

Chemical Composition of Some Roselle
(*Hibiscus sabdariffa*)
Genotypes



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DEDICATION

*To my father and mother,
to my family members
and my colleagues .*

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ABSTRACT

Six lines of Hibiscus sabdariffa were used in this study. The proximate analysis gave moisture 6.19%, 12.07% and 8.42%, ash 10.6%, 7.98% and 12.42%, protein 9.76%, 5.53% and 6.13%, oil 0.99%, 0.95% and 0.90%, fibre 10.74%, 11.73% and 12.17%, carbohydrate 61.64%, 61.76% and 60.43% for seasons 93/94, 94/95 and 95/96 respectively.

Physicochemical investigation of karkade calices gave the following results: reducing sugar 1.29%, 1.28% and 1.27%, non reducing sugar 0.87, 0.77 and 0.86, PH 3.1, 2.71 and 3.001, colour intensity 0.77, 0.72 and 0.51, insoluble solids 37.74%, 36.36% and 37.33% for seasons 93/94, 94/95 and 95/96 respectively. The value of vitamin C and acidity (organic acids) in season 93/94 was 92.06 mg/100g and 19.58% respectively. Organic acids as hibiscic acid, citric acid, vitamin C and oxalic acid was 25.51%, 14.15%, 92.69 mg/100g and 0.55% and 26.18%, 14.14%, 97.26 mg/100g and 0.56% for season 94/95 and 95/96 respectively.

Mineral contents for karkade calices for season 94/95 were 0.56%, 0.24%, 0.08%, 0.23%, 0.95% and 0.19% for Ca, Mg, Fe, Na, K and P respectively, while mineral content for season 95/96, 1.9%, 0.31%, 0.07 0.31%, 0.48%, 0.23%, 0.05%, 0.22%, 0.06%, 0.006%, 0.02%, and 0.03%. Ca, Mg, Fe, Na, K, P, Zn, Mn, Cu, Pb, Co and Cr respectively.

UV-visible spectrum showed the presence of the following pigments delphinidin -3-sambubioside and cyanidin -3-sambubioside. Total anthocyanin of karkade calices were found to be 0.89 to 1.4% and 0.99 to 1.6% for season 94/95 and 95/96 respectively. Acid hydrolysis of the pigments showed the presence of xylose and glucose in addition to the anthocyanidins delphinidin and cyanidin.

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Chapter one

INTRODUCTION

Roselle (Hibiscus sabdariffa) is known in the Sudan as karkade or Ingara. Other english names are East Indian sorrel and thorny mallow (Reaubourg and Monceaux, 1940). It belongs to the Family Malvaceae. Two botanical varieties are recognised: variety sabdariffa a bushy branched subshrub with red or green stems and red or pale yellow inflated edible calices; variety altissima, a tall unbranched plant 10 --16 ft high with fibrous spiny inedible calices grown for fibre (Purseglove, 1968).

Hibiscus sabdariffa is probably a native of West Africa and is now widely cultivated throughout the tropics. It has been taken to the new world by slave trade. It is confined to the tropics and tolerates poorer condition than jute.

There are two main types of karkade calices according to the commercial terminology used in the Sudan, namely El Rahad and El Fashir qualities. Karkade is grown in the Sudan as a cash crop in rainfed areas. The principal production area is in eastern Kordofan sands in the area encompassing, El Rahad and Umruaba. Internationally El Rahad variety is preferred. It is grown on a small scale around El Obied, in western Kordofan, near El Fashir, Nyala and in the southern Fung.

Its growing period ranges from 4 to 6 months. It can either be propagated from cutting or by direct seeding which is the best. Sudan is categorized as one of the largest exporter of Karkade in the world. A significant increase in the size of the crop has taken place during the last

three decades. In 1960 454 tons were exported for the total value of Ls. 67,829, whilst in 1969 1,512 tons were exported worth Ls. 546,298. At the same time the local consumption has increased. Germany is by far the largest importer and user of the crop (Percy, 1971).

Recently, however, karkade is grown as crop in some irrigated areas, planted crop in already existing agricultural projects. According to the “foreign trade statistical digest” of the bank of Sudan the quantity of exported dry karkade calices amounted to 9918 tons in the year 1993 at value of us 9099000. In 1994 the exported quantity amounted to 14243 tons at value of us 16065000. In 1995 the total exported quantity amounted to 32209 tons at value of us 12947000. During January - June 1996 the exported quantity amounted to 5994 tons at value of us 7897000.

Inspite of this tremendous increase in the production of karkade calices on the one hand, and the serious competition facing the Sudanese commodity in the international market on the other hand, very little work has been done to isolate the different type of varieties in order to select or hybridize a variety combining most of the desired characteristics.

The calyx is the most important part of the plant. It contains the valuable components which determine the quality of the product namely: colour (anthocyanins), flavour (organic acid) and aroma. The red acid succulent calices of variety sabdariffa are used for producing sorrel drink. They are also made into jellies and preservatives. The tender leaves and stalks are eaten as a salad and as a pot - herb. Variety altissima is grown for fibre in India, Java and the Phillipines. The root

has been reported as an aperient due to the presence of tartaric acid (Mclean, 1973).

As much as the calices are acidic in nature, they are considered as antiscorbutic factors and is said to be soothing caught (Watt and Breyer-Brandwijk, 1962).

The serious problem associated with processing of karkade into food product is the instability of the brilliant red colour imparted to food by the anthocyanin pigments contained in the calyx.

The present study is aimed at laying foundation for official standards and specification for new breeding lines of karkade. Therefore this research was carried out under the following objectives.

1. To study the proximate composition of the promoted lines.
2. To evaluate the chemical composition of the calices as a deterrent factor for further selection of the new inbred lines.

Chapter two

LITERATURE REVIEW

2.1 Chemical composition of karkade

Dried calices of roselle have been shown by Ibrahim *et al.* (1971) to have the following chemical composition:

moisture content	9 ----- 14%
ash	7 ----- 11%
protein	5.4 ----- 9.45%
titrable acidity	14 ----- 18.7%

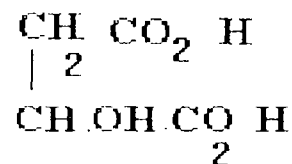
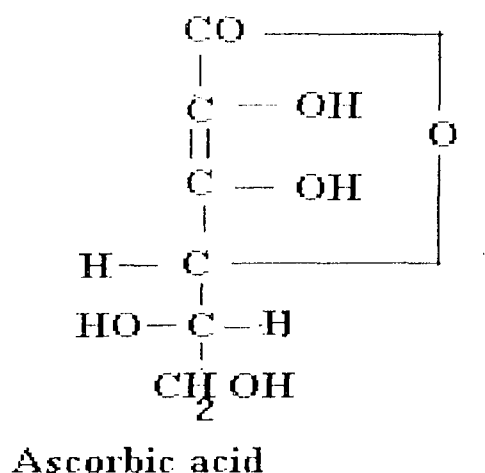
Also Reaunbourg and Monceaux (1940) pointed out that Hibiscus sabdariffa dried fruits of American and Abyssinian origin contained about 15% moisture, the dry matter consisted of 3.5% proteins, 12% ash and 16% reducing sugars.

2.1.1 Organic acid

Generally organic acids are water-soluble, colourless liquid or relatively low melting solids. The majority are non-volatile. Organic acids are classified chemically according to the number of carboxylic acid groups or to other functional groups present. Some of the organic acids shown to be present in aqueous extract of calices of Hibiscus sabdariffa are the following (Harborne, 1984).

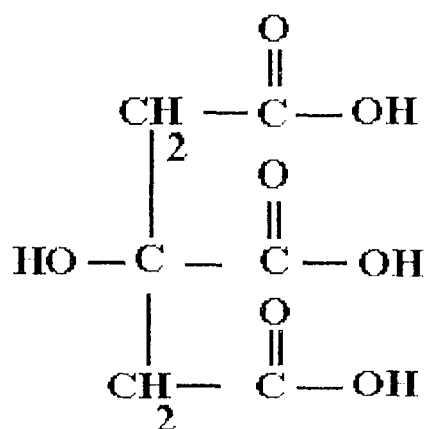
mono carboxylic acid

dicarboxylic acid



Malic acid

tri carboxylic acids



Citric acid

Flavour characteristics of roselle were found to be mainly in the acid content. The presence of citric, malic, succinic, lactic, tannic and oxalic acids have all been reported in roselle (Pritzker and Jungunz, 1937). Organic acids are important constituents of food products, not only as chelating agents for iron and copper but also for the control of pH and inhibition of enzymes (Sistrunk and Cash, 1973). These results

were confirmed by Brand (1939) who also detected oxalic acid found as the calcium salt in roselle of Egypt origin. Ibrahim *et al.* (1971), working on Sudanese roselle detected malic, oxalic, citric and 3-indolyl acetic acids. Hibiscic acid in the calices was found to be very soluble in water and alcohol and slightly soluble in ether. The amount of hibiscic acid in plant samples analyzed by Griebel (1939) was 13.6%. This result stands in agreement with the result obtained by Bachtez (1948) who found 15.3% in Mexican samples, and 14.6% in an Ethiopian sample.

The presence of vitamin C (L-ascorbic acid) in roselle has been confirmed by many analysts among them Mclean (1973) who showed that vitamin C concentration varies from 21 - to 89.4 mg/100g in both fresh and dried samples Ibrahim *et al.* (1971) reported ascorbic acid in roselle water extract with concentration of 7.12 mg/100g. Storing dry karkade calices for more than six months depletes them from vitamin C (Mario 1959, Addo 1981) According to Watt and Breyer (1962) the acids constitute about 13% of the whole karkade calices. The acids present are citric acid and hibiscic acid ($C_6H_6O_7$). Hibiscic acid was first confused with citric acid because of several reactions, especially those reactions involving colour changes which are the same. Griebel (1939) suggested that it was a lactone of hydroxy citric acid with two asymmetrical carbon atoms, with the formula ($C_6H_6O_7$). Other water soluble acids are malic acid, tartaric acid trace while oxalic, gallic, tannic and lactic acid are absent in the aqueous extract (Reabourg and Manceaux 1940). The total acidity in the dry calices can be expressed not only as hibiscic or citric, but also as tartaric acid (Schilcher 1976). Analysis of organic acids in calices showed their contents to range between 8.2 and 12.6% as reported by Milkowska and Strzelecka. (1995) .

2.1.2 Carbohydrates

Hamidi *et al.* (1966) detected galactose, galacturonic acid and rhamnose in the calices. Two other free sugars, glucose and arabinose were also detected in water extract by Ibrahim *et al.* (1971). The pectin content had been shown by Riaz (1969) to increase in both quantity and quality with sepal development during maturation, but it is unlikely to prove a commercial source of the material in competition with apple and citrus. The pectin content had been found to be only 3.19% but nevertheless gives the plant its mucilaginous character.

2.1.3 Proteins

Ibrahim *et al.* (1971) showed that the protein content of the whole calices of Sudanese varieties ranged between 7.05 -- 9.45% and thirteen amino acids were given by the protein hydrolyzate of the whole and spent calices, while the protein hydrolyzate from the water extract gave only nine amino acids out of the thirteen amino acids of which six are essential. Karamalla and Ali (1974) found that the protein content of mature karkade seed ranged from 27 -- 31%.

2.1.4 Ash

Analysis of non-volatile carbon-free residue from careful combustion gave about 9% of the calices on dry basis. The presence of sodium, calcium, magnesium, aluminum, manganese, iron and phosphate were reported by Mclean, (1973). The presence of manganese was indicated by the intense blue-green of the ash. Calcium is most copious followed by phosphorus and iron.

2.1.5 Fats

In an attempt to evaluate the local produce of karkade seeds with regard to their oil and protein content Karamalla *et al.*(1973) indicated that the stability of the karkade seed oil as compared to other edible Sudanese oils was fairly good. The seeds contain about 17% oil which is similar in properties to cottonseed oil. Cottonseed contains up to 25% while linseed contains up to 33 -- 43% (Cobley, 1956). Karamalla and Ali (1974) found that the oil content of the mature seed of karkade ranged from 19 -- 24%. The fatty acid composition of roselle seed was: myristic acid trace, palmitic acid 26.38%, stearic acid 4.80%, Oleic acid 35.84% and linoleic acid 32.97% (Rahamma, 1979).

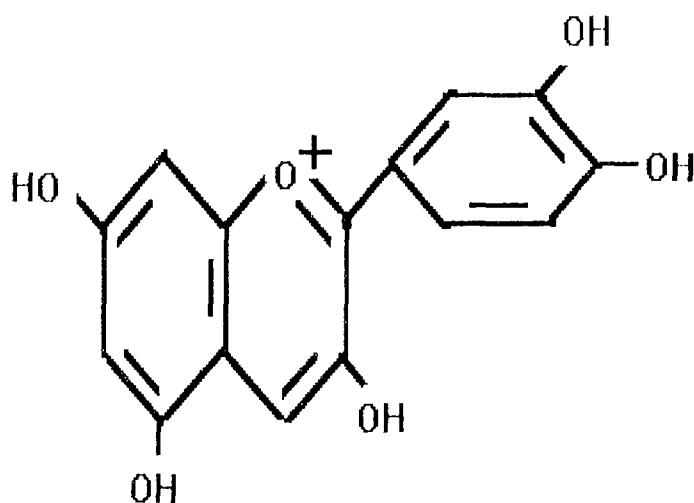
2.1.6 Tannins

Tannins are polymers of phenolic compounds with higher molecular weights (mol wt 500 -- 5000) containing sufficient phenolic hydroxyl groups to permit formation of stable crosslinks with proteins (Swain, 1965).

According to Tamir and Alumot (1969) tannins have the ability to bind proteins forming insoluble complexes, through the formation of hydrogen bonds between hydroxyl groups of tannins and polyphenols and the carbonyl groups peptide bonds of protein (Millic and Stojanovic, 1972). Therefore tannins precipitate protein from aqueous solution thus rendering plant proteins relatively indigestible and reducing enzymes activity (Goldstein and Swain, 1965; Van Sumere *et al.* 1975). Thus tannin are antinutritional factors in food and beverages. However study of type and quality of tannins in karkade is not known up to date.

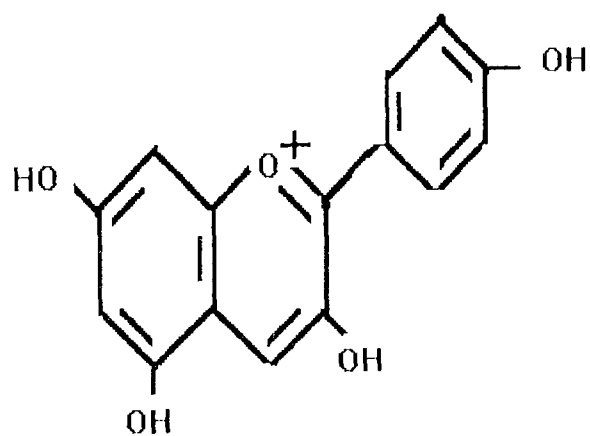
2-2 Pigments

Anthocyanins are the most important and wide spread group of colouring materials in plants. These intensely coloured water-soluble pigments are known to be responsible for nearly all the pink, scarlet, red, mauve, violet and blue colours in the petals and leaves of higher plants. Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenyl benzopyrylium (flavylium) salts. (Broillard, 1982). Anthocyanins are all based chemically on a single aromatic structure of 3, 5, 7, 3', 4' pentahydroxyl flavylium cation as show below.

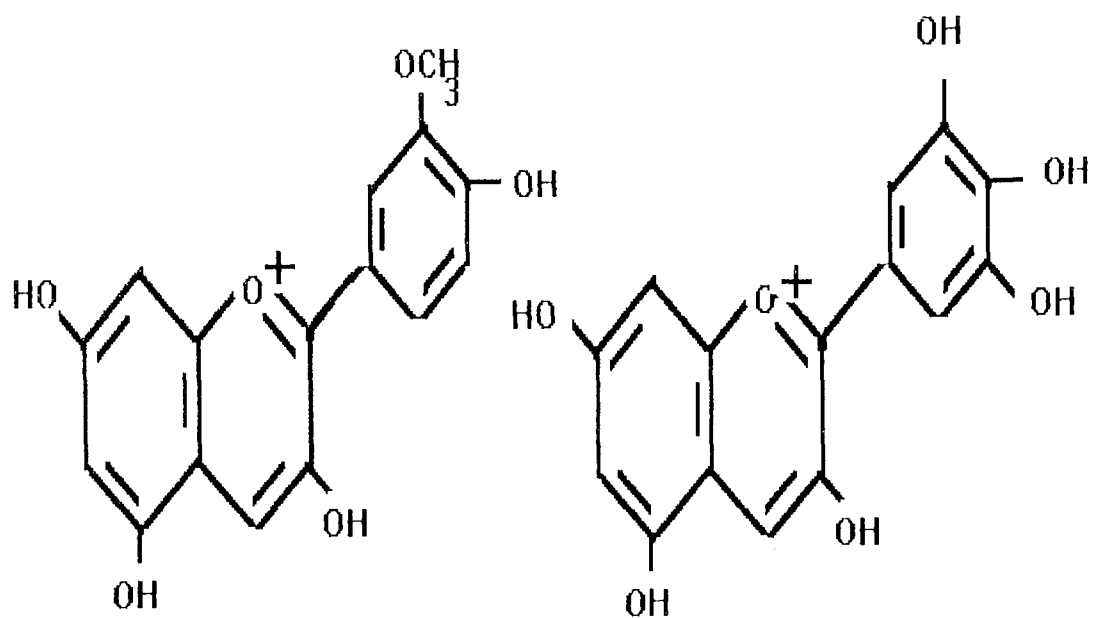


Cyanidin

In general, anthocyanidins do not accumulate in the plant (Harborne 1967) and the pigments occur in flowers and fruits mainly in the glycosylated form. The most common anthocyanidins can be shown as following (Markakis 1982).

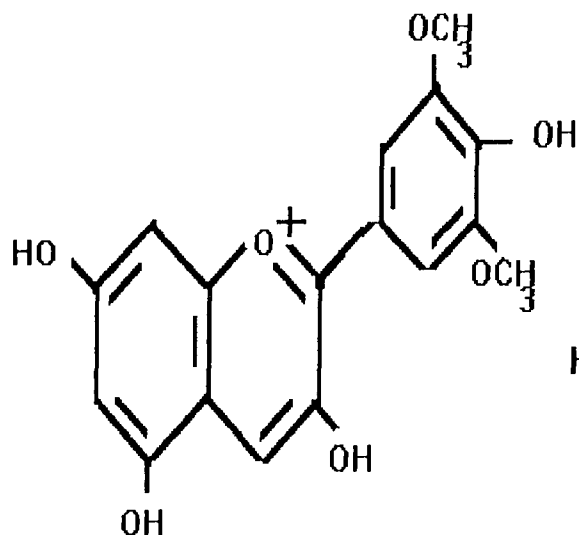


Pelargonidin

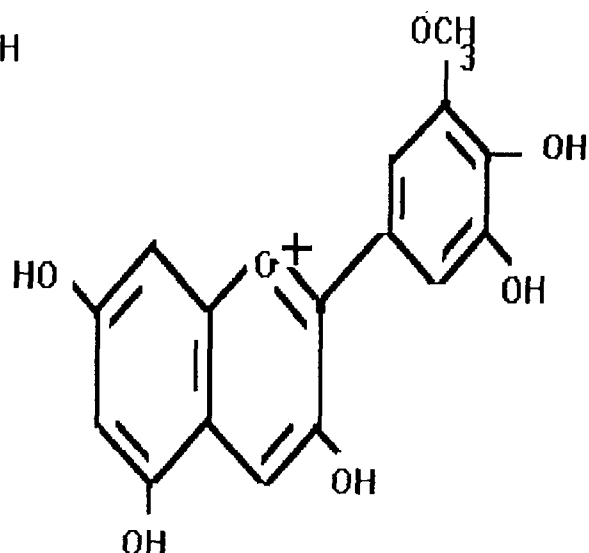


Peonidin

Delphinidin



Malvidin



petunidin

Anthocyanidins can be obtained from two different sources:

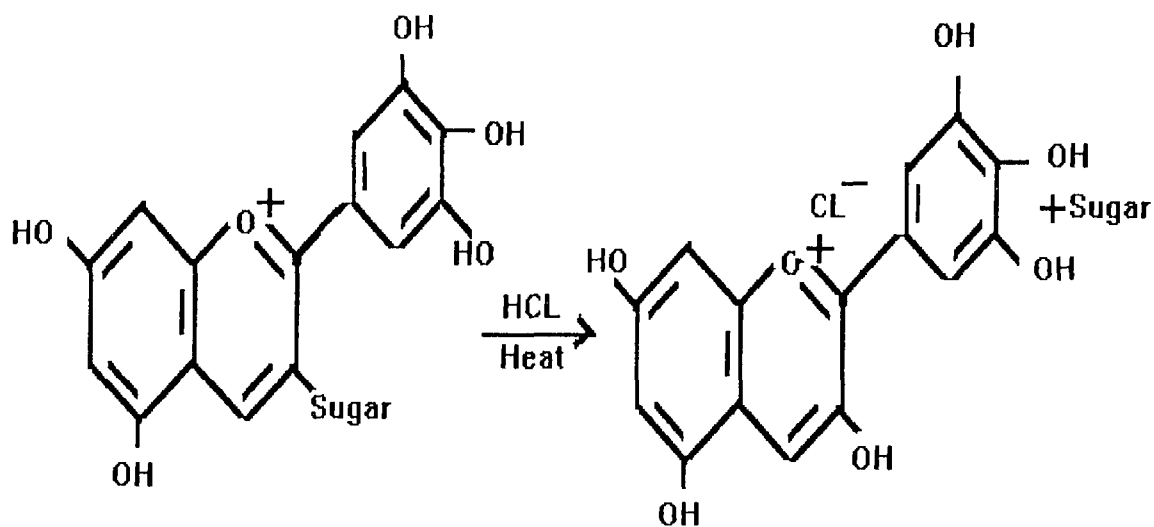
1- From the acid hydrolysis of naturally occurring coloured glycosides (anthocyanidins).

2 - From acid treatment of colourless leucoanthocyanidins.

The most common sugars associated with tannins are the monosaccharides glucose, galactose, rhamnose, arabinose and xylose. Di and trisaccharides also occur. The 3-hydroxyl group (see Fig 4) is always replaced by a sugar. When a second sugar is present, it is generally at C-5 and glycosylation of 7-, 3- and 5-hydroxyl groups, however, has also been demonstrated. Anthocyanins with more than two glucosidic

residues have been identified, e.g acylated delphinidin 3-rutinoside 5, 3, 5 triglucoside (Yoshitama, 1977) and acylated cyanidin 3, 7, 3-triglucoside (Yoshitama and Abe, 1977).

Often plant tissues will have a number of pigments composed of one or more anthocyanidins differing in the sugar moiety. These on complete hydrolysis with mineral acid, e.g hydrochloric acid, give anthocyanidin chlorides, as shown by the following example (Mayer, 1960).



Hydrolysis of anthocyanin

Yomamoto and Oshima (1932) obtained a crystalline anthocyanin from roselle which they called "hibscin". They pointed out the structure to be cyanidin 3- glucoside (cy-3-g). They changed it later (1936) to delphinidin-pentoside glucoside. Watt and Breyer (1962) also mentioned the presence of gossipetin and hibiscin. The pigment of roselle were further examined by Shibata and Furukawa (1969) who reported the

presence of cyanidin-3-glucoside and delphinidin-3-sambubioside. Forsyth and Simmonds (1954) also reported the presence of delphinidin and cyanidin in trimidal roselle. A more detailed work carried by Du and Francis (1973) using paper chromatography revealed the presence of more than these two pigments. Anthocyanins identified were cyanidin-3-glucoside which on complete hydrolysis gave cyanidin and glucose. Cyanidin-3-sambubioside was also present which was the second major anthocyanin. Delphinidin-3-glucoside was identified as a minor component. The most abundant anthocyanin in roselle and the pigment responsible for its reddish violet colour was delphinidin-3-sambubioside which on complete hydrolysis gave delphinidin, glucose and xylose. Three other pigments were not identified since they were present in trace amounts. The total anthocyanin content was calculated as 1.5g/100g on dry weight basis expressed in terms of delphinidin-3-glucoside. Deibner *et al.* (1965) reported that inclusion of water helps the extraction of the more hydrophilic anthocyanin which are present as a minor pigment in cranberries (Fuleki, 1967) and it also allows the use of ethanol recovered by distillation. The hydrochloric acid stabilizes the pigments and lowers the pH to the level where the absorbance of anthocyanin is at their maximum.

2.2.1 Factors affecting the stability of anthocyanins

2.2.1.1 Chemical structure

Not all anthocyanins appear to be equal in their resistance to the degrading effects of various agents. According to Robinson *et al.* (1966), the anthocyanidin diglucosides of stored New York wines were more

stable to decolourization than the corresponding monoglucosides. Van Buren *et al.* (1968) reported that the same diglucosides were more stable to heat and light than the monoglucosides. Starr and Francis (1968) found the galactosidic anthocyanins of cranberry juice to be more stable than the arabinosidic ones during storage of the juice. From the work of Harzdina *et al.* (1970), it appears that the stability of the anthocyanidin 3-, 5-diglucosides of grapes increases with an increasing degree of methoxylation but decreases with an increasing hydroxylation of the aglycone.

2.2.1.2 Temperature

The effect of temperature on the stability of anthocyanins in model systems and in food products has been studied by many investigators (Nebesky *et al.*, 1949, Meschter, 1953; Decareau *et al.* 1956; Markakis *et al.* 1957; Daravingas and Cain 1965; Segal and Negutz, 1969; Harzdina *et al.* 1970). Meschter (1953) showed that processing strawberry preserve at 100°C for 1 hr resulted in 50% destruction of the fruit anthocyanin and the half-life of the pigment was 1hr at 100°C. During storage of the preserve at 38°C the half life of the pigment was 10 days, while at 20 °C it was 54 days and by extrapolation the half-life of the anthocyanin would be 11 months at 0°C storage. Segal and Negutz (1969) formulated a logarithmic relationship between anthocyanin destruction and temperature, whereas Adams (1973) described a similar relationship between anthocyanin destruction and time of heating at a constant temperature. Saeed and Ahmed (1977) studied the stability of stored carbonated beverage from roselle. They found that the keeping

quality was 10 days at ambient temperature during summer time where the ambient temperature reaches its maximum (45 °C).

2.2.1.3 Ascorbic acid

Everett (1953) also pointed out the influence of ascorbic and dehydroascorbic acids on pigment degradation of strawberry products as being directly proportional to their concentration, ascorbic acid being more effective and that the reaction was catalysed by the presence of iron and copper. Iron and copper in themselves were not important contributors to the pigments loss mechanism, except in so far as they hastened the destruction of ascorbic acid. These destruction products in turn attack the pigment in an increased rate. Beattie *et al.* (1943) were the first to show that changes in colour occurred with progressive losses of ascorbic acid. Markakis *et al.* (1957) postulated that an interaction between ascorbic acid and anthocyanin may occur resulting in the loss of colour. Sondheimer and Kertesz, (1953) recognized the formation of hydrogen peroxide from ascorbic acid oxidation, which would react with anthocyanin to produce break-down products. They postulated two mechanisms for the pigment decolouration of strawberry juice.

2.2.1.4 PH

According to the work carried by Dravingas and Cain, (1968), raspberry anthocyanins which were cyanidin-3-diglucoside, cyanidin-3-glucoside and cyanidin-3-,5-rhamonoglucoside-5-glucoside, with cyanidin-3-diglucoside as the main component, the rate of degradation decreased as the pH was decreased. The maximum pigment retention was attained at pH 1.8 for strawberry anthocyanins.

2.2.1.5 Enzymes

Erlandson and Worlstad, (1972) worked on blanched and unblanched strawberry puree to ascertain the role of enzymes on anthocyanin degradation. They concluded that enzymatic degradation was negligible. They also concluded that oxygen had little effect on anthocyanin degradation. The pigment degradation rate was always greater in air than in nitrogen, the rate difference being small but relatively constant.

2.2.1.6 Light

The effect of light on the colour of bottled concord grape juice was noticed by Tressler and Pedersen, (1936). Van Buren *et al.* (1968) reported that acylated and methylated diglucosides were the most stable anthocyanins in wine exposed to light, non acylated diglucosides were less stable, and monoglucosides even less stable. Palamidis and Markakis, (1975) found that light accelerated the destruction of anthocyanins in carbonated beverage coloured with anthocyanins extracted from grape pomace.

2.2.1.7 Metals

Several multivalent metal ions can interact with anthocyanin possessing vicinal phenolic hydroxyls and shift the color of the pigment toward the blue end of the spectrum. The bathochromic shift caused by the addition of AlCl_3 is used as an analytical test in differentiating between cyanidin, petunidin and delphinidin on the one hand, and pelargonidin and malvidin on the other hand (Harborne, 1973).

2.3 Qualitative analysis of anthocyanins

2.3.1 Extraction

For plant materials, the extraction procedures have generally involved the use of acidic solvents. The plant sample is macerated in methanol containing a small amount of hydrochloric acid (up to 1%). The mixture may be blended or merely allowed to stand overnight in a refrigerator (Markakis 1982, Jackman *et al.* 1987). This is followed by filtration, and either precipitation of the pigment or removal of the solvent under vacuum at temperature not exceeding 50°C, so as to minimize pigment degradation.

2.3.2 Identification

Chromatographic and spectrophotometric techniques have been applied for identification of the anthocyanins and their breakdown products. The absorption spectra of anthocyanins and their aglycones in the visible and UV-regions have proved very useful in the identification of these pigments. Harborne (1958 a and 1967) published data giving the spectral characteristics of all the known anthocyanidins and most of the anthocyanins. Both anthocyanins and their aglycones (anthocyanidins) show two characteristic absorption maxima when in acid solution, a strong one in the visible region between 465 and 550 nm and a smaller one in the UV-region at about 275 nm. The wave length of the maximum absorption may provide valuable information as to the hydroxyl pattern of the specific aglycone in question. Glycosylation of the anthocyanins at the 3-position generally results in a hypsochromic shift in spectra, the

spectral characteristics of all the known anthocyanidins and most of the anthocyanins. Both anthocyanins and their aglycones (anthocyanidins) show two characteristic absorption maxima when in acid solution, a strong one in the visible region between 465 and 550 nm and a smaller one in the UV-region at about 275 nm. The wave length of the maximum absorption may provide valuable information as to the hydroxyl pattern of the specific aglycone in question. Glycosylation of the anthocyanins at the 3-position generally results in a hypsochromic shift in spectra, the nature of the specific sugar having no real effect (Harborne and Hall 1964). Measurement of the ratio of the absorbance at the UV-maximum to that at the visible-maximum, and the ratio of the absorbance at 440 nm to that at the visible-maximum have been shown to give valuable information regarding the extent and position of glycosidic substitution (Harborne 1958a; Timberlake and Bridle 1967). Of the chromatographic techniques used in anthocyanin identification paper and thin-layer chromatography (PC and TLC) have been the most popular. Harborne, (1958b and 1967) described chromatographic mobility (the R_f value) as "the most important characteristic for the identification of anthocyanin", and presented an extensive list of R_f values for all the available known pigments in a number of solvents, using paper chromatography.

2.3.3 Quantitative analysis of anthocyanins

The quantitative analysis of anthocyanin has been covered in a number of publications (Fuleki and Francis 1968, a, b, c, d; Francis 1982). In fresh fruit or vegetable juices there are usually very few interfering compounds that will absorb energy in the region of maximum absorption of anthocyanins (465 to 550 nm). The absorption

of pure pigments is often expressed as molar absorptivity which has been defined by Francis (1982) as the absorbance of one gram-mole of anthocyanin in one litre of solution. In determining that total anthocyanin content of an extract containing two or more distinct anthocyanins. Fuleki and Francis (1968c) suggested the use of a weighted average absorptivity. This, however, requires prior knowledge of the absorptivity of particular anthocyanins within the sample mixture. The anthocyanin pigments have normally been separated by paper or thin-layer chromatography (Fuleki and Francis 1968d), followed by photometric determination of the pigment. The use of HPLC for quantitative work has increased substantially in recent years due to its high resolving power of microgram amounts of anthocyanins without the need for extensive sample purification.

2.3.4 Anthocyanins of karkade

The major pigment in karkade calices has been identified by Sato *et al.* (1991) as delphinidin sambubioside. Its structure has been clearly identified as delphinidin 3-O-B-D-xylopyranosyl - (1→2)-B-O-glucopyranoside by NMR - spectroscopic method. On quantitative analysis by HPLC the same authors found the content of this major pigment was 1.1% in contrast to a value of 0.3% reported for the commercial roselle colour. Fuleki and Francis, (1973) identified cyanidin 3-sambubioside as the second major pigment, and two other minor pigments delphinidin 3-glucoside and cyanidin 3-glucoside. Milkowska-K (1995) reported that in seven samples of Hibiscus sabdariffa anthocyanin content was found to be between 0.37 and 0.66%. Hassan (1988) confirmed the presence of the two major

anthocyanins of karkade calices and found the ratio of delphinidin 3-sambubioside to cyanidin-3-sambubioside in four local varieties as follows:

light red variety	1:1.58
red (Gezira)	1:0.66
violet red (Rahad)	1:0.44
dark violet	1:0.49

Pouget *et al.* (1990) isolated two main anthocyanins, namely delphinidin-3-sambubioside or hibiscin, and cyanidin-3-sambubioside or gossypicyanin, from methanol extracts of calices of Hibiscus sabdariffa using column chromatography. These two pigments were identified using TLC, and their proportions were measured quantitatively by HPLC.

Chapter Three

MATERIALS AND METHODS

3.1 Materials

The materials used in this study consisted of six genotypes of roselle (Hibiscus sabdariffa) calices, seeds, leaves and stems of the six genotypes. These were developed at the Department of Agronomy, Faculty of Agriculture, University of Khartoum. The crops were of 1993, 1994 and 1995 seasons.

3.2 Methods

Each sample was ground using a mortar and pestle until a fine powder of the sample was obtained. Then different samples of the calices, seeds, stems, roots and leaves were subjected for the following chemical tests.

3.2.1 Moisture content (%)

Each powdered sample was mixed thoroughly. Then a subsample of 10g was heated in porcelain crucible in an oven at 105°C for 5hr, cooled in a dessicator and weighed. The procedure was repeated till a constant weight was obtained. The percentage moisture content was calculated according to the following formula :

Moisture content % =

$$\frac{\text{weight of moist sample} - \text{weight of dry sample} \times 100}{\text{weight of moist sample}}$$

3.2.2 Ash content (%)

One g of powdered sample was weighed in a dry porcelain crucible. The crucible was ignited in a furnace at 550°C for 6hr, until free from carbon. Then cooled in a dessiccator and weighed. The procedure was repeated to a constant weight of carbon free ash. The percentages of the total ash out of the dry sample were calculated according to the following rotation

Total ash content % =

$$\frac{\text{weight of ash} \times 100}{\text{weight of dry sample}}$$

3.2.3 Mineral composition

Eleven minerals namely sodium, potassium, calcium, manganese, zinc, magnesium, iron, phosphorus, cobalt, copper and lead were estimated according to the standard analytic methods using an atomic absorption Perkin Elmer model 3100.

3.2.4 Phosphorus content

Phosphorus content in each sample was determined according to the method described by Chapman and Pratt, (1961). Two ml of the mineral extract were pipetted into 50 ml volumetric flask. Ten ml of the ammonium molybdate-ammonium vanadate reagent (22.5g of $(\text{NH}_4)\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 400 ml distilled water + 1.23g ammonium vanadate in 300 ml boiling water + 250 ml conc HNO_3 , then diluted to 1 litre) were added. The contents of the flask were mixed and diluted to volume. The

density of the colour was read after 30 minutes at 470 nm using colorimeter (Lab System Analyser, filters, J Mitra-Bros pvt ltd). Phosphorus was determined from previously made standard curve.

Calculations:

$$P\% = \frac{\text{curve reading} \times \text{ash dilution} \times 100}{10^6 \times \text{oven dry weight of sample}}$$

3.2.5 Proteins(%)

Proteins content was determined using the standard Kjeldahl method (A.O.A.C 1984). 0.2g of each sample of karkade calices was digested in a small digestion flask using 0.8g of catalyst mixture (96% anhydrous sodium sulphate +4% cupric sulphate). 3.5ml of concentrated sulphuric acid were added to the flask and the contents were digested for 2 hrs till a colorless liquid was obtained. The digest was cooled, diluted and transferred to the distillation unit using minimum volume of distilled water and made alkaline with 20 ml of 40% aqueous sodium hydroxide solution. The ammonia was distilled into 10 ml of 2% boric acid solution for 5 - 10 minutes, then 3 drops of bromocresol-green methyl red mixed indicator were added. The apparatus was steamed out for 5 min, then the distillate was titrated with 0.02N HCl nitrogen and protein percentages were calculated according to the following equation

$$\text{Nitrogen \%} = \frac{V \times N_A \times 14.007 \times 100}{W}$$

where

V : the volume of titrant

NA : normality of the acid

W : weight of the sample (g)

$$\text{protein \%} = \text{nitrogen\%} \times 6.25$$

3.2.6 Crude oil

The crude oil of the samples of roselle calices was determined according to A.O.A.C (1984). The oil was determined for two grams of air-dried ground sample. Extraction of the oil from each sample was carried out by soxhlet using petroleum ether (b.p 50--70 °C) as solvent for six hours. After recovery of the solvent, the oil was dried in the oven at 105°C for two hours, then allowed to cool in a dessiccator, and finally weighed to constant weight.

$$\text{Crude oil (\%)} = \frac{(W_2 - W_1) \times 100}{S}$$

where

S : original weight of sample (g)

W₁ : weight of empty receiver (g)

W₂ : weight of receiver plus oil (g)

3.2.7 Crude fibre (%)

Crude fibre was determined according to A.O.A.C. (1984) using the fibertic system, 1010 heat extractor. About two grams of defatted sample were weighed. One hundred and fifty ml of the H_2SO_4 (conc. 7.3 ml/l) were added and then heated to boiling. The mixture was boiled for 30 minutes and then filtered. The residue was washed three times with hot water. Then 150 ml of pre-heated KOH (12.89/l) were added and then heated to boiling. Then the system was boiled for 30 minutes and then filtered. The residue was washed three times with hot water. It was dried under suction and then in an oven at 105°C overnight and then weighed (W_1). The residue was ashed in a muffle furnace at 550°C for three hours till a light grey ash was formed and then weighed to a weight (W).

Calculations:

$$\text{Crude fibre (\%)} = \frac{(W_1 - W_2) \times 100}{S}$$

where

S : original weight of sample

W_1 : weight of sample before ignition

W_2 : weight of sample after ignition

3.2.8 Total carbohydrates

The total carbohydrate was determined by the difference: 100 - (c.p% + c.f% + crude oil % + total ash% + moisture%). More over, calices were analysed to determine the following :

3.2.9 PH determination

PH of 10% solution were determined using a glass electrode Fisher pH meter. The pH meter was calibrated with a buffer solution at pH 4.0 (Ruck, 1963). Buffers were prepared from potassium hydrogen phthalate according to the A.O.A.C (1970) by dissolving 5 -- 128g in 500 distilled water.

3.2.10 Total acids

Total acids were determined by titrating 100 ml of 10% solution against 0.1N NaOH to pH 8.1. Total acids were expressed as citric according to the equation shown by Ruck (1963).

Total acidity % =

$$\frac{1/10 \times \text{eq. wt of citric acid} \times \text{normality of NaOH} \times \text{titre}}{\text{Wt. of sample}}$$

Equivalent wt of citric acid = 70.0

3.2.11 Ascorbic acid determination

Ascorbic acid was determined according to the method described by Evered, (1959) for highly coloured solution especially solutions containing red pigments.

Reagents :

N-bromo succinimide solution: A stock solution was prepared by dissolving 200 mg of the reagent in warm water, cooling and diluted to 100 ml, this solution is stable for a few days at 4°C . Just before use the stock solution was diluted (1+9) with distilled water. One ml of this solution is equivalent to 0.2 mg of ascorbic acid.

- **A cetic acid:** Glacial analytical reagent grade.

- **Potassium iodide solution:** 4% w/v - aqueous from iodate - free potassium iodide.

- **Diethyl ether:** (peroxide - free).

- **Standard ascorbic acid solution:**

0.2mg/ml freshly prepared by dissolving 50 mg of analytical grade ascorbic acid in 1% acetic acid and completing the volume to 250 ml.

Procedure :

The sample was diluted with 1% aqueous acetic acid until it contained from 0.4 - 1.0 mg of ascorbic acid/5 ml of solution. Five ml of the diluted sample were transeferred to a 6 inch × 1 inch test tube, 1 ml

of glacial acetic acid was added, mixed and 5 ml of potassium iodide solution were added and mixed again. Three ml of diethyl ether were added and the mixture was titrated with N-bromo succinimide solution (added from 10 ml semimicro burette).

The test tube was shaken vigorously after each addition of titrant, and the organic layer was allowed to separate. The end point was indicated by the first appearance of the brown colour of the liberated iodine in the upper ether layer, comparison against an untitrated mixture permitted easy establishment of the end point was indicated by the first appearance of the brown colour of the liberated iodine in the upper ether layer, comparison against an untitrated mixture permitted easy establishment of the end point. A blank was titrated in which 5 ml of potassium iodide plus a volume of water equivalent to the titre replaced the diluted sample. The volume of N-bromo succinimide solution necessary to impart a definite brown colour to the ether layer was determined. The N-bromo succinimide was standardised by titrating against 5 ml aliquots of standard ascorbic acid solution. The equivalent of ascorbic acid to 1 ml of N-bromo succinimide was obtained for each sample as follows:

$$\frac{\text{titre volume} \times \text{eq. N - bromosuccinimide} \times 100}{\text{wt of sample in g}}$$

3.2.12 Determination of the total acidity of karkade samples as hibiscic acid :

The total acidity of the sample was calculated according to the procedure described by Schilcher (1976). 50 ml of distilled water were added to 1g of the test sample and the mixture heated in water bath for 5 minutes under continuous shaking. The mixture was then filtered while hot through a fluted filter paper into a 50 ml volumetric flask. The residue on the filter paper was washed with hot distilled water to a volume less than 50 ml extract volume. After cooling to room temperature the volume was made up to 50 ml with room temperature distilled water. Volume of 10 ml of the test solution was pipetted into a tube and titrated with 0.1N NaOH to pH 7. The average volume of alkali from triplicate titration was recorded. Taking into consideration that 1ml of 0.1N NaOH is equivalent to 9.505 mg of hibiscic acid, the total acidity was calculated according to the following formula :

$$\text{Total acidity} = \frac{v \times e \times 5 \times 100}{g}$$

where

v : average volume of 0.1N Na OH solution used in titration.

e : milliequivalent weight of hibiscic acid.

5 : factor.

g : weight of karkade calices.

3.2.13 Estimation of sugars

Reducing and non reducing sugar were determined by using general method described in the A.O.A.C (1970) by Fehling methods.

3.2.14 Tannin content

Estimation of tannin for each sample was carried out using modified vaniline-HCl in methanol method as described by Price and Butler (1978).

Reagent :

Prepared daily by mixing equal volumes of 1% vaniline in methanol (w/v) and 8% con. HCl in methanol (v/v). These must be mixed just before use and should not be use after trace of colour appears.

Procedure :

Using 0.2g of the ground sample was put into a 100 ml conical flask. Ten ml of 1% con HCl in methanol (v/v) were added, shaken for 20 minutes using mechanical shaker and centrifuged at 2500 r.p.m for 5 minutes. One ml of the supernatant was pipetted into a test tube and 5 ml of vanillin-HCl reagent were added. The optical density by using a colorimeter (Lab System Analyser, Filters, J Mitor and Bros pvt. Ltd) at 500 nm after 20 minutes incubation at 30 °C for zero setting of the colorimeter. 1 ml of blank (1% methanol) was mixed with 5 ml 4% con. HCl in methanol (v/v) and 5 ml vanillin-HCl reagent in a test tube. The blank mixture was incubated, also at 30 °C for 20 minutes.

Calculation :

Tannin concentration was expressed as catechin equivalent (C.E) as follows:

$$\text{C.E. \%} = \frac{C \times 10 \times 100}{200}$$

where

C : concentration corresponding to the optical density.

10 : volume of extract in (ml).

200 : sample weight in (mg).

3.2.15 Oxalic acid determination

Oxalic acid was determined according to the method described by A.O.A.C (1980).

Reagents :

– Indicator paper (PH 3.5 -- 5.5).

– Potassium permanganate solution. Approx. 0.01 N was prepared fresh before use.

– Acetate buffer solution PH 4.5.

2.5 g anhydrous calcium chloride was dissolved in 50 ml acetic acid (1+1) and was added to solution of 33g sodium acetate trihydrate diluted to 50 ml.

–Wash liquid 12.5 g acetic acid was diluted to 250 ml with distilled water. Calcium oxalate powder was added, shaken and was let to stand. Addition and shaking were repeated till saturation. The wash liquid was stored in refrigerator. The amount needed was filtered just before use and was kept cold during filtration and use.

– Tungsto phosphoric acid reagent:

2.5g sodium tungstate dihydrate was dissolved in mixture of 4 ml orthophosphoric acid and 50 ml distilled water and was diluted to 100 ml with distilled water.

Preparation of sample :

Fifty grams of karkade were weighed and transferred to high speed blender, 100 ml water was added, the contents were homogenized for 15 minutes and, then cooled to room temperature.

Thirty five grams slurry were accurately weighed into 800 ml beaker, distilled water was added to bring total weight to 300 g, then 55 ml 6N HCl was added. Two drops caprylic alcohol were added, and the mixture was boiled for 15 minutes. After cooling, it was transferred quantitatively to 500 ml volumetric flask and it was diluted to volume with water. The contents were mixed and filtered through fast quantitative paper. The first 100 ml filtrate were discarded.

Precipitation of oxalic acid:

Twenty five of the filtrate were pipetted into 50 ml erlenmeyer flask, 5 ml tungstophosphoric acid reagent were added, mixed and left to

stand for 5 hours, then filtered through whatman no. (30) quantitative filter paper. Twenty ml filtrate were pipetted into 50 ml conical centrifuge tube and ammonium hydroxide was added drop wise to pH 4 - 4.5. Five ml buffer solution was added, and stirred with a glass rod. The rod was rinsed into centrifuge tube with small stream of water and was left to stand overnight. The contents were centrifuged for 15 minutes at 1700 rpm to compact precipitate. The supernatant was decanted carefully. The precipitate was washed in fine suspension with fine jet stream of 20 ml filtered cold wash liquid, centrifugation and decantation were repeated five ml H₂SO₄ (1+9) was added to the precipitate.

Determination by permanganate titration:

Sample and blank (5 ml H₂SO₄) (1+9) in 50 ml centrifuge tube, prepared solution was heated in a boiling water bath. One ml was then titrated against 0.01 N potassium permanganate until first

pink persists ≥ 30 sec

mg oxalic acid /100 g product

$$= \text{ml } 0.01\text{N KMnO}_4 \times \frac{(1350 \times \text{netwt} + 100\text{g})}{(\text{wt slurry taken} \times \text{netwt})}$$

where

1350 = 0.45 (mg anhyd. Oxalic acid equivalent to 1ml 0.01 N KMnO₄) \times (30/20 \times 500/25) (diluted factor) \times 100 (to convert to 100 g product).

3.2.16 Anthocyanin extraction

The plant sample was macerated in methanol containing a small amount of hydrochloric acid (up to 1%) (Markakis, 1982). This was followed by filtration and either precipitation of the pigment or removal of the solvent under vacuum at a temperature not exceeding 50°C, so as to minimize pigment degradation.

3.2.17 Determination of aglycone (anthocyanidins)

The aglycone and sugar portion of the pigment was identified by acid hydrolysis followed by thin layer chromatography. About three mls of extract of pigment can be transferred to a test tube, two ml of 2N HCl were added and the solution placed in a boiling water bath for 30 min. After cooling, the aglycone was extracted by the addition of 1 ml of diethyl ether. The upper diethyl ether layer was removed, dried on a watch glass, redissolved in methanol, and spotted on cellulose thin layer.

3.2.18 Absorption maximum of the pigments

For the purpose of determining the absorption maxima of the two bands, a small amount of the pigments was dissolved in 1% HCl in methanol. The pigment solution was preparatively streaked on two prewashed with BAW 4:1:5 TLC, and developed with which solvent for 7 hrs, the red and violet bands were eluted separately with 1% HCl in methanol and their UV-visible spectra of the two bands were run on a Perkin-Elmer Lambda 2 UV-vis spectrometer. The violet band gave maximum absorption at 538.3 -- 540 and the red band gave absorption at 530 -- 531.6 nm.

3.2.19 The determination of sugars

The aqueous solution remaining after removal of the diethyl ether containing the aglycone contained sugar. The HCl remaining in the aqueous layer interferes with the sugar determination was removed, using small portions of 10% di-n-octyl-methyl-amine in chloroform were used to remove the acid. The mixture usually has a purple tint colour, due to trace of aglycone, when all of the acid has been removed. Then the sugar solution was dried. Seven drops of water were added to the dried residue to dissolve all the sugar. The sample and reference sugars, arabinose, xylose, galactose, glucose and rhamnose were spotted on whatman N 0.1 papers and developed in BBPW and phenol. The developed papers were then dried thoroughly, since both phenol and pyridine have disagreeable odors. The dry chromatograms were then dipped in aniline--hydrogen phthalate sugar reagent (Partridge, 1945), dried and heated at 105 °C for 2 -- 3 min to locate the sugars which showed as reddish or brownish spots under visible light. They were even more visible under UV light. Usually were one, two or three sugars in 1:1 or 1:2 or 1:1 ratio. The ratio of intensity was estimated visually.

3.2.20 Optical density

A solution containing 1gm of karkade calices per 100 ml distilled water was used. The optical density which was used to indicate the colour intensity of the extract was measured at half hourly intervals for four hours. An EEL colorimeter adjusted at wave length 535 mu hand. The solution was kept continuously stirred by the use of amagnetic stirrer. Extraction was allowed to take place at room temperature (27 °C).

3.2.21 Insoluble solids

One gram of karkade calices per 100 ml distilled water allowed to extract overnight at room temperature (27 °C). The remainder of the calices after extraction were then dried and weighed. The difference in weight was expressed as a percentage of the original sample.

3.2.22 Total anthocyanin content

The total anthocyanin content was estimated by the method. Described by Du and Francis (1973). The total anthocyanin content of roselle was expressed as mg delphinidin-3-glucoside (Dp-3-G) per 100 g dry calices. The molar extinction coefficient of Dp-3-G established by Asen *et al.* (1959) was used in recalculating the $E_1^{1\%}$ cm at 543 nm. Triplicate determinations were made to give an average figure. Extracting solvent was 1% HCl/methanol instead of 0.1N HCl in ethanol in the original method. Absorbance was determined in spectronic 20 colourometer.

Procedure :

Five grams of the sample were macerated with 100 ml of solvent in a blender at full speed, and then transferred to 400 ml beaker, washing with 50 ml aliquots. The beaker was covered with parafilm and stored overnight at 4°C. The sample was filtered in a no. 1 whatman filter paper through abuchner funnel. The beaker as well as the residue in the filter paper were washed repeatedly until approximately 450 ml of the extract were collected, then transferred to 500 ml volumetric flask and completed to volume. 25ml aliquots were filtered through a fine porosity sintered glass filter or apolyvinyl chloride millipore filter.

These aliquots were diluted 10 times with the extracting solvent and then absorbance was determined at 543 nm.

Calculations :

The total optical density (T.O.D.) for 100 ml extract was calculated using the following equation :

$$(1) \quad T. O. D = O.D \times D. V \times V.F$$

where

O. D. : The absorbancy reading of the diluted sample (1cm cell).

D.V. : Diluted volume in ml of the diluted extract prepared for O. D measurement.

V.F. : Volume factor which corrects for the difference in size between 100 ml, the original volume (O.V) for which the calculation was made and that of the sample volume (S.V) or the volume of extract used for absorbancy measurement.

$$V.F. = \frac{O.V}{S.V} = \frac{100}{S.V}$$

since the T.O.D. for 100g is desired, the T.O.D. calculated for 100 ml extract should be brought up to this level using the following equation.

$$(2) \quad T.O.D/100g = T. O. D \text{ for } 100\text{ml extract} \times TEV/W$$

where

TEV : The total volume of the extract in ml obtained from the sample used for extraction.

W : The weight of the material in g used for extraction. The **T.O.D** values give the absorbance as if all the anthocyanin (ACY) present in the sample was concentrated in 1 ml of solution.

Since it is more convenient to calculate the ACY in mgs while **E** values were given in percentage basis (10mgs/1ml) solution, the **E/10** values are used in the calculation of total ACY. The ACY in mg/100ml extract is calculated from the calculated **T.O.D** value using the available $E_1^{1\%}$ cm value for D-3-G (2.9×10^4 which is 559).

$$(3) \text{ T ACY in mgs/100ml} = \frac{\text{T.O.A.}}{559/10} = \frac{\text{T.O.A.}}{55.9}$$

The above formulae reduce to

$$(4) \text{ T ACY in mgs/100g} = \text{OD} \times \text{DV} \times \frac{100}{\text{SV}} \times \frac{\text{TEV/W}}{55.9} \times \frac{1}{55.9}$$

3.3 Statistical analysis

Data obtained for the three seasons were analyzed by using arandomized complete block design (RCBD) according to the method of Gomez and Gomez (1981).

Chapter Four

RESULTS AND DISCUSSION

4. 1 Proximate analysis

4. 1. 1 Moisture content

Analysis of variance revealed significant differences among the six evaluated karkade lines in the moisture content of calices, seeds, leaves, stems and roots. As shown in Tables 1, 2 and 3 show the moisture contents for the six karkade lines investigated. The moisture content for season 93/94 ranged from 5.76% to 6.95% and a mean value of 6.19% and for season 94/95 ranged from 10.30% to 13.40% and a mean value of 12.07%. The moisture content for season 95/96 ranged from 5.40% to 9.93% and a mean value of 8.42%, which are in agreement with moisture values reported by Reaubourg and Monceax (1940) and Ibrahim *et al.* (1971). In season 93/94, line 18 was significantly ($p \leq 0.01$) lower in moisture content compared to other lines and that of line CV₁ was significantly ($p \leq 0.01$) higher than the other lines. In season 94/95 line G was significantly lower in moisture content, while line 24 was highly significant compared to other lines. In season 95/96, the moisture content of calices for the lines G, 15 and CV₂ were significantly ($P \leq 0.01$) higher compared to the remaining lines.

It is clear from Table 4 that the mean value of the moisture content for the karkade seeds is 2.61% with the minimum value of 1.56% and maximum value of 4.48%, which is in agreement with the data reported by Al Wandawi *et al.* (1984) and El Nour (1991).

Table 5 shows that the mean moisture content for the karkade leaf is 5.33%, with minimum value of 4.80% and maximum value of 5.95%, which is lower than the data reported by Mclean (1973).

Table 6 shows that the moisture content of karkade roots ranged from 4.61% to 5.05% and a mean value of 4.77%.

As shown in Table 7 the mean moisture content of karkade stems was 4.33% to 5.07%. Significant differences ($P \leq 0.01$) among the lines with regard to the moisture content of the seeds, leaves, stems and roots.

4.1.2 Ash content

Tables 1, 2 and 3 show the ash content of different lines of karkade calices for season 93/94, 94/95 and 95/96. The ash content of karkade calices ranged from 10.03% to 11.46%, 7.13% to 8.90% and 10.13% to 14.60 for seasons 93/94, 94/95 and 95/96 respectively. The results of season 94/95 are in agreement with the data reported by Ibrahim (1971), but the results of season 93/94 and 95/96 were higher. In season 93/94, line 15 was significantly ($P \leq 0.01$) higher in ash content compared to other lines.

In season 94/95 line 15 was significantly ($P \leq 0.01$) higher in ash content than all karkade lines. The lines CV₁, G and 24 were not significantly ($P > 0.01$) different in ash content. In season 95/96 the ash content of karkade calices of line CV₂ was significantly ($P \leq 0.01$) lower compared to the other lines, while line 18 was significantly ($P \leq 0.01$) higher in ash content than all karkade lines.

As shown in Table 4 the mean value of ash content for seeds was 5.89% with ranged of 5.44 % to 7.05% which is in agreement with the

data reported by El Nour (1991), but lower compared to values reported by El Adawy and Khalil (1994). As observed from Table 5 the mean ash content of karkade leaf was 10.14%. The minimum value was 8.45% and the maximum value was 12.45%, which is lower than the data reported by Mclean (1973). However, significant differences were observed in the ash content of seeds and leaves of the different karkade lines. The mean value of ash content for karkade roots was 4.27%, with minimum value of 3.85% and maximum value of 4.48% (Table 6).

As shown in Table 7, the mean ash content for karkade stems was 4.80% with a range of 3.76% to 5.95%. However, the stem ash content of lines 24, CV₂ and G were significantly ($P \leq 0.05$) lower compared to line CV₁.

4.1.3 Protein content

Tables 1, 2 and 3 show the protein content calices of different lines of karkade for seasons 93/94, 94/95 and 95/96. The protein content of karkade calices in the study ranged from 8.70% to 13.57%, 4.34% to 7.17% and 4.96% to 7.38% with the mean value of 9.76%, 5.53% and 6.13% for seasons 93/94, 94/95 and 95/96 respectively, which is in agreement with the protein values reported by Ibrahim *et al.* (1971).

In season 93/94 calices protein content of line 18 was significantly ($P \leq 0.01$) lower compared to other lines, whereas line G was significantly ($P \leq 0.01$) higher compared to other lines. In season 94/95, line 24 protein content of calices was found to be significantly ($P \leq 0.05$) higher than all other lines. while line CV₁ was significantly ($P \leq 0.05$) lower in calices protein content than all other karkade lines. In season 95/96 the protein content of karkade calices for lines CV₂, 24 and 18 were similar,

whereas line CV₁ was significantly ($P \leq 0.01$) lower and line 15 was significantly ($P \leq 0.01$) higher in protein content.

As shown in Table 4 the protein content of karkade seeds ranged from 26.87% to 30.20% and a mean value of 27.77%, which is good in agreement with the data obtained by Karamalla and Ali (1974) and the data reported by El Adawy and Khalil (1994).

Table 5 shows that the protein content of karkade leaves ranged from 9.80% to 14.20% and a mean value of 12.97%, which is in agreement with the data reported by Mclean (1973). The seed protein content of karkade line 24 was significantly ($P \leq 0.05$) higher compared to other lines. While line G was significantly ($P \leq 0.05$) lower than all other karkade lines. The leaf protein content of line 24 was significantly ($P \leq 0.01$) higher compared to the other lines, and line G was significantly ($P \leq 0.01$) lower than all other lines.

Table 6 gives the mean value of protein content of karkade roots (2.1%) with a minimum value of 1.99% and a maximum value of 2.21%. From Table 6 it can be seen that there was no significant difference among lines in protein content of karkade roots.

As shown in Table 7, the protein content of karkade stem ranged from 3.76% to 5.96% and a mean value of 4.64%. However, line CV₁ was significantly ($P \leq 0.01$) higher in stem protein content compared to other lines, while line 24 was significantly ($P \leq 0.01$) lower.

4.1.4 Oil content

Tables 1, 2 and 3 show the oil content of karkade calices for season 93/94, 94/95 and 95/96. The oil content of karkade calices ranged from

0.77% to 1.15%, 0.90% to 0.99% and 0.85% to 0.93% for the three seasons respectively. The mean values were 0.99%, 0.95% and 0.90% for seasons 93/94, 94/95, and 95/96 respectively. These means were higher than the data reported by Mclean (1973). In season 93/94 the calices oil content of line G was significantly ($P \leq 0.01$) lower to the other lines, while line CV₂ was significantly ($P \leq 0.01$) higher in oil content than all other lines. In season 94/95 the oil content of karkade calices of lines CV₁, G and 24 were significantly ($P \leq 0.01$) higher compared to the lines CV₂, 18 and 15. In season 95/96 the calices oil content of line CV₁, 24 and 18 were significantly ($P \leq 0.01$) higher in oil content compared to the remaining lines.

As shown in Table 4 the oil content of karkade seeds ranged from 19.46% to 24.04% and a mean value of 21.65%, which is in agreement with Karamalla and Ali, (1974); Mohiuddin and Zaidi, (1975); and Ahmed and Hudson, (1994). Line G seed oil was significantly ($P \leq 0.01$) lower compared to the other lines, while line 18 was significantly ($P \leq 0.01$) higher in seed oil content than the other lines.

As shown in Table 5 the oil content of karkade leaves ranged from 5.35% to 7.31% and a mean value of 6.49%, which is in agreement with the data reported by Mclean (1973). Line 24 was significantly ($P \leq 0.01$) lower in leaf oil content compared to other lines, and line G was significantly ($P \leq 0.01$) higher in leaf oil content than all other karkade lines.

Table 6 indicates that the oil content of karkade roots ranged from 0.65% -- 0.90% and a mean value of 0.79%. The root oil content of lines

CV₂ and 24 were similar, whereas line G was significantly ($P \leq 0.01$) lower. However, line CV₁ was markedly higher in the root oil content.

Table 7 shows that the oil content of karkade stems ranged from 3.95% to 4.32% and a mean value of 3.05%. However, stem oil content of line CV₂ was significantly ($P \leq 0.01$) lower compared to lines 18, 15 and G.

4. 1. 5 Crude fibre content

Data on crude fibre content of karkade calices, of different lines grown in season 93/94, 94/95 and 95/96 are presented in Tables 1, 2 and 3 respectively. The crude fibre of karkade calices ranged from 9.57% to 11.83%, 10.40% to 12.37% and 11.32% to 13.87% with the mean value 10.74%, 11.73% and 12.17% for seasons 93/94, 94/95 and 95/96 respectively. These results are in agreement with the data reported by Ismail (1985), but higher than the values reported by Mclean (1973).

As shown in Table 1, line CV₁ was significantly ($P \leq 0.01$) lower in calices crude fibre content compared to the other lines, while line 18 was significantly ($P \leq 0.01$) higher. As shown in Table 2, the crude fibre of the calices from the lines CV₁, CV₂, G and 24 were significantly ($P \leq 0.01$) higher compared to lines 18 and 15. In season 95/96 the calices crude fibre content of line 18 was significantly ($P \leq 0.01$) lower, but line 15 was significantly ($P < 0.01$) higher in calices crude fibre content. While the lines CV₂, G and 24 were similar in their calices crude fibre content.

Table 4 shows that crude fibre content of karkade seeds ranged from 17.39% to 19.19% and a mean value of 18.69%. The mean value is similar to the value reported by Al Wandawi *et al.* (1984), but is

slightly higher than that obtained by Mclean (1973). Line CV₂ was significantly ($P \leq 0.01$) lower in seed crude fibre compared to the other lines, while line G was significantly ($P \leq 0.01$) higher.

Table 5 shows that the crude fibre of karkade leaf ranged from 9.72% to 12.9% and a mean value of 10.70%, and the mean value in the present study is lower than the data reported by Mclean, (1973). The crude fibre of karkade leaves of line CV₂ was significantly ($P \leq 0.01$) lower compared to other lines and line G was significantly ($P \leq 0.01$) higher in crude fibre content than all lines.

Table 6 shows that the crude fibre content of karkade root ranged from 43.95% to 48.39% and a mean value of 46.62%, line CV₁ was significantly ($P \leq 0.01$) lower in root crude fibre compared to the other lines. While line CV₂ was significantly ($P \leq 0.01$) higher in root crude fibre content than all other lines.

Table 7 shows that the crude fibre of karkade stem ranged from 31.95% to 40.32% and a mean value of 36.05%. The stem crude fibre content of line 24 was significantly ($P \leq 0.01$) higher compared to other lines while line CV₁ was significantly ($P \leq 0.01$) lower in stem crude fibre content than all other lines.

4. 1. 6 Carbohydrate content

Tables 1, 2 and 3 show the carbohydrate content of the different lines of karkade calices for seasons 93/94, 94/95 and 95/96. The carbohydrate content of karkade calices ranged from 57.74% to 63.45%, 58.45% to 64.93% and from 54.87% to 65.83% with mean of 61.64%, 61.76% and 60.43% for seasons 93/94, 94/95 and 95/96

respectively. These results are in agreement with the data reported by Mclean (1973).

Table 1 shows that the carbohydrate content in season 93/94 of line G was significantly ($P \leq 0.05$) lower compared to the other lines. Whereas line 18 was significantly ($P \leq 0.05$) higher in carbohydrate content than all other karkade lines. From Table 2, it is clear that line 24 was significantly ($P \leq 0.01$) lower in carbohydrate content compared to other karkade lines, while line 18 was significantly ($P \leq 0.01$) higher in carbohydrate content than all other karkade lines. Table 3 shows that line 15 was significantly ($P \leq 0.05$) lowest in carbohydrate content of calices compared to other lines and line CV₁ was significantly ($P \leq 0.05$) highest in carbohydrate content of calices than all other lines for season 95/96.

As shows in Table 4, the carbohydrate content of karkade seeds ranged from 18.56% to 26.63% and a mean value of 23.88%, which is in agreement

with the data reported by Mclean (1973). Line G was significantly ($P \leq 0.01$) higher in carbohydrate content of karkade seed compared to the other lines, while line 24 was significantly ($P \leq 0.01$) lower in carbohydrate content of seeds than all other karkade lines.

Table 5 shows that the carbohydrate content of karkade leaves ranged from 52.38% to 56.62% and a mean value of 54.42%, which is in agreement with the data reported by Mclean (1973).

The leaf carbohydrate content of karkade of the lines G and 24 were significantly higher than that of the remaining lines.

From Table 6, it can be seen that the carbohydrate content of karkade root ranged from 39.79% to 43.67% and a mean value of 41.45%. The carbohydrate content of karkade roots of line CV₁ was significantly ($P \leq 0.01$) higher in carbohydrate content compared to other lines.

From Table 7, it can be seen that the carbohydrate content of karkade stems ranged from 47.11% to 52.77% and a mean value of 50.07%. Lines 24 and 18 had the same significantly high level of carbohydrate, while line CV₁ was significantly lower. The carbohydrate content varied considerably among the different parts of the karkade plant, ranging from 18.56% in the seed to 65.83% in the calices.

Table (1)

Analytical data of calices of some Hibiscus sabdariffa lines season 93/94.

Line	Moisture (%)	Ash (%)	Protein (%)	Crude fibre (%)	Oil extract (%)	Carbohydrate (%)
CV ₁	6.95 a	10.36 c	10.87 b	9.57f	0.97 e	61.28 d
CV ₂	6.02d	10.58 c	9.20 c	10.73 c	1.15 a	61.97 c
G	6.39 b	11.46 a	13.57 a	10.07 e	0.77f	57.74 e
24	6.19 c	10.77 b	8.77 cd	10.70 d	1.01d	62.57b
18	5.76 f	10.48 d	7.45 e	11.83 a	1.03 c	63.45 a
15	5.86 e	10.03 f	8.70 d	11.56 b	1.05 b	62.83 b
SE	± 0.02	± 0.02	± 0.2	± 0.01	± 0.01	± 0.24

* Values are mean (± SE).

* Means with the same letters are not significantly different.

Table (2)

Analytical data of calices of some Hibiscus sabdariffa lines season 94/95

Line	Moisture (%)	Ash (%)	Protein (%)	Oil extract (%)	Crude fibre (%)	Carbohydrate (%)
CV ₁	12.09 d	7.73 c	4.34 e	0.99 a	12.05 a	62.79 b
CV ₂	12.26 c	8.65 b	4.55 d	0.93 b	12.37 a	61.31 c
G	11.85 e	7.59 c	5.95 b	0.97 a	11.99 a	61.65 c
24	13.40 a	7.67 c	7.17 a	0.98 a	12.31 a	58.45 d
18	10.30 f	7.31 d	5.30 c	0.90 b	11.27 b	64.93 a
15	12.50 b	8.90 a	5.87 b	0.93 b	10.40 c	61.42 c
SE	± 0.06	± 0.1	± 0.07	± 0.02	± 0.22	± 0.4

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

Table (3)

Analytical data of calices of some Hibiscus sabdariffa lines season 95/96.

Line	Moisture (%)	Ash (%)	Protein (%)	Oil extract (%)	Fibre (%)	Carbohydrate (%)
CV ₁	5.40 d	12.73 c	4.96 d	0.91 ab	11.32 c	65.83 a
CV ₂	9.03 ab	10.13 f	6.53 b	0.85 c	12.24 b	61.21 b
G	9.93 a	11.20 e	5.27 c	0.93 a	12.19 b	60.47 bc
24	7.70 c	11.67 d	6.50 b	0.92 ab	12.37 b	61.46 b
18	8.60 bc	14.60 a	6.12 b	0.92 ab	11.05 d	58.71 c
15	9.83 a	14.20 b	7.38 a	0.90 b	13.87 a	54.87 d
SE	± 0.5	± 0.14	± 0.23	± 0.01	± 0.1	± 0.86

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

Table (4)

Analytical data of seeds of some Hibiscus sabdariffa lines.

Line	Moisture (%)	Ash (%)	Protein (%)	Crude fibre (%)	Oil extract (%)	Carbohydrate (%)
CV ₁	4.48 a	5.59 c	27.77 b	18.58 e	20.22 e	23.34 c
CV ₂	1.75 d	5.49 e	27.93 b	17.39 f	21.62 d	25.88 ab
G	1.57 e	7.05 a	25.83 c	19.30 a	19.46 f	26.63 a
24	4.39 b	5.54 d	30.20 a	19.19 b	22.11 c	18.56 d
18	1.88 c	6.23 b	26.87 bc	19.07 c	24.04 a	24.90 b
15	1.56 e	5.44 f	28.00 b	18.61 d	22.43 b	23.96 bc
SE	± 0.01	± 0.003	± 0.65	± 0.01	± 0.01	± 0.68

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

Table (5)

Analytical data of leaves of some Hibiscus sabdariffa lines.

Line	Moisture (%)	Ash (%)	Protein (%)	Crude fibre (%)	Oil extract (%)	Carbohydrate (%)
CV ₁	5.95 a	10.34 b	13.67 ab	10.22 c	7.03 c	53.09 c
CV ₂	5.76 a	10.32 c	13.57 ab	9.72 f	7.14 b	53.50 bc
G	4.82 b	8.45 f	9.80 c	12.99 a	7.31 a	56.62 a
24	4.80 b	9.24 e	14.20 a	10.03 d	5.35 f	56.38 a
18	5.60 a	10.05 d	13.70 ab	9.92 e	6.18 d	54.55 b
15	5.05 b	12.45 a	12.90 b	11.30 b	5.98 e	52.38 c
S E	± 0.22	± 0.01	± 0.52	± 0.01	± 0.01	± 0.5

* Values are means (\pm SE).

* Means with the same letters are not significantly differently.

Table (6)

Analytical data of roots of some Hibiscus sabdariffa lines.

Line	Moisture (%)	Ash (%)	Protein (%)	Crude fibre (%)	Oil extract (%)	Carbohydrate (%)
CV ₁	5.05 a	4.39 a	1.99 b	43.95 d	0.95 a	43.67 a
CV ₂	4.61 d	4.37 a	2.05 ab	48.39 a	0.78 b	39.79 d
G	4.75 b	3.85 b	2.14 ab	48.13 b	0.65 c	40.47 c
24	4.65 c	4.48 a	2.21 a	46.20 c	0.79 b	41.88 b
SE	± 0.004	± 0.15	± 0.08	± 0.05	± 0.01	± 0.08

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

Table (7)

Analytical data of stem of some Hibiscus sabdariffa lines.

Line	Moisture (%)	Ash (%)	Protein (%)	Crude fibre (%)	Oil extract (%)	Carbohydrate (%)
CV ₁	4.97 c	5.95 a	5.96 a	31.95 f	3.49 bc	52.77 a
CV ₂	4.33 f	3.93 bcd	3.93 e	40.09 b	3.17 c	48.03 c
G	5.03 b	4.50 bcd	4.50 d	36.11 c	3.85 ab	49.85 b
24	4.70 e	3.76 bcd	3.76 f	40.32 a	3.45 bc	47.11 d
18	5.07 a	5.14 b	5.14 b	32.20 e	4.10 a	52.62 a
15	4.81 d	5.52 bc	4.52 c	35.63 d	4.03 a	50.05 b
SE	± 0.01	± 0.01	± 0.01	± 0.03	± 0.22	± 0.28

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

4. 2 Physicochemical data on calices from some karkade lines

4. 2. 1 Free reducing sugar content

Tables 8, 9 and 10 show reducing sugar content of different lines of karkade calices for seasons 93/94, 94/95 and 95/96. Reducing sugar content of karkade calices in season 93/94 ranged from 1.25% to 1.40% and a mean value of 1.29%. Reducing sugar of karkade calices in season 94/95 ranged from 1.23% to 1.37% and a mean value of 1.28%, whereas in season 95/96 reducing sugar content of karkade calices were in the range 1.24% to 1.34% and a mean value of 1.27%. These results are lower than the data reported by Mclean (1973) and Brand (1942). In seasons 93/94, 94/95 and 95/96, the lines CV₂, 24, 18 and 15 contained the lowest level and line CV₁ the highest reducing sugar level.

4. 2. 2 Non reducing sugars

Tables 8, 9 and 10 show non reducing sugar content of the different lines of karkade calices in seasons 93/94, 94/95 and 95/96. Non reducing sugar content of karkade calices of season 93/94 ranged from 0.72% to 0.97% and a mean value of 0.87%. In season 94/95 ranged from 0.69% to 0.94% and a mean value of 0.86%) and in season 95/96 it was in the range of 0.70% to 0.93% and a mean value of 0.85%). These results are lower than the data reported by Ismail (1980).

There were a highly significant differences ($P \leq 0.01$) in non reducing sugar level between line G and the other lines in the three seasons.

4.2.3 PH value

As shown in Tables 8, 9 and 10 the pH of the different lines of karkade calices (2% solution) for seasons 93/94, 94/95 and 95/96 ranged from 2.95 to 3.42, 2.4 to 3.05 and 2.7 to 3.2 with the mean values of 3.17, 2.7 and 2.9 respectively. These results are in close agreement with those reported by Ibrahim *et al.* (1971). In season 93/94 there was no significant difference in pH value of karkade calices among the lines. However, significant differences exist among lines in season 94/95 and 95/96 with lines 24, CV₁ and CV₂ giving the lowest pH (highly acidic).

4.2.4 Anthocyanin content

Total anthocyanin content of karkade calices of different lines grown in seasons 94/95 and 95/96 are presented in Table 9 and 10 respectively. Anthocyanin content of pigmented lines ranged from 0.89 to 1.35% and 0.99 to 1.6% with the mean values 0.97% and 1.06% in seasons 94/95 and 95/96 respectively. Whereas in the white line the value was 0.001. The highest mean anthocyanin content was observed in line 24 in both seasons. These results are in agreement with the data reported by Du and Francis (1973), to higher than the data reported by Milkowaka and Strzelecka (1995).

There were significant differences ($P \leq 0.01$) in anthocyanin content of karkade calices in both seasons 94/95 and 95/96.

4.2.5 Colour intensity

Tables 8, 9 and 10 give colour intensity of karkade calices (1% solution; λ max 535) for seasons 93/94, 94/95 and 95/96. In the three

seasons line 24 had the highest value, which was significantly different ($P \leq 0.01$) in colour intensity from the other lines. While the minimum value was observed for line G. The lines CV₁ and CV₂ had the same colour intensity and they were significantly different from the remaining lines. The mean value of colour intensity (1% solution) of line 24 over the two seasons was 1.15%. This amount is equivalent to 342% of that reported for strawberry (Decareau, *et al.* 1956). Thus regarding the use of roselle calices as a colouring material, line 24 will be of great importance, whereas line G will be the least choice.

4.2.6 Insoluble solids (%)

Tables 8, 9 and 10 show insoluble solids of karkade calices for season 93/94, 94/95 and 95/96. Insoluble solids of karkade calices had the range from 32.30 to 40.05 %, 32.41 to 39.60% and 34.17 to 39.82% with the mean values of 37.74%, 36.36% and 37.33% for seasons 93/94, 94/95 and 95/96 respectively. Line G had high insoluble solids, while line 24 showed the least insoluble solids, which again puts line 24 at an advantage with respect to the other lines. In season 93/94 line E was significantly different in insoluble solids from the remaining lines which had similar high insoluble solids.

4. 2. 7 Tannin content

Tables 8, 9 and 10 show that tannin content of karkade calices for season 93/94, 94/95 and 95/96. In season 93/94 tannin content of karkade calices ranged from 0.39 to 0.58% for the pigmented lines (red). Albino line gave 0.004%. In season 94/95 the tannin content ranged from 0.38 to 0.57% for the pigmented line (red), while albino line G gave 0.004%.

In season 95/96 tannin content ranged from 0.38 to 0.56% for the pigmented line, while albino recorded 0.003%. The tannin content decreased significantly ($P \leq 0.05$) in the calices of line G.

Table (8): Physicochemical data of calices of some Hibiscus sabdariffa lines season 93/94

Line	Reducing sugars %	Non-reducing sugars %	Tannin %	pH value	Colour intensity %	Insoluble solid %
CV ₁	1.40 a	0.91 a	0.49 ab	3.13 a	0.76 ab	38.48 a
CV ₂	1.25 b	0.82 ab	0.39 ab	3.10 a	0.66 ab	38.98 a
G	1.35 ab	0.72 b	0.004 c	2.95 a	0.04 b	39.05 a
D	1.27 b	0.85 a	0.39 ab	3.33 a	0.58 ab	37.60 ab
B	1.25 b	0.95 a	0.39 ab	3.42 a	0.57 ab	40.05 a
E	1.25 b	0.96 a	0.58 a	3.10 a	1.98 a	32.3 b
SE	± 0.04	± 0.06	± 0.05	± 0.20	± 0.57	± 2.58

* Values are means (±SE)

* Means with the same letters are not significantly different.

Table (9) Physicochemical data of calices of some Hibiscus sabdariffa lines season 94/95

Line	Reducing sugars %	Non- reducing sugars %	Tannin %	pH value	Total n %	Colour intensity %	Insoluble solid %
CV ₁	1.38 a	0.91 b	0.48 ab	2.75 b	1.17 b	0.69 b	36.92 b
CV ₂	1.24 c	0.85 c	0.38 b	2.62 c	0.89 c	0.64 b	39.05 ab
G	1.31 b	0.70 d	0.004 c	2.58 c	0.001 d	0.03 d	39.60 a
24	1.24 c	0.95 a	0.57 a	2.43 d	1.35 a	1.90 a	32.41 c
18	1.27 c	0.92 ab	0.51 ab	2.85 b	1.15 b	0.52 c	36.98 b
15	1.24 c	0.84 c	0.40 ab	3.05 a	1.25 b	0.53 c	33.22 c
SE	± 0.01	± 0.01	± 0.08	± 0.05	± 0.04	± 0.03	± 1.10

* Values are means (±SE)

* Means with the same letters are not significantly different.

Table (10) : Physicochemical data of calices of some Hibiscus sabdariffa lines season 95/96.

Line	Reducing sugars %	Non-reducing sugars %	Tannin %	pH value	Total anthocyanin %	Colour intensity %	Insoluble solid %
CV ₁	1.35 a	0.90 a	0.43 b	2.80 bc	1.20 b	0.49 c	38.30 a
CV ₂	1.25 b	0.83 b	0.38 c	2.70 c	1.23 b	0.42 c	35.27 b
G	1.31 a	0.70 c	0.003 d	3.13 ab	0.001 d	0.02 d	39.82 a
24	1.20 b	0.93 a	0.56 a	2.98 abc	1.63 a	0.95 a	34.17 b
18	1.24 b	0.93 a	0.38 c	3.18 ab	1.30 b	0.60 b	39.33 a
15	1.24 b	0.83 b	0.38 c	3.25 a	0.99 c	0.60 b	37.07 a
SE	± 0.02	± 0.02	± 0.01	± 0.18	± 0.07	± 0.04	± 2.69

* Values are means (± SE)

* Means with the same letters are not significantly different.

4. 3 Organic acids in calices

4. 3. 1 Total acidity (as hibiscic acid)

Tables 12 and 13 show the total acidity as hibiscic acid of the calices of the different lines of karkade for seasons 94/95 and 95/96 respectively. The total acidity (as hibiscic acid) ranged from 20.77 to 28.53% and from 22.41 to 27.88% for seasons 94/95 and 95/96 respectively. The mean values of total acidity (as hibiscic acid) was 25.5% and 26.18% for seasons 94/95 and 95/96 respectively. These results are higher than those reported by Griebel (1939) and Batchez (1948). In season 94/95 there were significant differences in total acidity (as hibiscic acid) among different lines. In season 95/96 line 15 was significantly lower ($P \leq 0.01$) in total acidity compared to the other karkade lines, while lines Cv₁, CV₂, 24, G and 18 had high total acidity and no significant difference was observed among them.

4.3.2 Total acidity (as citric acid)

Tables 11, 12 and 13 show the total acidity expressed as citric acid for the different lines of karkade calices for seasons 93/94, 94/95 and 95/96. Total acids as citric acid in season 93/94 ranged from 17.43% to 21.84% with mean value of 19.59%. However, in season 94/95 the total acidity was lower than that in season 95/96. It ranged from 11.69% to 16.86% with mean value of 14.15%. In seasons 94/95 and 95/96 the highest value was observed for line G, whereas the lowest values were observed for line 18 and 15. In season 95/96 the total acidity content ranged from 11.63% (for line 18) to 16.79% (for line G) with an average value of 14.14%. In season 93/94 the total acidity (as citric acid) of the

lines CV₁ and CV₂ were significantly ($P \leq 0.05$) higher compared to the other lines except for line G. In seasons 94/95 and 95/96 total acidity (as citric acid) of line G was significantly ($P \leq 0.01$) higher compared to the other lines, while lines 15 and 18 were significantly lower than the other lines.

The mean value of the total acidity as citric acid of line G over the two seasons was 16.83%. This amount is equivalent to 29% of that reported for citric acid in citrus fruits (Harborne 1984).

4.2.3 Ascorbic acid (vitamin C)

Tables 11, 12 and 13 show the vitamin C content of different lines of karkade calices grown in seasons 93/94, 94/95 and 95/96. Vitamin C content of karkade calices grown in season 93/94 ranged from 66.4 to 122.84 mg/100g and a mean value of value 92.06 mg/100g. However, for seasons 94/95 and 95/96 it ranged from 65.9 to 120.6 mg/100g (mean value 92.69 mg/100g) and from 66.6 to 132.09 mg/100g and a mean value of 97.26 mg/100g respectively. In both seasons 94/95 and 95/96 the highest value was observed for line 24 and the lowest value for line G, those values were significantly different compared to other lines. In season 93/94 the vitamin C content for lines CV₁, CV₂ and E were significantly ($P \leq 0.05$) higher compared to the remaining lines. Present results are higher than those obtained by Reaubourg and Monceaux (1940) and Ibrahim *et al.* (1971). The mean value of vitamin C content of line 24 over the two seasons was 126.39 mg/100g. This amount is equivalent to 57.98%, 157.98% and 280.8% of that reported for Guava, Lemon and lime respectively. In addition this line could provide 4 times

the amount computed for daily requirements of vitamin C (30 mg/100g) as shown by Sukkar (1985).

4.2.4 Oxalic acid content

Tables 12 and 13 show the oxalate content of karkade calices from different lines grown in season 94/95 and 95/96. The oxalate content of karkade lines grown in season 94/95 ranged from 0.42 to 0.61% and a mean value of 0.55%. The highest value was observed in line CV₁, whereas the lowest value was observed in line 18 and 24. In season 95/96 the oxalate content of karkade calices ranged from 0.42 to 0.69% and a mean value of 0.57%. Line CV₁ gave the highest value, whereas line 24 gave the lowest value. The difference in oxalate content could be attributed to the nature of the line. Analysis of variance showed a significant differences ($P \leq 0.01$) among karkade lines grown in season 94/95 (Table 11) and in season 95/96 (Table 12). This variation is imported for selection for lines low in oxalate content recorded nutritionally. The mean value of oxalate content of the line 24 over the two seasons was 42 mg/100g. This amount is equivalent to 6.6% and 6.5% of that reported for leafy vegetables swisschard and purslane (Yousif 1993). It is noticed from Tables 8 and 11 that correlation coefficients between total acidity content and each of the tannin, color intensity, organic acid content and pH are positive and significant. Positive and significant correlation coefficients were obtained between tannin and colour intensity (0.79) as well as tannin content and the pH (0.40). On the other hand, organic acid content was negatively and significantly correlated with the pH (0.59) and colour intensity (0.29). Organic acid content was also negatively but non significantly correlated with tannin content. Thus, such negative relationships might be partly

due to the occurrence of competition among their pathway cycles for the same intermediate substrate. These findings are expected due to the fact that tannins, anthocyanins and acids are all acidic species.

Table 11

Organic acid of some Hibiscus sabdariffa lines season 93/94.

Line	Total acidity as citric acid (%)	Vitamin C (ascorbic acid) (mg/100g)
CV ₁	21.84 a	112.63 a
CV ₂	21.25 a	112.38 a
G	20.29 ab	66.60 b
D	17.43 b	66.40 b
B	18.45 b	71.50 b
E	18.27 b	122.84 a
SE	± 1.23	± 13.95

* Values are means (\pm SE).

*Means with the same letters are not significantly different.

Table (12)

Organic acids of calices of some Hibiscus sabdariffa lines season 94/95.

Line	Total acidity as citric acid (%)	Total acidity as hibiscic acid (%)	Vitamin C (ascorbic acid) (mg/100g)	Oxalate content (%)
CV ₁	14.66 b	25.05 d	98.52 c	0.61 a
CV ₂	14.72 b	26.24 bc	110.25 b	0.60 a
G	16.86 a	25.35 cd	65.90 c	0.54 b
24	15.16 b	28.53 a	120.69 a	0.48 c
18	11.83 c	27.12 b	81.21 d	0.42 c
15	11.69 c	20.77 e	79.58 d	0.62 a
SE	± 0.35	± 0.43	± 1.0	± 0.03

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

Table (13)

Organic acids of calices of some Hibiscus sabdariffa lines season 95/96.

Line	Total acidity as hibiscic acid (%)	Total acidity as citric acid (%)	Vitamin C (ascorbic acid) (mg/100g)	Oxalate content (%)
CV ₁	26.13 a	14.80 b	111.79 b	0.69 a
CV ₂	27.45 a	14.43 b	111.06 b	0.61 b
G	26.42 a	16.79 a	66.60 c	0.50 d
24	27.88 a	15.03 b	132.09 a	0.42 f
18	26.77 a	11.63 c	82.39 c	0.49 e
15	22.41 b	12.17 c	79.60 d	0.64 c
SE	± 1.31	± 0.33	± 0.49	± 0.06

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

4.4 Mineral content

The mineral content of karkade calices are shown in Tables 14 and 15 for seasons 94/95 and 95/96 respectively.

4.4.1 Calcium content

Table 14 shows that the calcium content of karkade calices for season 94/95 ranged from 0.53 to 0.57% and a mean value of 0.56%. The calcium content in season 95/96 (Table 15) ranged from 0.87 to 3.35% and a mean value of 1.94%. Further more, the mean values for the calcium content in season 95/96 were higher than that in season 94/95. This difference reflects the effect of the season. It may be noted that the first is a summer, and the second is a winter crop. Results of season 95/96 were similar to those reported by Ali *et al.*(1991). The mean value of calcium content of karkade calices, line 24, over the two seasons was 1960 mg/100g. This is equivalent to 1633% of that (120mgCa) obtained for cows fresh and whole milk (Sukkar,1985). The amount of calcium for daily requirements are 400 to 500 mg/day.

Table 16 indicates that the calcium content of karkade seeds ranged from 0.01 to 0.06% and a mean value of 0.02. These results are in agreement with those reported by Al Wandawi *et al.*(1984). In karkade leaves calcium content ranged from 1.03 to 2.30% and a mean value of 1.43%. These results are in greement with the data reported by Mclean (1973).

4.4.2 Magnesium content

Table 14 shows that the magnesium content of karkade calices grown in season 94/95 ranged from 0.24% to 0.25% and a mean value of 0.24%, whereas in season 95/96 the magnesium content ranged from 0.16% to 0.47% and a mean value of 0.32%. These results are in fair agreement with those obtained by Ali *et al.* (1991). In season 94/95, line 15 showed the lowest Mg content, whereas in season 95/96 lines 24 and CV₁ showed the lowest Mg content.

From Table 16, it can be seen the magnesium content of karkade seed ranged from 0.13% to 1.38% and a mean value of 0.36%. These results are higher than those reported by El Adawy and Khalil (1994). It is obvious that roselle lines show no significant differences in the magnesium content of seeds.

4.4.3 Potassium content

Table 14 and 15 show the potassium content of karkade calices in season 94/95 and 95/96 respectively. Data indicates significant ($P \leq 0.01$) difference among lines. The potassium content of season 94/95 and 95/96 ranged from 0.63% to 1.28% and 0.21% to 3.24% with the mean value 0.95% and 1.71% respectively. This wide variation in the values of potassium content may be attribute to the genetic factors. Present results are higher than the data reported by Hassan (1988).

Table 16 shows that the potassium content of karkade seeds ranged from 0.02 to 0.03% and a mean value of 0.02%. The results obtained here disagree with that of El Adawy and Khalil (1994). From Table 16, it is obvious that the potassium content of karkade leaves

ranged from 0.17 to 0.38% and a mean value of 0.26%. Different lines show significant variation ($P \leq 0.01$) for potassium content of seeds and leaves.

4.4.4 Sodium content

Tables 14 and 15 show that the sodium content of karkade calices ranged from 0.17% to 0.32% and 0.03% to 0.84% for seasons 94/95 and 95/96 respectively. The mean values of sodium content for calices were 0.23% and 0.31% for seasons 94/95 and 95/96 respectively. In seasons 94/95 and 95/96 calices sodium content of line CV₂ was significantly ($P \leq 0.01$) lower compared to the other lines.

From Table 14, it can be seen that the sodium content of karkade seeds ranged from 0.21% to 0.34% and a mean value of 0.27%. The results obtained here are slightly different than those reported by Al Wondawi *et al.* (1984). The sodium content of karkade leaves ranged from 0.13 to 0.20% and a mean value of 0.16% (Table 16). There were significant differences ($P \leq 0.01$) in sodium content among the lines for the seeds and the leaves.

4.4.5 Phosphorus content

Phosphorus content of karkade calices of different lines grown in season 94/95 and 95/96 are presented in Tables 14 and 15 respectively. The phosphorus content of karkade calices of the lines grown in season 94/95 ranged from 0.16% to 0.27% and a mean value of 0.19%. In season 95/96 the phosphorus content was found to be in the range of 0.16% to 0.33% and a mean value of 0.24%). These results are in agreement with the data reported by Mclean (1973). The mean value of

phosphorus content of line 24 over the two seasons was 302 mg/100g. This amount is equivalent to 331% of that (90mg P) reported for cows fresh and whole milk (Sukkar,1985).

This wide difference in the value of the total phosphorus content is due to the fact that phosphorus level in plant depend on differences among these lines in efficiency of phosphorus up take incorporation into organic compounds in the leaves and translocation to the calices. In this study the variation in total phosphorus may be due to genetic factors.

Analysis of variance showed significant differences ($P \leq 0.01$) among karkade lines grown in season 94/95 (Table 14) and also among karkade lines grown in season 95/96 (Table 15). The phosphorus content of karkade seeds (Table 16) ranged from 0.41% to 0.77% and a mean value of 0.48%. These results are in agreement with the data reported by Mclean (1973). The phosphorus content of karkade leaves ranged from 0.75% to 1.46% and a mean value of 1.17%. These results are higher compared to those obtained by Mclean (1973). Significant differences ($P \leq 0.01$) were noticed for phosphorus content in both seeds and leaves of the different lines.

4.4.6 Iron content

Tables 14 and 15 show iron content of calices of the six karkade lines grown in season 94/95 and 95/96 respectively. The iron content of calices of karkade lines grown in season 94/95 ranged from 0.04% to 0.16% and a mean value of 0.08%. In season 95/96 the iron content is found to be in the range of 0.03% to 0.14% and a mean value of 0.07%. These results are slightly higher than those reported by Mclean (1973).

The mean value of iron content of line CV₁ over the two seasons was 147.3 mg/100g. This is equivalent to 14730% of that (0.1mg Fe) reported for cows fresh and whole milk (Sukkar,1985). Line CV₁ could provide the amount computed for daily requirements of iron (10 mg for men and 15 mg for women). This result indicates that this line would be one of the richest sources of iron content, followed by line 24. In addition, significant differences in iron content were detected between the two seasons. Thus, calices iron content is affected by both genetic differences among the lines as well as the enviromental factors.

4.4.7 Manganese content

Table 15 shows that manganese content of karkade calices ranged from 0.03% to 0.54% with a mean value of 0.22%, which is similar to the data reported by Hassan (1988). Manganese content was highest in line 15 and lowest in line 18. Line CV₁ and 24 had the same Mn content and they were significantly different from the remaing lines. There was a highly significant difference ($P \leq 0.01$) in Mn content between line 15 and all other lines. Therefore Mn content is affected by line type.

4.4.8 Copper content

As shown in Table 15 copper content of karkade calices ranged from 0.03% to 0.09% and a mean value of 0.05%. These results are higher than the results reported by Hassan (1988). There is a highly significant differences ($P \leq 0.05$) in copper content between line 18 and all other lines. The difference in copper content between the lines may be attributed to the genetic variability among the lines.

4.4.9 Zinc content

Table 15 depicts zinc content of the different karkade calices ranged from 0.002% to 0.02% with the mean value of 0.01%. These results are in close agreement with the data reported by Hassan (1988). Line G and 18 had the same calices Zn content (0.02%) and they were significantly different from the remaining lines. There was a highly significant difference ($P \leq 0.01$) in Zn content between line 24 and all other lines.

4.4.10 Lead content

Table 15 gives the lead content of karkade calices, it ranged from 0.002% to 0.008% and a mean value of 0.005%. Lead content was significantly higher ($P \leq 0.01$) in line CV₂ and significantly lower in line 18, as compared to the other lines.

4.4.11 Cobalt content

As shown in Table 15 cobalt content of karkade calices ranged from 0.01% to 0.04% with the mean value of 0.02%. Line 18 shows the highest cobalt content, while line G and 24 show the lowest Co content.

4.4.12 Chromium content

Table 15 shows that chromium content of karkade calices ranged from 0.02% to 0.06% with a mean value of 0.03%. Line 18 was significantly higher ($P \leq 0.01$) in Cr content than all remaining lines.

In conclusion from the nutritional point of view, karkade calices are seen to be a rich source of calcium, magnesium and potassium. They are

also a fairly good source of iron and phosphorus, in addition to significant amounts of copper and zinc.

All lines are found to contain Zn, Mg, Cu, Cr and Co in more than trace amount (Table 15). These elements are required in small amounts mainly for their role in enzyme synthesis and enzyme action. Deficiencies, therefore, lead to metabolic disorders which mainly affect children and in the case of Zn, Mg and Cu interfere with growth as reported by Sukkar (1985).

Table (14)

Mineral content of Hibiscus sabdariffa calices season 94/95.

Line	Ca (%)	Mg (%)	Fe (%)	Na (%)	K (%)	P (%)
CV ₁	0.57 a	0.25 a	0.16 a	0.17 c	0.97 c	0.19 b
CV ₂	0.57 a	0.25 a	0.05 bc	0.12 d	0.63 f	0.16 d
G	0.57 a	0.25 a	0.14 ab	0.32 a	0.83 d	0.17 c
24	0.57 a	0.25 a	0.05 bc	0.23 b	1.28 a	0.27 a
18	0.53 a	0.24 b	0.04 c	0.21 b	1.23 b	0.19 b
15	0.57 a	0.23 c	0.06 bc	0.31 a	0.73 e	0.17 c
SE	± 0.02	± 0.001	± 0.04	± 0.01	± 0.16	± 0.004

* Values are means (\pm SE).

* Means with the same letters are not significantly different.

Table (15): Mineral content of Hibiscus sabdariffa calices (season 95/96).

Line	Mg %	Zn %	Mn %	Pb %	Cu %	Ca %	K %	Na %	Co %	Cr %	Fe %	P %
CV ₁	0.16 d	0.03 c	0.13 d	0.007 b	0.03 f	1.12 d	0.95 bc	0.09 cd	0.02 ab	0.02 b	0.14 a	0.16 e
CV ₂	0.47 a	0.08 b	0.26b	0.009 a	0.08 b	2.17 c	0.21 bcd	0.03 d	0.02 ab	0.02 b	0.09 b	0.29 b
G	0.20 c	0.02 a	0.21 c	0.005 c	0.04 d	0.89 e	0.94 bc	0.18 c	0.01 b	0.02 b	0.03 d	0.19 d
24	0.16 d	0.09 b	0.12 d	0.006 bc	0.03 e	3.35 a	2.39 b	0.59 b	0.01 b	0.02 b	0.07 c	0.33 a
18	0.46 a	0.02 a	0.03 e	0.003 e	0.09 a	0.87 e	3.24 a	0.84 a	0.04 a	0.06 a	0.04 d	0.19 d
15	0.43 b	0.04 bc	0.54 a	0.004 d	0.06 c	3.22 b	2.52 b	0.15 c	0.02 ab	0.02 b	0.05 cd	0.24 c
SE	± 0.004	± 0.002	± 0.008	± 0.0005	± 0.001	± 0.05	± 0.13	± 0.05	± 0.009	± 0.005	± 0.009	± 0.009

* Values are means (±SE)

* Means with the same letters are not significantly different.

Table (16): Mineral content of Hibiscus sabdariffa seeds and leaves (season 94/95).

Line	Seeds					Leaves			
	Na %	K %	Ca %	Mg %	P %	Na %	K %	Ca %	P %
CV ₁	0.34 a	0.02 b	0.01 a	0.16 a	0.77 a	0.18 c	0.35 ab	1.03 b	1.07 b
CV ₂	0.25 d	0.02 b	0.06 a	0.19 a	0.41 c	0.19 b	0.38 a	1.45 ab	1.14 b
G	0.26 cd	0.02 b	0.01 a	0.14 a	0.47 b	0.20 a	0.28 abc	1.35 b	0.75 c
24	0.21 e	0.03 a	0.01 a	1.38 a	0.41 c	0.13 e	0.23 bc	2.35 a	1.46 a
18	0.29 b	0.03 a	0.01 a	0.14 a	0.44 bc	0.14 d	0.17 c	1.24 b	1.43 a
15	0.27 c	0.02 b	0.01 a	0.19 a	0.41 c	0.13 e	0.30 abc	1.13 b	1.19 b
SE	± 0.003	± 0.001	± 0.03	± 0.69	± 0.02	± 0.001	± 0.06	± 0.41	± 0.05

* Values are means (±SE)

* Means with the same letters are not significantly different.

4.5 Pigments

4.5.1 Types of anthocyanins

Roselle pigment hydrolysates run on TLC (Fig 12) produced two major anthocyanidins, red and violet pigments. Table 17 shows the red spot had an R_f value of 31 - 37 (PC in BAW). UV-visible spectrum the red pigment has $\lambda_{\text{max}} = 530$ nm. On the other hand, the violet had an R_f value of 23 - 26 (PC in BAW) and showed UV-visible spectrum at $\lambda_{\text{max}} = 540$ nm. Figures 1 to 11 show UV-absorption spectra with λ_{max} ca 281 and visible absorption spectra with λ_{max} absorbance of the red and violet bands at 530 and 540 respectively for the two major anthocyanidins obtained from the hydrolysis of the original pigment present in the calyx. These results indicate that the red and the violet bands are cyanidin and delphinidin respectively. These are supported by the work of Du and Francis (1973) who detected two compounds for roselle calices pigment. Furthermore, the absorbance ratio at 440 nm to the maximum visible absorbance ($A_{440}/A_{\text{vis.max}}$) was 24 to 25 for the red spot, whereas it was 19 - 22 for the violet spot (Table 18).

The ratio of the red to violet pigments differed in the different karkade lines. The ratio obtained indicated that the concentration of delphinidin was higher than that of cyanidin. The concentration of delphinidin is highest in line 24 and lowest in line CV₁. These characteristics further confirm that the red spot is cyanidin while the violet is delphinidin (Table 18).

Fig (13) shows sugar constituents of pigment hydrolyest of calices of the six lines of karkade. It is clear that these pigments are xylose and glucose glycosides of cyanidin and delphinidin. They could be mono or biosides. These results are in agreement with the data reported by Du and Francis, (1973 a and b). Thus the main anthocyanin pigments responsible for karkade calyx colour are cyanidin 3-sambubioside and delphinidin 3-sambubioside.

Table (17)

Rf values of the major two bands of six lines of Hibiscus sabdariffa BAW (4:1:5 v/v).

Line	Red band	Violet band	Authentic markers	
CV ₁	37	26	cn-3-G from v-dentatum* (Du and Francis, 1973 a)	36
CV ₂	33	26	cn-3 G al from c. mas (Du and Francis, 1973 b)	33
24	31	23	Dp-3-G from pomegranates** (Harborne, 1967)	23
18	36	23	cn-3 G x from v-dentatum*** (Du and Francis, 1973 b)	33
15	33	25		

* cyanidin 3-glucoside.

** delphinidin 3-glucoside.

*** cyanidin 3-sambubioside.

Table (18): The ratio of the absorbency at 440nm to that maximum visible absorbency (A 440/Avis - max.) for anthocyanidin from roselle calices and the ratio of the concentration of delphinidin to cyanidin .

Line	CV ₁	CV ₂	G	24	18	15
	Red - violet	Red - violet	White	Red - violet	Red - violet	Red - violet
Evis - max.	529.6 539.7	530 539.4	325.9	530 540	530 539.2	530 538.5
A440/Evis	25 20	24 22	-----	25 20	25 22	25 19
delphinidin/ cyanidin ratio	1: 0.97	1:0.73	-----	1:0.69	1:78	1:0.97

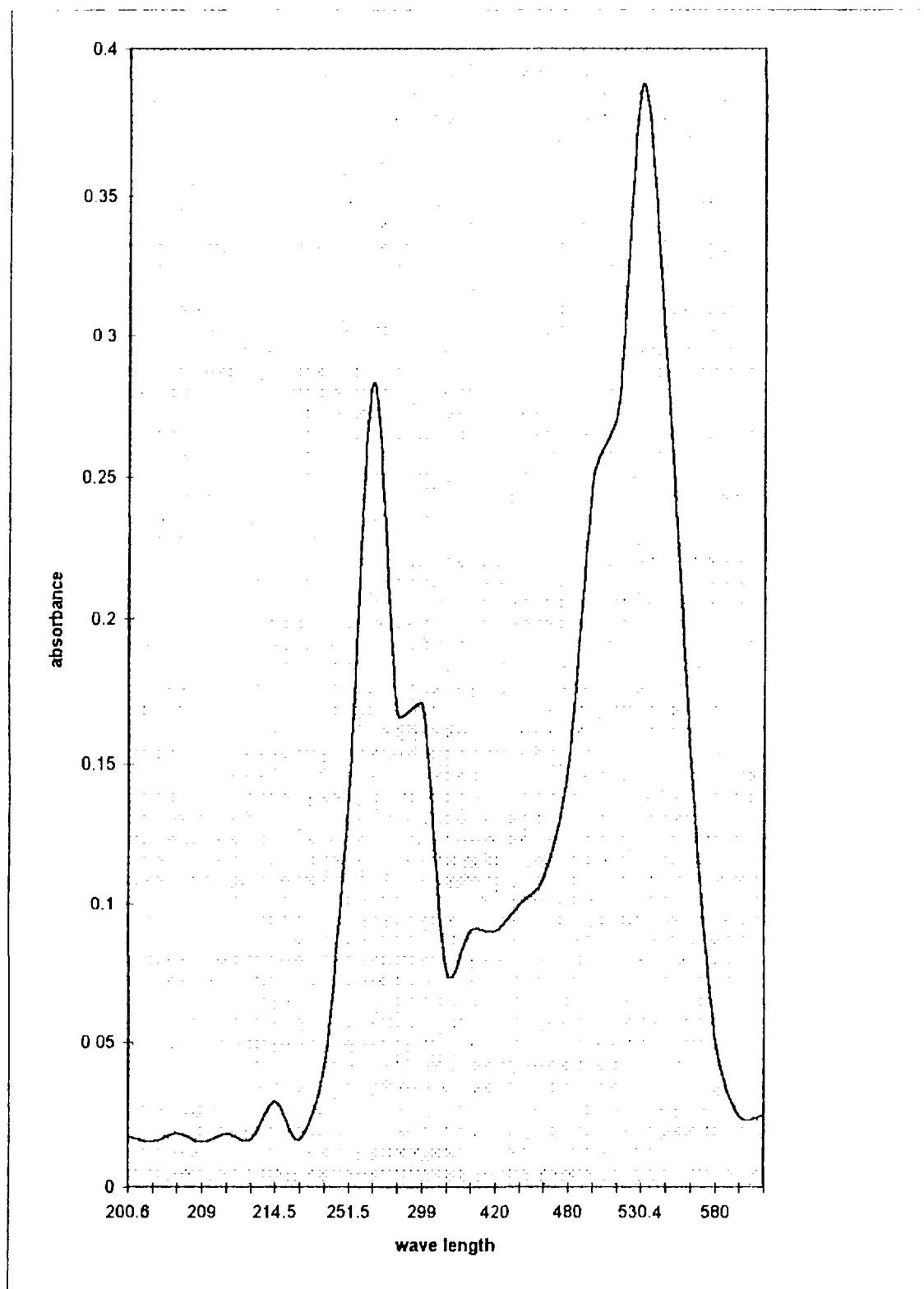


Fig (1) UV - visible spectrum of anthocyanidin (cyanidin red band)
in methanol for roselle line 24

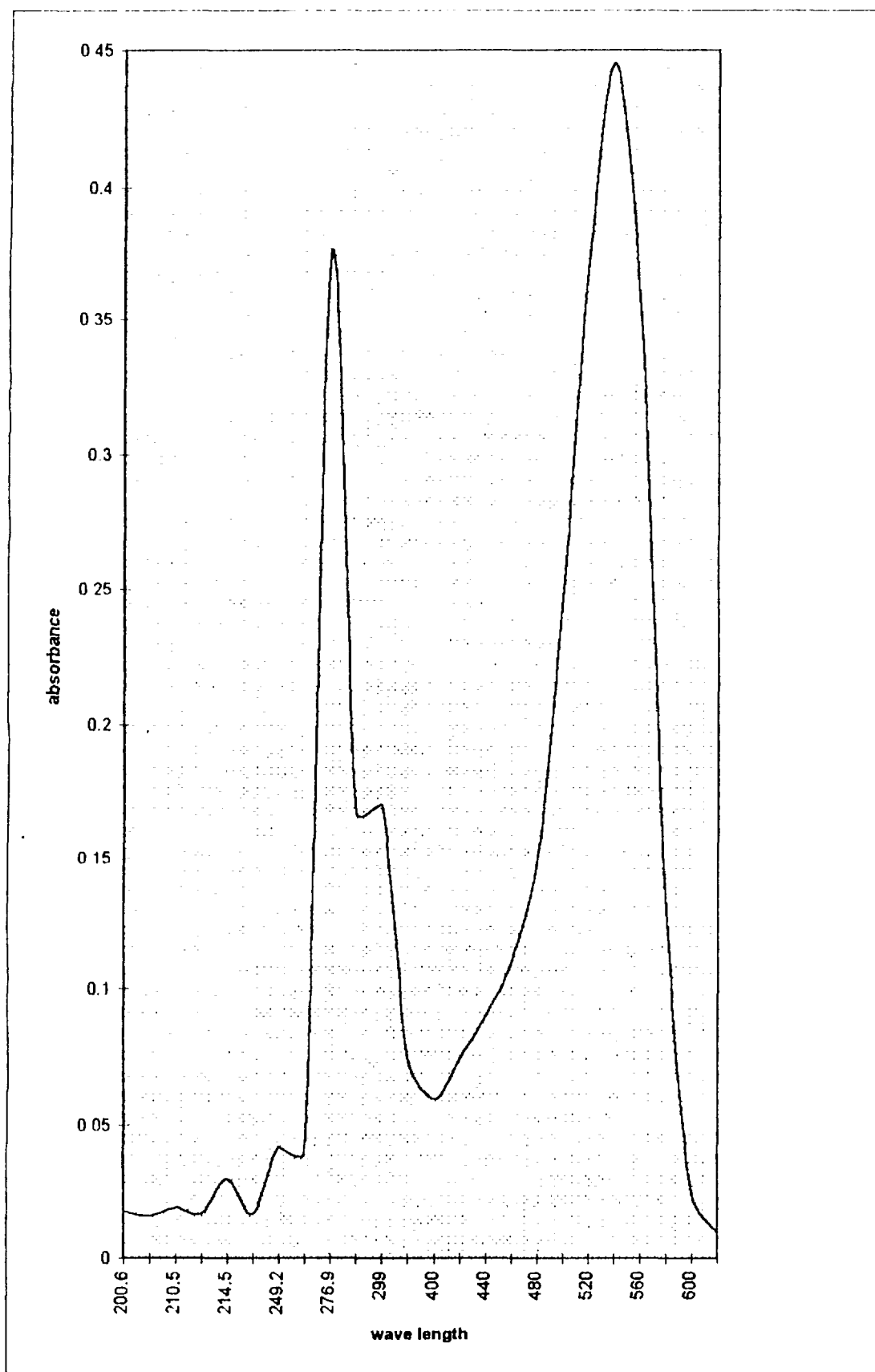


Fig (2) UV - visible spectrum of anthocyanidin (delphinidin violet band)
in methanol for roselle line 24

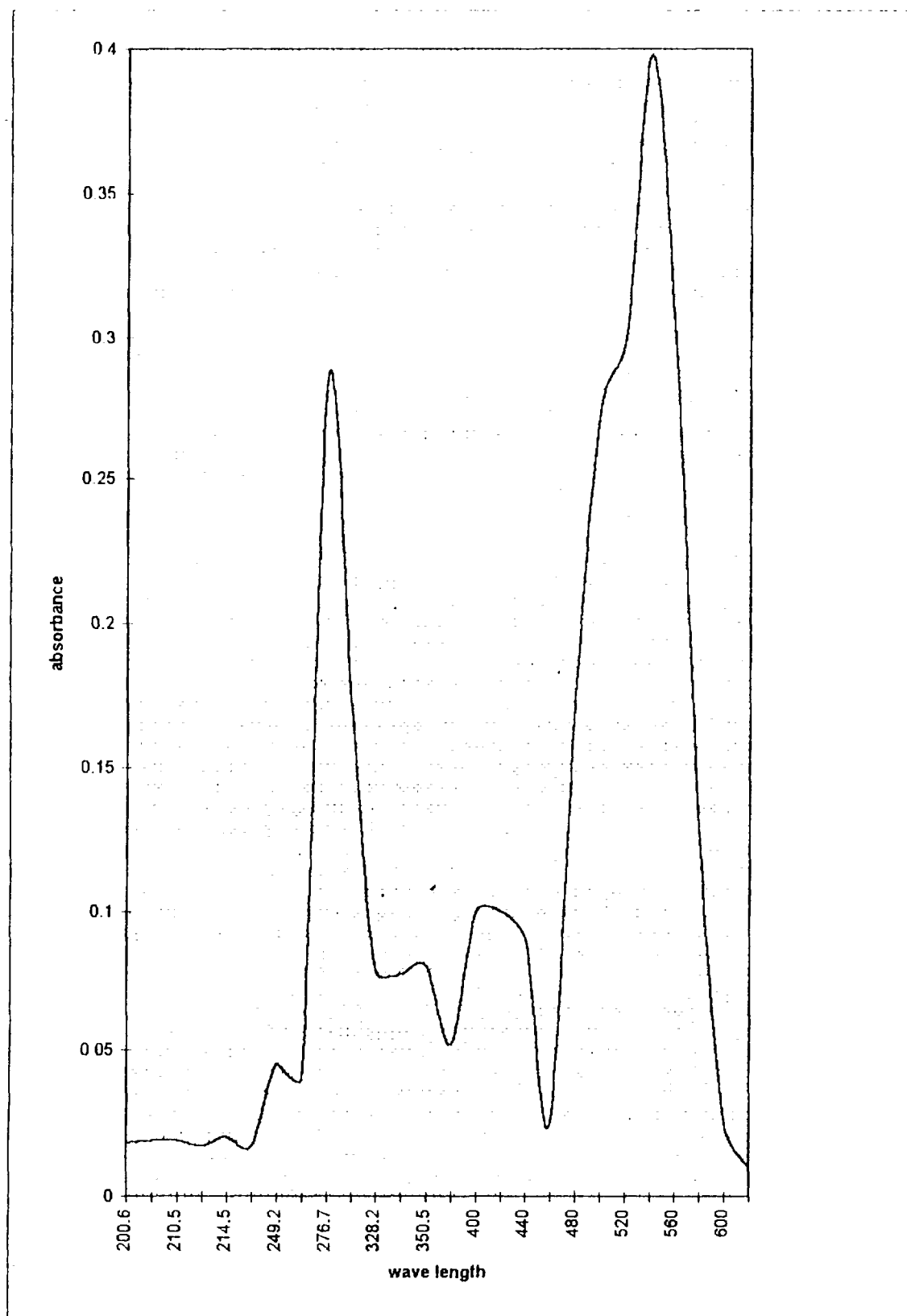


Fig (3) UV - visible spectrum of anthocyanidin (delphinidin violet band)
in methanol for roselle line 18

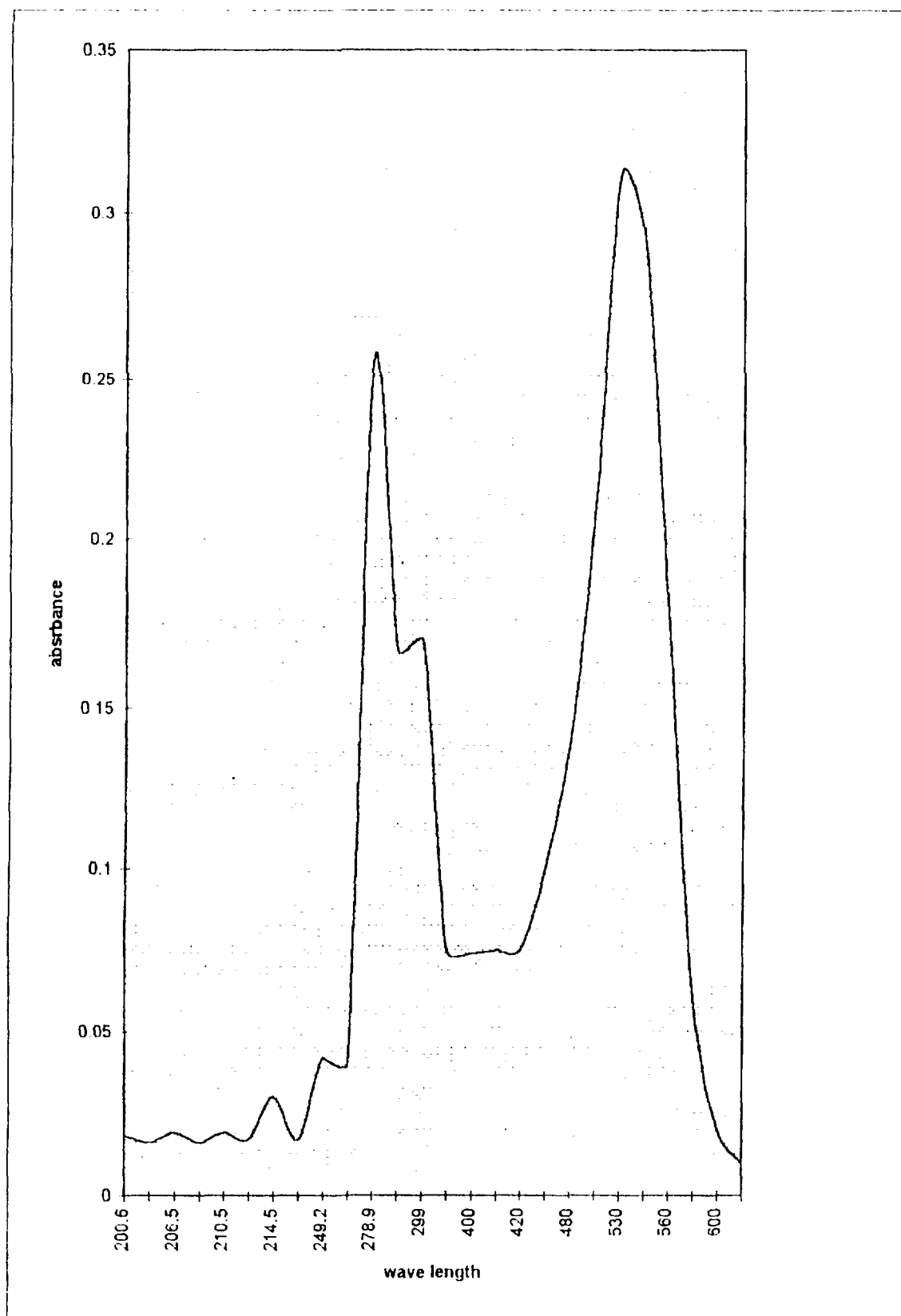


Fig (4) UV - visible spectrum of anthocyanidin (cyanidin 'red band)
in methanol for roselle line 18

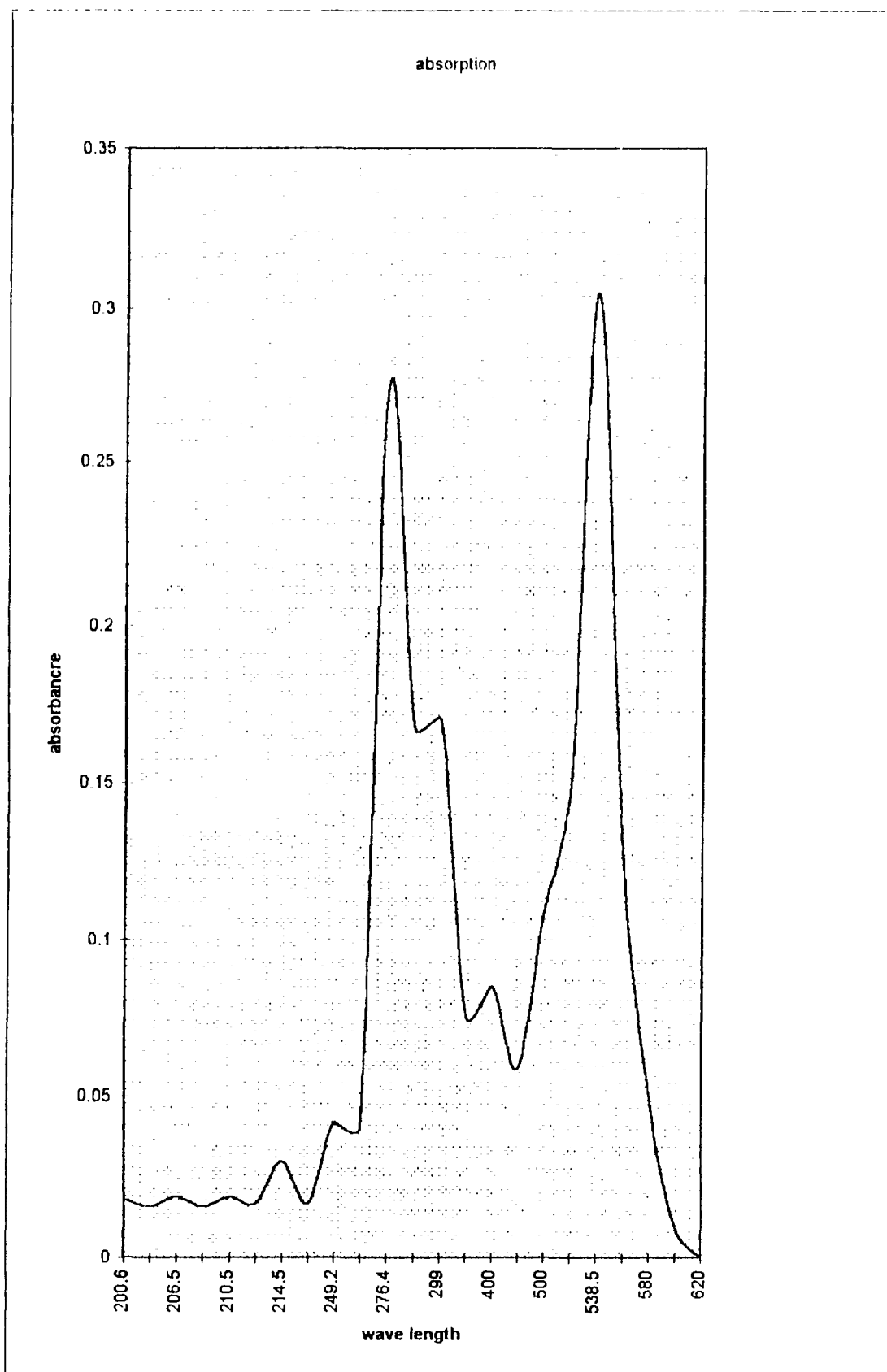


Fig (5) UV - visible spectrum of anthocyanidin (delphinidin :violet band)
in methanol for roselle line 15

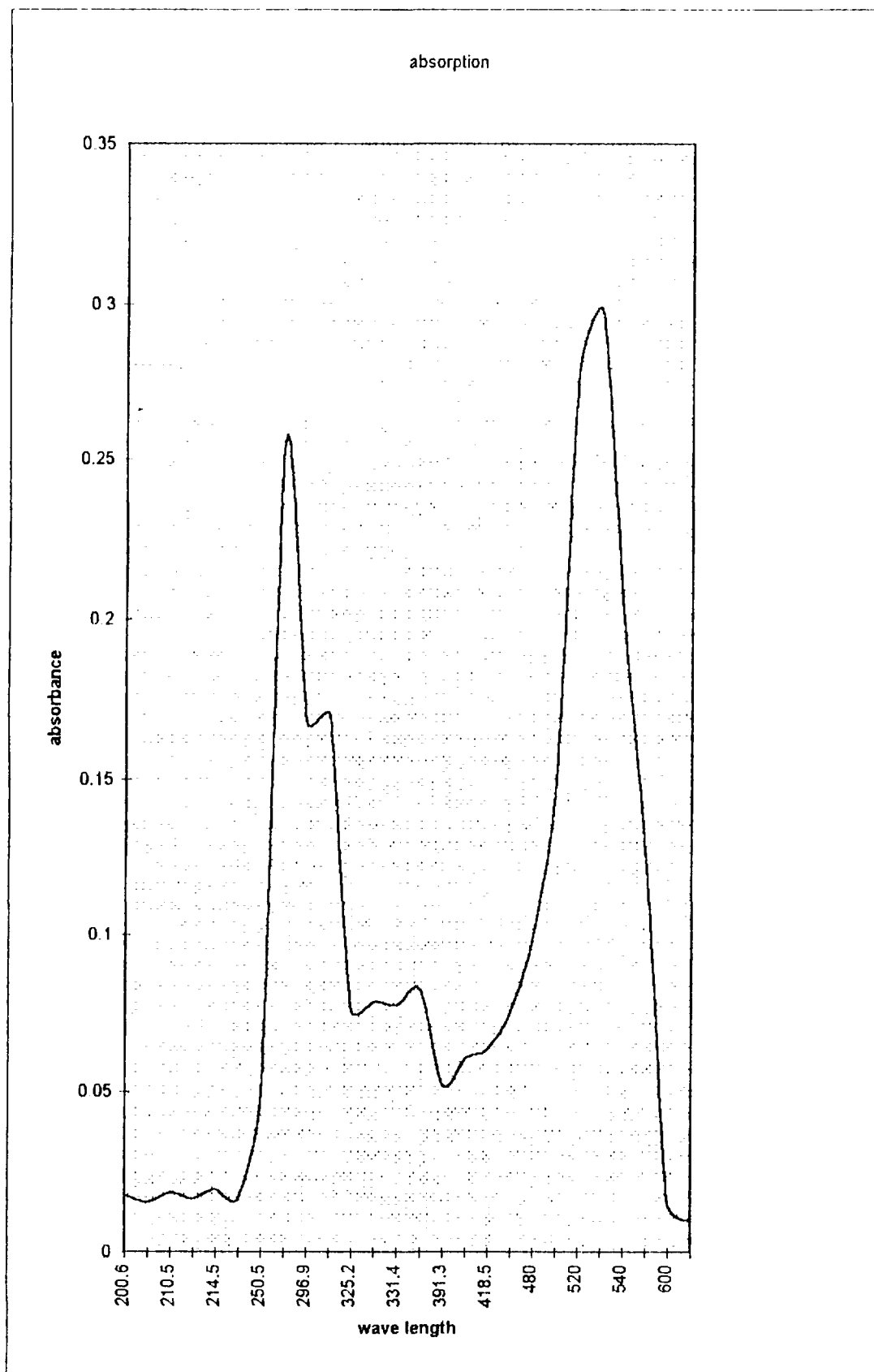


Fig (6) UV - visible spectrum of anthocyanidin (cyanidin .red band)
in methanol for roselle line 15

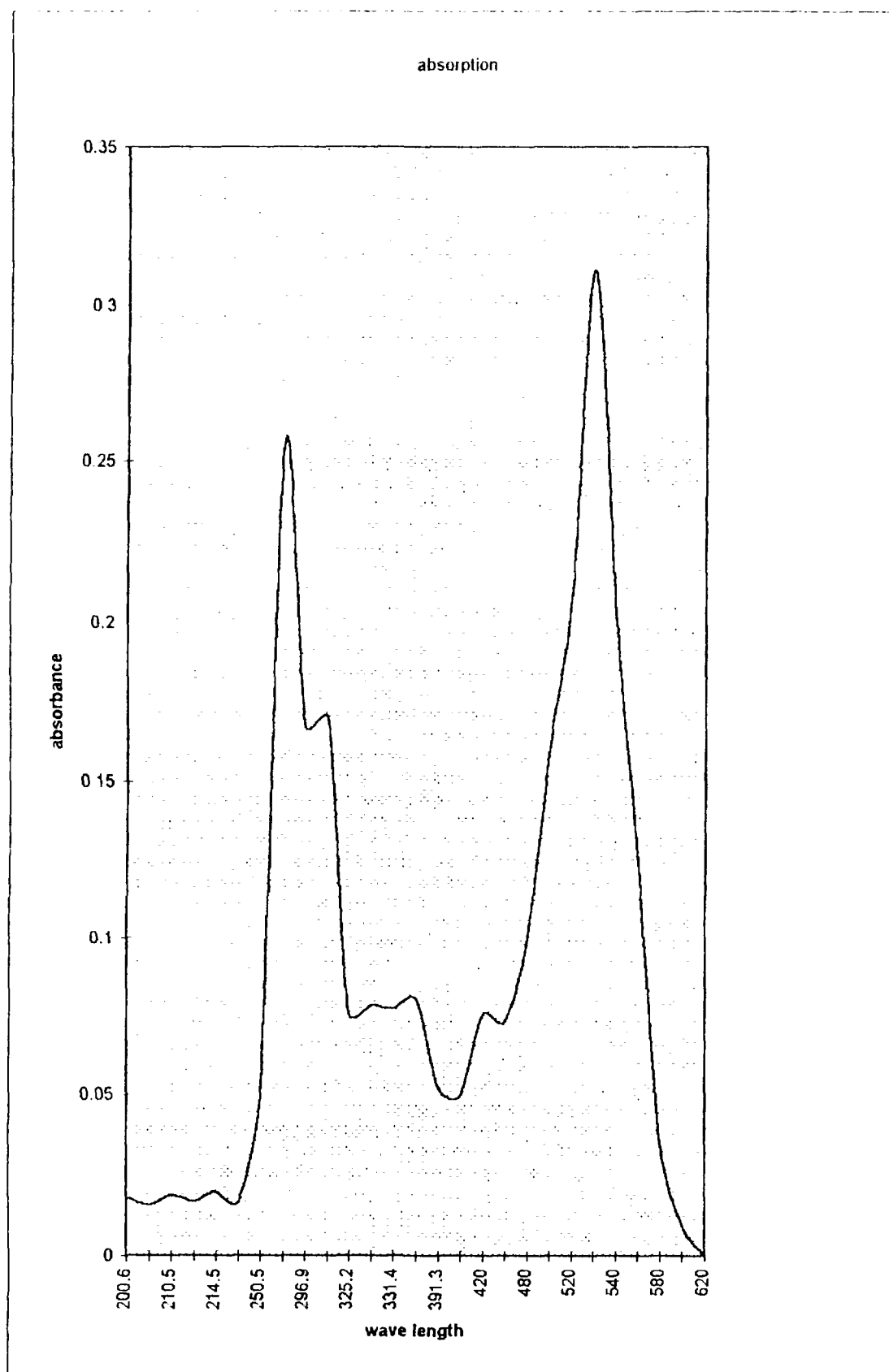


Fig (7) UV - visible spectrum of anthocyanidin (cyanidin red band) in methanol for roselle line cv -2

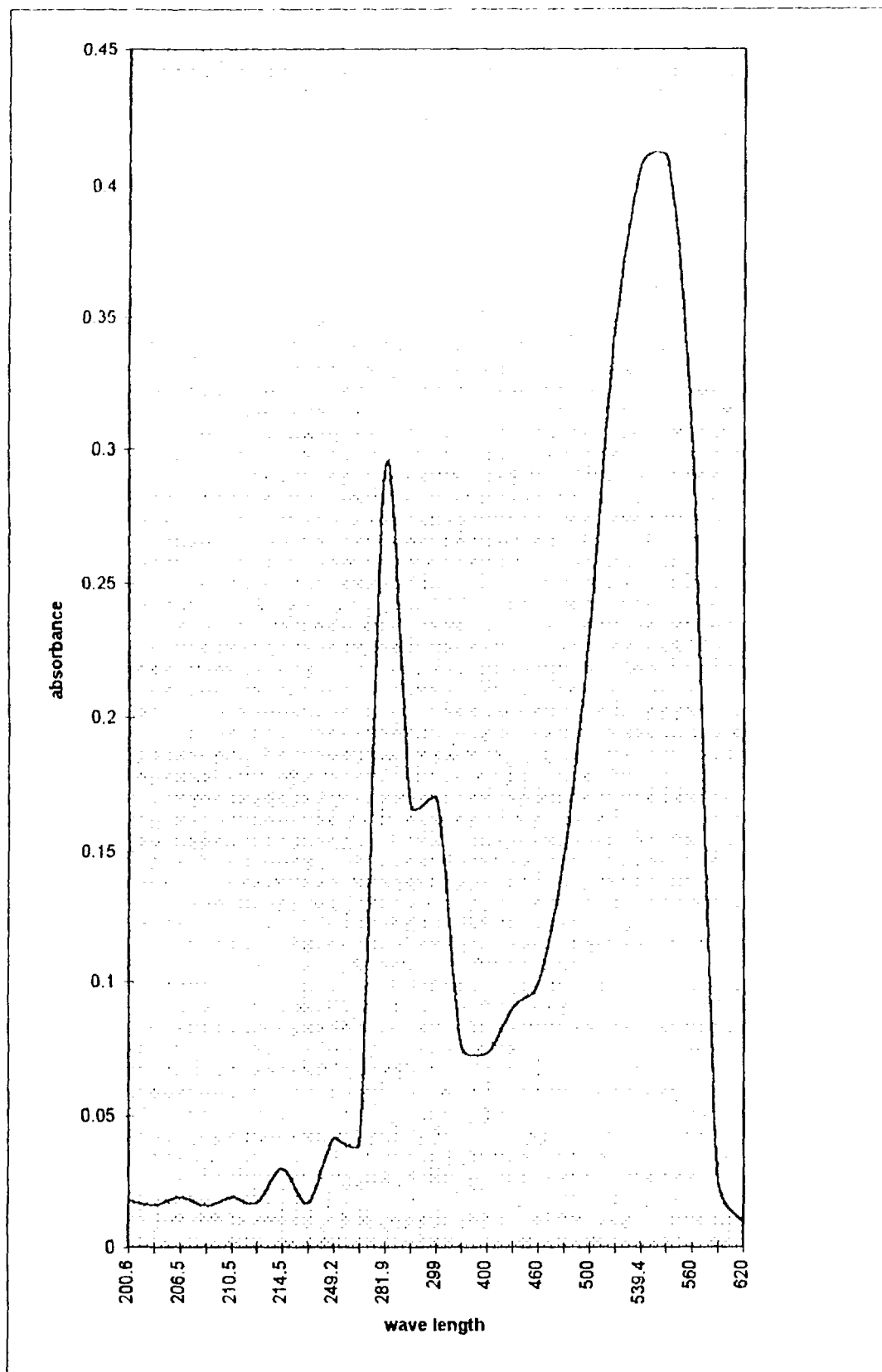


Fig (8) UV - visible spectrum of anthocyanidin (delphinidin :violet band) in methanol for roselle line cv - 2

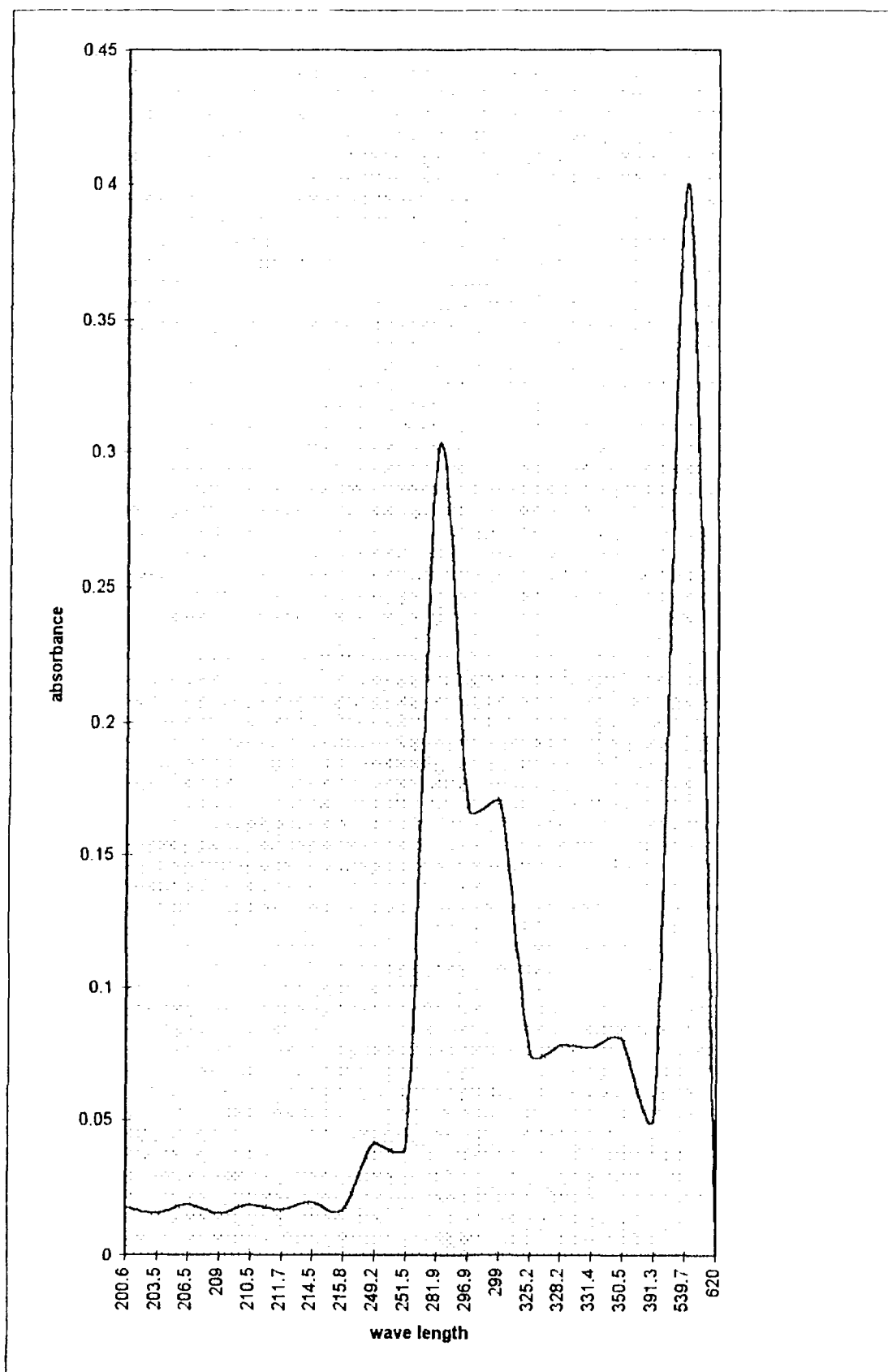


Fig (9) UV - visible spectrum of anthocyanidin (delphinidin violet band)
in methanol for roselle line cv - 1

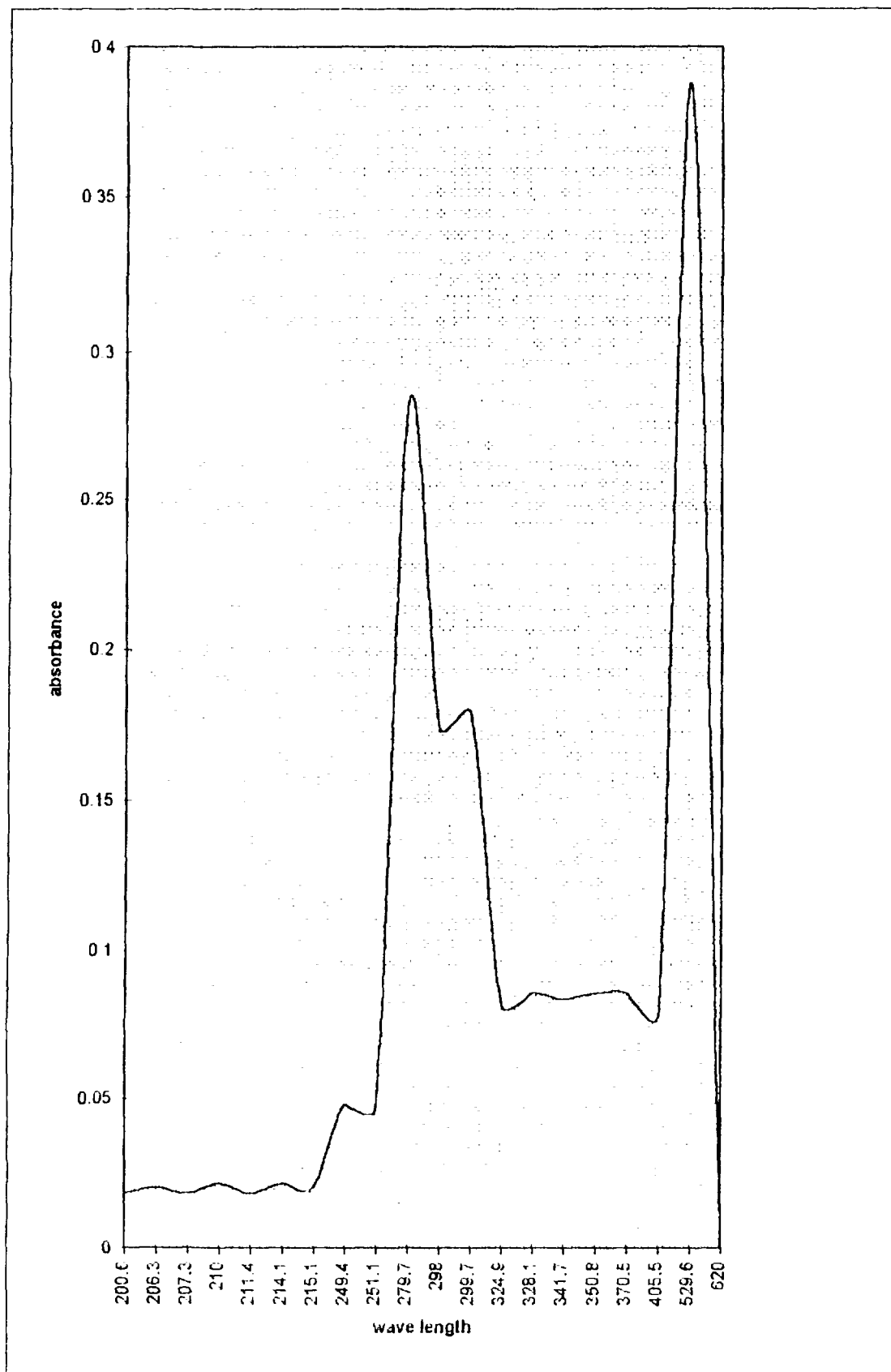


Fig (10) UV - visible spectrum of anthocyanidin (cyanidin :red band)
in methanol for roselle line cv-1

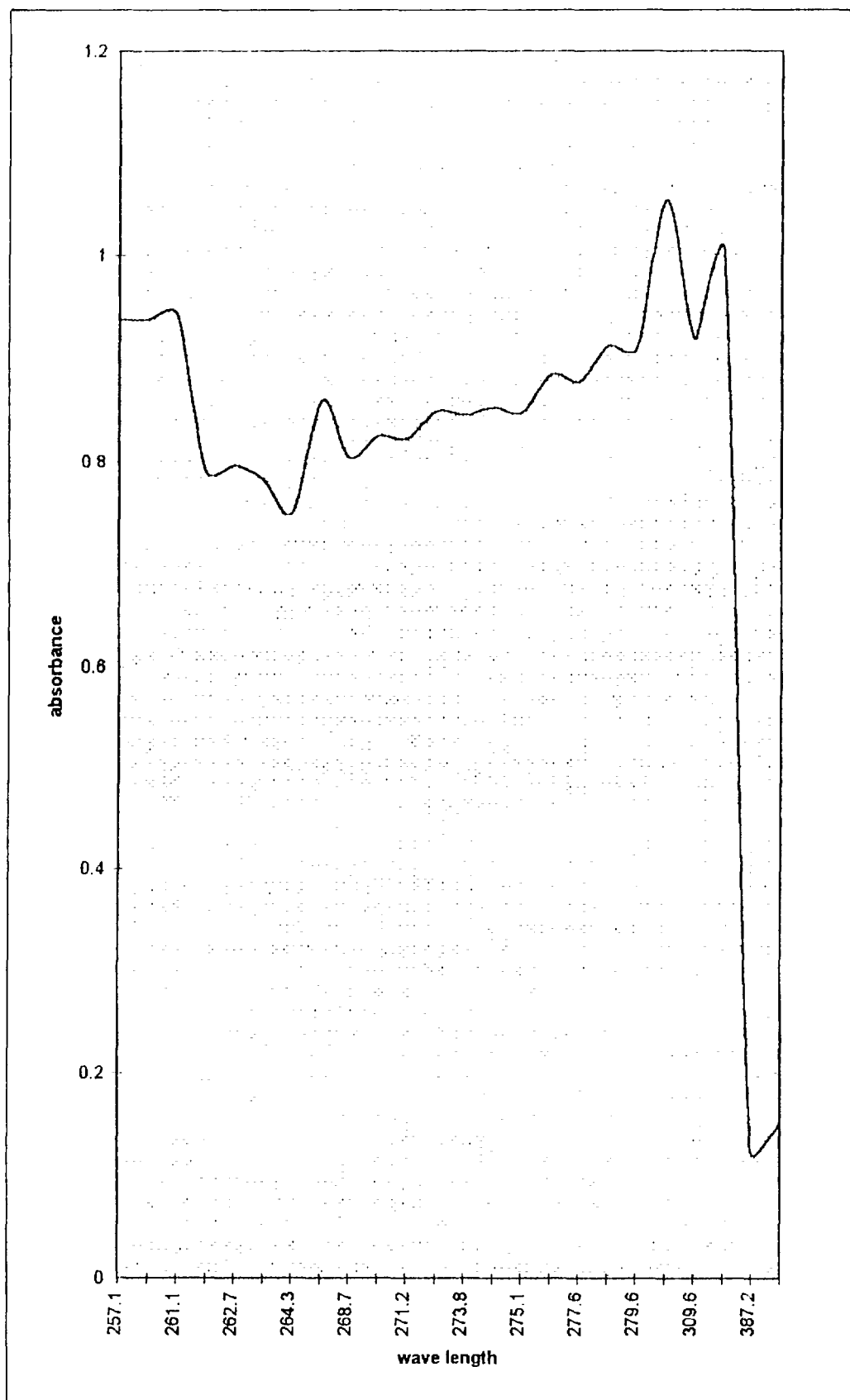


Fig (11) UV - visible spectrum of anthocyanidin in methanol for roselle line G

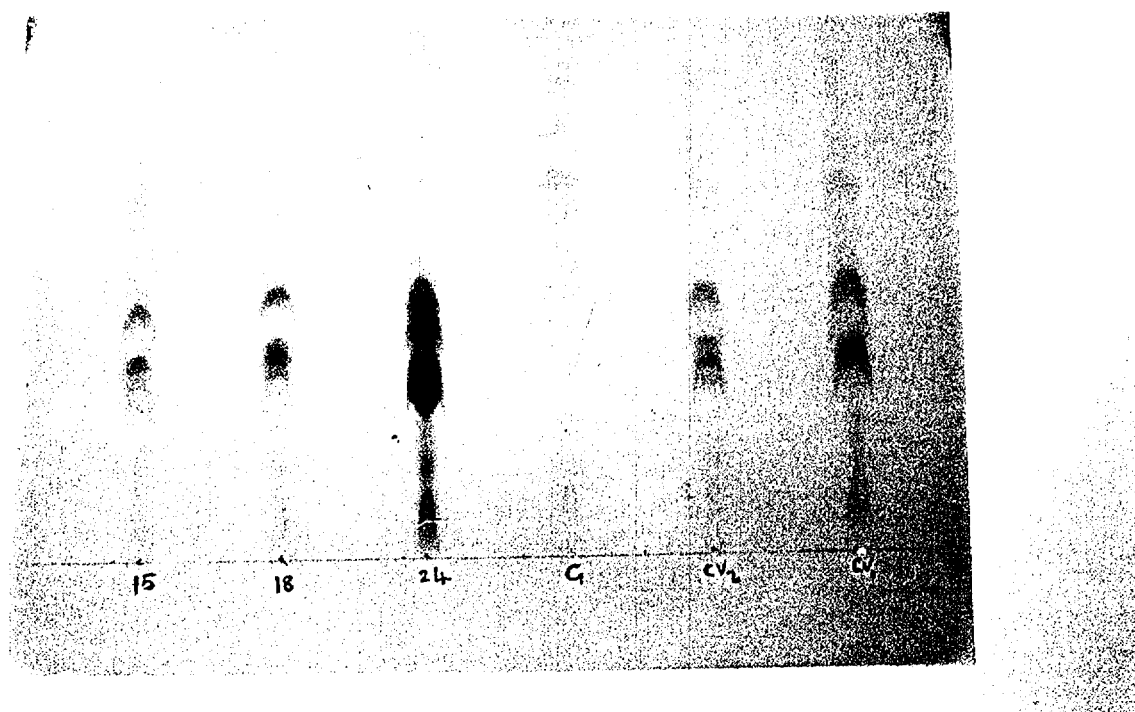


Fig 12
Thin layer chromatography of the major karkade pigments
solvent BAW 4:1:5 (upper layer)

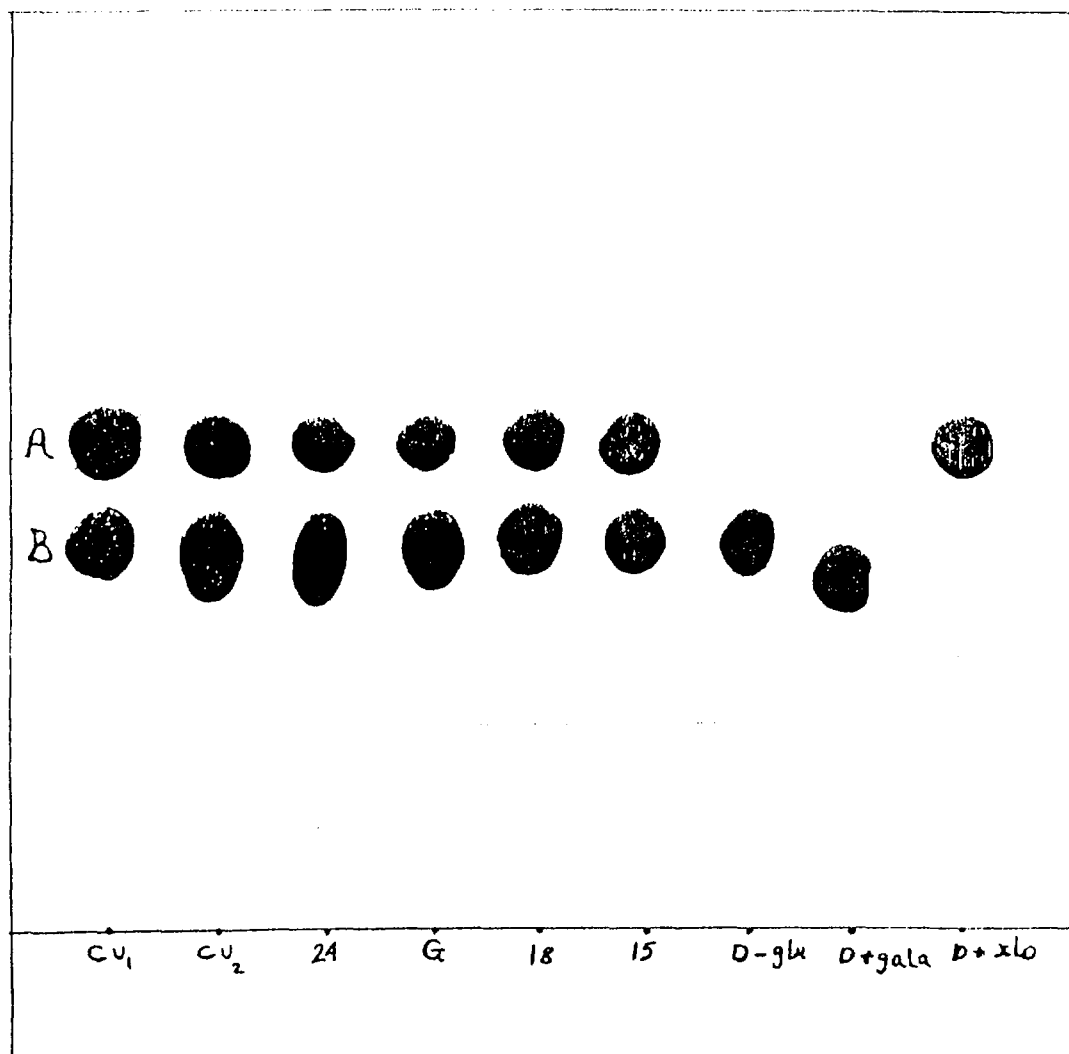


Fig 13

Thin layer chromatography of sugars after acid hydrolysis of
roselle pigments .

A :D+ xylose B :D- glucose

solvent : n- butanol - benzene - pyridine - water

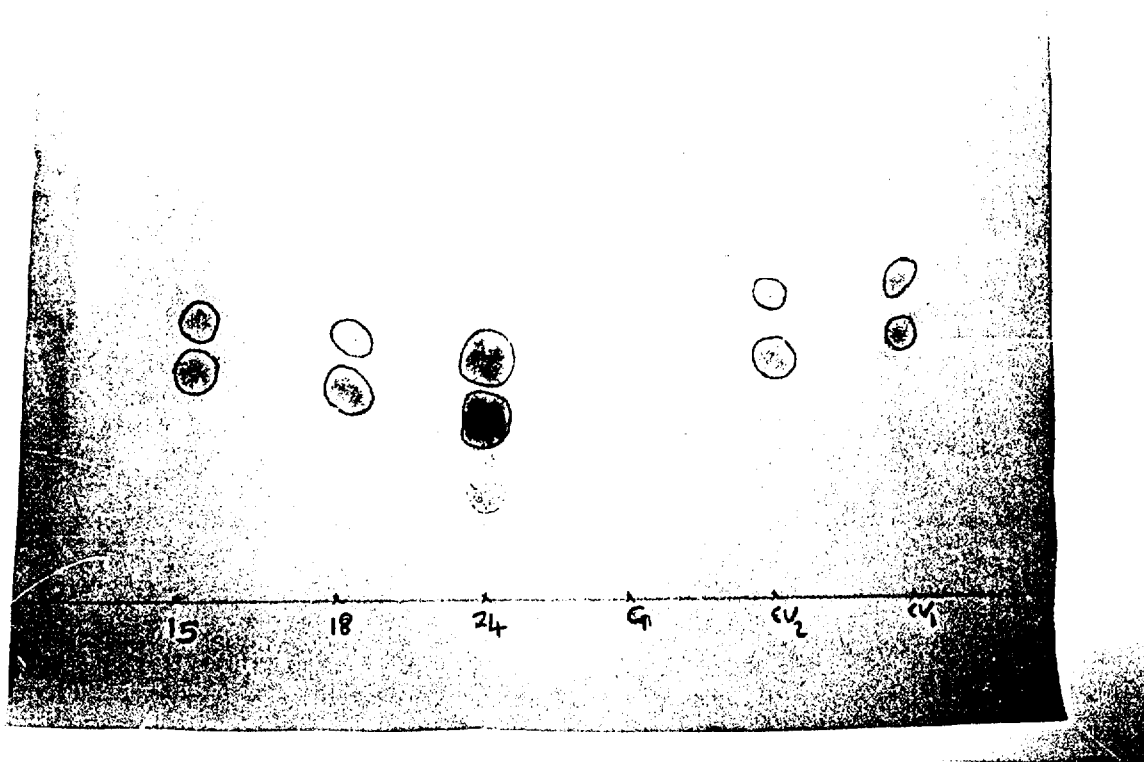


Fig 14
Paper chromatography of the major karkade pigments
solvent BAW 4:1:5 (upper layer)

SUMMARY

Six lines of karkade calices were evaluated for crude fibre, crude protein, carbohydrate, total acids as citric acid and hibiscic acid, pH value, reducing sugars, non-reducing sugars, tannin content, total anthocyanins and ascorbic acid content.

Analytical data obtained from this study demonstrate that there is a wide variation among the lines particularly in the important parameters for food industry viz anthocyanin content (highest in line 24), vitamin C (highest in line 24), oxalate content (lowest in line 24 and line 18).

Calcium is found to be highest in line 24, iron highest in line CV₁ and phosphorus highest in line 24. Leaves of the line 24 was found to contain the highest level of calcium and phosphorus compared to the other lines. Therefore line 24 has very desivable characters for utilization in the food industry, particularly for its considerable amounts of phosphorus and calcium, in addition to the other previous characters such as Vitamin C, anthocyanin content, colour intensity and lower oxalate and insoluble solid contents.

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PLATES

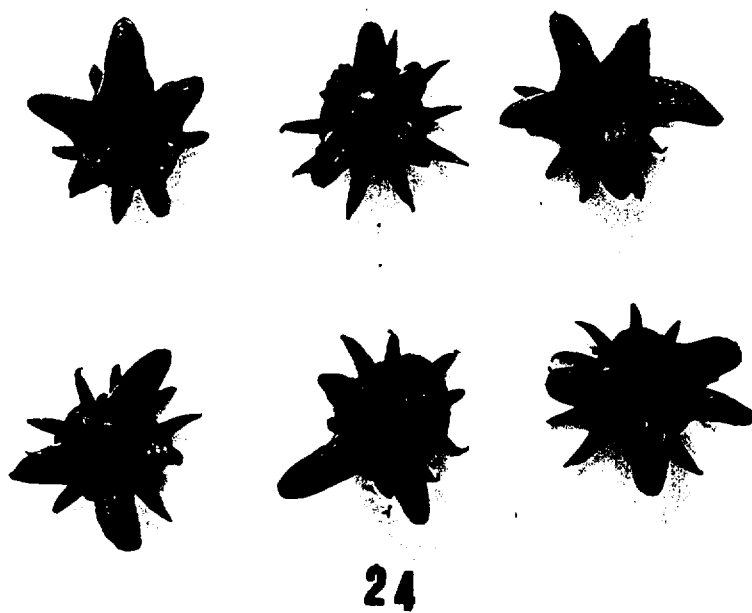


Plate 1: Roselle calices line 24

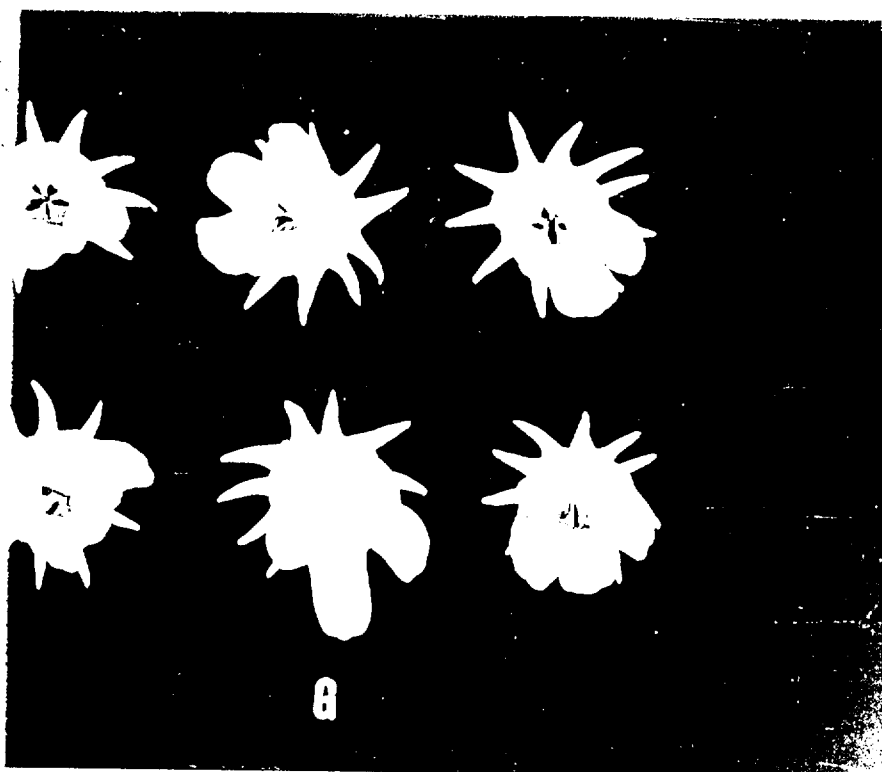


Plate 2: Roselle calices line G



Plate 3: Roselle calices line CV_1

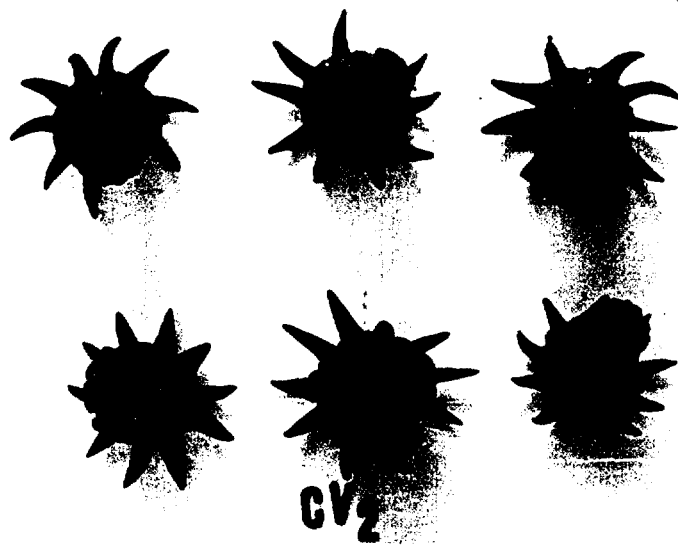


Plate 4: Roselle calices line CV_2

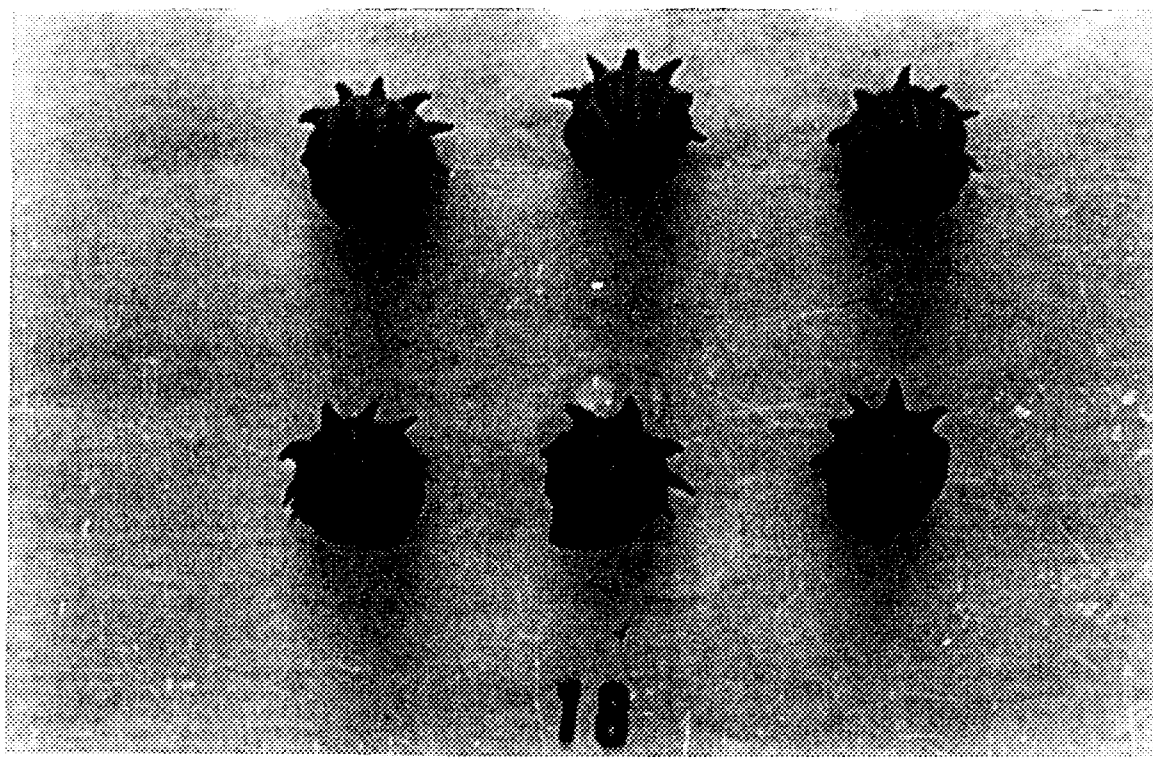


Plate 5: Roselle calices line 18

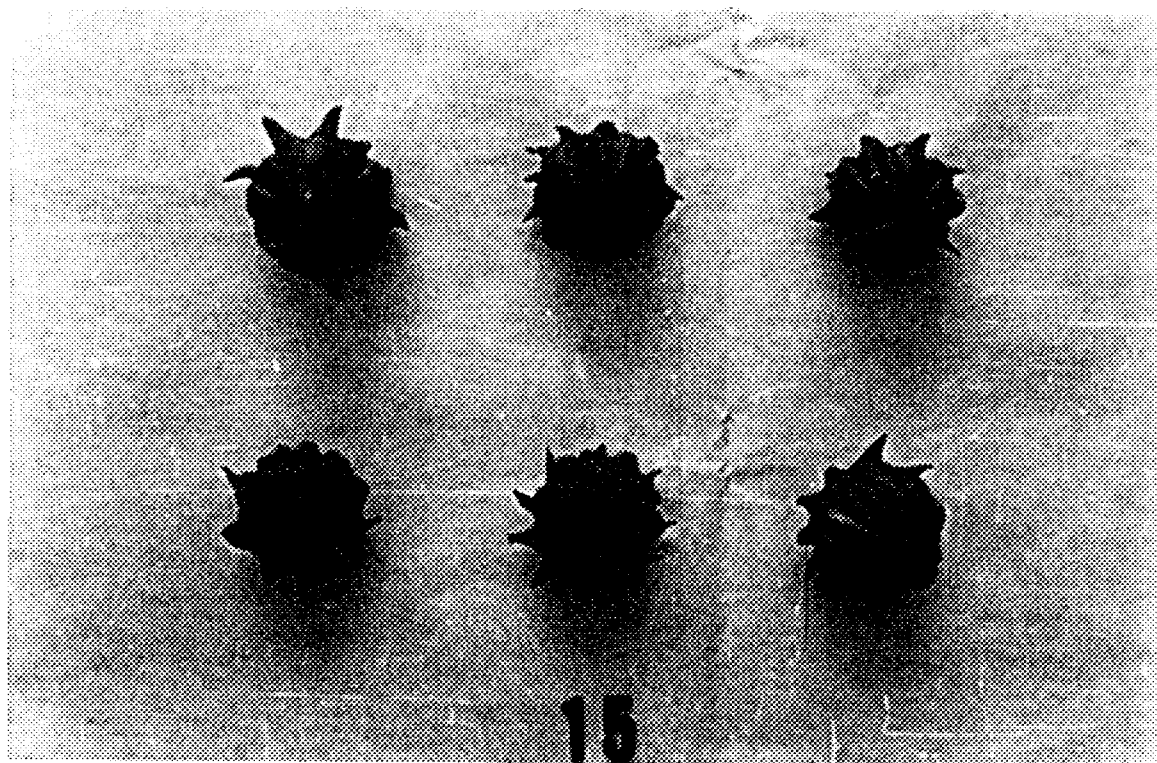


Plate 6: Roselle calices line 15