Effect of Soil Types and Cultivars on Chemical Constituents of Roselle (*Hibiscus sabdariffa* L.) under growing conditions of Sudan.

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Abstract

The effect of two soil types, namely Shambat soil (Vertisol) and Soba soil (Entisol) on the performance of four cultivars of *Hibiscus sabdariffa* L. (namely: Rahad, Fashir, Kass and Abiad)- was studied by evaluating some of its constituents as, organic acids (citric, ascorbic and tartaric), Anthocyanines (malvidin and pelargonidin), pH and essential amino acids under the semi -arid conditions of Sudan. HPLC and amino acid analyzer were used in this study. It was found that, there was a significant effect of type of soil on pH, organic acids and essential amino acids content where, Shambat soil gave significantly higher pH values, total organic acids, and amino acids than those grown in Soba soil. But, there was a significant difference between soil types on organic acids separately, where, cultivars grown in Soba soil gave significantly higher amount of citric and ascorbic acid content than those grown in Shambat soil, while tartaric acid was the highest in cultivars cultivated in Shambat soil. Anthocyanines also were significantly affected by type of soil, where Malvidin was found to be higher in Shambat soil than Soba soil, but pelargonidin was higher in Soba soil, but there were no significant effect on total anthocyanines. With respect to cultivars it was found that, there was no significant different among cultivars in the pH, while the organic acids were significantly affected, where, Fashir cultivar contains the highest amount of total organic acids. On the other hand, Rahad cultivar gave significantly the highest amount of the Anthocyanines and essential amino acids content among all other cultivars.

Key words: *Hibiscus sabdariffa,* Roselle, soil type, Amino acids, Organic acids, Anthocyanines, pH.

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Introduction

Hibiscus sabdariffa L., (Roselle) has more than 300 species distributed in tropical and subtropical regions all around the world, it is known in the Sudan as Karkade or Angara, Main cultivars of Karkade calyces; locally known in the Sudan as EL Rahad, EL Fashir, Abiad and Kass. Karkade is widely used as food to make jellies, jam and beverages (Nasir, 2004). Odigie et al. (2003) reported that aqueous extract of *Hibiscus sabdariffa* L. attenuates hypertension and reverses cardiac hypertrophy and Essa (2007) reported that alcoholic extract of *Hibiscus sabdariffa* L. has an anti- hyperammonemic and antioxidant effects on brain tissues.

This plant has gained an interest for study because of the high demand in the world market and is expected to replace chemical products in many industries and medicinal uses.

Types of soils may affect the chemical constituents of some plants, such as organic acids, pH, anthocyanines and amino acids. Salinity found to decrease productivity of many crops and it could be reduced or alliviated by increasing fertility of the soil (El-Tilib, 1993). Aziz, (1992) reported that *Hibiscus sabdariffa* L.(Roselle, Karkade) can grow in different types of soils and environmental conditions such as high temperature and salinity. Modawi, et al. (1984) found that there was small variation in chemical constituents of volatile oil of *Cymbopogon proximus* L. in different location in the Sudan (Markyat, Butana, Jubel Kone and North mountain) due to differences in soils type. Siribel et al. (2004) studied the effect of two soil types, namely Shambat and Gerif soil on the performance of two lines of *Catharanthus roseous* L. and they found that Gerif soil gave better herbage yield than Shambat soil while alkaloid content was not significantly affected by soil types.

Objectives

The objective of this study was to assess the effect of different soil types and variation in cultivars on the chemical constituents of *Hibiscus sabdariffa* L.

Materials and methods

Experiment

The experiment was executed at the experimental farm of Medicinal and Aromatic Plants Research Institute at Shambat, Sudan (Latitude 15° 40′ N, Longitude 32° 32′ and 360m above sea level). The climate is semi-arid with low relative humidity and daily mean air temperature

ranging from 25 to 40 °C in summer, and 15 to 21 °C in winter. It was carried out on two soil types, namely, Shambat soil (Vertisol), classified as fine montomorillonitic, isohyperthermic, Entic Chromustert and Soba soil (Entisol), which is classified as medium, loamy mixed isohyperthermic, Typic Torrifluvent, it is salty affected soil. The Physico-chemical properties of the two soil types are presented in Table-1.

Four cultivars of *Hibiscus sabdariffa* L. namely Rahad, Fashir, Kass and Abiad were cultivated in the demonstration farms of Medicinal And Aromatic Plant Research Institute at Shambat and Soba (Sudan), in year 2005. Plants were identified in the department of plant taxonomy in the same institute. At mature stage calyces were collected, dried and kept in carton bags for extraction.

The design used was split plot design in which cultivars assigned to the main plot and soil types assigned to the sub-plot. Each treatment was replicated two times. The data were statistically analyzed and Duncan Multiple Range Test was used for the mean separation.

Determination of pH values and organic acids

2.5g of each sample were macerated in 25ml hot distilled water at 100°C for organic acids and for pH values (A.O.A.C., 2000).

Organic acids were determined using HPLC (Shimadzu corporation (Koyoto, Japan) pumps (LC-10 ADVP), Degasser (DGU-14A) UV detector (SPD-10 AVP), system controller (Scl-10 AVP), according to the method described by Withy et al. (1993). Authentic compounds of organic acids were obtained from Chemistry of Natural and Microbial Product Laboratory National Research Centre, Egypt, (ascorbic acid 3 mg, citric acid 5mg and tartaric acid 5.5mg).

Determination of pH

pH value was determined using pH meter Hanna instrument.

Determination of essential amino acids

5g of the test samples were macerated in 50% alcohol until all pigment was extracted and concentrated under reduced pressure at 40°C. 10 ml NaCl (10%) was added to the extract, stirred for one hour then 10 ml of trichloroacetic were added and filtrated. The precipitate was collected by centrifugation, washed and dried in a desiccator.

20 mg of protein were refluxed with 6 NHCl (10ml) for 20 hrs and the acid removed by evaporation under reduced pressure, the residue was dissolved in 10 % isopropanol for amino

acids identification using the method of (Baily, 1967), using (Eppendorf-Germany Lc 3000) Amino acid analyzer.

Determination of Anthocyanins

According to the method described by Durker, and Jones, (1969), Methanol with 1ml HCl was added to 5 gm of sample until all pigments were extracted. The extract was dried and 20 ml of 6NHCl were added to the dried extract and then reflux for about one hour, after that 20 ml amyle alcohol were added to form two layers, the upper layer (organic layer) was separated and analyzed using HPLC. (Shimadzu corporation (Koyoto, Japan) pumps (LC-10 ADVP), Degasser (DGU-14A) UV detector (SPD-10 AVP), system controller (Scl-10 AVP), according to the method described by Withy et al. (1993). Authentics compound of Anthocyanines were obtained from the Central laboratory of The National research centre, Egypt, as malvidin 5mm and pelargonidin 10mm.

Result and Discussion

Effect of soil type

Shambat soil cultivated cultivars showed significantly higher pH values than those of Soba soil (Table 2.). This was attributed to the good physical conditions and high pH of Shambat soil, which reflected on the plant characteristics.

Organic acids were greatly influenced by soil type (Table 3.). Soba soil gave significantly better ascorbic and citric acid content than Shambat soil, while tartaric acid was significantly higher in those grown in Shambat soil. This also may be attributed to the high pH in Shambat soil. These findings are in conformity with those obtained in previous studies of Modawi et al. (1984).

The essential amino acids (Threonine, Valine, Methionine, Leucine, Isoleucine, Phenylalanine, Histidine, Lycine, and Arginin) as illustrated in table 4 were significantly affected by type of soil where, Shambat soil grown plants gave significantly higher essential amino acids than Soba soil. This may be attributed to the difference in soil content, where Soba soil is salt affected while Shambat is not (Table 1), this also may attributed to the favourable conditions of Shambat soil while Soba soil which suffer from salinity may adversely affect the plant. This finding corroborates the previous findings of El.Tilib et al. (1993).

With respect to the Anthocyanines (Malvidin and Pelargonidin), it was found that they were significantly affected by soil type, where Malvidin was higher in those grown in Shambat soil than Soba soil. While Pelargonidin was higher in those grown in Soba soil than Shambat soil,but there was no significant effect on total anthocyanines as The synthesis of anthocyanins depends mainly on Light which shown to be the most important environmental factor influencing anthocyanin biosynthesis in plants (Grisebach, 1982). And there is no difference in light intensity between the two areas of the present research (Shambat and Soba).

Table 1. Some chemical and physical properties of Soba and Shambat soil. Depth 0.30cm

Sample	**Sand %**	**silt %**	**clay %**	**CEC Meq/100g**	**O.C %**	**Mg meq L^{-1}**	**SAR**
Shambat	30.5	22.5	47	50.6	0.79	4.0	0.6
Soba	44	20.5	35.5	45	0.31	1.26	27.41
Sample	**N %**	**P ppm**	**Na meq L^{-1}**	**K meq L^{-1}**	**Ca meq L^{-1}**	**pH (paste)**	**Ec dS m^{-1}**
Shambat	0.05	0.9	1.7	0.3	3.5	8.5	4.5
Soba	0.02	2.59	39.6	0.11	3.0	7.8	6.3

Soil samples were analyzed in Laboratories of the Department of Soil chemistry, Faculty of Agriculture, University of Khartoum (Shambat).

Table 2. Effect of Soil Type on pH of four Cultivars of *Hibiscus sabdariffa*

Soil *T*ype / Cultivars	Soba soil	Shambat soil	Mean cultivars Effect
1-Rahad	1.63 *	2.14	1.89 a
2-Fashir	1.77	1.86	1.82 a
3-Kass	1.50	2.20	1.85 a
4-Abiad	1.81	1.95	1.88 a
Mean pH for soil	1.68 b	2.04 a	

**mean of three replicates.*
Means followed by similar letter on the same rows and columns are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Table 3. Effect of Soil Type on Organic Acids (O.A.) of *Hibiscus sabdariffa* (%)

Soil type / (O. A)	Soba soil	Shambat soil	Mean O. acid effect
Citric acid	16.93*	14.75	15.84 b
Ascorbic acid	15.07	10.29	12.68 b
Tartaric acid	19.75	45.24	32.50 a
Mean soil effect	17.25 b	23.43 a	

Table 4. Effect of Soil Type on Essential Amino Acids (E.A.A.) of *Hibiscus sabdariffa* L. (µg/ml)

Soil type / (E.A.A.)	Soba soil	Shambat soil	Mean Essential Amino-acid effect
Threonine	30.97*	38.96	34.97 a
Valine	14.37	21.65	18.00 c
Methionine	01.47	07.49	04.48 e
Leucine	13.50	15.70	14.60 d
Isoleucine	32.75	40.50	36.62 a
Phenylanaline	21.75	35.05	28.40 b
Histidine	34.88	32.90	33.89 a
Lycine	32.69	24.73	28.71 b
Argnine	19.05	20.15	19.60 c
Mean soil effect	22.35 b	26.35 a	

Table 5. Effect of Soil Type on Anthocyanines of *Hibiscus sabdariffa* L. (%)

Soil type / *Anthocyanines*	*Soba soil*	*Shambat soil*	*Mean Anthocyanines effect*
Malvidin	19.85* b	23.45 a	21.65 b
Pelargonidin	26.17 a	23.97 b	25.57 a
Mean soil effect	23.51a	23.61 a	

Effect of Cultivars

Table 2. shows that, there was no significant difference in the pH values among the four cultivars, while table 6. shows that, Fashir cultivars gave significantly higher citric and ascorbic acid than others, and Abiad gave the lowest, Moreover, it was found that tartaric acid content of Abiad cultivar was the highest, while Kass cultivar was the lowest These results were on line with previously reported observations that light colored hibiscus is more acidic than dark colored (Mandour et al. (1979) and, Alshoosh (1997).

Table 6. Effect of Cultivars on Organic Acids (O. A) of *Hibiscus sabdariffa* L. (%)

Cultivars / *(O. A.)*	*Rahad*	*Fashir*	*Kass*	*Abiad*	*Mean (O. A.) effect*
Citric acid	17.84 a	14.47bc	18.65 a	12.40 c	15.84 b
Ascorbic acid	14.88 a	19.54 b	15.99 a	10.32 b	12.68 b
Tartaric acid	25.09 c	44.17 a	22.86 c	37.86 b	32.49 a
Mean Cultivars effect	19.27 b	24.73 a	19.16 b	20.21 b	

The essential amino acids, as illustrated in table 7. were significantly affected by cultivar where, Rahad and Abiad cultivars gave significantly higher essential amino acids than Fashir and Kass. This may be attributed to their genetic characteristics.

With respect to the Anthocyanines (Malvidin and Pelargonidin), it was found that they were significantly affected by cultivar, where Rahad gave higher Anthocyanines than the other cultivars.

Table 7. Effect of Cultivars on Essential Amino Acids (E.A.A.) of *Hibiscus sabdariffa* L. (µg/ml)

Cultivars / (E.A.A.)	Rahad	Fashir	Kass	Abiad	Mean *(E.A.A.)* effect
Threonine	36.39	35.04	30.19	38.22	34.97 a
Valine	18.56	19.34	15.49	18.65	18.00 c
Methionine	01.76	02.20	12.77	01.18	04.48 e
Leucine	16.90	13.94	12.49	15.06	14.60 d
Isoleucine	39.60	36.20	32.94	37.75	36.62 a
Phenylanaline	29.56	28.84	23.95	31.20	28.40 b
Histidine	37.40	31.05	32.45	34.65	33.89 a
Lycine	37.50	18.45	30.65	28.23	28.71 b
Argnine	18.90	19.12	19.17	21.23	19.61 c
Mean Cultivar effect	26.29 a	22.69 b	22.23 b	24.99 a	

Table 8. Effect of Cultivars on Anthocyanines of *Hibiscus sabdariffa* L.(%)

Cultivars / Anthocyanines	Rahad	Abiad	Kass	Fashir	Mean Anthocyanines effect
Malvidin	30.49	16.38	18.97	20.36	21.61 b
Pelargonidin	36.90	20.58	27.24	26.76	27.87 a
Mean Cultivar effect	33.71a	18.48 b	23.11b	23.56 b	

Conclusion:

This work comes to conclude that *Hibiscus sabdariffa* L. conistituents is highly affected by type of soil and cultivars and the performance was the best when grown in Shambat soil.

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