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ORGANIC ACIDS COMPOSITION OF DIFFERENT PARTS OF THE MEDICINAL PLANT – ROSELLE (*HIBISCUS SABDARIFFA*)

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ABSTRACT

Roselle (*Hibiscus sabdariffa var. sabdariffa*) is known as a folk medicinal plant . The main parts used are flowers and calyces. These parts also used in hot and cold drinks and jams. Oxalic acid which is important health concern present in these parts. This study was conducted to investigate the presence of oxalic and other acids in calyces, flowers, leaves, petiols, seed pots etc. The results revealed that, the organic acids, oxalic, malic, tartaric, ascorbic, and citric were found in all these parts. Oxalic acid was found in decreasing order as follows; petioles (86.77 %), green leaves (74.30 %), calyces (44.60 %), seed pods (19.28%) . The levels of other organic acids in these plant parts have also been determined. The importance of these findings arises from the fact that although healthy individuals can safely consume this acid in moderation but in vulnerable individuals such as those with kidney disorders, osteoporosis or rheumatoid arthritis are typically advised to avoid this acid, even if there is no story or cause to be concerned about, consuming parts containing dangerously high concentrations of oxalic acid, must be avoided due to its poisonous nature because sometimes ,consuming even dilute amounts of oxalic acid can rapidly " crack " the casein in various dairy products .Therefore , the infusion beverage or Roselle tea must contain low to moderate amounts of oxalic acid per serving . The other organic acids with some health and / or beneficial characteristics were also found in all plant parts of Roselle and their profiles were varied within the plant parts .The aqueous extracts of some plant acidifying agents was also compared with that of Roselle.

Key Words: Roselle (*Hibiscus sabdariffa* L.), Plant Parts, Organic Acid Profiles.

INTRODUCTION

Organic acids as we employ the term in this study, are those acids that result from the synthetic activities of plants and animals, as distinct from decomposition ,like uric acid etc. They have a very pleasing flavor relished by everyone. The plant which has been subjected to this study belongs to the genus *Hibiscus* has more than 350 species distributed in tropical and subtropical regions around the world and many are believed to have certain medicinal properties and have been used in traditional medicine for many centuries. However, the species *Hibiscus sabdariffa* comprises a large number of cultivated types classified broadly under two varieties, H. *sabdariffa var. sabdariffa and H. sabdariffa var . altissima Wester* .The former is generally bushy and pigmented and cultivated for the edible calyces , the latter includes tall growing , un-branched types bearing inedible calyces and mainly cultivated for the stem fiber (Gautam RD, 2014) . The former, is known by several different names, such as Roselle, rosella, red sorrel, herbal tea, sour tea, cranberry, Karkade etc. The plant used in this study belong to the former variety which is known as a popular medicinal plant (the main parts used are flowers and calyces) which has been shown to relax the uterus and for treating indigestion (control of diarrhea, loss of appetite, circulation disorders, as well as anti-bacterial and anti-

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oxidant properties (Tsai PJ *et al*., 2002; Tee PL *et al*., 2002) , lowers the blood pressure (Odigie LP *et al*., 2003; Mckay DL *et al*., 2013; Tabassum N and Ahmad F, 2011; Odigie IP *et al*., 2003), and it can suppress significantly blood lipid levels including triglycerides and total cholesterol (Chau JW *et al*., 2004) . A study in mice found that *H. sabdariffa* can prevent liver damage caused by paracetamol (Ali BH *et al*., 2003), the active ingredient in Tylenol and a number of other pain – killers which is known to cause serious damage in high enough doses . The mild laxative effect of the calyces and seeds of Roselle and also their ability to increase urination attributed to two diuretic contents, ascorbic acid and glycolic acid. Moreover, because of its citric acid contents, the water extract of calyces is used as cooling herb providing relief during hot weather by increasing the blood to the skin surface. The aqueous extracts of Roselle calyces were found to exert anti - diabetic activity (Dahiru D *et al*., 2003; Hansawasdi C *et al*., 2003). The anthocyanin pigments which are water – soluble main coloring agents in the calyces were found to possess potential as a cancer chemo - preventive agent against tumor promotion ((Dahiru D *et al*., 2003). The tea prepared from the calyces was found to be rich in red color and tart in taste and it is naturally caffeine free. For many of the conditions discussed above, treatment with prescription or over –the counter medication is also available. Our previous study (Al-Wandawi H *et al*., 1984) have shown that the whole mature seeds of Roselle contain protein (25.20) and lipids (21.10) and eighteen amino acids and many elements detected and quantified for the first time: and the most abundant essential amino acids were found to be leosine , lysine, while oleic acid was the most predominant fatty acid followed by palmitic and stearic acids, and gossypol found only as traces. Evaluation of the safety of the seeds by our group was also conducted for the first time Farju IB and Al-Wandawi H (1983) and it was found that Roselle seeds are nutritive , safe , and may possibly have an anti-atherosclerosis effect when included in diet. This latter effect was also reported by Farjou IB and Al-Wandawi H (1983). The review of literature revealed that the water extract of calyces and flowers have been and still in use in folk medicine as crude, nevertheless, with the exception of a few controversial reports no relevant data are available regarding the organic acid composition of these and other parts of this plant ,and no data are also available regarding the effect of the methods used for preparation of the hot and cold drinks on the acids profiles in the part used . In the present study we report on the organic acids composition of the different parts of Roselle plant. Finally, the organic acids composition of the aqueous solutions of Roselle parts were compared with some other traditional sources used as cold and or hot drinks.

MATERIALS AND METHODS

The seeds were sown during early April in a . nearby field in Baghdad area .Flowering and fruit formation continued until early October.

Sample preparation

Experiments were carried on using fresh and air - dried samples representing different plant parts.

Extraction and identification of the organic acids

As a routine procedure, equal samples (30 g each) was mixed with 350 ml distilled water and blended for 10 minutes using an electric blender and filtered using Whatman No. 4 filter paper . The filter cake was re-extracted with 100 ml distilled water .The filtrate was centrifuged for 10 min at 8000 rpm. The supernatant was adjusted to 25 ml. and the pH was determined. The organic acids of each sample were determined by HPLC technique using AOAC, method – 986. 13 (2000) on a Shimadzu Prominance20 A System.

RESULTS

The results presented in (Table .1) show the organic acids profiles in different fresh plant parts of Roselle .It can be seen that in calyces ,malic acid is the predominant acid (47.06 %), followed by oxalic acid (44.60), tartaric (3.37) ,ascorbic (2.75 %) and citric acid (trace) . In green leaves , the most predominant acid is oxalic acid (74.30 %), followed by malic acid (21.79 %) , tartaric acid (2.30 %) , ascorbic acid (1.37 % and citric acid (0.21) .The organic acids profile in petioles is as follows: oxalic acid is found at its highest level (86.77), malic acid (10.38 %) , tartaric acid (1. 05 %) , ascorbic acid (0.87 %), and citric acid (0.76 %) .In seed pods, the most striking point was the presence of citric acid at a very level where it accounted to (73.26 %), while oxalic and malic acids dropped to their lowest values (19.28 %) and (1.98 %) respectively and ascorbic acid was accounted to (1.78 %). The data presented in (Table 2) show acid profiles in calyces in fresh and state. The data indicate un expected increase in citric acid (from trace to 15.09 %) and significant drop of malic acid from (47.06 to 9.63 %), while the increase in the levels of other acids is attributed to the difference in the moisture content between fresh and dry calyces. The results presented in (Table3) shows the comparison between the organic acid profiles of petioles at fresh and dry state. It can be seen that citric acid shows unexpected increase at dry state(51.09 %) compared to (0.46 %) at fresh state .Oxalic acid and malic acid behaved in controversial way (39.42 % and 3.55 %) at dry state compared to (86.77 % and 10.88 %) at fresh state respectively. Tartaric acid and ascorbic acid behaved in the expected way. The organic acid composition of fresh and dry petioles is shown in (Table4) . Malic ,tartaric and ascorbic acid expected increase at dry state (due to the difference in moisture content), while citric and oxalic acids behaved in a peculiar way .Thus, the former dropped from 73.74 % at fresh state to 32.18% at dry state ; while

oxalic acid increased from (18.65 to 54.91%). The results presented in (Table 5) indicate that, with the exception oxalic acid (which showed about 6 % increase in leaves without petioles),the presence of petioles has increased the levels of other acids .The results presented in (Table 6) show that in immature fresh seedpods , citric acid is dominant and accounts to (73.74 %) of the total detected acids ,followed by oxalic acid (18.65%) , while in dry immature seedpods oxalic acid was dominant and accounted to (54.91 %), followed by citric acid (32.18%), other acids were significantly increased in dry immature seed pods. Table (7) shows the effect of temperature used to extract the organic acids from the dry green leaves (without petioles). It can be seen the water extract at 100 ^{O}C resulted in the highest level of oxalic acid (93.61 %) compared to (74%) 25 ^{o} C. Malic acid level was dropped from (21.74 %) to only 0.72 at ^{o} C 100 .The results presented in (Table 8) show the final pH of aqueous solutions prepared from some conventional plants acidifying agents used as direct hot and or cold drinks almost after adding a sweetening agents .The Roselle calyces was found to be the most acidic source . The data presented in (Table 9) compare the organic acid profiles of Roselle calyces with most popular and acidic fruits as cold and or hot drinks mixed with tea. The results reveal that Roselle calyces contain the highest level of oxalic acid (63.67 %) of the total organic acid , followed by sour tea (31.16 %) , sour currant (10.58 %), lime (0.23 %) , and sour lemon (0%).

Table 1. Organic Acid Composition * of Different Fresh Parts of (*Hibiscus sabdariffa L.) plant*

Pant part	Citric acid	Oxalic acid	Malic acid	Tartaric acid	Ascorbic acid
Calyces	Trace	44.60	47.06	3.37	2.75
Green leaves	0.21	74.30	21.79	2.30	1.37
Petioles	0.76	86.77	10.83	1.05	0.87
Seed pods	73.26	19.28	1.98	3.74	1.78

* per cent (% of total organic acid detected .

Table 2. The Organic Acid Composition* of Fresh and Dry Calyces of Roselle

	Citric acid	oxalic acid	Malic acid	Tartaric acid	Ascorbic acid
Fresh calyces	Trace	44,60	47.06	3.37	2.75
Dry calyces	15.09	63.47	9.63	3.06	3.13

*Per cent (% of total organic acids detected .

Table 3.The Organic Acid Composition* of Fresh and Dry Petioles of *Roselle*

	Citric acid	Oxalic acid	Malic acid	Tartaric acid	Ascorbic acid
Fresh petioles	0.46	86.77	10.83	1.05	0.87
Dry petioles	51.69	39.42	3.55	2.20	3.12

*Per cent (%) of total organic acids detected .

Table 4. The Organic Acids Composition* of Fresh and Dry Green Petioles of Roselle

	Citric acid	Oxalic acid	Malic acid	Tartaric acid	Ascorbic acid
Green fresh petioles	73.74	18.65	2.21	3.32	2.05
Green –dry petioles	32.18	54.91	5.39	5.65	2.85

Per cent (%) of total detected organic acids .

Table 5. The Organic Acids Composition* of Green leaves of Roselle with and without Petioles Soaked with Stirring in Boiling Water for 10 minutes

	Citric acid	Oxalic acid	Malic acid	Tartaric acid	Ascorbic acid
Green leaves with petioles	7.54	85.00	4.72	0.89	1.07
Green leaves without petioles	4.61	91.00	3.06	0.73	0.22

*Per cent (%) of total detected organic acids .

Table 6 . Organic Acid Composition *of Mature and Immature Seedpods 0f *Roselle*

	Citric acid	Oxalic acid	Malic acid	tartaric acid	ascorbic acid
Immature fresh seedpods	73.74	18.65	2.21	3.32	2.05
Immature dry seedpods	32.18	54.91	4.39	5.65	2.85

*Per cent (%) of total detected organic acids. . .

Table 7. The Organic Acid Profile*of Green Leaves of Roselle without petioles Extracted with Water at Different Temperatures

Temperature, ^{o}C	citric acid	oxalic acid	malic acid	tartaric acid	ascorbic acid
25	0.21	74.00	21.79	2.30	1.37
45	Trace	76.30	22.37	Trace	1.31
100	2.46	93.61	3.91	1.52	0.72

*Per cent (%) of total detected organic acids .

Table 8. The pH Values of Aqueous Extract of Some Conventional Acidifying Agents from Plant Sources are compared with Roselle calyces.

Source	Methods of extraction	pH
Roselle calyces	3.37 g + 300 ml D.W., boiling for5 min and filtered	2.616
Lime	3.37 g+ 300 ml D.W., boiling for5 min and filtered.	2.875
Sour lemon	3.37+ 300 ml D.W., boiling for 5 min and filtered .	3.175
Sour currant	3.37 + 300 ml D.W., boiling for 5 min and filtered .	3.211
Sour tea	3.37g +300 ml boiling for 5 min and filtered .	3.071

D.W. = distilled water .

Table 9. Comparison of the organic acid composition of Roselle with most popular plant produces locally used as hot and or cold drinks

Source	Citric Acid	Oxalic Acid	Malic Acid	Tartaric Acid	Ascorbic Acid
Roselle calyces	15.09	63.47	9.63	3.o6	3.13
Lime	2.38	0.23	0.59	76.46	20.30
Sour lemon	0	0	53.82	23.90	22.26
Sour currant	9.00	10.58	3.55	67.73	18.13
Sour tea	0	31.16	1.98	12.23	57.54

* % of detected organic acids

Fig 1. General view of Roselle plants at the flowering stage

Fig 2. Close view shows flowers and flashy calyces

Fig 3. A seed pod of Roselle covered by calyces

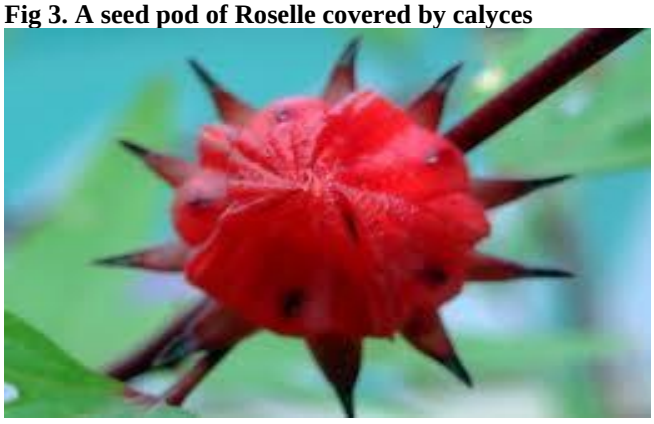


Fig 4. A seed pod surrounded by calyces

Fig 5. Roselle plant at flower stage showing leaves with long petioles

Fig 6. Development process of Roselle leaves

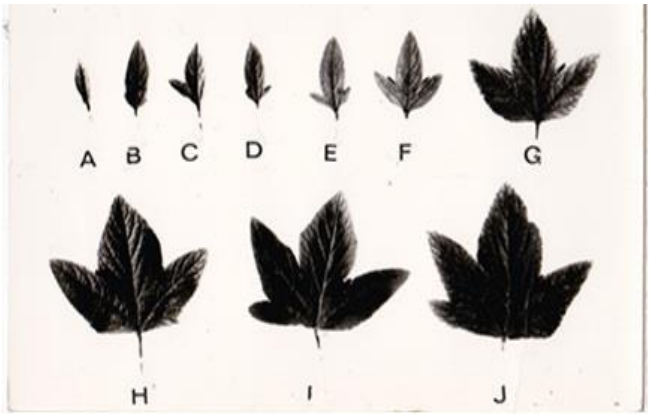
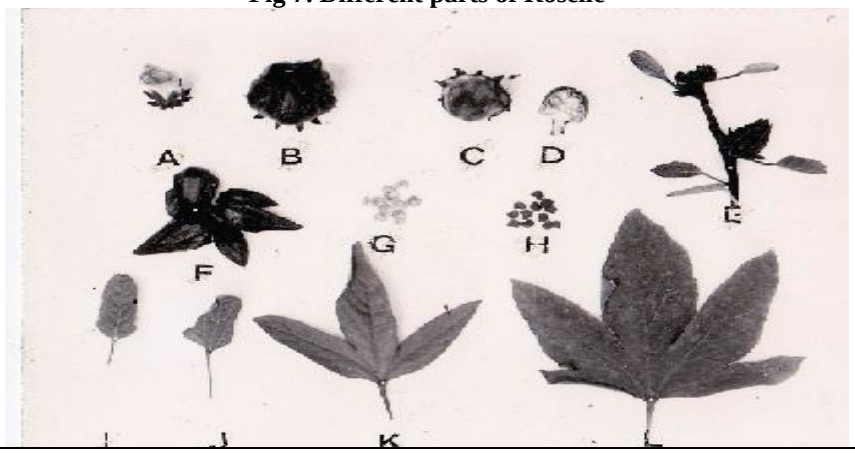


Fig 7. Different parts of Roselle



DISCUSSION AND CONCLUSIONS

Nowadays, Roselle is known as medicinal plant and it has also applications in foods and cosmetics. Hibiscus tea is infusion from the dry calyces consumed hot and cold by people around the world .The calyx is not flower petals, but rather the leaves which form specially shaped like a cup below it , the tea is made from these, not the flower petals. On the other hand, people consuming Roselle plant parts may not know that those people who have health conditions or diseases need to take care when juicing or dinking. The calyx, and leaves of true Roselle (Hibiscus sabdariffa var. sabdariffa) are acid and closely resemble the cranberry .The plant is mostly appreciated for its anthocyanins and organic acids contents. The present study showed that the organic acids, oxalic, malic, tartaric, ascorbic and citric were found to exist in varying percentages in all parts of Roselle, with oxalic acid as the most predominant acid .Healthy individuals can safely consume oxalic acid in moderation ,but those with kidney disorders, osteoporosis or rheumatoid arthritis are typically advised to avoid this acid ,Even if there is no story or cause to be concerned about. A dilute amounts of oxalic acid can rapidly crack the casein in various dairy products . Malic acid , is known to possesses many health benefits , such as increasing body's energy, maintaining oral heath ,reducing the risk of toxic metal poisoning and promoting younger skin by providing remedies for hyper pigmentation. Malic acid is also effective in stimulating the production of saliva and acting as an oral antiseptic benefiting oral hygiene by reducing harmful bacteria. Tartaric acid may be used in pharmaceutical and bakery industries .It has also has some applications in cement, plaster, gypsum ,and for polishing and cleaning metals .Ascorbic acid is well known for its anti - a scorbutic, antioxidant ,and free radicals scavenger. It is used most often for preventing and treating common cold, gum diseases, acne and other skin infections .Citric acid is the main ingredients in food and beverage industry, and a common ingredient in face packs and skin products. It prevents the formation of kidney stones; its antioxidant properties prevent cancer tumor form and obesity. The calyces of Roselle are used as a laxative, anti-carcinogenic, antihypertensive, and cholesterol lowering medicine. Also exhibit great antioxidant activity, lower hepatoxicity and reduce fever. The leaves and flowers can also be used in medicinal applications as a soothing reagent for cough, poor appetite and antibacterial reagent.

It is well documented that, all most all parts of the plant (including the seeds) are considered diuretic and anti - scorbutic .The steams and branches can be used in the production of twine and cord known as " rosella hemp " .For many conditions discussed above claims are supported in some scientific studies or trials, therefore, treatment with prescription or over –the counter medication is also available. Nevertheless, people who have health conditions need to take care when using any part of this plant. For instance, due to the hypotensive effect caution is expressed when using with other blood lowering medicine. The organic acids contents of the different parts which have been subjected to this investigation are also important. In addition the pH values of aqueous extract of some natural plant acidifying agents was compared with that or Roselle calyces.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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