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Effect of the Glycoside Extract of *Nerium oleander* L. Flowers on the *Arabidopsis thaliana* Colombia-0

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ABSTRACT

The effect of *Nerium oleander* flower glycoside extract on *Arabidopsis thaliana* (Colombia-0) was investigated at different concentrations viz., 0, 2, 4, 6 and 10 g/ml. According to biochemical and morphological features, glycoside compounds reduced leaf area, highest value, leaf number and plant height. Chlorophyll pigments had maximum value in the control (4.2 mg/g) and then decreased in the treatments. The effect of glycoside chemical compounds varied between the control and treatment groups. The number of glycoside compounds of *A. thaliana* revealed 34 chemical compounds, whereas the treatment recorded 20, 12, 9 and 5 chemical compounds in 2, 4, 6 and 10%, respectively. The main compounds in control treatment were 8-Nonene-1-nitrile (25.46%), 2.alpha., 4a.beta., 8a.beta. Decahydro-2-naphthalenol was 13.38%. As well as in 2% was 2.alpha., 4a.beta., 8a.beta. Decahydro-2-naphthalenol (28.78%), 8-Nonene-1-nitrile was 14.59%. Similarly, in 4% concentration treatment main compound was 8-Nonene-1-nitrile 32.56%, pyrazine, tetramethyl 26.62%. Main compound in 6% concentration 8-Nonene-1-nitrile was 36.85%. The analysis of the glycoside extract of the 10% treatment showed the highest value ceten 27.41%, and 8-Nonene-1-nitil by 20.07%.

Key words : Biochemical, chlorophyll, *Nerium oleander, Arabidopsis thaliana*, GC-MS analysis

INTRODUCTION

Nerium oleander is a widespread plant in Iraq, growing in most sorts of gardens and orchards. It contains active chemical compounds and is used medicinally. *N. oleander* has chemical compounds such as glycosides, triterpenoids, steroids and flavonoids which interred with metabolism process (Al-Saadi *et al.*, 2017). *N. oleander* is a well-known medicinal plant, particularly in India and China, and recent pharmacological studies have revealed the presence of a variety of chemical components, including antioxidants anti-ulcer, antifungal, anti-cancer, anti-inflammatory agents, liver remedies, diabetes treatment and analgesics (Al-azem *et al.*, 2019). The presence of several cardiac glycosides is known to cause the poisonous impact of the oleander plant on other creatures (Farkhondeh *et al.,* 2020).

During the plant growth stage, many secondary metabolites are produced to serve cellular and physiological functions. Evidence indicates that these products are response to external conditions and that the concentration and type of this product depend on the type of plant, genotype, growth stage and environmental factors (Isah, 2019). The chemical compounds have importance in balancing biotic and abiotic stresses and affect other organisms in terms of growth, physiological functions and behaviour (van Loon, 2016). These chemicals are important for pharmaceutical research and drug development (Qader *et al.*, 2017; Aljazy *et al.*, 2019; Al-Tamimi *et al.*, 2020). Natural products which affect plant growth and germination are called an allelochemical (Mushtaq *et al.,* 2020). Allelochemicals are released to the environment during the plant life cycle due to biotic or abiotic stress such as drought, radiation, temperature, low nutrients, competition, disease, antimicrobials, anti-weed and insecticides and insect infestation (Bhargava *et al.*, 2020; Mushtaq *et al.,* 2020). The aim of this study was to estimate the effect of glycosides extract of *N. oleander* flowers on the growth, morphological, biochemical and quality and quantity of chemical compounds of *A. thaliana*.

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MATERIALS AND METHODS

Flowers *of N. oleander* were collected at morning from the garden of general places in different regions of Basrah city, and washed with distilled water and dried, then ground into fine powder and stored until use. For preparation of the glycoside extraction, 200 ml of ethyl alcohol was added to 20 g of dry ground flowers and heated at 70°C for two min. Added 100 ml of distilled water to the mixture and heated for 5 min at 70°C, then filtered to obtain the filtrate. Two hundred ml of 60% ethyl alcohol was added to remaining precipitate to obtain the largest amount of extract. It was boiled for another 5 min and then filtered. The extract was concentrated to 20 ml. 10% (weight/volume in distilled water) lead acetate was added and left for 10 min with light stirring to filter to remove the precipitate. 10% (weight/volume distilled water) 20 ml of sodium phosphate was added with light stirring to remove lead acetate residues before filtering and disposing of the precipitate. One hundred ml of chloroform was added three times with shaking. The lower part was disposed of. The product was dried to obtain the glycoside extract. Treatments for concentration were made by dissolving the extract in distilled water viz., 0, 2, 4, 6 and 10/100 g/ml.

Arabidopsis thaliana (Colombia-0) seeds were obtained from Carolina (USA). It was germinated and grown in growth chamber for two months (January and February 2020) by using cylinder pots 12 × 12 cm in soil mixture containing sand and peat (1:1). Experiment was done under controlled condition in growth chamber at 20-22°C, light period 18/6 (light/dark), light intensity 6000 lux, humidity 50-70%, irrigated with Hogland solution (1.6 g/l) to field capacity. After four weeks of growth, glycoside extractions of *N. oleander* flower were added as : control drenched in water and concentrations treatments 2, 4, 6 and 10% (weight/volume) by dissolving the extract in distilled water and used for irrigation.

After four weeks of treatment, morphological characteristics such as leaf area (cm^2), plant height (cm), number of siliquae, and inflorescence stem emerges were measured (day). Total chlorophyll was determined. One g of fresh leaf material was ground in 25 ml of 80% acetone, and centrifuged at 5000 rpm for 5 min. The extracted solution's absorbance was measured at 645 and 663 nm in comparison to a blank acetone solvent as :

Total chlorophyll = $[20.2 \times A_{645} + 8.02 \times A_{663} \times$ sample size (ml)]/[1000 x fresh weight (g)]

The glycosidic extract was analyzed by GC-MS technique (Mass Spectrometry-Gas Chromatography) device type GC Shimadzu 2010 using Agilent technologies 7890B GC coupled to an Agilent technologies 5977A MSD at Nahran Omar/Basrah Oil Company Laboratories. The column temperature started at 40°C for 5 min and then rised at a rate of 10°C/min to reach 300°C for 20 min. The spectra of the curves were diagnosed by the Spectral Library (NIST, 2014 and Wiley 9 Library GC Method/Retention Index Library).

All the data were analyzed using the One-Way ANOVA and the L. S. D (Least Significant Difference) test at a significant level of $0.05 \geq P$ followed by treatment means with the computer software SPSS 17.

RESULTS AND DISCUSSION

The results of leaf area showed that highest value was recorded in control treatment 1.14 cm^2, while it was 0.63 cm^2 in 2% treatment and 10% was the least value 0.52 cm^2 (Fig. 1A). The increased leaf area may be attributable to the antagonistic action inhibiting cell division and leaf size (Hussein *et al.*, 2020). Plant height average (Fig. 1B) showed no significant difference among 0, 2, 4 and 6% treatments, but decreased in 10% treatment. The highest value of leaf number in control 9.7 cm and the least number was 5.57 cm in 10% treatment (Fig. 1C). Branch number (Fig. 1D) decreased in 6 and 10% but showed no significant difference in 0, 2 and 4%. The maximum number of flowers was 9.32 in treatment 4% (Fig. 1E) and then decreased in 6 and 10%.

Allelochemicals had both positive and negative effects in small doses. They had a stimulating effect, while excessive amounts had an inhibitory effect. Plants also made changes in plant metabolism and gene expression in response to stress (Hussein *et al.*, 2020). Highest value of chlorophyll was recorded in control 4.2 mg/g then decreased as treatments concentration increased and the least value was 2.65 mg/g in 10% treatment (Fig. 1F). Most

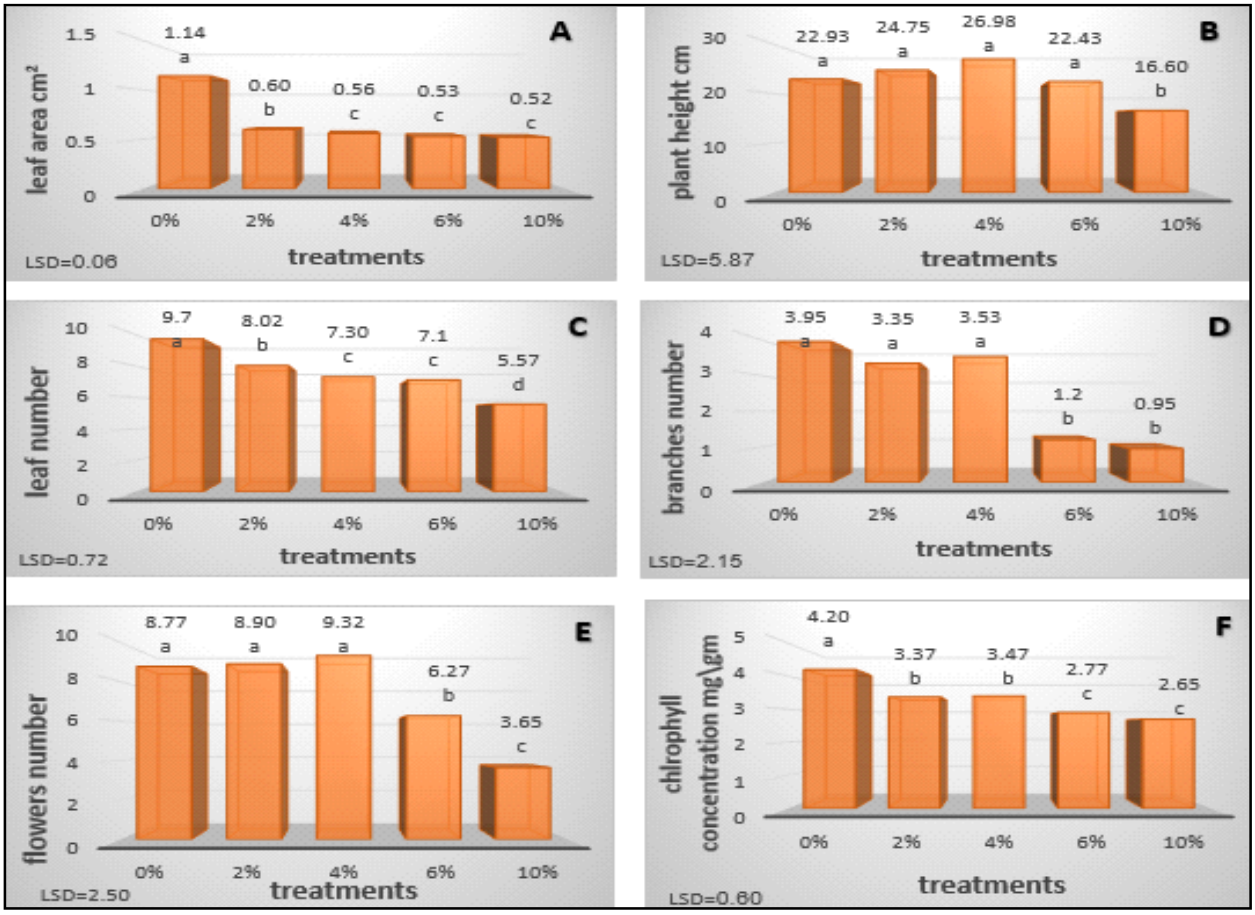

Fig. 1. Allelopathic effect of glycoside of *N. oleander* flowers in (A) leaf area, (B) plant height, (C) leaf number, (D) branch number, (E) flower number and (F) chlorophyll content mg/g.

of the allelochemicals affected chlorophyll concentrations in plants (Al-Abbawy *et al.*, 2020).

The glycoside extract of *N. oleander* flowers showed the presence of 30 chemical compounds, the highest was 16.63% in 5-Hydroxymethylfurfural, 2-Propyn-ol, 3-(trimethylsilyl)-1 with 12.81%, furfural was 9.72%, and the lowest Octanoic acid, 4,6-dimethyl-, methyl ester 1.01% (Table 1; Fig. 2). *N. oleander* flowers extract had 5-Hydroxymethylfurfural, which resulted from dehydration of reducing sugars, recorded the highest percentage and the results agreed with (Hameed *et al.*, 2015; Abdul-Rasool *et al.*, 2017). Methyl esters had an antagonistic effect on some weeds (Anwar *et al.*, 2021). Furfural produced by removing water from the xylose found in large quantities in the hemicellulose portion of the lignocellulosic (Mathew *et al.*, 2018). It was toxic to the skin, respiratory system and digestive system. Further, considered as one of the so-called chemical platform compounds, meaning that it was considered as a basis for manufacturing other chemical compounds. John *et al.* (2017) found that hydrolysis of sugar bagasse residues containing furfural and hydroxymethyl furfural caused negative allelopathic effect on the cell wall of plant. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4 was also found in *N. oleander*.

A. thaliana recorded the highest proportion of 8-Nonene-1-nitrile as 25.46%, 6H-Purin-6-one,1,7-dihydro- and 2.alpha., 4a.beta, 8a.beta. Decahydro-2-naphthalenol was 13.38%, and thiazole, 5-methyl 11.9%. Five chemical compounds were found associated between *N. oleander* extract of *A. thaliana* and all treatments. Furfural compound was 6.88% in the 2% treatment and 5.5% in the 4% concentration, while it did not appear in the *A. thaliana* control and the rest of concentrations (Table 2; Fig. 3). The results reported 0.56% in *N. oleander* extract of 1-tetradecene, while it was 5.47 and 17.45% in

Table 1. Chemical compounds identified in the glycoside extract of *N. oleander* flowers using GC-MS technique

S. No.	Retention time	Area (%)	Formula	Name of chemical compound
1.	4.273	9.72	$C_5H_4O_2$	Furfural
2.	6.167	2.72	$C_6H_6O_2$	Ethanone, 1-(2-furanyl)-
3.	7.484	3.99	$C_6H_6O_2$	2-Furancarboxaldehyde, 5-methyl-
4.	8.544	1.74	$C_6H_{15}NO$	3-Isopropoxypropylamine
5.	9.314	1.65	$C_5H_8O_3$	Oxirane-2-carboxylic acid, ethyl ester
6.	9.758	1.44	$C_8H_{16}O_2$	Butanoic acid, 3-methyl-, 1-methyl ethyl ester
7.	10.052	3.39	$C_{14}H_{20}O_{10}$	1-Deoxy-1-piperidinocarbothioamido-.beta.-d-glucopyranose 2,3,4,6- tetraacetate
8.	10.615	1.57	$C_5H_{12}O$	Hydroperoxide, 1-methylbutyl
9.	10.792	1.72	$C_7H_{10}O_2$	1-Methoxy-3-keto-4-methyl-1,4-pentadiene
10.	11.211	7.7	C6H8O4	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-
11.	12.348	2.84	$C_8H_{16}NO_7$	Butanal, dimethylhydrazone
12.	12.49	1.67	$C_8H_{12}O_5$	exo-1,2-O-Ethylidene-.alpha.-d-erythrofuranose
13.	12.749	16.63	$C_6H_6O_3$	5-Hydroxymethylfurfural
14.	13.534	2.86	C_6H_6OS	4-Mercaptophenol
15.	13.824	0.72	$C_6H_{14}N_2$	1,3-Cyclohexanediamine
16.	13.824	0.72	C_4H_3NS	Thiocyanic acid, 2-propynyl ester
17.	13.824	0.72	$C_6H_{14}O_2$	6-Acetyl-.beta.-d-mannose
18.	14.613	0.56	$C_{14}H_{28}$	1-Tetradecene
19.	15.89	0.91	$C_9H_{16}O$	3-Octen-2-one, 7-methyl-
20.	16.06	0.14	$C_8H_{12}O_2$	2,4-Pentanedione, 3-(2-propenyl)-
21.	16.62	4.82	$C_{10}H1_7NO$	N-Cyclooct-4-enylacetamide
22.	17.078	1.01	$C_{18}H_{36}$	1-Octadecene
23.	18.42	12.81	$C_7H_{12}O_7$	Methyl(methyl4-O-methyl-alpha.-d-mannopyranoside) uronate
24.	18.746	5.74	$C_2H_{14}O_6$	2-Methyl-d-glucose
25.	18.746	5.74	$C_7H_{14}O_6$	2-O-Methyl-D-mannopyranosa
26.	19.206	1.01	$C_{11}H_{22}O_2$	Octanoic acid, 4,6-dimethyl-, methyl ester
27.	21.002	0.5	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
28.	23.127	0.29	$C_{18}H_{36}$	3-Eicosane,(E)
29.	27.695	3.92	$C_{22}H_{43}NO$	13-Docosenamide, (Z)-
30.	32.094	3.92	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-

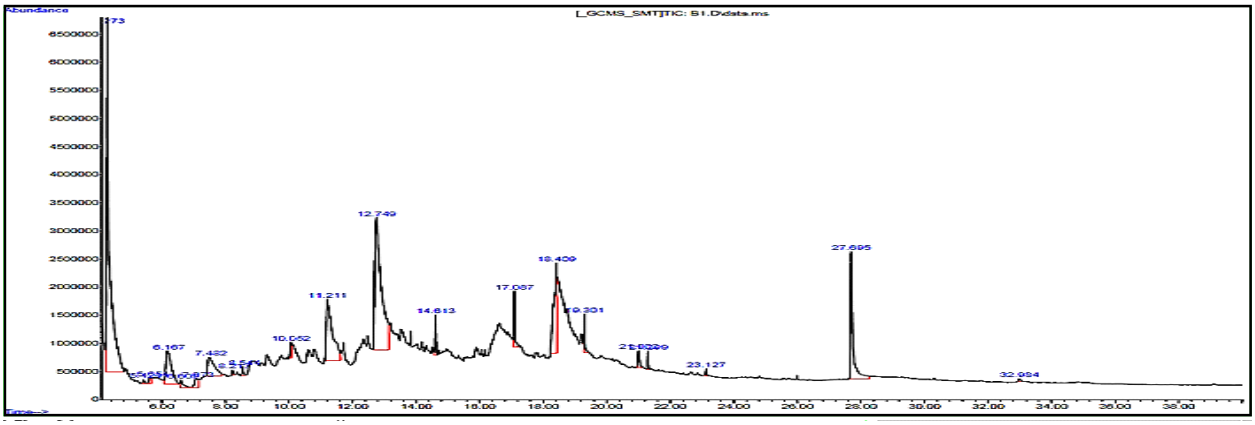

Fig. 2. Chemical compounds identified in the glycoside extract of *N. oleander* using GC-MS technique.

6 and 10%, respectively. Further, 1-tetradecene was found in small amount 1.69%. The results showed the *A. thaliana* exposure with *N. oleander* increased with increased concentration : it was 2.67, 3.51, 6.17 and 15.82% in 2, 4, 6 and 10% concentrations, respectively. 9-Octadecenamide (Z) recorded 5.06 and 9.34% in 2 and 4%, respectively, which recorded 3.92% in the *N. oleander* extract and in *A. thaliana* extract was 4.45% (Table 2). 8-Nonene-1-nitrile in the *A. thaliana* control agreed with Dubey *et al.* (2021), nitrile compounds increased with increasing the concentration, due to growth and age of *A. thaliana*, however, isothiocyanate decreased in the last three weeks of plant life. Allyl isothiocyanate was related with growth inhibition as a defensive state for the plant when exposed to plant eaters or bacteria (Sporsheim *et al.*, 2015). Allyl isothiocyanate was identified in several *Brassica* genus. *A. thaliana* contained glucosinolate, which was

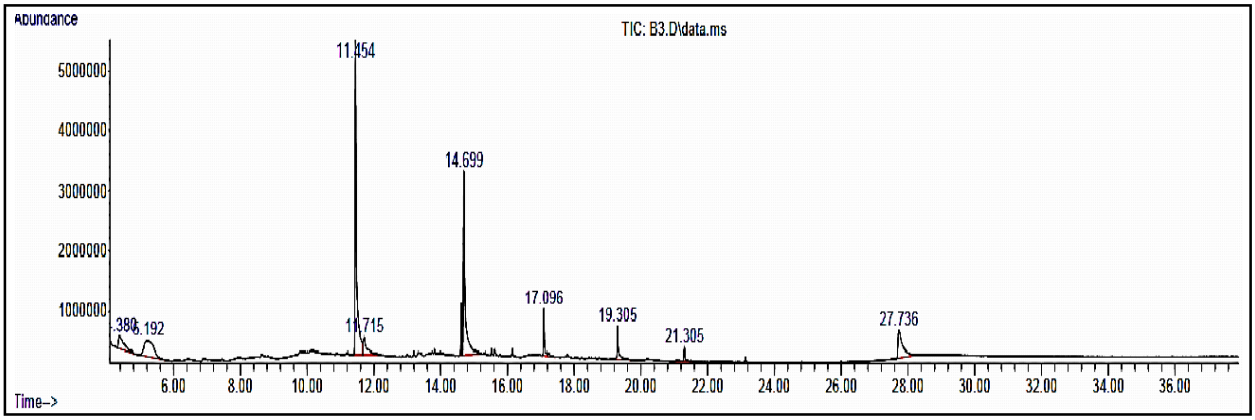

Fig. 3. Chemical compounds identified in *A. thaliana* treated with 4% concentration of glycoside extract using GC-MS technique.

an inactive secondary metabolite. However, after hydrolysis, the enzyme thyioglucosidase, also known as myrosinase, converted it to isothiocyanate and the interaction with an epithiospecificer protein led to the formation of epithioniles or nitril. Myrosinase was stored separately from glucosinolate and the reaction occurred upon tissue breakdown. Pyrazine and thiazole, 5-methyl-, tetramethyl had medical importance in cardiovascular and neurological diseases (Abdu-Rahem *et al.*, 2021).

The analysis of the glycoside extract of *N. oleander* flowers on *A. thaliana* revealed that the 2% concentration contained 20 chemical compounds, the highest percentage was 28.78 in 2.alpha, 4a.beta., 8a.beta. Decahydro-2-naphthalenol, 8 Nonene-1-nitrile was 14.59%, cyclopropane, isothiocyanato-and allyl isothiocyanate 13.59% (Table 2; Fig. 4). In 4% 12 compounds were found but recorded only 9 chemical compounds with a concentration of 6%, 8-Nonene-1-nitrile having the highest percentage of 36.85%, 1H-Imidazole, 4,5-dimethyl 16.16%, and cyclohexene, 4,4,5-trifluoro 15.32%. 10% concentration contained five compounds, the highest was ceten 27.41%, followed by the compound 8-Nonene-1-nitil 20.07%. 1-Tetradecene was 17.45% and 1-Octadecene was 15.82% (Table 2; Figs. 3-5). Some chemical compounds, 1-Dodecene, Furfural, 1-Eicosene, galacto-heptulose, d-Glycero-d-galacto-heptose, and D- allose were not found in the control treatment. However, it was present in all concentration 2, 4, 6 and 10%. 1-Dodecene was reported by González-Cabanelas *et al.* (2015). Furfural compounds appeared in the treatment 2 and 4%, which may be absorbed from the glycoside extraction or produced in the plant. Hexadecenoic acid was considered as an antimicrobial (Tyagi and Agarwal, 2017). The 6% treatment contained 1-H-Imidazole, 4,5-dimethyl- which was recorded in some plants as *Cyperus rotundus* (Abo-Altemen *et al.*, 2019). The number of chemical compounds decreased as the concentration increased, which could be

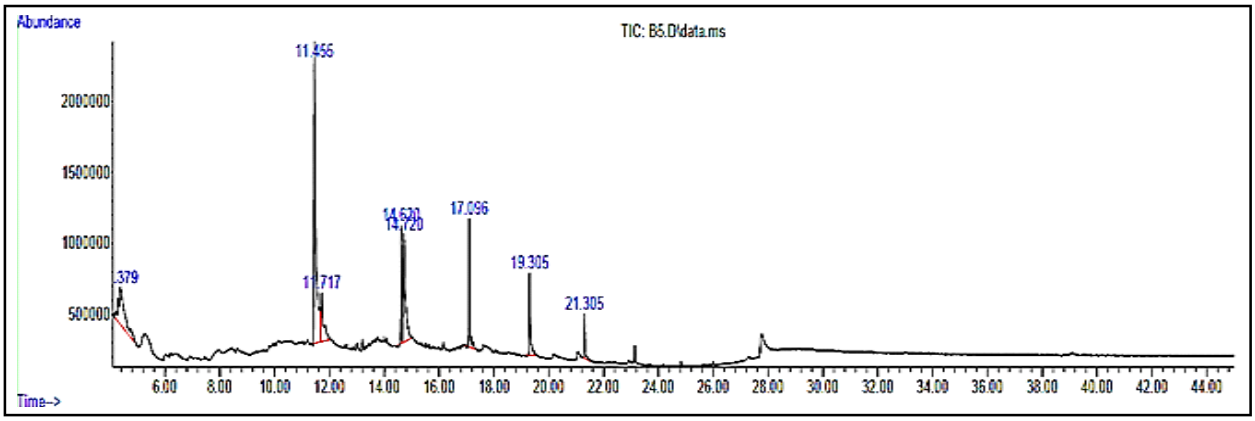

Fig. 4. Chemical compounds identified in *A. thaliana* treated with 6% concentration of glycoside extract by GC-MS technique.

Table 2. Chemical compounds of glycoside extract treatment of *A. thaliana* using GC-MS technique

S. No.	Treatments					Chemical compounds
	10%	6%	4%	2%	0%	
1.	-	-	11.53	13.59	1.8	Cyclopropane, isothiocyanato-
2.	-	-	11.53	13.59	1.8	Allyl Isothiocyanate
3.	-	-	-	-	11.9	Thiazole, 5-methyl-
4.	-	-	-	-	5.54	Dimethyl Sulfoxide
5.	-	-	-	-	2.64	Pyrazine, 2,5-dimethyl-
6.	-	-	-	-	0.9	Butyrolactone
7.	-	-	-	-	2.99	Formic acid phenyl ester
8.	-	-	-	-	2.99	2,4,6-Cycloheptatrien-1-one, 2-hydroxy-
9.	-	-	-	-	2.05	Formic acid phenyl ester
10.	-	-	-	-	2.04	Phenol
11.	-	-	-	-	0.44	7-Octene-1-nitrile
12.	-	-	-	-	0.36	1-Butanethiol, 4-(methylthio)-
13.	-	-	-	-	3.81	2-Pyrrolidinone
14.	20.07	36.85	32.56	14.95	25.46	8-Nonene-1-nitrile
15.	-	-	-	-	2.41	Erythrol
16.	-	-	-	-	0.68	Azacyclohexane, 3-[1pyrrolidyl]-
17.	-	-	-	-	1.38	Indole
18.	-	-	-	0.43	1.37	Cyclohexasiloxane,dodecamethyl-
19.	17.45	5.47	-	-	1.69	1-Tetradecene
20.	-	-	-	-	13.38	Pyrazine, tetramethyl-
21.	-	-	-	-	13.38	6H-Purin-6-one, 1,7-dihydro-
22.	-	-	-	-	5.88	.beta.-D-Glucopyranose, 1,6-anhydro-
23.	-	-	-	-	5,88	Ethyl .alpha.-d-glucopyranoside
24.	-	-	-	-	1.24	D-Allose
25.	-	-	-	-	1.24	.beta.-D-Glucopyranose, 1,6-anhydrO-
26.	-	-	-	-	1.24	Propanoic acid, 3-hydroxy-
27.	-	-	-	-	3	.alpha.-D-Glucopyranoside, methyl
28.	15.82	6.17	3.51	2.67	2.43	1-Octadecene
29.	-	-	-	-	2.43	E-15-Heptadecenal
30.	-	2.79	1.74	-	2.79	Cycloeicosane
31.	-	-	4.45	9.43	5.06	13-Docosenamide, (Z)-
32.	-	-	9.43	5.06	4.45	9-Octadecenamide, (Z)-
33.	-	-	5.5	6.88	-	Furfural
34.	-	-	-	1.87	-	Pyrrolid-2-one-5-carboxylic acid, N-methyl-, ethyl ester
35.	-	-	-	1.97	-	Galacto-heptulose,
36.	19.25	9.15	4.94	3.37	-	1-Dodecene
37.	-	-	-	1.84	-	Propargyl ethyl sulfide
38.	-	-	-	0.8	-	Dodecane, 1-chloro-
39.	27.41	8.09	4.18	3.55	3.27	Cetene
40.	-	-	-	1.14	-	1-Eicosene
41.	-	-	-		-	Cyclohexadecane, 1,2-diethyl-
42.	-	-	-	0.94	-	Butyl citrate
43.	-	-	-	6.3	-	Hexadecanoic acid, 2-hydroxy-1-(hy droxymethyl) ethyl ester
44.	-	-	-	2.43	-	Bis(2-ethylhexyl) phthalate
45.	-	-	-	3.8	-	Octadecanoic acid, 2,3-dihydroxypropyl ester
46.	-	16.16	5.5	-	-	1H-Imidazole, 4,5-dimethyl-
47.	-	-	26.62	28.78	13.38	2.alpha.,4a.beta., 8a.beta.-Decahydro-2-naphthalenol
48.	-	9.15	-	-	-	Cyclopentane, butyl-
49.	-	15.32	-	-	-	Cyclohexene, 4,4,5-trifluoro-
50.	-		-	-	-	Benzoic acid, 2-mercapto-

related to the plant's production of secondary metabolites; by undergoing process of changing the same compound for the polar and non-polar groups, thus increasing the compounds derived from the same compound such as bind to an SH group or an amino acid (Sporsheim *et al.*, 2015). 8-Nonene-1-nitrile showed decrease in treatments 2 and 10% and increase in 4 and 6%, while in the control treatment it was recorded 25.46%. Nitrile compounds had a role in plant resistance. Presence of 1-Tetradecene was agreed with (Rankic *et al.,* 2021). Ceten increased with the increase of concentration. It was 3.55, 4.18, 8.09 and 27.41, respectively, for treatments 2, 4, 6 and 10%, while it scored 3.27% and the

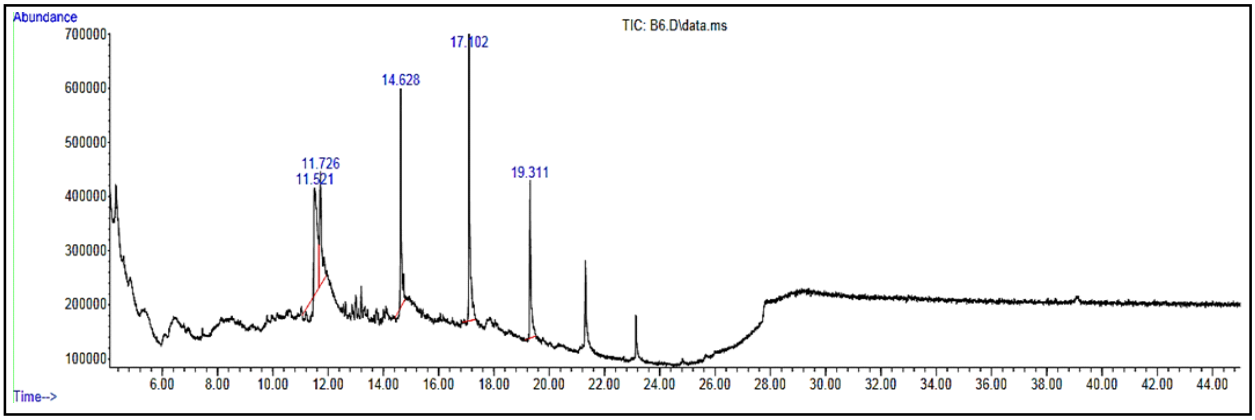

Fig. 5. Chemical compounds identified in *A. thaliana* treated with a concentration of 10% of the glycoside extract using GC-MS technique.

compound had medical importance as an antioxidant and against cancer (Sunil *et al.*, 2018).

REFERENCES

Abdu-Rahem, R. L., Ahmad, A.K. and Abachi, F. T. (2021). Synthesis and medicinal attributes of thiazole derivatives : A review. *Syst. Rev. Pharm.* **12** : 290-329.

Abdul-Rasool, S. I., Al-Saadi, S. A. and Al-Derawi, K. H. (2017). Studying the alcoholic extracts effects of *Juglons regia* L. bark and *Nerium oleander* L. leaves on males' tissues and females' ovaries of the albino mice. Thesis, University of Basrah-College of Science.

Abo-Altemen, R. A., Al-Shammari, A. M. and Shawkat, M. S. (2019). GC-MS analysis and chemical composition identification of *Cyperus rotundus* L. from Iraq temperature function for a long-term district heat demand forecast. *Ene. Procedia.* **157** : 1462-1474.

Al-Abbawy, D., Al-Sweid, Z. and Al-Saady, S. A. (2020). Effects of salinity stress on biochemical and anatomical characteristics of *Ceratophyllum demersum* L. *Eurasia J. Biosci.* **14** : 5219-5225.

Al-azem, D., Al-Derawi, K. H. and Al-Saadi, S. A. (2019). The protective effects of *Syzygium aromaticum* essential oil extract against methotrexate induced hepatic and renal toxicity in rats. *J. Pure Appl. Microbiol.* **13** : 01-11.

Aljazy, N. A., Al-Mossawi, A. E. B. H. and Al-Rikabi, A. K. (2019). Study of antibacterial activity of some date seed extracts. *Basrah J. Agric. Sci.* **32** : 247-257.

Al-Saadi, S. A. A. M., Al-Derawi, K. H. and Abd Alrasool, S. (2017). The effect of steroidal extract of *Nerium oleander* on the liver tissue structure of albino mice. *IOSR J. Phar. Biol. Sci.* **12** : 52-55.

Al-Tamimi, W. H., Al-Saadi, S. A. A. M. and Burghal, A. (2020). Antibacterial activity and GC-MS analysis of baltic amber against pathogenic bacteria. *Int. J. Adv. Sci. Tech.* **29** : 611-618.

Anwar, T., Qureshi, H., Mahnashi , M. H., Kabir , F., Parveen, N., Ahmed . D., Afzal, U., Batool, H., Awais , M., Alyami, S. A. and Alhaider, H. A. (2021). Bioherbicidal ability and weed management of allelopathic methyl esters from *Lantana camara*. *Saudi J. Biol. Sci.* **28** : 4365-4374.

Bhargava, P., Gupta, N., Kumar, R. and Vats, S. (2020). Plants and microbes : Bioresources for sustainable development and biocontrol. In : *Plant Microbe Symbiosis*. pp. 153-176. Springer, Cham.

Dubey, S., Guignard, F., Pellaud, S., Pedrazzetti, M., van der Schuren, A., Gaume, A. and Dubey, O. (2021). Isothiocyanate derivatives of glucosinolates as efficient natural fungicides. *Phyto Front.* **1** : 40-50.

Farkhondeh, T., Kianmehr, M., Kazemi, T., Samarghandian, S. and Khazdair, M. R. (2020). Toxicity effects of *Nerium oleander*, basic and clinical evidence : A comprehensive review. *Human Exptl. Toxicol.* **39** : 773-784.

Gonzalez-Cabanelas, D., Wright, L. P., Paetz, C., Onkokesung, N., Gershenzon, J., Rodriguez-Concepcion, M. and Phillips, M. A. (2015). The diversion of 2-C-methyl-D-erythritol-2,4-cyclodiphosphate from the 2-C-methyl-D-erythritol 4-phosphate pathway to hemiterpene glycosides mediates stress responses in *Arabidopsis thaliana*. *Plant J.* **82** : 122-137.

Hameed, I. H., Jasim, H., Kareem, M. A. and Hussein, A. O. (2015). Alkaloid constitution of *Nerium oleander* using gas chromatography-mass spectroscopy (GC-MS). *J. Med. Plant Res.* **9** : 326-334.

Hussein, W. M., Abdul-Qadir, L. H. and Al-Kaaby, H. K. (2020). Rapid and cost-effective protocol for planting *Arabidopsis thaliana* L. in Iraq. *J. Basrah Res. Sci.* **46** : 159-169.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biol. Res. J.* **52** : 01-25.

John, I., Muthukumar, K. and Arunagiri, A. (2017). A review on the potential of citrus waste for D-Limonene, pectin and bioethanol production. *Int. J. Green Energy* **14** : 599-612.

Mathew, A. K., Abraham, A., Mallapureddy, K. K. and Sukumaran, R. K. (2018). Lignocellulosic biorefinery wastes or resources? In : *Waste Biorefinery*. Elsevier, Amsterdam. pp. 267-297.

Mushtaq, W., Siddiqui, M. B. and Hakeem, K. R. (2020). Mechanism of action of allelochemicals. In : *Allelopathy*. pp. 61-66. Springer, Cham.

Qader, K. O., Al-Saadi, S. A. M. and Al-Saadi, T. A. (2017). Chemical composition of *Myrtus communis* L. (Myrtaceae) fruits. *J. App. Life Sci. Int.* **12** : 1-8.

Rankic, I., Zelinka, R., Ridoskova, A., Gagic, M., Pelcova, P. and Huska, D. (2021). Nano/ microparticles in conjunction with microalgae extract as novel insecticides against Mealworm beetles, *Tenebrio molitor*. *Sci. Reports* **11** : 1-9.

Sporsheim, B., Overby, A. and Bones, A. A. (2015). Allyl isothiocyanate inhibits actin-dependent intracellular transport in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **16** : 29134-29147.

Sunil, K. S., Suma, K., Ashika, B. D., Roy, C. L., Naresh, S. and Balasubramanian, S. (2018). GC-MS and FTIR analysis on the methanolic extraco of *Coriandrum sativum* leaves. *European J. Phar. Med. Res.* **5** : 454-460.

Tyagi, S. T. and Agarwal, M. (2017). Activity source Dr. Duke's phytochemical and ethnobotanical databases. In : *GC-MS Analysis of Invasive Aquatic Weed, Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.). *Int. J. Cur. Phar. Res.* **9** : 111-117.

van Loon, L. C. (2016). The intelligent behaviour of plants. *Trends Plant Sci.* **21** : 286-294.