorus plant (Figure 1), belongs to *Cistaceae* family, the family consists of 8 genera and 180 species, with 5 genera native to the Mediterranean area (*Cistus, Fumara, Halimium, Helianthemum,* and *Tuberaria*). Traditionally, a number of *Cistus* species have been used in Mediterranean folk medicine as herbal tea infusions for healing digestive problems and colds, as extracts for the treatment of diseases, and as fragrances [1]. This genus is made up of various pioneer species, characterized by their ability to proliferate in areas exposed to drought, with stony and infertile soils. The plants of the family are very resistant pyrophilic plants that are able to regenerate quickly after forest fires due to the seeds' increased germination capacity after exposure to high temperatures [2].



Corresponding author Email: Zmnajah@elmergib.edu.ly

Figure 1. Cistus Parviflorus plant

Chemical analysis of different *Cistus* species' tissues showed different chemical classes, including diterpenes, which are usually detected in *Cistus monspeliensis* L. and *Cistus libanotis* L. These plants are also recognized to contain multiple compounds from different chemical classes such as flavonoids, coumarins, terpene derivatives, hydrocarbons. Many studies have reported on the phytochemicals in extracts of different *Cistus* species from several regions [3].

A phytochemical investigation of leaves of *Cistus parviflorus* led to the isolation of 18 compounds, most of them are flavonoids such as ; kaempferol 3-O-(3",6"-di-O-E-pcoumaroyl) – β – D - glucopyranoside , scopoletin, kaempferol 3 – O - (3"-O-E-pcoumaroyl)- β - D-glucopyranoside, kaempferol 3 - O- (6"-O-E-p-coumaroyl) - β - D -3-O-β-D-glucopyranoside glucopyranoside kaempferol , kaempferol 3-0α-L-• rhamnopyranosyl - $(1\rightarrow 2)$ - $(6"-O-E-p-coumaroyl) - \beta - D$ -glucopyranoside, methyl 3-O-β-D-glucopyranoside , quercetin flavogallonate quercetin 3-O-β-Dand galactopyranoside [4].

The composition of the essential oils of nine populations of *Cistus parviflorus* L., from Crete (Greece) and their interpopulation variability, were investigated by GC-MS. 114 compounds were identified representing of 85-96% an average of oil Carvacrol, carvophyllene oxide, α -epi-cadinol, abietatriene, composition. 4-epidehydroabietol, dehydro abietol, cis-ferruginol and manoyl oxide mixture of isomers are the main constituents, while oxygenated sesquiterpenes as well as labdane diterpenes have been found [5].

II. Materials and Methods

A. Plant Material

Cistus Parviflorus plant (aireal part) was collected from Gaser Khiar region (East of the capital Tripoli, 32°46'26"N 13°46'56"E) in flowering time, March of 2022. The plant was identified by plant taxonomist from Botany department, Science Faculty, Elmergib University. The plant material was cleaned and foreign materials were removed, dried in the shade then grinded to a fine powder using electrical blender, the powder was stored in airtight container and stored at room temperature until further use.

B. Extraction method for phytochemical screening

10.00 g of the plant powder and 200 ml of the solvent was put in 250 ml flask, then stirred for 24 hours using Jenway 1002 Stirrer machine at room temperature to keep most of the low boiling point components. Different polarity solvents were used to guarantee extraction of all types of chemical classes, the solvents were; ethyl acetate, ethanol, chloroform and hexane. Aqueous extract was prepared by heating 10.0 g of the powder in 200 ml of water at 70 °C for 20 min., after cooling, the mixture was filtered and kept in the fridge. All extracts were filtered then concentrated using water bath then kept until using for phytochemical screening process illustrated by Harborne [6].

C. DPPH Radical scavenging method

Free radical scavenging activity of different extracts of studied plant were measured by 1, 1-Di Phenyl-2-Picryl Hydrazyl (DPPH). In brief, 0.10 m*M* solution of DPPH in ethanol was prepared. The solution (1.0 ml) was added to 3.0 ml. of different extracts in ethanol at different concentration (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 μ g/ml). Here, only those extracts are used which are solubilize in ethanol and their various concentrations were

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prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. then, absorbance was measured at 517 nm by using spectrophotometer (UV-VIS milton roy). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC₅₀ value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity [7]. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or inhibition Percent = $A_0 - A_1 / A_0 \times 100$.

Where A_0 the Absorbance of control reaction, A_1 the Absorbance in of test sample.

D. GC-MS protocol

10.0 ml of ethanol was added to 2.0 g of a homogenized powder sample, the mixture was shaken vigorously for 60 min. to transfer phytochemicals from the sample matrix into the organic layer. The extract was centrifuged and the supernatant was collected and filtered through 0.20 μ m syringe to remove particulate matter. The filtered extract was concentrated using rotary evaporation. The dried concentrated extract was dissolved in 5.0 ml ethanol, then, 1.0 μ l of reconstituted sample was injected into the GC injection port using a microliter syringe [8].

The chemical composition of ethanolic extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 μ m film thickness). The column oven temperature was initially held at 50 °C and then increased by 5 °C /min to 230 °C hold for 2 min. increased to the final temperature 290 °C by 30 °C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260 °C respectively; Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min. and diluted samples of 1.0 μ l were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

III. Results and Discussion

A. Phytochemical screening

The phytochemical content of aerial parts extract of *Cistus Parvilorus* was investigated with different solvents, the results were summarized in Table (1). Alkaloids, carbohydrates, glycosides, saponines, phytosterols, phenols, tannins, flavonoids, proteins, terpenes and quinones, all were present in different solvent extracts.

Test	Extract	EtOAc	EtOH	CH ₃ Cl	Aqueous	Hexane
	Wagner	•	-	-	+	-
Alkaloids	Dragendrof	-	+	-	+	+
Carbohydrates	Molish	+	+	+	+	+
Reducing sugars	Fehling	+	+	+	+	-
Glycosides	Keller-Kelani	+	+	-	+	-
Sapor	-	+	-	+	-	
Director actor and in	Salkowsky	+	+	-	+	-
Phytosterols	Liberman	+	+	-	+	+
Phenols		-	+	-	+	-
Tannins		-	-	-	+	-
Florencido	Basic test	+	+	+	+	-
Flavonoids	Lead acetate	-	+	-	+	-
Proteins	Xanthoprotein	+	+	+	+	-
	Nenhydrin	-	+	-	+	-
Terpenes		-	+	-	+	-
Diterp	-	+	-	+	-	
Quino	-	+	-	+	-	
Anthraqu	-	-	-	-	-	

+ : Present, - : Absent

All the phytoconstituents were present in aqueous extract except anthraquinones which were absent in all extracts, carbohydrates were present in all extracts, flavonoids, proteins and reducing sugars were present in all extracts except hexane extract. Phytosterols were present in all extracts except chloroform extract, tannins were present in ethanolic extract only, alkaloids were absent in ethyl acetate and chloroform extracts, cardiac glycosides were absent in chloroform and hexane extracts. Saponines, phenols, amino acids, terpenes and quinones were absent in ethyl acetate, chloroform and hexane extracts. Aqueous and ethanolic extracts yielded more metabolites compared to ethyl acetate, hexane and chloroform extracts, while hexane extract yielded less metabolites.

Thetannins in plant species act as astringent, antioxidants, free radical scavengers, promote healing of wounds and effective in peptic ulcers while presence of reducing sugars in these plants has a reductive property [9]. Terpenoids act as cardio protective and antioxidant. Steroids are frequently used as a signaling molecules and decrease fluidity of membranes [10]. Glycosides are characterized by their actions on contractile forces of cardiac muscle and saponins show anti-fungal, antibacterial, anti-protozoal and lipid lowering effects. Saponins present in all plant species, and work as a lipid lowering agent as well as has anthelmentic and antibacterial activity [11]. Phenolic compounds widely distributed in plant kingdom and have been reported to exert multiple biological effects, including antioxidant, free radical

scavenging abilities, anti-inflammatory and anti-carcinogenic. Due to presence of phenolic compounds, the plant might play role in the prevention of several chronic diseases such as cardiovascular disease, cancer, diabetes, bacterial and parasitic infections [12]. Flavonoids can also inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide synthase, which are supposed to be involved in free radical generation, thereby resulting in decreased oxidative damage of macromolecules [13].

B. Antioxidation analysis

The free radicals are mainly derived from oxygen and nitrogen, and are produced in our body systems, exposure to physicochemical conditions or could be related to some diseases. Free radicals can cause adverse effects on lipids, proteins and oligonucleotides including DNAs and RNAs and also involved in aging and a many human diseases. The use of plant extracts with antioxidant activity can be of great significance in the treatment of many diseases [14]. Plants are able to produce a large number of antioxidants to manage the oxidative stress resulted from the sunbeams and oxyge. Antioxidants are the compounds or materials which can effectively catch the free radicals and reduce the occurrence of damage induced by the oxidative stress [15].

Ascorbic acid, commonly known as vitamin C, is one of the basic and best-known compounds necessary for the proper functioning of the human body, and it's one of the basic low-molecular antioxidants functioning in the human body. It takes part in the regulation reactive oxygen species (ROS) levels and the effectiveness of other antioxidants [16].

Aerial part ethanolic extract of *C. parviflorus* plant showed better antioxidant potential when compared with the standard, ascorbic acid by DPPH scavenging assay method. The better antioxidant agents should have lower IC_{50} value, comparing with super scavenging agent, ascorbic acid. IC₅₀ value of *C. parviflorus* plant was 3.95 µg/ml, lower than ascorbic acid with 13.49 µg/ml, the result clearly indicates that, *C. parviflorus* aerial part is a strong and promising natural antioxidation agent.

Kalpoutzakis and co-workers reported excellent DPPH radical scavenging of *C. parviflorus* collected from Greek Island of Crete, with $18.5 \pm 0.6 \mu \text{g/ml}$ (Reference compound; Dimethyl sulphoxide DMSO), the most effective DPPH radical scavengers IC₅₀ <50 µg/ml) [17], another study by Akyuz *et. al.* illustrated good antioxidation activity of *C. parviflorus* with $5.55 \pm 0.41 \mu \text{g/ml}$ (Reference compound BHT, IC₅₀ value; $5.83 \pm 0.2 \mu \text{g/ml}$) [18].

C. GC-MS analysis

GC-MS analysis of ethanolic extract of aerial part of *Cistus Pavriflorus* produced complex spectrum as shown in Figure 2.

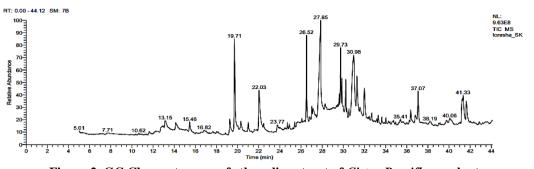


Figure 2. GC Chromatogram of ethanolic extract of Cistus Parviflorus plant

The identification of the compounds was based on the comparison of the retention times and molecular weights to those found in NIST spectra database, the compounds were very diverse; unsaturated fatty acids and their esters, substituted aromatic hydrocarbon, substituted heterocyclic aromatic compounds and wide range of phenolic compounds. The highest concentration compounds (with high peak area) and most important compounds were listed in Table (2).

No.	RT	Compound Name	Area %	MW	MF
1	13.14	Thymol	1.21	150	C ₁₀ H ₁₄ O
2	14.15	(E) -2-Methoxy-5-(1-Propenyl) Phenol	1.35	164	$C_{10}H_{12}O_2$
3	15.46	4-(<i>N</i> -Methylamino)-6,7-(1,2,3,4- Tetrahydro-1,1,4,4-Tetramethylbenzo) Indol	1.07	256	$C_{17}H_{24}N_2$
4	19.26	<i>N</i> -Ethyl-6-Chloro-2,4- Diamine -1,3,5- Trizine	1.62	173	C ₅ H ₈ ClN ₅
5	19.71	7-Chloro-3-Methylquinoline-8-Carboxylic acid	1.21	221	C ₁₁ H ₈ ClNO ₂
6	19.71	2-Ethylidene-6-Methyl- 3,5-Heptadienal	1.21	150	$C_{10}H_{14}O$
7	20.31	Retinol (vitamin A1)	0.99	286	C ₂₀ H ₃₀ O
8	21.00	Cis-3-Octyl-Oxiraneoctanoic acid	1.09	298	$C_{18}H_{34}O_3$
9	22.03	1-Naphthaleneacetic acid, methyl ester	4.52	200	$C_{13}H_{12}O_2$
10	23.76	19,20-Dihydroxy-(19S)-Curan-17-oic acid, methyl ester	0.70	358	$C_{20}H_{26}N_2O_4$
11	24.71	Lactaropallidine (Sesquiterpene)	0.48	252	$C_{15}H_{24}O_3$
12	25.39	Digitoxine (phytosterol)	0.56	764	$C_{41}H_{64}O_{13}$
13	26.52	Hexadecanoic acid, methyl ester	7.33	270	$C_{17}H_{34}O_2$
14	26.52	14-Methyl Pentadecanoic acid, methyl ester	7.33	270	$C_{17}H_{34}O_2$
15	27.02	5,7,3',4',5'-Pentamethoxyflavone	1.15	372	$C_{20}H_{20}O_7$
16	27.02	Selagine (sesquiterpene alkaloid)	1.15	242	$C_{15}H_{18}N_2O$
17	27.85	n-Hexadecanoic acid	10.75	256	$C_{16}H_{32}O_2$

Table 2. Compounds identified from GC-MS analysis of Cistus Pavriflorus ethanolic
extract

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18	27.85	l-(+)-Ascorbic acid, 2,6-dihexadecanoate	10.75	652	C ₃₈ H ₆₈ O ₈	
19	29.73	10-Octadecenoic acid, methyl ester	5.06	296	$C_{19}H_{36}O_2$	
20	30.23	Octadecanoic acid methyl ester (Methyl Stearate)	3.20	298	$C_{19}H_{38}O_2$	
21	30.23	16-Methyl heptadecanoic methyl ester	3.20	298	$C_{19}H_{38}O_2$	
22	30.98	Oleic Acid	11.72	282	$C_{18}H_{34}O_2$	
23	30.98	Trans-13-Octadecenoic acid	11.72	282	$C_{18}H_{34}O_2$	
24	30.98	Cis-13-Octadecenoic acid	11.72	282	$C_{18}H_{34}O_2$	
25	30.98	Cis-Vaccenic acid	11.72	282	$C_{18}H_{34}O_2$	
26	31.58	3',4',7-Trimethoy Quercetin (flavonoid)	0.82	344	C ₁₈ H ₁₆ O ₇	
27	31.58	Gibberelline A22, methyl ester	0.82	360	$C_{20}H_{24}O_{6}$	
28	31.99	Allogibberic acid (plant hormone)	3.46	284	$C_{18}H_{20}O_3$	
29	33.30	Isochiapin B (sesquiterpene lactone)	0.71	346	$C_{19}H_{22}O_{6}$	
30	36.38	13-Docosenoic acid, methyl ester (Methyl Erucate)	1.46	352	$C_{23}H_{44}O_2$	
31	36.38	13-Docosenoic acid (Erucic acid)	1.46	338	$C_{22}H_{42}O_2$	
32	37.07	3',8,8'-Trimethoxy-3-piperidyl-2,2'- binaphthalene-1,1',4,4'-Tetrone	3.32	487	C ₂₈ H ₂₅ NO ₇	
33	37.07	9-(2',2'-Dimethylpropanoilhydrazono)-3,6- dichloro-2,7-bis-[2-(diethylamino)- ethoxy]fluorene	3.32	576	$C_{30}H_{42}Cl_2N_4O_3$	
34	37.07	2,6-Dimethyl- <i>N</i> -(2-Methyl-β -Phenylbenzyl) Aniline	3.32	301	C ₂₂ H ₂₃ N	
35	37.07	6,8 -Di-β-D-Glucopyranosyl -5,7- Dihydroxy- 2-(3,4-Dihydroxyphenyl)-4H-1- Benzopyran-4-One	3.32	610	$C_{27}H_{30}O_{16}$	
36	39.77	Ethyl iso-Allocholate (sterol)	0.51	463	$C_{26}H_{44}O_5$	
37	39.77	Tetraneurine A – Diol	0.51	280	$C_{15}H_{20}O_5$	
38	40.07	Cholan-24-oic acid	0.87	504	$C_{29}H_{44}O_7$	
39	41.33	Ancistrocline (Naphthyl Isoquinoline alkaloids)	3.00	436	C ₂₇ H ₃₄ NO ₄	
40	41.33	1,2-Dimethyl-3-(3-Bromo-4-Methyl-2H- Pyran-2-on-6-yl) Indolizidine Dicarboxylate	3.00	419	C ₁₈ H ₁₄ BrNO ₆	
41	41.33	2-(3,5-Dibromo-2-Methoxyphenyl)-1H- Pyrrol-3,4-Dicarboxylic Acid	3.00	417	C ₁₃ H ₉ Br ₂ NO ₅	
42	41.33	Dibromo-N-Phthaloyl Norvaline, methyl ester	3.00	417	$C_{14}H_{13}Br_2NO_4$	
43	41.33	3-(Acetyloxy)-16,17-epoxy-6-methyl - (3β,16β)- Pregn-5-en-20-One	3.00	386	$C_{24}H_{34}O_4$	
44	41.64	Tocospiro A(tocopherol)	2.27	462	$C_{29}H_{50}O_4$	
45	41.64	Dimethoxycurcumin (polyphenol)	2.27	396	$C_{23}H_{24}O_{6}$	
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Firstly, five fatty acids were the most abundant in the ethanolic extract with 11.72% peak area, the acids are; Oleic acid ((Z)-9-Octadecenoic acid), *trans*-13-Octadecenoic acid, *cis*-13-Octadecenoic acid and *cis*-Vaccenic acid.

Oleic acid is a fatty acid consists of 18 carbons and one double bond found on the 9th carbon, the systematic name is *cis*-9-Octadecenoic acid, the acid occurs naturally in various animal and vegetable fats and oils, and found to have antibacterial activity, particularly in inhibiting the growth of several Gram-positive bacterial species [19], in addition to antifungal, anti-inflammatory, antioxidation properties [20].

Cis-vaccenic acid is a kind of trans-fatty acid (omega-7 fatty acid) found in human milk, known for its various biological effects like antibacterial and hypolipidemic effects in rats [21].

Trans-13- Octadecenoic acid is an unsaturated long chain fatty acid with a trans-double bond at position C-13, well known for anti-inflammatory, antiamdrogenic, dermatitigenic, anaemiagenic, insecticides properties and used also as a flavor [22]. *Cis*-13- Octadecenoic acid has therapeutic uses in medicine and some applications in surgery [23].

Next, another fatty acid recorded high concentration in the spectrum, Hexadecanoic acid (10.75%), its methyl ester (with peak area of 7.33%), the acid has reported Cardioprotective effect [24], and anti-inflammatory activity [25]. 1-(+) -Ascorbic acid, 2,6-dihexadecanoate (10.75%) and 14-Methyl Pentadecanoic acid methyl ester (7.33%), Ascorbic acid is often used for treating common cold, gum disease, acne and other skin infections, bronchitis, stomach ulcers, tuberculosis, dysentery, boils and wounds, in addition to well-known properties of prevention of prevent glaucoma, cataracts, gallbladder disease, dental cavities, constipation, hay fever, asthma, arthritis, back pain, diabetes, chronic fatigue syndrome, osteoporosis and boosting the immune system [26], 14-Methyl Pentadecanoic acid methyl ester (7.33%) belongs to fatty acids family and has antibacterial and antifungal activity [27], 10-Octadecenoic acid methyl ester (5.06%), fatty acid ester well known for Antibacterial, antifungal, antioxidant, decrease blood cholesterol actions [28].

Next, 1-Naphthaleneacetic acid methyl ester with peak area of 4.52%, 1-Naphthaleneacetic acid methyl ester is a cyclic peptide that has been shown to inhibit the production of cortisol, which leads to an increase in growth hormone. It also inhibits the activity of protein kinase domains and can be used as a chemical pesticide [29]. Octadecanoic acid methyl ester (known also as Methyl stearate) recorded 3.20% peak area, reported to have antimicrobial activity [30].

Interesting compounds with low concentrations were observed in the chromatogram; Retinol and Digitoxine (eluted at 20.31 and 25.39 min. with peak area of 0.99, 0.48 and 0.56% respectively). Retinol, well known member of vitamin A family and known as vitamin A_1 , fat-soluble vitamin used as a dietary supplement, retinol improves fine wrinkles associated with natural aging. Significant induction of glycosaminoglycan, which is known to retain substantial water [31]. Digitoxin is a phytosterol and cardiac glycoside, used for heart failure treatment and certain kinds of heart arrhythmia [32].

An interesting alkaloid came out at 27.02 min. with 1.15% peak area identified as Selagine, (more commonly known as Huperzine A) is a naturally-occurring sesquiterpene alkaloid compound extracted from different plants. Huperzine A used for treatment of neurological conditions such as Alzheimer's disease [33]. The sesquiterpene lactone Isochiapin B was

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came out at 33.30 min. with 0.71% peak area, it has been used to treat arthritis, tonsillitis, and other ailments by Chinese medicine [34].

13-Docosenoic acid and its methyl ester (also known as Erucic acid and methyl Erucate) came out at 36.38 min. with 1.46 % peak area. Erucic acid is a fatty acid used in treatment of Adrenoleukodystrophy, exerts antioxidant and anti-inflammatory effects, and may act positively in Multiple Sclerosis and Alzheimer's Disease [35].

Ethyl isoallocholate is a sterol compound, came out at 39.77 min. with 0.51% peak area, used as an antibacterial, antioxidant, antitumor, cancer preventive, pesticide and chemo preventive agent [36]. Tetraneurine-A diol, also came out at 39.77 min. with 0.51% peak area, known to posses Antifeedant and Pesticide properties [37].

One of the interesting identified compounds was Ancistrocline derivative, (+) (1*R*,3*S*)-5-(4,5-Dimethoxy-2-Methyl-1-Naphthyl)-6,8-Dimethoxy-1,2,3-Trimethyl-1,2,3,4-Tetrahydro iso quinoline [(+)-O-Methyl Ancistrocline, 3%). Ancistrocline belongs to Naphthyl Isoquinoline alkaloids (NIQAs) family. The structurally unique NIQAs are characterized by a biaryl system consisting of naphthalene and an iso-quinoline moiety. Bringmannn and Pokorny proved the unusual molecular structure of achiral biaryl axis is produced by an unparalleled biosynthesis of iso-quinoline alkaloids from acetic acid units [38].

Ancistrocline extracted from *A. tectorius* (an African species) exhibited anticancer activity [39], also, Ancistrocline was extracted from stem of Indian variety *Ancistrocladus heyneanus* and proved to work as agent with minimum cytotoxicity to normal cells [40].

Tocospiro A, a tocopherol family member came out at 41.64 min. with 2.27% peak area, tocopherol is one of the most abundant natural antioxidants, with excellent biological activity that protect cellular membranes and increase stability in fat and oil [41].

The last compound in the chromatogram with 2.27% peak area was Dimethoxycurcumin, a derivative of well-known polyphenol curcumin, which exhibits anti-carcinogenic, anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities. Dimethoxy curcumin, demonstrates powerful anti-inflammatory activity and could work as an alternative to curcumin, due to its superior bioavailability and comparable efficacy [42].

Limited researches in literature mentioned GC-MS study of the studied plant, most of them focused on volatile oil of the plant. Tetik and co-workers identified Seventy-six compound from Water distilled essential oil of *Cistus parviflorus* from Turkey. $8-\alpha-13$ -oxy-14-ene-epilabdane (18.2%), manoyl oxide (9.1%) and δ -cadinene (6%) were characterized as major constituents [43].

A study in Greece focused on GC-MS analysis of essential oils of nine populations of *Cistus parviflorus*. 114 compounds were identified representing an average of 85-96% of oil composition. Labdane diterpenes, carvacrol, caryophyllene oxide, alpha-epi-cadinol, abietatriene, 4-epi-dehydroabietol, dehydro abietol, cis-ferruginol and manoyl oxide mixture of isomers are the main constituents, while oxygenated sesquiterpenes and diterpenes have been found in high percentage [5].

IV. Conclusion

The performed analyses on ethanolic extract of *Cistus parviflorus* confirmed the presence of wide range of useful phytochemical compounds justifying the medicinal use of the plant. An excellent antioxidation activity of the plant is very encouraging for more research in drugs discovery area to explore the potent of the phytocomponents identified by GC-MS analysis.

Extra research work also recommended for individual plant parts especially leaves and flowers.

V. Acknoledgemnets

The authors are thankful to Elmergib university for supporting the research.

VI. References

(1) D. Papaefthimiou, A. Papanikolaou, V. Falara, S. Kostas, *et al.*, "Genus *Cistus*: a model for exploring labdane-type diterpenes' biosynthesis and a natural source of high value products with biological, aromatic, and pharmacological properties," *Frontiers in chemistry*, vol. 2, pp. 35 -50, 2014.

(2) F. J. Alvarez-Martinez, F. Borrás-Rocher, V. Micol, and E. Barrajon-Catalan, "Artificial intelligence applied to improve scientific reviews: The antibacterial activity of *Cistus* plants as proof of concept," *Antibiotics*, vol. 12, no. 2, pp. 327-359, 2023.

(3) I. Zalegh, M. Akssira, M. Bourhia, F. Mellouki, *et al.*, "A review on *Cistus* sp.: phytochemical and antimicrobial activities," *Plants*, vol. 10, no. 6, pp. 1214-1245, 2021.

(4) X. Fang, R. Wang, S. Sun, X. Liu, *et al.*, "Chemical constituents from the leaves of *Cistus parviflorus*," *Journal of Chinese Pharmaceutical Sciences*, vol. 27, no. 1, pp. 40-50, 2018.

(5) D. Angelopoulou, C. Demetzos, and D. Perdetzoglou, "An interpopulation study of the essential oils of *Cistus parviflorus* L. growing in Crete (Greece)," *Biochemical systematics and ecology*, vol. 29, no. 4, pp. 405-415, 2001.

(6) P. M. Dey, and J. B. Harborne, *Methods in plant biochemistry: Plant phenolics*, London, UK, Academic Press Ltd., 1989, ch. 1, pp. 552-559.

(7) N. Liang, and D. Kitts, "Antioxidant property of coffee components: assessment of methods that define mechanisms of action," *Molecules*, vol. 19, no. 11, 19180-19208, 2014

(8) K. R. Asha, S. Priyanga, S. Hemmalakshmi, and K. Devaki, "GC-MS analysis of the ethanolic extract of the whole plant *Drosera indica* L.," *International Journal of Pharmacognocy and Phytochemical Research*, vol. 9, no. 5, pp. 685-688, 2017.

(9) N. S. Rajurkar, and K. Gaikwad, "Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris* leaves," *Journal of chemical and pharmaceutical research*, vol. 4, no. 1, pp. 365-374, 2012.

- (10) D. Sadava, D. M. Hillis, H. C. Heller, and M. R. Berenbaum, *Life: The Science of Biology*, 9th ed., USA, Sinauer Associates, Inc., 2011.
- (11) P. B. Ayoola, and A. Adeyeye, "Phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves," *Int. J. Rec. Res. Appl. Stud. (ijrras,*), vol. 5, no. 3, pp. 325-328, 2010.
- (12) A. Canini, D. Alesiani, G. D'Arcangelo, and P. Tagliatesta, "Gas chromatography-mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf," *Journal of food composition and analysis*, vol. 20, no. 7, pp. 584-590, 2007.
- (13) F. Mumtaz, S. M. Raza, Z. Ahmad, A. Iftikhar, *et. al.*, "Qualitative phytochemical analysis of some selected medicinal plants occurring in local area of Faisalabad, Pakistan," *Journal of Pharmacy and Alternative Medicine*, vol. 3, no. 3, pp. 5-10, 2014.
- (14) S. G. Alsabri, A. E. Zetrini, N. B. Ermeli, S. B. Mohamed, *et. al.*, "Study of eight medicinal plants for antioxidant activities," *Journal of Chemical and Pharmaceutical Research*, vol. 4, no. 8, pp. 4028-403, 2012.
- (15) M. Alshamsi, A. Alnuaimi, R. Alshamsi, A. Senthilkumar, *et al.*, "Phytochemical analysis and antioxidant activity of *Haloxylon salicarnicum*, *Ochradenus arabicus* and *Tamarix nilotica*," J. Phytol, vol. 11, pp. 47-50, 2019.

Volume (28) Issue 02 (2024)

- (16) A. Akbari, G. Jelodar, S. Nazifi, and J. Sajedianfard, "An Overview of the Characteristics and Function of Vitamin C in Various Tissues: Relying on its Antioxidant Function," *Zahedan J. Res. Med. Sci.*, vol. 18, no. 11, pp. e4037, 2016.
- (17) E. Kalpoutzakis, T. Chatzimitakos, V. Athanasiadis, S. Mitakou, *et al.*, "Determination of the Total Phenolics Content and Antioxidant Activity of Extracts from Parts of Plants from the Greek Island of Crete," *Plants*, vol. 12, no. 5, pp.1092-1107, 2023.
- (18) Y. Inan, S. Akyuz, I. Kurt-Celep, E. Celep, *et al.*, "Influence of in *vitro* human digestion simulation on the phenolics contents and biological activities of the aqueous extracts from turkish *cistus* species," *Molecules*, vol. 26, no. 17, pp. 5322-5338, 2021.
- (19) D. P. Speert, L. W. Wannamaker, E. D. Gray, and C. C. Clawson, "Bactericidal effect of oleic acid on group A *streptococci*: mechanism of action," *Infection and immunity*, vol. 26, no. 3, pp. 1202-1210, 1979.
- (20) O. I. Awonyemi, M. S. Abegunde, and T. E Olabiran, "Analysis of bioactive compounds from *Raphia taedigera* using gas chromatography-mass spectrometry," *Eur. Chem. Commun.*, vol. 2, no. 8, pp. 933-944, 2020.
- (21) K. Hamazaki, N. Suzuki, K. Kitamura, A. Hattori, *et al.*, "Is vaccenic acid (18: 1t n-7) associated with an increased incidence of hip fracture, An explanation for the calcium paradox," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 109, no. 8-12, 2016.
- (22) C. Lans, and T. Van Asseldonk, "Dr. Duke's phytochemical and ethnobotanical databases, a cornerstone in the validation of ethnoveterinary medicinal plants, as demonstrated by data on pets in British Columbia," *Medicinal and aromatic plants of North America*, 2020, pp. 219-246.
- (23) A. Sunita, K. Ganesh, and M. Sonam, "Screening and evaluation of bioactive components of *Cenchrus ciliaris L*. by GC-MS analysis," *Int. Res. J. Pharm.*, vol. 8, no. 6, pp. 69-76, 2017.
- (24) L. A. Lima, S. Johann, P. S. Cisalpino, L. P. Pimenta, et al., "In vitro antifungal activity of fatty acid methyl esters of the seeds of Annona cornifolia A. St. Hil. (Annonaceae) against pathogenic fungus Paracoccidioides brasiliensis.," Rev. Soc. Bras. Med. Trop., vol. 44, no. 6, pp.777-780, 2011.
- (25) Y. J. Shen, Y. C. Shen, W. S. Lee, and K. T. Yang, "Methyl palmitate protects heart against ischemia/reperfusion-induced injury through G-protein coupled receptor 40-mediated activation of the PI3K/AKT pathway," *European Journal of Pharmacology*, vol. 905, pp.174183-174185, 2021.
- (26) U. O. Igwe and U. F. Okwunodulu, JCPS, vol. 2, no. 1, 554-560, 2014.
- (27) B. Ghazala, M. Shameel, M. Choudhary, S. Shahzad, *et al.*, "Phytochemistry and bioactivity of *Tetraspora (Volvocophyta)* from Sindh," *Pakistan Journal of Botany*, vol. 36, no. 3, 531-548, 2004.
- (28) S. F. Asghar, H. U. Habib-ur-Rehman, M. I. Choudahry, and A. U. Atta-ur-Rahman, "Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of *Iris germanica*," *Inter. J. Genetics Molecular Biol*, vol. 3, pp. 95-100, 2012.
- (29) www.biosynth.com/p/FN31611/2876-78-0-1-(13.09.2024).
- (30) C. Chandekar, and M. Madhugiri, "Antimicrobial potential of leaves of *Psidium guajava*," *The Bioscan*, vol. 6, no. 4, pp. 557-561, 2011.

Volume (28) Issue 02 (2024)

- (31) R. Kafi, H. S. Kwak and W. E Schumacher, "Improvement of Naturally Aged Skin with Vitamin A (Retinol)," *Arch. Dermatol.*, vol. 143, no. 5, pp. 606-612, 2007.
- (32) G. G. Belz, K. Breithaupt-Grögler, and Osowski U, "Treatment of congestive heart failure--current status of use of digitoxin," *European Journal of Clinical Investigation*, vol. 31, no. (Suppl. 2), pp. 10-7, 2001.
- (33) A. Zangara, "The psychopharmacology of huperzine A: an alkaloid with cognitive enhancing and neuroprotective properties of interest in the treatment of Alzheimer's disease," *Pharmacology, Biochemistry, and Behavior*, vol. 75, no. 3, pp. 675–686, 2003.
- (34) R. Krishnan, K. V. Kannan, and K. Murugan, "Antifungal activity of the ethanolic extracts of *Marchantia linearis* Lehm. and Lindenb. against some pathogenic fungi," *J. Aquatic Biol. & Fisheries*, vol. 2, pp. 556-563, 2014.
- (35) A. M. Altinoz, and A. Ozpinar, "PPAR-δ and erucic acid in multiple sclerosis and Alzheimer's Disease. Likely benefits in terms of immunity and metabolism," *International Immunopharmacology*, vol. 69, pp. 245-256, 2019.
- (36) Duke, U.S. Department of Agriculture, Agricultural Research Service [Online]. Available: http://phytochem.nal.usda.gov, 1992-1996, (13.09.2024).
- (37) M. S. Abdelhamid, E. I. Kondratenko, and N. A. Lomteva, "GC-MS analysis of phytocomponents in the ethanolic extract of *Nelumbo nucifera* seeds from Russia," *J. App. Pharm. Sci.*, vol. 5, no. 4, pp. 115-118, 2015.
- (38) G. Bringmann, and F. Pokorny, "The naphthylisoquinoline alkaloids. In *The alkaloids: chemistry and pharmacology*, New York, Academic Press, 1995, vol. 46, pp. 127-271.
- (39) G. Bringmann, and L. Kinzinger, "(+)-Ancistrocline, a naphthylisoquinoline alkaloid from *Ancistrocladus tectorius*," *Phytochemistry*, vol. 31, no. 9, pp. 3297-3299, 1992.
- (40) A. K. Karn, R. Dharmatti, M. Sharon, M. Sharon, *et al.*, "Cytotoxicity and anti-cancer activity of ancistrocline: a naphthyl iso-quinoline alkaloid extracted from the stem of *Ancistrocladus heyneanus*," *Der Chemica Sinica*, vol. 6, no. 2, pp. 35-44, 2015.
- (41) M. H. Bruscatto, R. C. Zambiazi, M. Sganzerla, V. R. Pestana, *et al.* "Degradation of Tocopherols in Rice Bran Oil Submitted to Heating at Different Temperatures," *Journal of Chromatographic Science*, vol. 47, no. 9, pp. 762-765, 2009.
- (42) R. Patwardhan, R. Checker, D. Sharma, V. Kohli, *et al.*, "Dimethoxycurcumin, a metabolically stable analogue of curcumin, exhibits anti-inflammatory activities in murine and human lymphocytes," *Biochemical Pharmacology*, vol. 82, no. 6, pp. 642-657, 2011.
- (43) M. Ogutveren, and S. S. Tetik, "Composition of the essential oil of *Cistus parviflorus* L. from Turkey," *Journal of Essential Oil Research*, vol. 16, no. 2, pp. 115-116, 2004.