

STUDIES ON THE BIOCHEMICAL CONSTITUENTS  
OF ROSELLE PLANT

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Thesis

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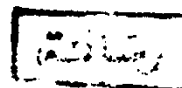
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## I. INTRODUCTION

Roselle, (Hibiscus Sabdariffa) belongs to the plant family Malvaceae L.,. It is an annual or sometimes perennial bushy subshrub 5-7 feet height, with a slightly branched, erect, smooth and often purplish stem,

It is native to tropic Africa (Kirby 1963) or to India (Brouk 1975). Roselle can be grown in fairly wide range of climatic conditions, so, it is found in a large number of countries.

It is cultivated for the fleshy calyx from which, Jelly, Jam, a kind of tea named "Kerkedeh", etc., are prepared (Kirby 1963).

The tender young leaves and shoots of roselle are eaten as salad or as pot herb in some countries (Brouk 1975).

The calyces decoction or infusion may be used as an hybotensive agent, since it lowers blood pressure without producing side effect (Sharaf 1962). The roselle extract may be used as antispasmodic to the intestine and uterus, anthelmintic in tiniasis, and antibacterial.

Nowadays, the whole of the world give a great attention to the substances produced from natural sources instead of the synthetic ones, regarding to the injurious Side-effects caused after using those synthetics. So roselle calyces may be considered as a source of natural red colorant for foods, such as fruit jellis, jams, since the color is being retained for 6 months of storage at 82-4°F., with some evident darkening (Esslen 1973). It can be concluded that roselle is an important dietetic and therapeutic factor.

In Egypt (A.R.E.), the roselle plant was first cultivated in upper Egypt, especially in the governorate of Asswan. Regarding the old thought that Harkadé grows well in the hot regions, since it is a tropical plant, it was still cultivated in upper Egypt, until this thought was rectified. It has been made clear that roselle sepals can be produced in A.R.E. even in the coolest temperature climate of the north Egypt and attaining a high quality as those produced in warm regions (Osman, 1970). For this reason cultivators gave a particular attention



to this crop, owing to its possibility to grow successfully in different localities in A.R.E.

The aim of this study is to investigate biochemical changes during fruit formation in relation to the color, acidity and related character as they are the major participates in forming the taste of the commercial commodity designated as sepals.

## II. REVIEW OF LITERATURE

### 1. Chemical composition of Roselle Plant:

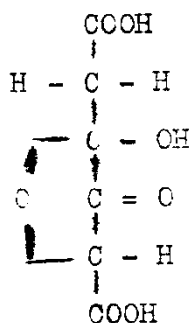
#### a) Organic acids:

Angelo Catiglioni (1934) reported that the calyx of roselle plant is being citric acid rather than tartaric acid.

Leupin (1935) showed that the acid taste of the drug prepared from roselle was found by fractional microsublimation to be owing to large quantities of oxalic, malic, citric and tartaric acids.

Indovina and Compotummino (1938) examined the extract of roselle cultivated in Sicily and they reported that it consisted essentially of a mixture of citric acid (12.5-16.8%), malic (2.1-3.1%) and traces of tartaric acid could also be detected.

Gribel (1939) obtained a new acid from roselle calyces by extraction with 95% alcohol, precipitation with lead acetate, filtration, and crystalization. The previous mentioned acid was given the name of Hibiscus acid and identified by the same investigator (1943) as (L)-allo hydroxy citric acid having the following formula.



Hibiscus acid ( $\text{C}_6\text{H}_6\text{O}_7$ )

Leclerc (1938) reported that the infusion of Karkadé is a refreshing beverage containing malic, citric and tartaric acids.

Reaubourg and Monceux (1940) showed that the water soluble solids of roselle dried fruit of American and Abyssinian origin consist citric (77%), malic (22%) and traces of tartaric acid. However, oxalic, gallic, tannic and lactic acids were absent in the aqueous extract.

Bachstey (1948) obtained Hibiscus acid from the dried flowers of Mexican and Abyssinian roselles. The content of this acid was amounted 14.6% and 15.3% respectively in the two varieties. Tzu-sheng Tung (1963) showed that, the dried

flowers petals of roselle plant were extracted with ethanol in a soxhlet apparatus. The extract was purified by using columns impregnated with cation and anion resins and fractionated by paper chromatography. The chromatogram showed 4 spots of identified acid, i.e. glycolic, citric, tartaric, and oxalic as well as one unknown spot with Rf (0.44). Malic acid could not be detected.

Rosita (1964) mentioned that the presence of citric acid was determined by paper chromatography in infusions and extracts of roselle plant grown in the Sudan at the Eritrean border. The possible presence of malic and malonic acids was indicated from the chromatograms, Compared with known samples.

Alshaarawi (1967) found that roselle calyces contained 20% of acids calculated as citric acid.

Diab (1968) examined roselle sepals by extracting the acids with boiling water and filtration. The extract was fractionated by paper chromatography, five yellow spots were located

in the chromatogram. Malic, tartaric and citric acid were identical with respect to the reference acid.

Osman and Hussein (1969) studied the suitable harvest stage of roselle sepals and found that the acidity (i.e. 20-25.2%) increased at 10-15 days after the fruit setting, while the fruit weight (sepals + ovary) reached the maximum at 22 days after the fruit setting. The investigators suggested to harvest roselle sepals 20-25 days after fruit setting.

Osman and Violet Jacob (1970) studied the relation between the cultivation region and the chemical composition of light red variety of roselle cultivated in different localities in Egypt. The analysis revealed that the acidity calculated as citric acid ranged from 16% to 20%.

Kerharo (1971) reported that the concentration of organic acids was increased in sepals of roselle after flowering, citric acid was the predominant acid (12-17%) but malic and tartaric acids were present in few amount. The dried flowers contained high concentration of hibiscus acid.

Osman and Hussein, (1972) studied the chemical composition of three strains of roselle cultivated in Egypt the first was chracterized by its light red sepals and high acid content, the second by the dark red sepals, big fruits and moderate acidity, and the third by dark red sepals, small fruits and low amount of acids.

They found that the acid percentages were 16.4, 14 and 11% in the first, second and third strains respectively.

El-Bedawi (1973) investigated the chemical composition of light and dark roselle sepals from Aswan and Sudan regions, and found that the acidity was 18.94% in light red sepals whereas the dark red being 16.28-16.20% calculated as citric acid, chromatographic fractionation, and identification proved the presence of oxalic, citric, ascorbic, and tartaric acids while malic acid was absent, However oxalic acid was detected only in the light roselle calyces.

Lin (1976) studied the carboxilic acids in roselle by paper chromatography, and showed that citric, tartaric, and oxalic acids were identified.

Schilche (1976) mentioned that the analysis of *H. Sabdariffa* flowers from various sources suggested requirements for a minimum content of acids in commercial preparations of 20 mg% (based on hibiscus acid).

b) Coloring matter:

Yamamoto and Oshima (1932) obtained the so called Hibiscin chloride  $C_{20} H_{19} Cl_4 \cdot 5H_2O$  they mentioned that this compound was probably cyanidine monoside, by hydrolysis they obtained cyanidine chloride  $C_{15} H_{11} O_6 Cl$  together with a pentose.

In a complementary work of the same investigators (1936) they reported that hibiscin was not cyanidine pentoside because of the incomplete purification. They obtained dephinidine chloride with glucose and small amount of aldopentose.

Reaubourg and Moncouc (1940) declared that the extract of roselle dried calyces of American and of Abyssinian origin contains red pigment. The red aqueous or alcoholic extract turns green in the presence of alkali. The other dye-stuff is anthocyanin.