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ORIGINAL ARTICLE

ACTIVE COMPOUNDS ANALYSIS IN FIVE ROSELLE VARIETIES USING GC/MS

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Abstract : Field trial was laid out at private field belongings to Fallujah city 45 km west Baghdad, Iraq. Five genotypes of Roselle *Hibiscus sabdariffa* (malvaceae) were sown in clayey loam soil in summer during June 17, 2018. These genotypes were heet- red, heet-black, oreib, Aswan and sudan-3. Genotypes were randomly distributed within RCBD design with three replicates. GC/MS analysis was done to characterize the active compounds in calyx extract for each genotype. Results pointed that GC/MS separated 22 active compounds in heet-red calyx extract in which the highest active compound was 2, 5-pyrrolidinedione with area of 23.71% at RT of 11.708 min. In heet-black, 28 active compounds were separated with highest of 2-acetyl-5-methyl-isoxazolidin-5-ylmethyl ester of 17.24% at RT of 16.860 min, 29 active compounds with highest phenyl-1, 2-diamine of 20.55% area at RT of 12.633 min In oreib, 23 active compounds with highest 2-acetyl-5-methyl-isoxazolin-5-ylmethyl ester of 23.90% area at RT of 16.863 min. In Aswan and 15 active compounds were isolated which gave highest area of 23.19 for 2.5-pyrrolidinedione at RT of 11.682 min in sudan-3. GC/MS analysis proved that genotypes differed in number of active compounds and quality these compounds. Thus, GC/MS could be exploited to characterize the genotypes of different crops. Therefore, these genotypes could be subjected to breeding project to transfer genetic material to each other.

***Key words*:** Active compounds analysis, Roselle varieties, GC/MS, Randomized complete block design (RCBD).

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1. Introduction

Hibiscus sabdariffa L. (Hs), also known as Roselle is considered as an interesting crop that used to develop the some tropical agricultural regions which is easily growing from seeds, could be sown as one part crop that introduced in multi-cropping systems and exploited as food and fiber. In China, its seeds are extracted for their oil and the plant calyx is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. Additionally, it is used in the pharmaceutical and food industries [Da-Costa-Rocha *et al*. (2014)]. Its ripe calyces contain active compounds like gossypetin, anthocyanin, and the glycoside hibiscin that possess potential as diuretic, antiscorbutic, antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, purgative and resolving [Islam (2019)]. Naeem *et al*. (2019) showed that the roselle seeds oil extracted by solvent gave the highest oil content and extraction rate (17.98 and 98.34%, respectively). The red variety had a greatest leaf area (94.25±0.310cm^2) and petiole length (6.50±0.620cm). The varieties were varied in minerals and vitamins depended on variety and plant parts. However, the nutrients were higher in the red variety [Ilodibia *et al*. (2019)]. Others need nitrogen to improve their performance [Al-Hasan and Najeeb (2011)]. HPLC analysis revealed two phenolic acids, 16 flavonoids and four anthocyanins in petal of *H. sabdariffa*. The major compounds were gossypetin, hibiscetin, quercetin and sabdaretin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside were the major anthocyanins [Pacome *et al*. (2014)]. Totally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was identified 85

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volatiles, mainly aldehydes, alcohols, ketones, furans, and acids indicating lipid oxidation in stored roselle [Juhari and Petersen (2018)]. Farag *et al*. (2015) completely mapped for volatiles, sugars and organic acids distribution in two Hibiscus flower cultivars and its fermented product using GC/MS, which identified 104 volatiles in Aswan and Sudan-1 varieties. Inikpi *et al*. (2014) indicted that GC/MS identified the main compounds in essential oil of roselle were hexadecanoic acid (64.3%) and linoleic acid (22.7%). In Cuba, Eighty-one volatile compounds were identified in the aroma concentrate, of which linalool and aterpineol were found to be the major constituents using GC/MS [Pino *et al*. (2006)]. In Mexico, Avalos-Martinez *et al*. (2018) characterized 104 essential aromatic compounds using GC/MS in four cultivars namely 4Q4, Puebla Precoz, UAN16-1 and sudan, the compounds *viz*, 2-fufural, 5-methyl-2-fufural, hexanal E-2-hexenal, 5-methyl-2(3H)-furanone and etc. Alara and Abdulrahman (2019) identified 16 chemical compounds were identified in the oils from *H. sabdariffa* flower. The main identified compounds were fatty acids and esters using GC/MS. Therefore, this field trial was accomplished to estimate the difference in the active compounds among five roselle cultivars using GC/MS over Iraqi conditions.

2. Materials and Methods

2.1 Trial lay out

A field trial was conducted during seasons 2018/2019 under low rainfed in Fallujah, west Baghdad, Iraq. The climate of the area is arid and semi-arid. The soil is clayey loamy with low fertility. Rainfall ranged between 50 and 150 mm. Average maximum daily temperatures varied between 30 - 35°C annually. The trial laid out in a Randomized complete block design (RCBD) with three replicates. The trial unit was 4×3.6 meters. Each plot contained 6 rows of four meters length. The rows are 60 cm apart and within row relied on the needed spacing .Treatments consisted of five varieties *viz*., sudan-3, oreib, Aswan, heet black and red heet. Sowing was applied on June 17, 2018. Crop practices like weeding and irrigation were managed as and when needed. Urea containing 46% N was used as source of nitrogen. Fertilizer was applied at 4 weeks after sowing (WAS) for 250 kg urea.ha^{-1} [Al-Hilfy *et al*. (2017)], the dose was halved and applied at 4 and 8 WAS. Nitrogen was applied in a band on one side of Roselle rows, at 5 cm from the plant.

2.2 Calyx Extract preparation

100 g of Roselle petals previously freeze dried were extracted in 200 ml of methanol+100 ml distilled water +50 ml chloroform acidified with trifluoroacetic acid 0.1% (v/v) for 24 h at 4°C. Macerated extract was put in shaker for 24 hours. Thereafter, extract was filtered successively on cotton wool and Whatman paper. After vacuum evaporation of the mixture solvents in BÜCHI Rota vapor R-114 at 38°C, we obtained a dry extract. Two hundred milliliters (200 ml) of distilled water were added to the dry extract and the aqueous extract was submitted to a filtration on gel XAD7, in order to eliminate sugars and chlorophyll pigments. The water obtained after filtration was discarded. 100 mL of methanol 100 % were poured over the gel X-AD7 and the methanolic filtrate obtained was evaporated to dry with Rota vapor R-114 at 38°C and dissolved again in a 100 mL of water. This filtrate was lyophilized with the freeze dryer Christ Alpha 1-2. The dried extract obtained represents the petals crude extract of Roselle which was used to achieve the different analyses [Pacome *et al.* (2014)].

2.3 GC/MS Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil was performed on a Hewlett Packard Gas Chromatograph HP6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm) under the same condition as the GC column. The oven temperature was programmed from 70-240°C at the rate of 5°C/min. The ion source was set at 240°C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. The scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC/MS.

2.4 Statistical Analysis

Areas of peaks were analyzed using PCA, to assess the differences among the five roselle cultivars. The areas represented the active compounds characterized by GC/MS. PCA was applied on data of each compound that were normalized to extract various axes (PCAs) that reduced the dimensionality of original values. Principal component analysis was done using comprehensive online software called METABOANALYST 4.0.

3. Results and Discussion

3.1 Predominant compounds

The Technique of GC/MS was exploited to separate and characterize the active components in five cultivars of roselle those grew in Iraqi environment. The five cultivars were Sudan-3, oreib (local), Aswan, Hit (black) and Hit (red). Results in Table 1 pointed that there are differences among these varieties in both quality and quantity. Sudan-3 has 15 active components; oreib has 29 and Aswan has 23, Heet (black) has 28 active components and Heet (red) has 22 active components for each cultivar. However, the oreib (local), grown in Iraq environment, has 29 active components. While Sudan-3 has the lowest number of components. Aswan possessed 9 dominant compounds, followed by heet-black of 8 compounds, Sudan-3 and oreib of 7 compounds, for both whereas heet-red had the lowest 6 compounds. GC/MS for aswan calyx extract gave highest area of 23.90%, while heet-black had the lowest highest area of 17.24%. In contrast, the highest lowest area was recorded in Sudan-3 calyx extract of 0.86%. The smallest lowest area was registered in aswan of 0.15%. The lowest starting time was reported in aswan calyx extract of 4.647 min. The biggest starting time was 7.950 min in heet-red. From other hand, the highest ending time was registered in heet-red of 28.286 min, whereas, the lowest ending time was recorded in aswan of 23.065 min.

The five varieties were similar in three components *viz*., propanedioic, Butanedioic and pentanoic. 4-ketopimelic acid is consecutively found in oreib with RT (12.055 min.) area% of 0.91 and height (0.88%), in Aswan of 13.439 min. 0.32% area% and 0.56% height.in heet- black of 13.442 min, area% 0.92 and height % 1.44%, in heet-red of 13.443, area 1.51%, height of 2.27%, for each cultivar, respectively, whereas, the compound is not found in Sudan-3.

Principal component analysis (PCA) revealed that the active compounds were various in five roselle cultivars (Fig. 1). The two PCAs interpreted 71.1% from variance in relative to five cultivars and active compounds. Thus, PCA_1 explained 50.1% and PCA_2 21%. So, E -2-Ethoxyethenyl acetate and 3-Heptenoic acid were the major active compounds of Sudan-3. 1-Ethyl 4-methyl succinate was the main compound in heet-black. 3- Pentenoic acid was the predominant

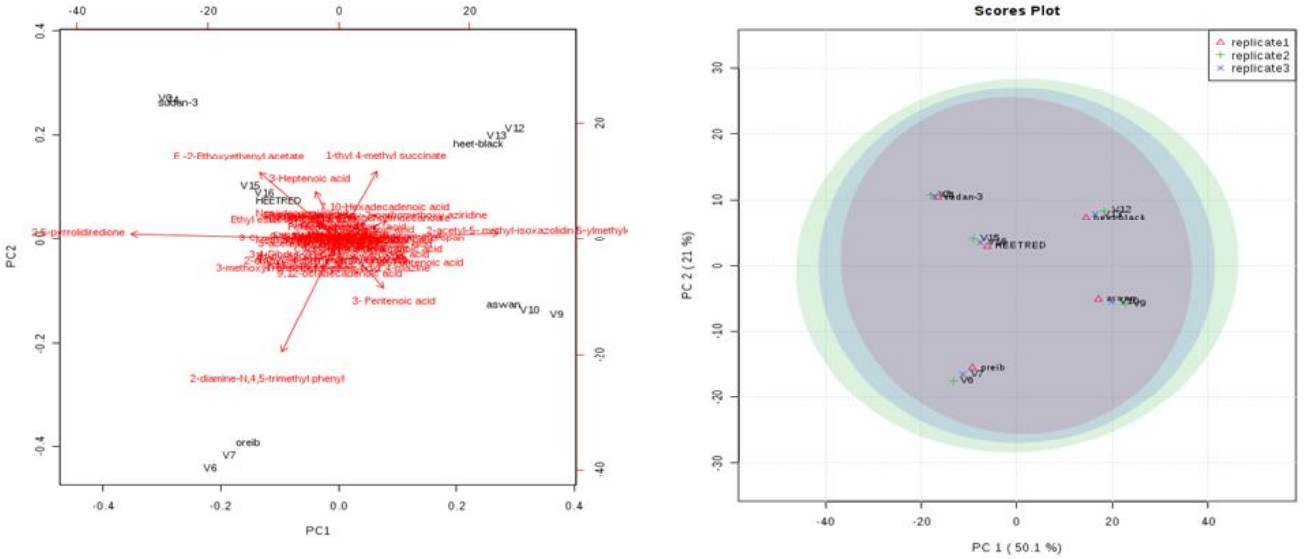

Fig. 1: Behavior of roselle cultivars over replicates (right), biplot of two PCAs of active compounds over five roselle cultivars (left)

Table 1: Five cultivars of roselle, number of compounds and the dominant compounds.

Cultivars	Compounds number	Dominant compound	Highest area	Lowest area	Starting time	Ending time
Sudan3	15	7	23.19	0.86	7.932	23.068
oreib	29	7	20.55	0.18	7.433	23.066
Aswan	23	9	23.90	0.15	4.647	23.065
Heet-red	22	6	23.71	0.69	7.950	28.286
Heet-black	28	8	17.24	0.31	7.435	23.067

Table 2: Behavior of active components relies on RT, area and height in five roselle cultivars.

pk#	sudan-3			oreib			aswan			heet-black			heet-red		
1	Name structure	R.T.	Area %	Name structure	R.T.	Area %	Name structure	R.T.	Area %	Name structure	R.T.	Area %	Name structure	R.T.	Area %
2	Propanedioic acid	7.932	3.43	Propanedioic acid	7.433	0.24	2-furancarbox-aldehyde	4.647	0.5	2H-pyrrol-one	7.435	0.76	Propanedioic acid	7.95	3.05
3	Butanedioic acid	9.064	1.81	Unknown	7.951	3.11	2-formyl-5-methy furan	6.907	0.15	Butanedioic acid	7.947	2.17	Butanedioic acid	9.071	2.12
4	3-Heptenoic acid	9.851	8.73	1-ethyl-3-methyl-2-methylmalonate	9.077	3	Propanedioic acid	7.947	0.51	Propanedioic acid	8.713	0.4	Unknown	9.744	2.14
5	3,4-Dihdro-2H-pyran-2-caroxylic acid	10.746	3.85	Unknown	9.749	1.84	3- Pentenoic acid	9.908	15.02	1-thyl3-methyl 2-methyl-malonate	9.07	1.03	3- Pentenoic acid	9.867	6.62
6	2- Butanedioic acid	11.44	5.81	3- Pentenoic acid	9.867	4.93	3,4-Dihdro-2H-pyran-2-caroxylic acid	10.825	5.77	3-actoxy-3 -hydroxypro-pionic	9.755	1.07	3,4-Dihdro-2H-pyran-2-caroxylic acid	10.805	3.56
7	2,5-pyrrolidi-redione	11.682	23.19	Butanedioic acid	10.14	0.45	2- Pentenoic acid	11.505	12.54	4-methoxycy-cloheptanonic	9.866	4	2,5-pyrrolidine dione	11.708	23.71
8	E -2-Ethoxy-ethenyl acetate	12.595	16.59	3-acetoxy-3-hydroxy propionic acid	10.6	1.01	1-thyl 4-methyl succinate	11.687	5.48	3,4-Dihdro-2H-pyran-2-caroxylic acid	10.81	1.63	1-Ethyl 4-methyl succinate	12.612	15.99
9	Ethyl ester levulinic acid	16.743	10.7	3,4-Dihdro-2H-pyran-2-caroxylic acid	10.813	4.05	5-hydroxy methyl fur fural	12.06	1	2- Propanedioic acid	11.46	3.19	4-ketopimelic	13.443	1.59
10	5H-1,4-Dioxepin	17.09	2.68	1-thyl 4-methyl succinate	11.162	1.01	Pyrrolidin-5-onc	12.333	4.25	1-Ethyl 4-methyl succinate	11.713	16.21	N-methoxy-2-carbomethoxy aziridine	16.289	3.09
11	Pentanoic acid	17.358	2.38	Trans-Butenedioic acid	11.466	4.19	Butanedioic acid	12.59	2.69	1H-pyrazolc-3- carboxylic acid	12.303	1.07	Ethyl laevulinate	16.775	9.77
12	Dodencanoic acid	18.891	3.71	Unknown	11.716	20.18	4-ketopimleic	13.439	0.32	Butanedioic acid	12.609	8.41	2-acetyl-5-methyl-isoxazo-lidin 5-ylmethy-lester	17.105	2.63

Table 2 continued...

Table 2 continued...

13	Nonadecanoic acid	19.457	6.38	4-ketopimeic acid	12.055	0.91	Methyl 5-oxo-2-pyrrolidine-carbxylate	14.641	1.21	4-ketopimeic acid	13.442	0.92	5H-1,4-Dioxepin	17.373	1.92
14	9,12-octadeca-dienoic acid	20.964	4.42	1H-pyrazolc-3 -carboxylic acid	12.316	2.25	unknown	16.3	2.15	Unknown	16.354	11.94	Octadecanoic acid	18.89	4.05
15	6-Tetradecene	21.44	5.49	2-diamine-N, 4,5-trimethyl phenyl	12.632	20.55	2-acetyl-5-methyl-isoxa-zolidin 5-ylmethylester	16.863	23.9	2-acetyl-5-methyl-isoxa-zolidin 5-ylmethylester	16.86	17.24	Nonadecanoic acid	19.45	3.62
16	Unknown	23.068	0.86	3-methoxyl-6-methyl-6-phenyl -1,2,4-triazine	13.459	3.4	o-ethyloxinc	17.158	5.26	Unknown	16.947	1.06	Ethyltridecanoic acid	19.546	0.64
17				N-methoxy-2-carbomethoxy aziridine	16.289	1.76	Ethyl laevulinate	17.42	4.47	Unknown	17.079	0.71	9-dodecanoiate	20.863	0.78
18				{3-(actyl oxy)-4, 5-di hydro-5-iso-xazolyl}methyl	16.777	6.71	3-[Cl-methyl-1H -1,2,3,4-tetrazol 5-ydthio]-propan	17.756	1.05	Oxomalonic acid	17.158	4.26	9,12-Octadecan-oic acid	20.964	5.16
19				2-thoxy-4-methyltetrahy-dropyran	17.115	2.91	Cyclopentan-etriedecanoic acid	18.893	3.27	Ethyl laevulinate	17.418	3.91	9,12-15-Octad-ecanoic acid	21.164	0.87
20				5H-1,4-Dioxepin	17.386	2.32	Nonadecanoic acid	19.448	1.26	Methyl13-cyclopentyltri-decanoate	18.895	4.55	Unknown	21.436	3.37
21				Octadecanoic acid	18.895	3.61	9,12-octade-cadenoic acid	20.967	5.95	Nonadecanoic acid	19.438	1.03	Cycloundecene (z)	21.55	1.38
22				Nonadecanoic acid	19.441	1.38	7,10,12-Hexa decadenoic acid	21.168	1.57	Ethyltride-canoiate	19.545	0.8	Hydrooxylamine	23.064	0.69
23				Ethyl tricdecanoate	19.549	0.81	Unknown	21.549	1.21	Unknown	20.864	1.07	Androstan-3-one	28.286	3.27
24				9-Dodecanoic acid	29.865	0.51	Unknown	23.065	0.49	7,10-Hexa decadenoic acid	20.968	6.94			

Table 2 continued...

Table 2 continued...

25			9,12-octa decadenoic acid	20.967	4.36			7,10,13-Hexa decadenoic acid	21.168	1.85		
26			7,10,12-Hexa decadenoic acid	21.167	0.84			Unknown	21.412	1.24		
27			4-tetradecane	21.423	1.58			Unknown	21.55	0.92		
28			Unknown	21.554	1.61			5-pentadecen-7-yne	21.751	0.31		
29			4-tetradecan-b-yne	21.754	0.18			Unknown	23.067	1.33		
30			2-methyl butane	23.066	0.3							

compound in aswan. 2-diamine-N,4,5-trimethyl phenyl was the main compound in oreib. 2, 5-pyrrolidinedione was the major compound in heet-red.

3.2 Cultivars GC/MS

In Table 2, GC/MS technique separated 15 active components in Sudan-3, where it characterized 14 compounds. But it did not recognize the last one that possessed height% of 1.15, area% 0.86 and RT 23.068 min). Pyrrolidinedione gave the highest proportions in active components according to area and height are 23.19%, 21.35%, followed by E-2-ethoxyethenyl acetate of area = 16.59 height = 16.76% and Ethyl ester levulinic with area = 10.70% height = 8.58%. The lowest proportion is achieved for Butanedioic: area = 1.81% heights = 2.78%, from characterized active compounds in this cultivar. Table 2 shows the behavior of active components to retention time, peak area and height in oreib (the local) type. The GC-MS technique separated 29 active components. Thus, 27 active components were recognized. Two active components remained unrecognized had area% of 0.18, 3.11%, height of 0.34, 4.28% and RT of 7.951, 21.554 min, for each unknown compound. Dominant components were 2-diamine-N, 4, 5-trimethyl phenyl with area% of 22.55%, height=15.64%, 3-(acetyloxy) - 4, 5 di hydro-5-isoxazolyl methyl had area of 6.71%, height of 5.05% Pentenoic with area of 5.24%, height of 4.93%. While the lowest proportion was to 4-tetradecan-6-yne: area of 4.93 %, height of 5.24% at RT of 21.754min from characterized active compounds in this cultivar.

Table 2 shows the behavior of diagnosed active components relied on retention time, peak area and height in Aswan variety, which referred that GC/MS isolated 23 active compounds. This technique characterized 20 active components. However, 3 active components were remained unrecognized. 2-acetyl-5-methyl-isoxazolidin-5-ylmethylester possessed the highest GC/MS indices as area of 23.90%, height 13.12% and RT of 16.863 min, 3-pentenoic acid with area of 15.02%, height of 12.05% and RT of 9.908 min, 2-pentenoic acid with area of 12.54%, height of 9.98% and RT of 11.505 min. The lowest proportion was to 2-formyl-5-methylfuran with area of 0.15%, height of 0.30% and RT of 6.907. It is extracted from results in Table 2 that Heet black variety contained 28 active components with Retention time started from 7.435 ended at 23.067 minutes. The GC-MS technique diagnosed 21 active components. Seven components

Table 3: Area means of active compounds in five Roselle cultivars.

Cultivars compounds	Sudan-3	Oreib	Aswan	Heet-black	Heet-red
2-furancarboxaldehyde	nd	Nd	0.665	nd	nd
2-formyl-5-methy furan	nd	Nd	0.225	nd	nd
2H-pyrrol-one	nd	Nd	Nd	1	nd
Propanedioic acid	3.99	0.32	0.665	0.52	3.58
Butanedioic acid	2.295	3.695	3.36	8.565	2.5
1-thyl3-methyl2-methylmalonate	nd	3.57	Nd	1.325	nd
3-acetoxy-3-hydroxypropionic	nd	1.785	Nd	1.06	1.97
3-Heptenoic acid	8.89	nd	Nd	4.25	nd
4-methoxycycloheptanonic	nd	nd	Nd	nd	nd
3- Pentenoic acid	nd	5.085	13.535	nd	6.785
3,4-Dihdro-2H-pyran-2-caroxylic acid	3.7	4.16	5.86	1.62	3.825
1-thyl 4-methyl succinate	5.67	0.89	6.265	14.44	14.965
2- Butanedioic acid	nd	nd	Nd	nd	nd
2- Propanedioic acid	nd	nd	Nd	3.13	nd
Trans- Butenedioic acid	nd	3.84	Nd	nd	nd
2- Pentenoic acid	nd	nd	11.26	1.27	nd
2,5-pyrrolidiredione	22.27	17.91	Nd	nd	21.435
4-ketopimelic acid	nd	0.895	0.44	1.18	1.93
5-hydroxy methyl fur fural	nd	nd	1.095	nd	nd
1H-pyrazolc-3- carboxylic acid	nd	2.495	Nd	nd	nd
Pyrrolidin-5-onc	nd	nd	4.8	nd	nd
E -2-Ethoxyethenyl acetate	16.675	nd	nd	nd	nd
2-diamine-N,4,5-trimethyl phenyl	nd	18.095	nd	nd	nd
3-methoxyl -6-methyl-6-phenyl-1,2,4-triazine	nd	4.03	nd	nd	nd
Methyl 5-oxo-2-pyrrolidinecarbxylate	nd	nd	0.97	nd	nd
N-methoxy-2-carbomethoxy aziridine	nd	1.955	2.54	10.47	3.22
Ethyl ester levulinic acid	9.64	5.88	4.675	3.93	8.45
{3-(acetyl oxy)-4,5-di hydro-5-isoxazolyl}methyl	nd	nd	nd	nd	nd
2-acetyl-5- methyl-isoxazolidin 5-ylmethylester	nd	nd	18.51	13.555	2.785
5H-1,4-Dioxepin	2.895	2.49	nd	1.13	2.43
2-ethoxy-4-methyltetrahydropyran	nd	3.035	nd	nd	nd
Oxomalonic acid	nd	nd	5.515	4.73	nd
Pentanoic acid	2.62	nd	nd	nd	nd
3-[Cl-methyl-1H-1,2,3,4-tetrazol5-ydthio]-propan	nd	nd	1.37	0.86	nd
Octadecanoic acid	nd	4.55	nd	nd	5.285
Dodencanoic acid	4.255	nd	nd	nd	nd
Cyclopentanetriedecanoic acid	nd	nd	4.59	nd	nd
Methyl13-cyclopentyltridecanoate	nd	nd	nd	5.67	nd
Nonadecanoic acid	6.085	1.445	1.415	1.095	3.725
Ethyl tricdecanoate	nd	1.125	nd	0.995	0.855
9,12-octadecadenoic acid	5.225	5.395	7.875	nd	nd
9-dodecanoiate	nd	nd	nd	1.305	6.59
9-Dodecanoic acid	nd	0.69	nd	nd	1.01
7,10-Hexadecadenoic acid	nd	nd	nd	8.475	nd
9,12-15-Octadecanoic acid	nd	nd	nd	nd	1.13
7,10,12-Hexadecadenoic acid	nd	1.165	2.24	2.13	nd

Table 3 continued...

Table 3 continued...

4-tetradecane	nd	1.33	nd	1.02	nd
6-Tetradecene	nd	nd	nd	nd	2.935
Cycloundecene(z)	nd	1.735	1.56	1.205	1.51
5-pentadecen-7-yne	nd	nd	nd	0.42	nd
4-tetradecan-b-yne	4.805	0.26	nd	nd	nd
Hydrooxylamine	nd	nd	0.7	1.74	0.89
2-methyl butane	1.005	0.485	nd	nd	nd
Androstan-3-one	nd	nd	nd	nd	2.445

still undiagnosed. 2-acetyl-methyl-isoxazolidin-5-ylmethylster achieved highest area% of 17.244%, height% of 9.87% and RT of 16.860 min, followed by 1-ethyl 4-methyl succinate with area% of 16.21%, height% of 12.67% and RT of 11.713 min, Butanedioic acid with area% of 8.41%/ height% of 8.72% and RT of 12.609 min, Whereas 5-pentaadecen-7-yne possessed the lowest GC/MS indices as area of 0.31%, height of 0.53% and RT of 21.751 min. Furthermore, undiagnosed components registered highest area of 11.94%, height of 9.00% and RT of 16,354 min. while lowest undiagnosed compounds indices of GC/MS were area of 0.71%, height of 1.01% and RT of 17.079 min.

Table 2 shows that Calyx extract of Heet (red) variety contained 22 active components. Retention time started from 7.950 ended 28.286 minutes in separated column. So, the GC-MS technique diagnosed 20 active components. While two components are still undiagnosed. 2, 5-pyrrolidinedione recorded highest area of 23.71%, height of 19.16% and RT of 11.708 min, followed by 1-ethyl 4-methyl succinate with area of 15.99%, height 13.94% and RT of 12.612 min, Ethyl laevulinate with area of 9.77%, height of 7.13% and RT of 16.775, whereas Ethyltridecanoic acid gave The lowest GC/MS indices as area of 0.64%, height of 1.07% at RT of 19.546 min from isolated active compounds in roselle calyx extract. From other hand, the two Undiagnosed components registered area of 2.14, 3.37%, height of 1.80, 2.50% at RT 9.744 and 21.436 min for each compound, respectively.

4. Discussion

Roselle is member of vulgarism higher plants species. This plant is being wealthy in some pigments and protocatechuic acid such as anthocyanins. The dried calyces contain the flavonoids gossypetine, hibiscetine and sabdaretine. The major pigment, formerly reported as hibiscine has been identified as daphniphylline. Consequently, these compounds relied on genetic materials namely cultivars and ecotypes. So, some genotypes of roselle contain a pigment that brightens a brilliant red color for making culinary products from this plant; others are totally green. The GC/MS analyses for active compounds showed interesting differences between the five cultivars. It is ,therefore, possible to study the correlations between these parameter variations. The physicochemical in four roselle genotypes significantly differed as genotypes differed as they applied multivariate techniques like PCA and FDA. Phenotypic traits (not published) could contribute high variability among roselle genotypes [Ilodibia *et al*. (2019)]. Certain genotypes also were differed to growth inputs as nitrogen and phosphorus and stage growth led to active compounds might differ. Thus, Abbas and Ali (2011) pointed that vit C was increased as roselle genotype was differed by different NPK levels. Furthermore, Pacome *et al*. (2014) stated that the some differences of active compounds in aqueous extract of Roselle petals might be due to genetic variation, edaphic factor and solvent of extraction. Farag *et al*. (2015) revealed that although essential components were often distinguished as reduction of saccharides and amino acids resulted in degradation products as interaction thereby drying at high temperatures, they had in fact been previously characterized in roselle petals that dried in air [Chen *et al.* (1998)]. Such a wealth of furfural products in *Hibiscus* essential components is similar to mediate for the fresh and roasted Aromatic that involved in its flowers. Some genotypes are different in their enzymatic system that induced the biosynthesis of some secondary compounds like gossypetin via potassium and nitrogen which is necessary for translocation carbohydrates to metabolic regions. The genetic components are the effectiveness of crops. So, the certain genotypes represent the performance of given crop and the interaction of these genotypes by environments which are very important to extract the ability of genotypes for highest level. Thus, certain

genotypes are different in response to these factors in field as maximizing secondary metabolites built in the these genotypes as absorption and ionization increased depended on SCC- system capability constant of these genotypes. Likely, many of these compounds were previously recorded. Where, Avalos-Marinez *et al*. (2018) reported many of above active constituents and mentioned that these compounds were different as cultivars were different as 2-fufural and 5-methyl-2-fufural.

4. Conclusion

Multivariate analysis initially revealed that the 5 cultivars of calices of *H. sabdariffa* represented significant differences in their components of active chemicals. Chemicals parameters of each cultivar were recognized by principal components analysis. Thus, parameters combinations that were not associated and facilitatively to be measured allowed a good discriminative representativeness among the 5 cultivars of calices and led to accurately yield classification. Thus, these results showed that the cultivars of calices could be authenticated from very main physicochemical analyses. Further, study had to be applied over other genotypes of *H. sabdariffa* across multienvironment and their results had to be validated thereby a higher number of samples. Moreover, phenolic could be interested to be analyzed so as to precisely categorize the cultivars. Finally, results could use as assistant tool in breeding project for improving the Roselle production. Furthermore, proper taxonomic detection and recognition could be enhanced in other plant species.

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