Chapter one

1. Introduction:

Roselle (Hibiscus sabdariffa) is known in the Sudan as Kerkrade orIngara. Other English names are East Indian sorrel and thorny mallow(Reaubourg and Monteux, 1940). It belongs to ـthe Family Malvaceae.Two botanical varieties are recognized: variety sabdariffa a bushybranched subshrub with red or green stems and red or pale yellowinflated edible calices; variety latissimi, a tall unbranched plant 10—16ft. high with fibrous spiny inedible calices grown for fiber (Purseglove,1968).Hibiscus sabdariffa is probably a native of West Africa and is nowwidely cultivated throughout the tropics. It has been taken to the newworld by slaveـ trade. It is confined to the tropics and tolerates poorercondition than jut .There are two main types of Kerkrade calices according to thecommercial terminology used in the Sudan, namely £1 Rashad and ElFisher qualitie, Kerkrade is grown in the Sudan as a cash crop inrain fedareas. The principal production area is in eastern Kordofanians in the area encompassing, El Raghad and Umruaba. International ElRaghad variety is preferred. It is grown on a small scale aroundEl Obied, in western Kordofan, near El Fashir, Nyala and in thesouthern Fung. Its growing period ranges from 4 to 6 months. It can either bepropagated from cutting or by direct seeding which is the best. Sudan iscategorized as one of the largest exporter of Kerkrade in the world. Significantincrease in the size of the crop has taken place during the last167,829 three decades. In 1960 454 tons were exported for the total value of Ls.67829, whilst in 1969 1,512 tons were exported worth Ls. 546,298. Atthe same time the local consumption has increased. Germany is by farthe largest importer and user of the crop (Percy, 1971).Recently, however, Kerkrade is grown as crop in some irrigatedareas, planted crop in already existing agricultural projects. Accordingto the "foreign trade statistical digest" of the bank of Sudan the quantityof exported dry Kerkrade calices amounted to 9918 tons in the year 1993at value of us 9099000. In 1994 the exported quantity amounted to14243 tons at value of us 16065000. In 1995 the

Total exported quantity amounted to 32209 tons at value of us 12947000. During January – June1996 the exported quantity amounted to 5994 tons at value of us7897000.In spite of this tremendous increase in the production of Kerkradecalices on the one hand, and the serious competition facing the Sudanese commodity in the international market on the other hand, very littlework has been done to isolate the differed type of varieties in order toselect 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 productnamely: color (anthocyanin's), flavor (organic acid) and aroma. Thered acid succulent calices of variety sabdariffa are used for producing sorrel drink. They are also made into jellies and preservatives. Thetender leaves and stalks are eaten as a salad and as a pot - herb. Varietylatissimus is grown for fiber in India, Java and the Philippines. The roothas 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 asantiscorbutic factors and is said to be soothing caught (Watt and BreyerBrandwijk, 1962).The serious problem associated with processing of Kerkrade intofood product is the instability of the brilliant red color imparted to foodby the anthocyanin pigments contained in the calyx.The present study is aimed at laying foundation for officialstandards and specification for new breeding lines of Kerkrade.

Therefore, this research was carried out under the following objectives.

- To extraction the pigments(Anthocyanin).
- To using anthocyanin as food colour.
- To study intensity of colour by using different typesof solvent.

Chapter two

2. Literature review

2.1Chemical composition of Kerkrade :

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

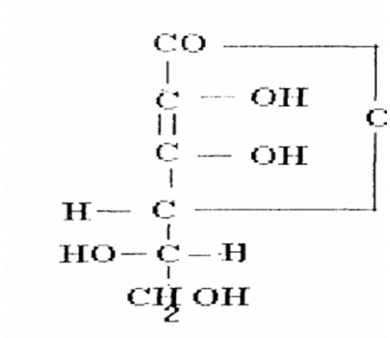
Moisture content	9 14%
Ash	7 11%
Protein	5.4 9.45%
Terrible acidity	14 18.7%

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

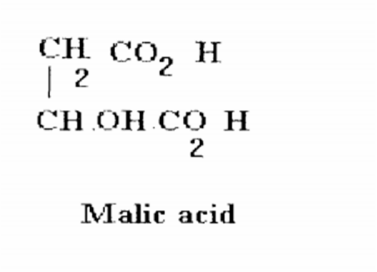
2.1.1Organic acid

Generally organic acids are water-soluble, colorless liquid orrelatively low melting solids. The majority are non-volatile. Organicacids are classified chemically according to the number of carboxylicacid groups or to other functional groups present. Some of the organic

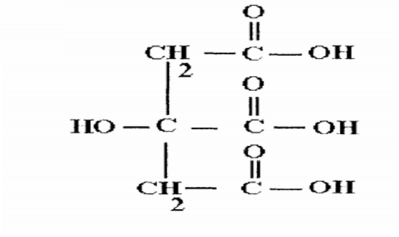
Acids shown to be present in aqueous extract of calices of Hibiscussabdariffa are the followingacids shown to be present in aqueous extract of calices of Hibiscussabdariffa are the following



Ascorbic acid



Malic acid



Citric acid

Flavor characteristics of Roselle were found to be mainly in the acid content. The presence of citric, malic, succinic, lactic, tannic andoxalic acids have all been reported in Roselle (Pritzker and Jungunz.1937). Organic acids are important constituents of food products, notonly as chelating agents for iron and copper but also for the control ofPH and inhibition of enzymes (Sistrunk and Cash, 1973). These resultswere confirmed by Brand (1939) who also detected oxalic acid found asthe calcium salt in Roselle of Egyptian origin. Ibrahim et al. (1971),working on Sudanese Roselle detected malic, oxalic, citric and 3-indolylacetic acids. Hibiscus acid in the calices was found to be very soluble inwater and alcohol and slightly soluble in ether. The amount of Hibiscusacid in plant samples analyzed by Griebel (1939) was 13.6%. Resultstands in agreement with the result obtained by Bachtez (1948) whofound 15.3% in Mexican samples and 14.6% in an Ethiopian sample.

The presence of vitamin C (L-ascorbic acid) in roselle has beenconfirmed by many analysts among them Mclean (1973) who showedthat vitamin C concentration varies from 21 - to 89.4 mg/100g in bothfresh and dried samples Ibrahim et al. (1971) reported ascorbic acid in roselle water extract with concentration of 7.12 mg/l00g. Storing dryKerkrade calices for more than six months depletes them from vitamin C(Mario 1959, Addo 1981) According to(Watt and Breyer1962) the acidsconstitute about 13% of the whole Kerkrade calices. The acids present

arecitric acid and hibiscus acid ($C_6H_6O_7$). hibiscus acid was first confusedwith citric acid because of several reactions, especially those reactionsinvolving color changes which are the same.(Griebel 1939) suggestedthat it was a lactone of hydroxyl citric acid with two asymmetric carbonatoms, with the formula ($C_6H_6O_7$). Other water soluble acids are malicacid, tartaric acid trace while oxalic, gallic, tannic and lactic acid areabsent in the aqueous extract (Reabourg and Manteaux 1940). The totalacidity in the dry calices can be expressed not only as hibiscus or citric,but also as tartaric acid (Schilcher 1976). Analysis of organic acids incalices showed their contents to range between 8.2 and 12.6% as reported by (Murkowski and Strzelecka).

2.1.2Carbohydrates

(Hamidi et al. 1966) detected galactose, galacturonic acid andrhamnose in the calices. Two other free sugars, glucose and arabinosewere also detected in water extract by(Ibrahim et al. 1971). The pectincontent 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 andcitrus. The pectin content had been found to be only 3.19% but never theless gives the plant its mucilaginous character.

2.1.3 Proteins

(Ibrahim et al. 1971) showed that the protein content of the wholecalices of Sudanese varieties ranged between 7.05 -- 9.45% and thirteenamino acids were given by the protein hydrolyzate of the whole andspent calices, while the protein hydrolyzate from the water extract gaveonly nine amino acids out of the thirteen amino acids of which six areessential. (Karamalla and Ali 1974) found that the protein content ofmature Kerkrade seed ranged from 27 — 31 %.

2.1.4 Ash

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

2.1.5 Fats

In an attempt to evaluate the local produce of Kerkrade seeds withregard to their oil and protein content Karamalla etc. on. (1973) indicatedthat the stability of the Kerkrade seed oil as compared to other edibleSudanese oils was fairly good. The seeds contain about 17% oil which issimilar in properties to cottonseed oil. Cottonseed contains up to 25%while linseed contains up to 33 — 43% (Cobley, 1956). Karamalla andAli (1974) found that the oil content of the mature seed of Kerkraderanged from 19 ~ 24%. The fatty acid composition of Roselle seed was:

Meristicacidtrace, palmitic acid 26.38%, stearic acid 4.80%, Oleic acid35.84% and linoleic acid 32.97% (Rahamma, 1979).

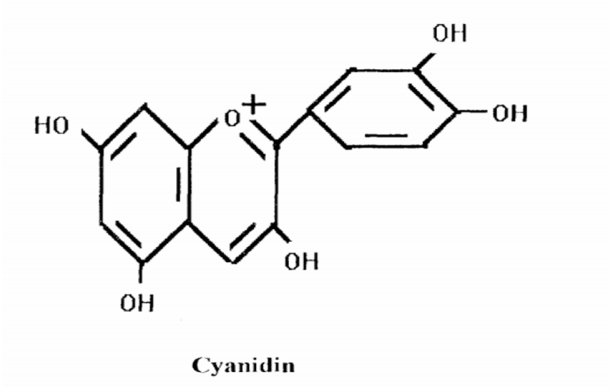
2.1.6 Tannins

Tannins are polymers of phenolic compounds with higher molecularweights (mol wt. 500 — 5000) containing sufficient phenolic hydroxylgroups to permit formation of stable crosslinks with proteins (Swain,1965).According to Tamir and Alumot (1969) tannins have the ability tobind proteins forming insoluble complexes, through the formation ofhydrogen bonds between hydroxyl groups of tannins and polyphenolsand the carbonyl groups peptide bonds of protein (Millie and Stojanovic,1972). Therefore, tannin precipitate protein from aqueous solution thusrendering plant proteins relatively indigestible and reducing enzymesactivity (Goldstein and Swain, 1965; Van Sumer et al. 1975). Thustannin areantinational factors in food and beverages. However, studyof type and quality of tannins in Kerkrade is not known up to date.

2.2 Pigments

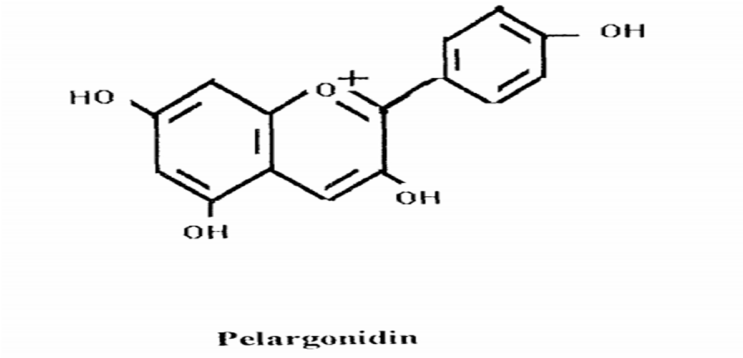
Anthocyanins are the most important and wide spread group ofcolouring materials in plants. These intensely colored water-solublepigments are known to be responsible for nearly all the pink, scarlet,red, mauve, violet and blue colors in the petals and leaves of higherplants. Anthocyaninsare glycosylated polyhydroxy and polymethoxyderivatives of 2-phenyl .

Benzopyrylium (flavylium) salts. (Broillard, 1982). Anthocyanins are all based chemically on a single aromaticstructure of 3, 5, 7, 3, 4 pent hydroxyl flavylium cation as show below

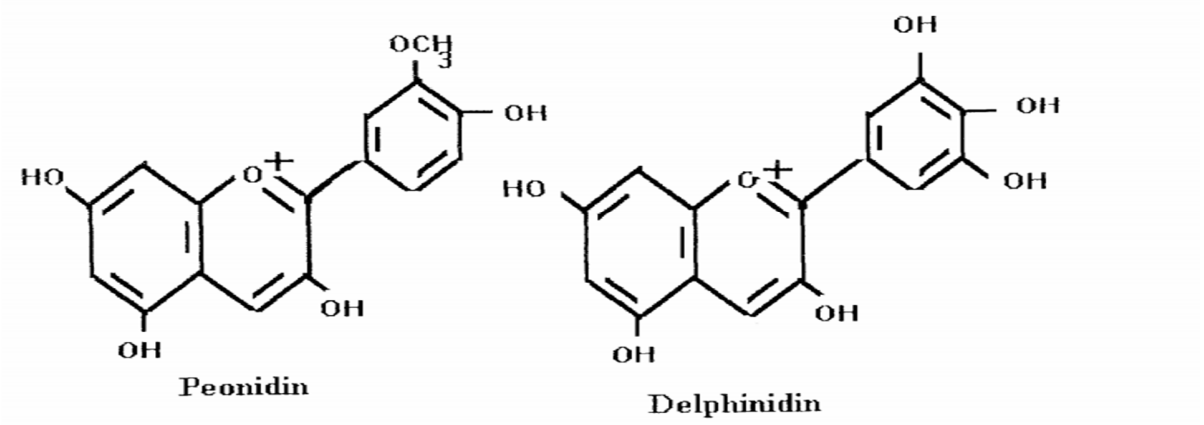


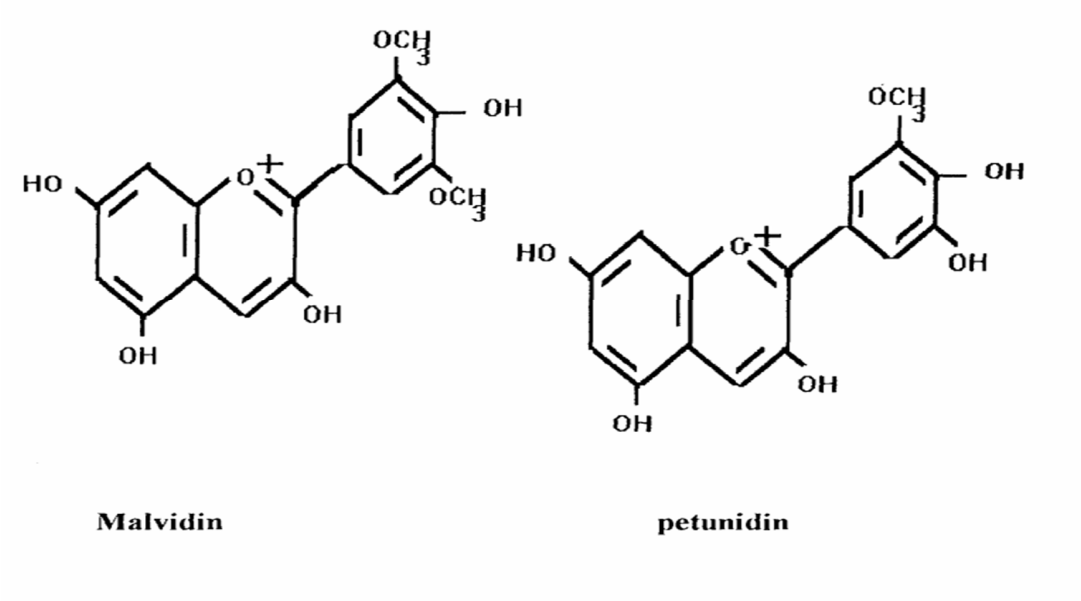
Cyanidin

In general, anthocyanin's do not accumulate in the plant (Harborne1967) and the pigments occur in flowers and fruits mainly in theglycosylated form. The most common anthocyanin's can be show asfollowing (Markakis 1982).



Pelargonidin



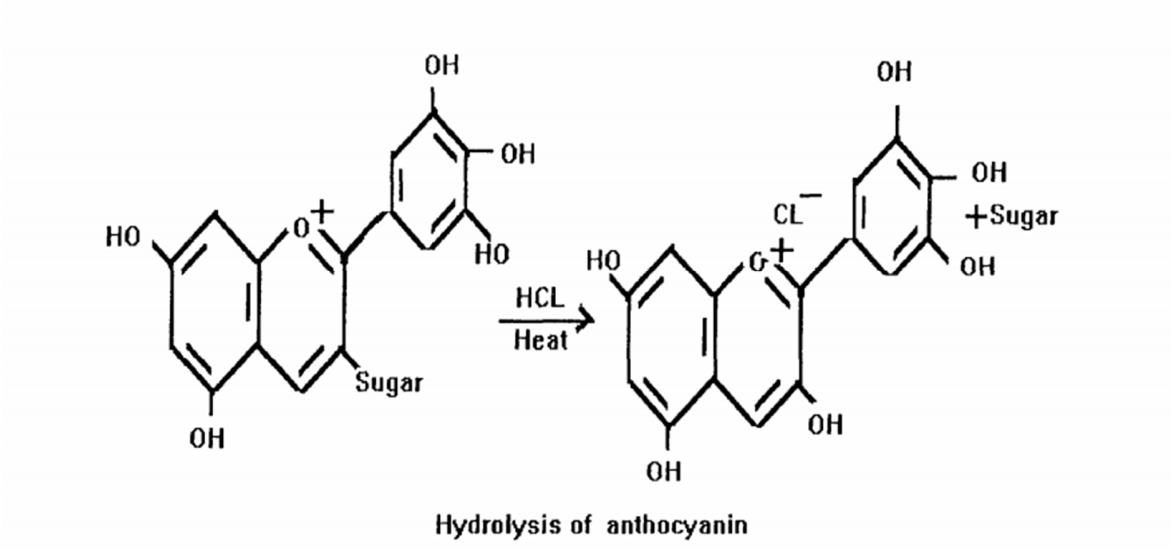


Malvidin **petunidin**

Anthocyainidins can be obtained from two different sources:

1- From the acid hydrolysis of naturally occurringcoloredglycosides (anthocyainidins).

2 - From acid treatment of colorless leucoanthocyanidins.The most common sugars associated with tannins are themonosaccharides glucose, galactose, arabinose and xylose. Diand trisaccha rides also occur. The 3-hydroxyl group (see Fig 4) is alwaysreplaced by a sugar. When A second sugar is present, it is generally atC-5 and glycosylation of 7-, 3- and 5-hydroxyl groups, however, has alsobeen demonstrated. Anthocyanins with more than two glucosidicallresidues have been identified, e.g.acrylatedelphinine 3-rutinoside 5, 3,5 triglucoside (Yoshitama, 1977) and acylated cyanidin 3, 7, 3triglucoside (Yoshitama and Abe, 1977).Often plant tissues will have a number of pigments composed ofone or more anthocyanidins differing in the sugar moiety. These oncomplete hydrolysis with mineral acid, e.g. hydrochloric acid, giveanthocyanin chlorides, as shown by the following example (Mayer,1960).



Hydrolysis of anthocyanin

(Yom moto and Oshawa1932) obtained a crystalline anthocyaninfrom Roselle which they called "hissing". They pointed out the structureto be cyaniding 3- glucoside (cy-3-g). They changed it later todelphinidin-pentoside glucoside.(Watt and Breyer 1962) also mentionedthe presence of gossipetin and hibiscus. The pigment of Rosellewasfurther examined by(Shibata and Furukawa 1969) who reported thepresence of cyanidin-3-glucoside and delphindin-3-sambubioside.(Forsyth and Simmonds 1954) also reported the presence of delphinineand cyaniding in trimidal Roselle. A more detailed work carried by(Duand Francis 1973) usingpaper chromatography revealed the presenceof more than these two pigments. Anthocyanins identified werecyanidin-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 aminor component. The most abundant anthocyanin in Roselle and thepigment responsible for its reddish violet color wasdelphindin-3-sambubioside which on complete hydrolysis gavedelphinine, glucose and xylose. Three other pigments were notidentified since they were present in trace amounts. The totalanthocyanin content was calculated as 1.5g/100g on dry weight basisexpressed in terms of delphinidin-3-glucoside.(Deibner et al. 1965)reported that inclusion of water helps the extraction of the morehydrophilic anthocyanin which are present as a minor pigment incranberries (Fuleki, 1967) and it also allows the use of ethanolrecovered by distillation. The hydrochloric acid

Stabilizes the pigmentsand lowers the pH to the level where the absorbance of anthocyanin is attheir maximum.

2.2.1 Factors affecting the stability of anthocyanin

2.2.1.1 Chemical structure

Not all anthocyaninappears to be equal in their resistance to thedegrading effects of various agents. According to(Robinson etal. 1966),the anthocyanin diglucosides of stored New York wines were morestable to decolourization than the corresponding monoglucosides.(Van Bursn et al. 1968) reported that the same diglucosides were more stableto heat and light than the monoglucosides. Starr and Francis (1968)found the galactosidic anthocyanin of cranberry juice to be more stablethan the arabinosidic ones during storage of the juice. From the work of(Hardin et al. 1970), it appears that the stability of the anthocyanin3-, 5-diglucosides of grapes increases with an increasing degree ofmethylation but decreases with an increasing hydroxylation of theaglycone.

2.2.1.2 Temperature

The effect of temperature on the stability of anthocyanins in modelsystems and in food products has been studied by many investigators(Nebesky et al, 1949, Meschter, 1953; Decareau et al. 1956; Markakis etal. 1957; Daravingas and Cain 1965; Segal and Negutz, 1969; Harzdinaet al. 1970).(Meschter1953) showed that processing strawberrypreserve at 100°C for 1 hr. resulted in 50% destruction of the fruitanthocyanin and the half-life of the pigment was hr. at 100°C. Duringstorage 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 theanthocyanin would be 11 months at O°C storage.(Segal and Negate1969) formulated a logarithmic relationship between anthocyanindestruction and temperature, whereas (Adams 1973) described asimilarrelationship between anthocyanin destruction and time of heating at aconstant temperature.

(Saeed and Ahmed 1977) studied the stability ofstored carbonated beverage from roselle. They found that the keepingquality was 10 days at ambient temperature during summer time wherethe ambient temperature reaches its maximum (45 °C).

2.2.1.3 Ascorbic acid

(Everett 1953) also pointed out the influence of ascorbic anddehydroascorbic acids on pigment degradation of strawberry productsas being directly proportional to their concentration, ascorbic acid beingmore effective and that the reaction was catalyzed by the presence of iron and copper. Iron and copper in themselves were not importantcontributes to the pigments loss mechanism, except in so far as theyhastened the destruction of ascorbic acid. These destruction products inturn attack the pigment in an increased rate.(Beattie teal. 1943) werethe first to show that changes in colour occurred with progressive lossesof ascorbic acid.

(Markakis et ah 1957) postulated that an interactionbetween ascorbic acid and anthocyanin may occur resulting in the loss ofcolour.(Sondheim and Kerecz, 1953) recognized the formation ofhydrogen peroxide from ascorbic acid oxidation, which would react with Anthocyanin to produce break-down products. They postulated twomechanisms for the pigment discoloration of strawberry juice.

2.2.1.4 PH

According to the work carried by Driving's and(Cain, 1968),raspberry anthocyanins which were cyaniding -3-diglucoside, cyanidin-3-glucoside and cyanidin-3-,5-rhamonoglucoside-5-glucoside,with cyanidin-3-diglucoside as the main component, the rate ofdegradation decreased as the pH was decreased. The maximum pigmentretention! was attained at pH 1.8 for strawberry anthocyans.

2.2.1.5 Enzymes

(Erlandson and Worlstad, 1972) worked on blanched andunbranched strawberry puree to certain the role of enzymes onanthocyanin degradation. They concluded that enzymatic degradationwas negligible. They also concluded that oxygen had little effect on anthocyanin

degradation. The pigment degradation rate was alwaysgreater in air than in nitrogen, the rate difference being small butrelatively constant.

2.2.1.6 Light

The effect of light on the colour of bottled concord grape juice wasnoticed by(Trestle and Pedersen, 1936). Van Buren et al. (1968)reported that acrylate and methylated glucosides were the most stableanthocyanins in wine exposed to light, non acylated diglucosides were

less stable, and monoglucosides even less stable.(Palamidis andMarkakis, 1975) found that light accelerated the destruction ofanthocyanins in carbonated beverage colored with anthocyanins extracted from grape pomace.

2.2.1.7 Metals

Several multivalent metal ions can interact with anthocyaninpossessing vicinal phenolic hydroxyls and shift the color of the pigmenttoward the blue end of the spectrum. The bathochromic shift caused bythe addition of $AlCL_{l3}$ is used as an analytical test in differentiating

between cyanidin, petunidin and delphinidin on the one hand, andpelargonidin and malvidin on the other hand (Harborne, 1973).

2.3 Flavonoids:

Phenolic compounds constitute one of the main classes of secondary metabolites. They display a large range of structures and they are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties and

they also contribute to the nutritional qualities of fruits and vegetables. The most importantNatural pigments are carotenoids which are tetrapyrrole derivatives of naturally occurring phenolic compounds ubiquitously distributed in plant kingdom. Among these compounds, flavonoids constitute one of the most ubiquitous groups of all plant phenolics. So far, over 8,000 varieties of flavonoids have been identified1. Until ~50 years ago, information on the working mechanisms of flavonoids was scare. But it has been widely known for centuries that compounds of plant origin possess a broad spectrum of biological activity2. In 1930, Szent-

Gyorgyi isolated a new substance from oranges and classified it as vitamin P but later, it became clear that this substance was actually a flavonoid3. Flavonoids drew greater attention from researchers with the discovery of the French Paradox, i.e., the decrease incidence of cadio-vascular disease observed in the Mediterranean population which was associated with red wine consumption, and a greater amount of saturated fat the average diet than in other countries3.

2.3.1 Structure and Classification offlavonoids:

Flavonoids occur as aglycones, glycosides and methylated derivatives4. In plants, flavonoids aglycones (i.e., flavonoids without attached sugar) occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus: two six-membered rings linked with a three carbon unit which may or may not be a part of a third ring5. For convenience, the rings are labeled A, B, and C (see Fig 1). The individual carbon atoms are based on a numbering system which uses ordinary numerals for the A and C and "primed" numerals for B-ring (1). Primed modified numbering system is not used for chalcones(2) and theyare flavones derivatives (6): the pterocarpans and the rotenoids6. The different ways to close this ring associated with the different oxidation degrees of ring A provide the various classes of flavonoids. The six-membered ring condensed with the benzene ring is either a γ-prone (flavones (1) flavones (3)) or its dihydroderivative (flavanones (4) and flavan-3-ols (5)). The position of the benzenoid substituent divides the flavonoids into two classes: flavonoids (1) (2-position) and isoflavonoids (6) (3-position). Most flavonoids occur naturally associated with sugar in conjugated form and, within any one class, may be characterized as monoglycosidic, diglycosidic, etc. The glycosidic linkage is normally located at position 3 or 7 and the carbohydrate unit can be L-rhamnose, Dglucose, glucorhamnose, galactose or arabinose

2.4 Spectroscopy Analysis:

Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain rang of wavelength. Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits. Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical

engineering, clinical applications, industrial applications, etc.). Any application that deals with chemical substances or materials can use this technique. In biochemistry, for example, it is used to determine enzyme-catalyzed reactions. In clinical applications, it is used to examine blood or

Tissues for clinical diagnosis. There are also several variations of the spectrophotometry such as atomic absorption spectrophotometry and atomic emission spectrophotometry.

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types:

• UV-visible spectrophotometer: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
• IR spectrophotometer: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed color. For instance, a solution sample that absorbs light over all visible ranges (i.e., transmits none of visible wavelengths) appears black in theory. On the other hand, if all visible wavelengths are transmitted (i.e., absorbs nothing), the solution sample appears white. If a solution sample absorbs red light (~700 nm), it appears green because green is the complementary color of red. Visible spectrophotometers, in practice, use a prism to narrow down a certain range of wavelength (to filter out other wavelengths) so that the particular beam of light is passed through a solution sample.

2.4.1UV-visible spectrophotometer

Devices and mechanism

Figure 1 illustrates the basic structure of spectrophotometers. It consists of a light source, a collimator, a monochromatic, a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a digital display or a meter. Detailed mechanism is described below. Figure 2 shows a sample spectrophotometer (Model: Spectronic 20D)

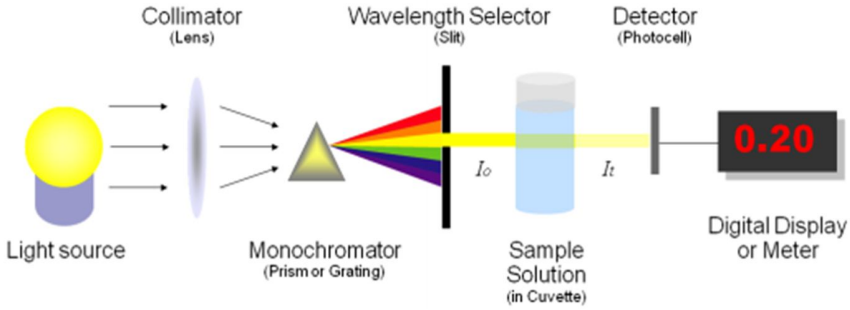


Figure 1: Basic structure of spectrophotometers (illustrated by HeesungShim)

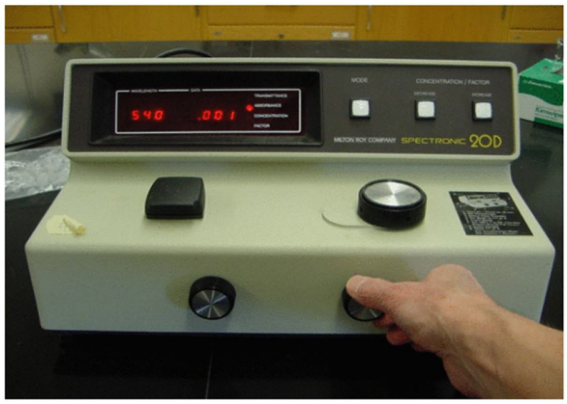
A spectrophotometer, in general, consists of two devices; a spectrometer and a photometer. A spectrometer is advice that produces, typically disperses and measures light. A photometer indicates the photoelectric detector that measures the intensity of light.

• Spectrometer: It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromatic (prism) to split it into several component wavelengths (spectrum). Then a wavelength selector (slit) transmits only the desired wavelengths, as shown in Figure 1.

• Photometer: After the desired range of wavelength of light passes through the solution of a sample in cuvette, the photometer detects the amount of photons that is absorbed and then sends a signal to a galvanometer or a digital display, as illustrated in Figure

Figure 2: A single wavelength spectrophotometer

You need a spectrometer to produce a variety of wavelengths because different compounds absorb best at different wavelengths. For example, p-nitro phenol (acid form) has the maximum absorbance at approximately 320 nm and p-nitrophenolate (basic form) absorb best at 400nm, as shown in Figure 3.



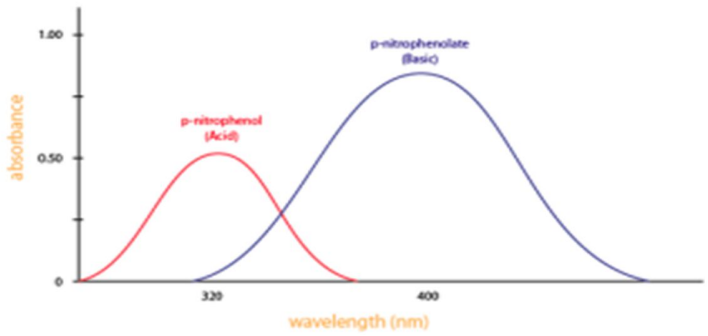


Figure 3: Absorbance of two different compounds

Looking at the graph that measures absorbance and wavelength, an isosbestic point can also be observed. An isosbestic point is the wavelength in which the absorbance's of two or more species are the same. The appearance of an isosbestic point in reaction demonstrates that an intermediate is NOT required to form a product from a reactant. Figure 4 shows an example of an isosbestic point.

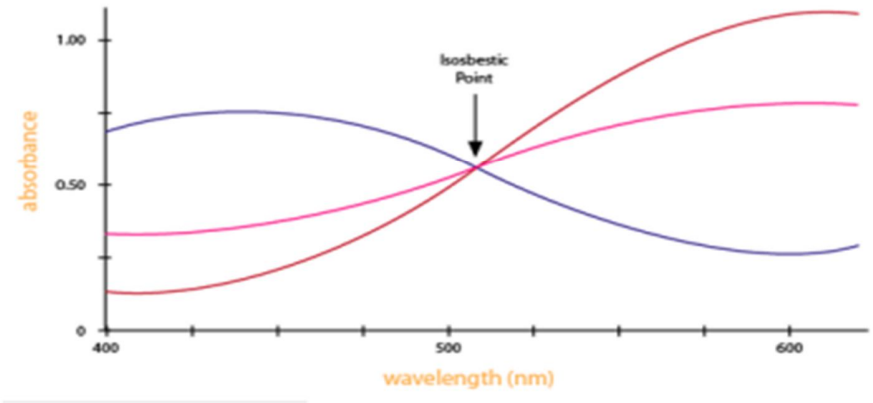


Figure 4: An example of isosbestic point

Referring back to Figure 1 (and Figure 5), the amount of photons that goes through the cuvette and into the detector is dependent on the length of the cuvette and the concentration of the sample. Once you know the intensity of light after it passes through the cuvette, you can relate it to transmittance (T). Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation: Transmittance (T) = $I\mathrm{t}/I_o$

Where it is the light intensity after the beam of light passes through the cuvette and Io is the light intensity before the beam of light passes through the cuvette. Transmittance is related to absorption by the expression:

$$\text{Absorbance (A)} = -\log(T) = -\log {}^{It}/_{Io}$$

Where absorbance stands for the amount of photons that is absorbed. With the amount of absorbance known from the above equation, you can determine the unknown concentration of the sample by using Beer-Lambert Law. Figure 5 illustrates transmittance of light through

a sample. The length (L) is used for Beer-Lambert Law described below

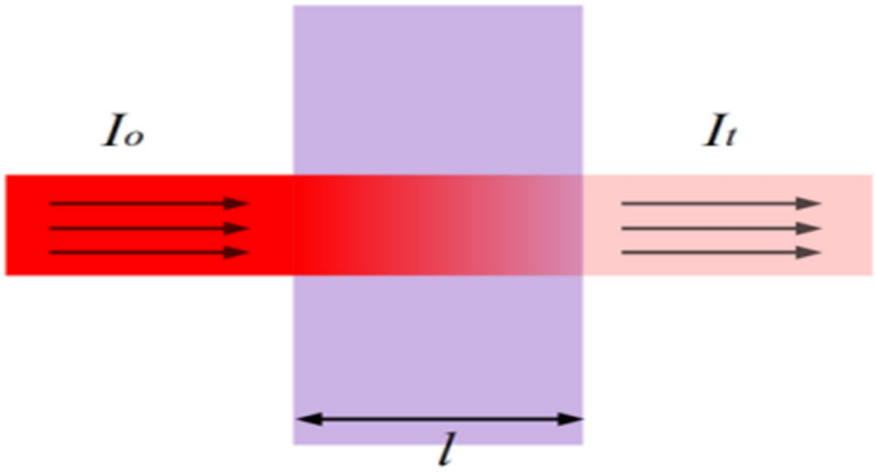


Figure 5: Transmittance (illustrated by Heesung Shim)

Beer-Lambert Law

Also known as Beer's Law states that there is a linear relationship between the absorbance and the concentration of a sample. For this reason, Beer's Law can *only* be applied when there is a linear relationship. Beer's Law is written as:

$$A = \text{epsilon } lc$$

Where

- A is the measure of absorbance (no units)
- Epsilon is the molar extinction coefficient or molar absorptivity (or absorption coefficient,
  - I is the path length,
- C is the concentration.

The molar extinction coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units for (epsilon) must cancel out the units of length and concentration. As a result, (epsilon) has the units: L·mol-1·cm-1. The path length is measured in centimeters. Because a standard spectrometer uses a cuvette that is 1 cm in width, (l) is always assumed to equal 1 cm. Since absorption, (epsilon), and path length are known, we can calculate the concentration (c) of the sample.

2.4.2Infrared (IR) Spectroscopy :

Infrared (IR) spectroscopy is a chemical analytical technique, which measures the infrared intensity versus wavelength (wavenumber) of light. Based upon the wavenumber, infrared light can be categorized as far infrared (4 ~ 400cm-1), mid infrared (400 ~ 4,000cm-1) and near infrared (4,000 ~ 14,000cm-1).

Infrared spectroscopy detects the vibration characteristics of chemical functional groups in a sample. When an infrared light interacts with the matter, chemical bonds will stretch, contract and bend. As a result, a chemical functional group tends to adsorb infrared radiation in a specific wavenumber range regardless of the structure of the rest of the molecule. For example, the C=O stretch of a carbonyl group appears at around 1700cm-1 in a variety of molecules. Hence, the correlation of the band wavenumber position with the chemical structure is used to identify a functional group in a sample.

The wave number positions where functional groups adsorb are consistent, despite the effect of temperature, pressure, sampling, or change in the molecule structure in other parts of the molecules. Thus the presence of specific functional groups can be monitored by these types of

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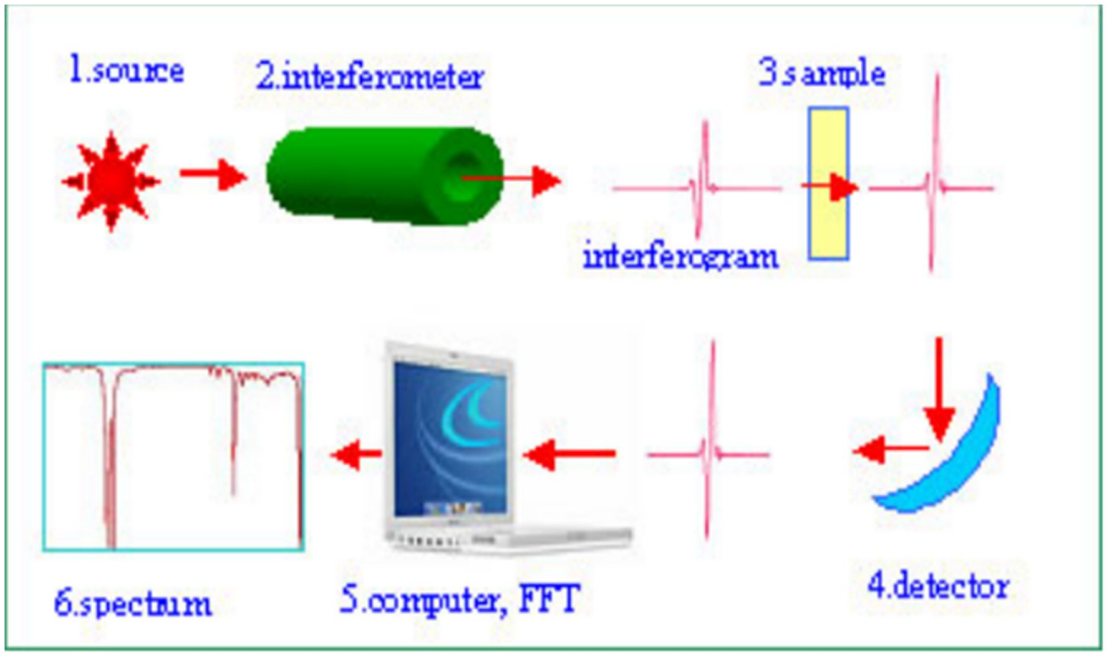
The early-stage IR instrument is of the dispersive type, which uses a prism or a grating monochromator. The dispersive instrument is characteristic of a slow scanning. A Fourier Transform Infrared (FTIR) spectrometer obtains infrared spectra by first collecting an interferogram of a sample signal with an interferometer, which measures all of infrared frequencies simultaneously. An FTIR Spectrometer acquires and digitizes the interferogram, performs the FT function, and outputs the spectrum.



Fig(6)showthe schematic illustration of IR system

An interferometer utilizes a beamsplitter to split the incoming infrared beam into two optical beams. One beam reflects off of a flat mirror which is fixed in place. Another beam reflects off of a flat mirror which travels a very short distance (typically a few millimeters) away from the b ea-msplitter. The two beams reflect off of their respective mirrors and are recombined when th ey meet together at the beamsplitter. The re-combined signal results from the "interfering" with each other Consequently, the resulting signal is called interferogram, which has every infr- ared frequency "encoded" into it When the interferogram signal is transmitted through or refl- ected off of the sample surface, the specific frequencies of energy are adsorbed by the sample due to the excited vibration of function groups in molecules The infrared signal after interactio- n with the sample is uniquely characteristic of the sampleThe beam finally arrives at the detect-

or and is measure by the detector. The detected interferogram can not be directly interpreted. t has to bedecoded withawellknown mathematical technique in term of Fourier Transformation The computer can perform the Fourier transformation calculation and present an infrared spec- trum, which plots adsorbance (or transmittance) versus wavenumber. When an interferogram

is Fourier transformed a single beam spectrum is generated A single

beam spectrum is a plot of raw detector response versus wavenumber. A single beams obtained without a sample is called a background spectrum, which is induced by the instrument and the environments Characteristic bands around 3500 cm1 and 1630 cm1 are atmospheric water vapor, and the bands at 2350 cm1 and 667 cm1 are attributed to carbon dioxide A background spectrum must always be run when analyzing samples by FTIR When an interferogram is measured with a sample and Fourier transforme_ a sample single beam spectru is obtained It looks similar to the background spectrum exce_pt that the sample peaks a re supermposed upon the instrumental and atmospheric contributions to he spectrum To elimi nate these contributio_ns, the sample single beam spectrum must be normalized against the background spectrum

Consequently, a transmittance spectruis obtained as follows.

$$\%T = \frac{Io}{I}$$

Where:

%T is transmittance; I am the intensity measured with a sample in the beam (from the sample single beam spectrum); Io is the intensity measured from the back ground spectrum The absorbance spectrum can be calculated from the transmittance spectrum using the following equation.

$$\mathbf{A = -\log T}$$

Where

A is the absorbance.

The final transmittanceabsorbance spectrum should be devoid of all instrumental and environm _ental contributions, and only present the features of the sample. If the concentrations of gases such as water vapor and carbon dioxide in the instrument are the same when the background

and sample spectra are obtaine their contributions to the spectrum will ratio out exactly and their bands will not occur If the concentrations of these gases are different when the backgroun and sample spectra are obtained, their bands will appear in the sample spectrum.

Chapter Three

3. 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

3.2 Apparatus

Spectrophotometer (UK, Genway 6505),In frared spectroscopy, Mortar,Beakers, Funnel

3.3 Method

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 followingchemical tests.

3.5Antliocyanins 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 removedof the solvent under vacuum at a temperature not exceeding 50°C, so as to minimize pigment degradation.

3.6 Absorption maximum of the pigments

For the purpose of determining the absorption maxima of the One bands, a small amount of the pigments was dissolved in 1% HCLmethanol, and developed with which solvent for 7 hrs., the red bands were eluted in methanol and their UV-visible spectra of theonebands were run on Aperkin-El mer 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.

3.7 Optical density

A solution contain in 1 gm. of Kerkrade 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 a magnetic stirrer. Extraction was allowed to take place at room temperature.

3.8Optical density by using Different solvent :

A solution containing1 gm. of Kerkrade per 100 ml distilled water and using anther different solvent (methanol, ethanol) 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 a magnetic stirrer. Extraction was allowed to take place at room temperature.

3.9 Characterization of the function group by using the Infrared spectroscopy:

A solution containing 1 gm. of Kerkrade per100ml (85methonol, 15 HCL) was using the optical density which was used to indicate theInfrared spectroscopy.

Take amount of the sample solution and then put it in the sample cell and choose the spectrum choice and give the spectrum.

Chapter four

4. Result and Discussion:

4.1 Absorption maximum of the pigments

We were found the one band 1 which it appears in range from 530 - 531.6 nm and not found band this is indictor the compound belong the group three 3 or group2 of the flavonoids also absent of conjugate system because no double bond between 2and 3 carbon atom.

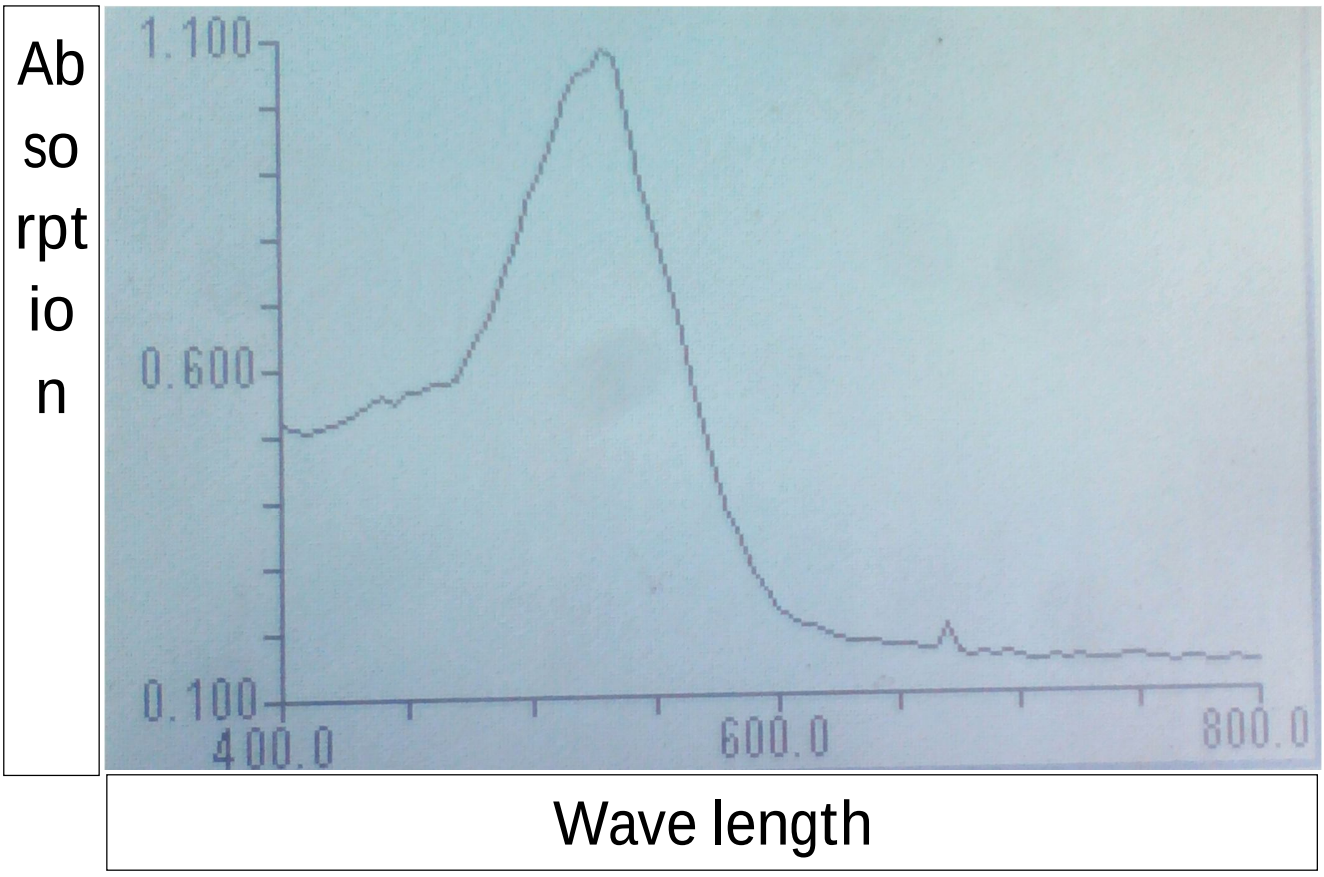


Fig (7) show UV-vis spectrometer the red band gave absorption at 530 - 531.6 nm

4.2 Colour intensity in different solvent:

In this experiment we used different type of the solvents to determine the colour intensity and the values of adsorption shown infollowing table:

Type of solvent	Wavelength nm	Value of the adsorption
water	530	2.148
methanol	530	2.390
ethanol	530	1.094

Table (1) show the Value of the adsorption by using different of solvent

3.4 Infrared (IR) Spectroscopy

Table 2 show the range of Function group in Infrared (IR) SpectroscopyWhich is show in figer 3.4. To determine exactly this compound belong to each group 2or 3 we used IR. We did not found the range from 1680 to 1880 that is indicateof absent of carbonyl group also that mean this compound belong to group 3 exactly itwas Anthocyanin.

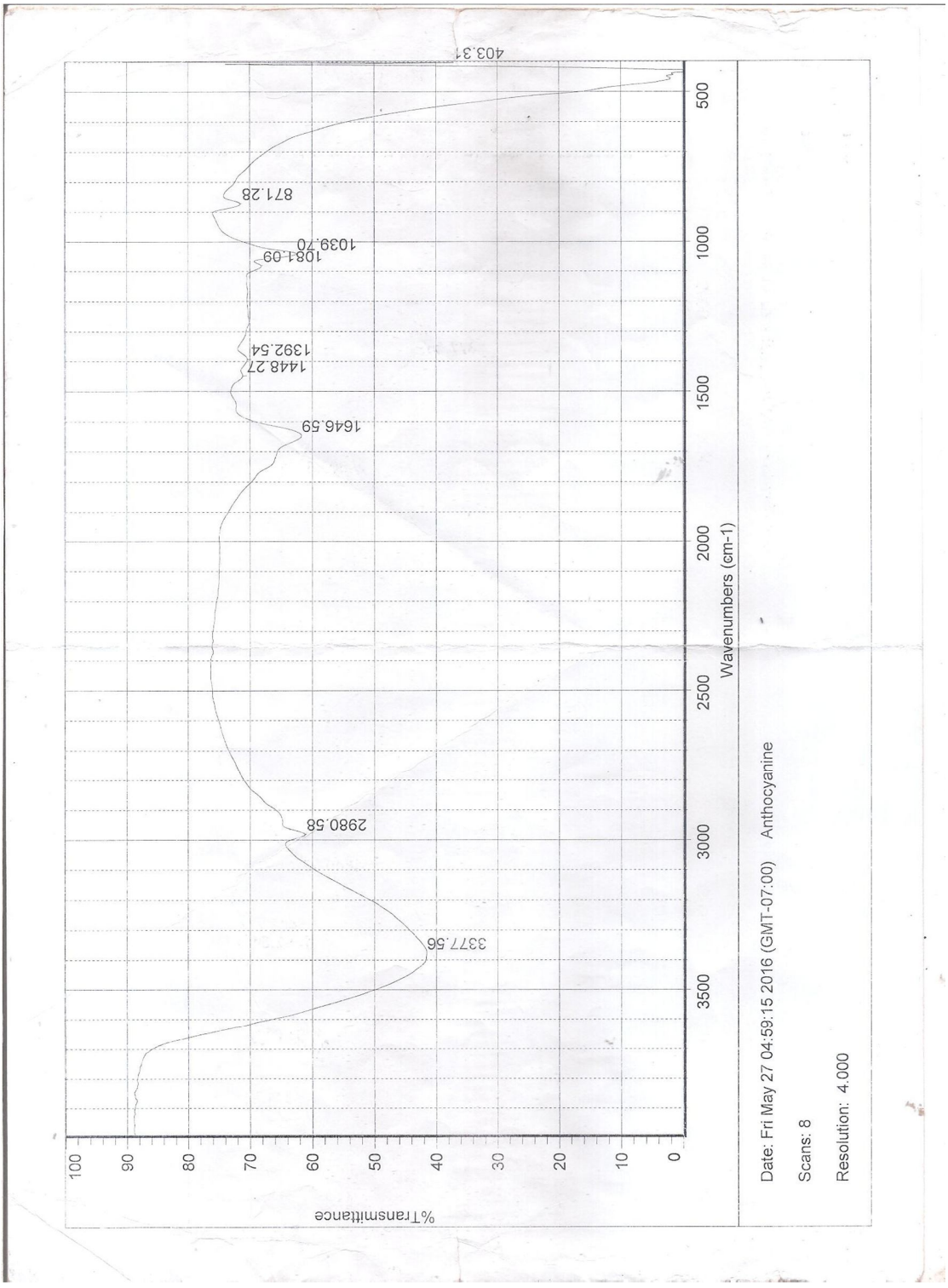
Function group	Rang
O H (s)	38000 - 2700
C—O (s)	1000 - 1100
C—H (s)	3000 - 2850
C — H (b)	1375
C =C aromatic (s)	1445 - 1500

Table 2 show the range of Function group in Infrared (IR) Spectroscopy

Where:

S stretching

B bending



Figures (8) showFunction group in Infrared (IR) Spectroscopy

Conclusion and Recommendation :

This study concluded that

- The anthocyanin has been successfully extracted from Kerkrade.
- The maximum wavelength has been measured (530).
- The colour intensity of anthocyanins has been determined.
- The functional group of the pigment has been characterized.

It is recommended that:

The anthocyanins can be used as food colour if it extracted it by safely way, it can use as cancer cell apoptosis.

Chapter Five

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