INVESTIGATING THE SUITABILITY OF *HIBISCUS SABDARIFFA* CALYX EXTRACT AS COLOURING AGENT FOR PAEDIATRIC SYRUPS

BY

GRACE FRIMPONG (MRS)

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICS, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF PHARMACY (PHARMACEUTICS)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

COLLEGE OF HEALTH SCIENCES

JULY 2008

ABSTRACT

Effects of temperature, light and pH on formulated paediatric syrups: - Paracetamol, Chloroquine phosphate, Diphenhydramine and Paediatric cough linctus, using *Hibiscus sabdariffa* calyx extract as colouring agent, were investigated.

Paediatric syrups formulated with amaranth as colouring agent, and used as a standard in the experiments, had comparable stabilities with *Hibiscus sabdariffa* at room temperature and at 37^0C

Stabilities of the paediatric syrups on exposure to light showed that syrups. Chloroquine phosphate and Diphenhydramine were stable, whether coloured with amaranth or with *Hibiscus sabdariffa* calyx extract.

Syrups Paracetamol and Paediatric Cough Linctus were unstable on exposure to light, hence the need to protect from light by use of amber bottles.

pH stability for syrups coloured with *Hibiscus sabdariffa* extract was poor compared with those coloured with amaranth. Formulations buffered at pH 5.0 showed marked stability over the four month test period.

Microbiological tests performed on *Hibiscus sabdariffa* calyx extract proved to have antimicrobial properties, but very little anti-fungal action. Methanolic extract of *H. sabdariffa* had better anti- microbial activity.

The colour value of *Hibiscus sabdariffa* extract was determined and found to be within the BP range of not less than 0.25. The colour value for *Hibicus sabdariffa* being found to be 0.26. That of amaranth was 0.46. *Hibicus sabdariffa* extract retained its colour value within BP standards for up to six months.

The macroscopic, microscopic and physico-chemical properties of both test and standard colour agents were also investigated and found to have effects on colour stability.

Colour extact from *Hibiscus sabdarrifa* calyces was found to be a suitable colouring agent for the four paediatric syrups, at room temperature and at 37^0C; and when stored in amber bottles, and buffered at pH 5.0

ACKNOWLEDGEMENTS

My profound gratitude goes to the Lord Almighty, without whom I could not have done this work. I have much pleasure in thanking my supervisor, Dr. J. Adotey, whose suggestions and very useful comments have been of much help.

Many thanks also go to my second supervisor, Dr. Dwomoh-Fokuo and to the Head of Department of Pharmaceutics: - Dr. Kwakye.

Special thanks go to Dr. Woode of the Pharmacology Department who supplied very useful information on the plant, *Hibiscus sabdariffa*.

The following are also very well appreciated: Mr. Pobee, Mr. Charles Manful, Mr. Gbedemah and Mr. Adu of the Pharmaceutics Department; Mr. Sarpeh of Exotic Gardens, Fumesua, Laura Atua and Mrs. Hannah Adzraku of the Horticultural Department, KNUST; and Mr. Rashid of the Pharmaceutical Chemistry Department GOD RICHLY BLESS YOU ALL!!!

DECLARATION

I hereby declare that this submission is my own work towards the M. Pharm degree, and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University except where due acknowledgement has been made in the text.

Grace Frimpong (Mrs)	…….	……..
20010107	Signature	Date

Certified by:

……

Supervisor

……	……	……
Head of Department	Signature	Date

DEDICATION

Dedicated to our Lovely children MARIA, ISAAC AND HANNAH FRIMPONG

TABLE OF CONTENTS

CHAPTER THREE – RESULTS, COMMENTS AND DISCUSSION

LIST OF TABLES

FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 General Introduction / Justification of Research

Colouring agents have long been used in foods, cosmetics, textiles and pharmaceutical dosage forms, in an attempt to improve the appearance of the product or material[(1)] Customers first look at the colour of a product, which more often than not gives an indication of product quality and freshness. [(2).]

Colouring agents are also used in medical preparations with the aim of improving their acceptability to patients. Such uses are however widely controlled and this has resulted in restrictions on the extent to which colouring agents may be used. The search for colouring agents[(3).] with minimal or no toxic side effects has led to the discovery of several plant parts yielding various colours for food, cosmetics, textiles and some pharmaceutical dosage forms. Plant colour has been found to contain flavanoids[(4).]. These are polyphenolic compounds that act as pigments, imparting colour, often yellow or red to flowers, fruits and at times calyces. They have a wide range of actions and many medical uses. They are antioxidant and especially useful in maintaining healthy circulation. Some flavanoids also have an anti-inflammatory, antiviral, and liver-protective activity. Flavanoids such as hesperidin and rutin, found in many plants, notably lemon *(Citrus limon)* and buckwheat *(Fagopyrum esculentum)* strengthen capillaries and prevents leakage into surrounding tissues. Isoflavanoids, found for example in red clovers (Trifidium pretense), are estrogenic and valuable in treating menopausal symptoms.

The plant *Hibiscus sabdariffa* is very popular among the peoples of Northern Ghana, where the leaves are used in soups; and calyces for soft drinks and also used medicinally. It has been found to possess several health benefits [(6).]

The calyces have a very rich red colour, which this study aims at investigating its suitability as a colouring agent in pharmaceutical syrups.

Amaranth widely used as colouring agent in pharmaceuticals has been found to be carcinogenic[(3)], If the colour extract from *Hibiscus sabdariffa* is found suitable as a pharmaceutical colouring agent, then amaranth can be replaced with this natural source of colour, promoting the health of our people.

In recent times, several colouring agents of plant origin have gained entry into International Pharmaceutical Reference Books, such as the British Pharmacopoeia, United States Pharmacopoeia and Martindale. Examples of such plants are – red rose petals, red poppy petals, red cherry, tumeric and saffron.[3]

1.1 Objective of Research

1. To identify the plant whose calyces contain the colouring agent of interest.
2. To determine the physic-chemical properties of the dried calyces of *Hibiscus sabdariffa*, using B.P. standards
3. To formulate paediatric syrups (paracetamol, chloroquine phosphate, Diphenhydramine (paediatric), Cough Lintus paediatric) with extract of *Hibiscus sabdariffa.*
4. To determine the colour value of fresh *Hibiscus sabdariffa* extract and upon storage.
5. To determine whether *Hibiscus sabdariffa* calyces has anti-microbial properties
6. To carry out temperature, light and pH stability tests on the formulations coloured with *Hibiscus sabdariffa* and with amaranth.

1.2 Scope

This would involve:-

1. Extraction of *Hibiscus sabdariffa* calyces
2. Determination of anti-microbial activity of *Hibiscus sabdariffa* extract on selected micro-organisms
3. Determination of the most appropriate concentration of extract to be used in coloring the paediatric syrups formulated.
4. Determination of maximum storage time of *Hibiscus sabdariffa* extract.
5. Determination of optimum temperature for storage of formulations coloured with *Hibiscus sabdariffa* extract.
6. Investigation of sensitivity to light of formulations coloured with *Hibiscus sabdariffa* calyx extract
7. Determination of optimum pH for formulations coloured with *Hibiscus sabdariffa* extract.

1.3 Literature Review

1.3.1 Plant Sources of Colour

Below are some examples of plant sources of colour for food, some pharmaceutical dosage forms, cosmetics, textiles and leather [3], [5].

A. FOOD COLOURANTS

i. Crocus sativus

ii. Cochlospermum tinctorium

iii. Curcuma longa

iv. Beta vulgaris var. rubra

B. MEDICINE COLOURANTS

i. Rubus idaeus

ii. Rosa gallica

iii. Prunus cerasus

iv. *Papaver rhoeas*

C. TEXTILE COLOURANTS

i. Angeissus leicarpus

ii. Bridella feruginea

iii. Jatropha curcas

iv. Psidium guajava

D. COSMETIC COLOURANTS

i. Lawsonia inermis

ii. Daniella ogea

iii. Pterocarpus evinaceaus

iv. *Stereopernium kunthianum*

E. LEATHER COLOURANTS

i. Hibiscus rosa-sinensis

ii. Adansonia digitata

iii. Acacia scorpoides

iv. Fossypium arboretum

F. POTTERY COLOURANTS

i. Bridelia micrantha

ii. Alchornia cordifolia

iii. *Rhizophora spp.*

A. Food Colourants

- ***Crocus sativus***

The common name for this plant is saffron. It belongs to the Iridaceae family. The dried stigmas and tops of the styles of this plant yields a yellow dye for food (and also for cosmetics), it is also a flavouring agent. In some circles, it is considered to be a god. It was once widely used for colouring medicines.

Description

It is a perennial plant growing to about 23cm from a bulb-like corm. It has narrow leaves and mauve to purple flowers with 3 deep red thread-like stigmas.

Habitat and Cultivation

It is native to India, the Balkans and Eastern Mediteranean. Saffron is cultivated in India, Spain, France, Italy and the Middle East. It is harvested in early autumn and then dried.

Constituents

Saffron contains a volatile oil composed of terpenes, terpene alcohols and esters. The herb also contains bitter glycosides, such as crocines and picrocine. Carotenoids and vitamins B1, and B2 are also present.

History and Folkore

In the past, saffron was credited with an immense array of health benefits. In Ancient Greece and Rome, it was used not only within medicine and cookery, but as a cosmetic dye. Saffron peaked in popularly as a medicinal herb in Europe during the late Middle Ages. An example of the herb's acclaim is provided by the herbalist Christopher Catton. "Saffron has power to quicken the spirits, and the virtue thereof pierces by and by to the heart, provoking laughter and merriment".

Medicinal uses

It has the ability to induce menstruation, treat period pain and chronic uterine bleeding. It also calms indigestion and colic. In large doses, it may induce abortion, and as such, only normal amounts used in cooking should be used during pregnancy.

- ***Cochlospermum tinctorium***

Some local names of the plant are: kokrosabia (Ashanti); Kakalito (Ewe), Bole (Chumbulu). Biberetugu or Bibere tutugu (Dagbani). It belongs to the Cochlospermaceae family.

Habitat

Savannah woodland:- it appears to thrive on rocky and stony soil. Localities, include Nsuta, near Mampong in Ashanti, Northern territories such as Navrongo and Yendi. Its distribution is from Senegal to Chad region, Sudan and Uganda.

Description

A shrub, up to 30 in, high, with a large irregular root from which flower – stalks arise when the plant is leafless. The leaves are very variable, up to 4 in long, more or less deeply 3-5 digitately lobed, becoming bronzed. Flowers appear around October and are large, golden yellow, common appearing near ground after annual bush fires and before stems appear. The fruits are oboviod, about 3 in long, with 3-5 placentas, containing numerous seeds covered with closely adhering cotton – like hairs, to help wind pollination.

Uses

The root is used to flavour as well as colour the oil in which food is cooked. The roots have been found to contain sugar, tannins and an alkaloid.

Other uses include the floss being used for stuffing pillows.

The yellow dye from the roots is also used to dye cloth, thread, leather, mats, and to colour shea butter. It stains the mouth. The bark affords a useful fibre.

Medicinal Properties

The root, mixed with shea butter and other oils, is applied for burns. A root decoction or infusion is taken with other herbs for stomach troubles and urethral discharges.

The Moshi of Upper Ivory Coast use the roots for indigestion and stomach pains. Local applications of the powdered root in water or millet – beer are used to cure bites of poisonous snakes. The root decoction is also drunk for worms, and fevers in general.

- ***Curcuma longa***

The common name for this plants is Turmeric, or Natural Yellow. It belongs to the family Zingibearceae.

Description

A perennial reaching 90cm (3ft), with a short stem, lanceshaped leaves and a knobby rhizome, whose bright yellow colour is used as a spice, and for colouring food.

Habitat and Cultivation

Turmeric is native to India and Southern Asia, and is cultivated throughout Southern and Eastern Asia. It is propagated by cuttings from the root, and needs well-drained soil and a humid climate. The rhizome is unearthed in winter.

Key Constituents

Turmeric contains volatile oil (3-5%), including zingiberen and turmerone. It also contains curcumin, bitter principles and resin.

Research

Despite its longstanding use in India and China, the therapeutic actions of turmeric were not researched until recent decades when there was an upsurge of interest foods and medicines that lower cholesterol levels or have antioxidant properties (i.e neutralize harmful free radicals). Research since the early 1970's mainly in India, has confirmed turmeric's traditional actions and revealed potential new uses for it.

Medicinal Properties

During the last two decades, turmeric's ancient use a treatment for digestive and liver problems has been largely confirmed by scientific research. The herb has also been shown to inhibit blood clotting, relieve inflammatory conditions and help lower cholesterol levels. Turmeric is a powerful anti-inflammatory agent. It has an even stronger action than hydrocortisone according to research studies conducted between 1971 and 1991.

When applied to the skin and exposed to sunlight, turmeric is strongly antibacterial. Curcumin is the constituent responsible for this action. Curcumin is also more strongly antioxidant than vitamin E.

Chinese clinical trails in 1987 indicate that turmeric lowers cholestoral levels. Turmeric may be a valuable preventive remedy for those at risk of developing cancer; but more research work is needed.

Research has shown that turmeric has anti-coagulant action keeping the blood thin. It also increases bile production and flow. It has a protective action on the stomach and liver, Turmeric improves the action of the liver and is a traditional remedy for jaundice in both Ayurvedic and Chinese herbal medicine.

It is also an ancient herb for digestive problems such as gastritis and acidity, helping to increase mucous production and protect the stomach.

The herb alleviates nausea. Even though turmeric does not relieve pain, its anti-inflammatory action makes it useful for arthritis and other inflammatory conditions such as asthma and eczema.

Due to its anti-inflammatory, blood-thinning and cholestoral – lowering properties, turmeric is now used to reduce the risk of strokes and heart attacks.
Applied to the skin, turmeric is useful in treating a number of conditions, including psoriasis, and fungal infections such as athelete's foot.

- ***Beta vulgaris* var, rubra**

The common name for this plant is Red Beet or Betroot Red, it belongs to the family Chemopodiaceae.

Description

It is a perennial, with swollen edible red root, upright shoots, large deep green leaves, tinged with red spikes and green-petalled flowers.

Habitat and Cultivation

Sea beat, (the wild sub-species) is native to Coastal regions of Europe, North Africa and Asia from Turkey to the East Indies. The red variety (var rubra) are cultivated world wide as a vegetable, while the white beet is also extensively cultivated as a source of sugar.

Part Used:- The Root,

Constituents

Red beet contains betanin – an anthrocyanin, similar to those found in red wine which is partly responsible for red beet's immune – enhancing effect (The white beet contains bataine, which promotes the regeneration of liver cells and the metabolism of fat cells).

History and Folklore

Dioscondes, in the Materia medica of the 1st century AD, recommends the following prescription for clearing the head and relieving ear ache:- "mix beet root juice with honey and sniff it up the nose". The herbalist also notes that a decoction of the leaves and roots removes dandruff and nits (eggs of lice). Sugar was first extracted from white beet in 1760 by the Berlin apothecary Margraff.

Medical actions and uses

Red beet 'juice is used in colouring food. The juice is also thought to stimulate the immune system. However, it must be taken in very large quantities – at least a litre a day. Red beet juice is prescribed by herbalists as part of a cancer – treatment regime. (White beet acts, to support the liver, bile ducts and gall bladder, influencing fat metabolism and helping to lower blood fat levels). The red dye obtained from the root of Red beet is used in colouring food, as well cosmetics.

B. MEDICINE COLOURANTS

- *Rubus idaeus*

The common name for this plant is Raspberry. It belongs to the family Rosacea.

Description: It is deciduous shrub growing to 2m (6feet). It has woody stems with pickles, pale green leaves with 3-7 leaflets, white flowers and red berries.

Habitat and Cultivation

The plant is native to Europe and Asia. It grows wild and is cultivated in many temperate regions. The leaves are collected in early summer; and the fruit when ripe in summer.

Parts used:- leaves and fruit

Constituents: - Raspberry leaves contain polypeptides, flavanoids and tannins. The fruit contains pectin, fruit sugars, fruit acids and vitamins A, B1' and C.

History and Folklore: In 1735, the Irish herbalist K' Eogh described uses for raspberry a such:- An application of the flower brushed with honey is beneficial for inflammation of the eyes, burning fever and boils. The fruit is good for the heart and disease of the mouth".

Medicinal Actions and Uses

Raspberry leaves are mainly used to encourage easy labour. While the specific mode of action is unknown, the leaves are thought to strengthen the longitudinal muscles of the uterus, increasing the force of contractions and thereby hastening childbirth, a decoction of raspberry leaves may be used to relieve diarrhea. The leaves also find use as an astringent; external remedy – as an eyewash for conjunctivitis, a mouth wash for mouth problems, or a lotion for ulcers, wounds or excessive vaginal discharge. Caution however should be taken in its use, during the early stages of pregnancy.

The Argentinian Pharmacopoeia includes concentrated Raspberry juice and Raspberry syrup for colouring medicines. It may also be used to colour food stuffs.

- ***Rosa gallica***

The common name is Rose. It belongs to the family Rosacea.

There are two main varieties: - The white and red varieties.

Description:- It is a deciduous shrub growing to about 1.5m (5ft). It has a smooth stem, sharp thorns, serrated leaves with 2.3 pairs of leaflets and scarlet tips.

Habitat and Cultivation

Native to the Middle East, the rose is not found in the wild except as a garden escape. It has been cultivated for at least 3,000 years. The flowers are gathered in summer.

Parts Used: Flowers

Constituents

Rose contains a volatile oil consisting of geranoil, nerol, citronellol, geranic acid and other terpenes, and man y other substances.

History and Folklore

The rose comes originally from Iran and has been cultivated since antiquity. Sappho[(4).], the 6th century BC Greek poet, described the red rose as the "Queen of Flowers". In Rome, it was much used in festivities and the petals were consumed as food. Rosewater was prepared by the Arab physician Avicenna (AD 980-1037). During the Middle Ages and the Renaissance, the rose was esteemed as a remedy for depression.

Medicinal Actions and Uses

The rose is currently used in herbal medicine, but it is probably time for a re-evaluation of its medicinal benefits. The essential oil, called "attar of rose" is used in aromatherapy as a mild sedative, antidepressant and anti-inflammatory remedy[(4).].

Preparations from Rose petals reduce high cholestoral levels, when taken internally. Rose petals have been employed usually as an acid infusion as a colouring agent. Rosewater is mildly astringent and makes a valuable lotion for inflammatory sore eyes.

- ***Prunus cerasus*:** The common name for this plant is cherry, of the family Rosaceae.

Description: A beautiful tree with brown bark which is smooth when young and rough when old. It has oval-shaped, toothed leaves, white flowers, and fruits whose colour varies from light red to dark purple.

Parts Used: The fruits and the stalks (peduncles). The fruit yields a red dye

Habitat and Cultivation

All the several and varieties of cherry trees, or better said cultivated cherry trees, come from the wild cherry tree (*Prunus arium*) which is native to Asia Minor.

Cultivated cherries are usually less sour than wild ones, thus fulfilling the palate of people accustomed to mildly intense, sweet flavours of Western diet. However, cultivated cherries

contain a smaller proportion of medicinally-active components than wild ones. Both varieties, nevertheless can be referred to as true medicinal food.

The cherry tree (Prunus cerasus) renders a highly desirable fruit used in confectionary. In Great Britain and North America, a hybrid species known as Dukecherry (*Prunus gonduouini*) is cultivated; because it is sweeter.

Constitutions and Medicinal Uses

Cherries contain a balanced combination of active components which make this fruit an excellent, medicine and food.

Sugars: The sugar in cherry fruit is easily metabolized even by diabetic patients. The proportion varies in a range of 3-15%.

Vitamins – Carotene (provitamin A) is present in measurable amounts, as well as vitamin B and Vitamin C.

Minerals – Present are Iron, calcium, phosphorous, sulphur, sodium and potassium. Trace elements such as zinc, copper, manganese, and cobalt, give cherries their invigorating properties.

Natural acids – Malic, succinic and citric acids are present. The acid flavour of cherries depends on the percentage of these acids, which act as stimulants on the digestive glands, and as a blood depurative.

Soluble bran – Cherries contain small amounts of bran , which gives cherries mild laxative properties.

Flavavoids – They make cherries mildly diuretic.

Salicylic acid – This is present in small quantities, and may be responsible for cherries' mild anti-inflammatory and anti-arthritic properties.

The peduncles of the fruits (cherry stalks) contain mineral salts (mainly potassium salts) and flavanoids. These components may be responsible for the diuretic properties.

Cherries have the ability to decrease the sensation of hunger, thus helping obese people to lose weight. Cherries are very useful in the treatment of gout and arthritis due to their ability to decrease uric acid levels in the blood.

Inflammation of the urinary pathways (pyelonephritis or cystitis), caused by chronic infection can be treated with cherries. Cherries may be used to treat chronic constipation, caused by intestinal atony, since they have a mild laxative and invigorating effect on the digestive system.

Papaver rhoes

The common name is Red poppy. It belongs to the Papaveraceae family.

Description

It is a delicate hairy-stemmed annual, growing to about 90cm (3ft). It has lance – shaped leaves. Its flowers are 4-petalled and red, with black anthers and small rounded seed capsules.

Habitat and Cultivation

Red poppy is native to Europe, North Africa and temperature regions of Asia, and is naturalized in North and South America. It thrives on cultivated land and on road sides.

Constituents

Red poppy contain alkaloids including papaverine, rhoeadine, isorhoeadine, meconic acid and mokocyanin. Mucilage and tannnis are also present. The alkaloids are similar to those in the opium poppy (*Papaver somniferum*), but are much milder.

Parts used – Flowers which yield a red dye.

History and Folkore

Agnus Castus[(4).], wrote in the 14th century records that, “if a man has any pain about his eye or has a migraine, he should take this herb, meddle it with oil and anoint the forehead, it shall amend the pain and destroy the migraine”.

Medicinal Actions and Uses

Red poppy flowers are mildly analgesic and sedative, and have long been used in European herbal medicine, particularly for ailments in children and the elderly. Chiefly employed as a mild pain reliever and as a treatment for irritable coughs, red poppy also helps reduce nervous overactivity. The herb may be used in the treatment of insomnia, general irritability, coughs – especially paroxysmal coughs – and asthma.

Red poppy petal has been used as colouring agent, usually in the form of a syrup. It appears in the following pharmacopoeias:- Argentina, Belgium, France, Portugal and Spain. [4].

C. TEXTILE COLOURANTS

- ***Anogeissus leiocarpus***

The leaves of this plant yields a yellow dye. It belongs to the Combretaceae family. Some local names include: Kwawu – Osakanea (desert lamp), Ga: Sakane, Ewe – Tsetse, Dagbani – Kankanli.

Habitat – It is common is Savannah and fringing forests, frequently gregarious.

Localities – The plant can be found in the drier parts of the Volta River area; It can also found in Ashanti and Northern Territories.

Its distribution is from Senegal to Camerooms, Sudan and Upper Nile Region; and also found in Abyssinea and Eritrea.

Description – A tall tree of graceful habit, up to 70 feet high; girth 8ft at breast height, bark grey turning to blackish later, slash yellow and streaky exuding a dark – coloured gum, twigs delicate and drooping; leaves up to 3 x 1½ in, elliptic to ovate – lanceolate, often eaten by caterpillars, and downy when young. The flowers which appear in (Feb, August – September) are creamy white, in balls; fruits small, cone-like, readily breaking up into numerous seeds with 2-winged appendages.

Some Medicinal Properties and Uses

The pulped roots are applied to wounds in the Ivory Coast. A bark infusion is applied to sores on the feet. The powdered bark is rubbed on the gums to relieve tooth ache. A decoction of the young bark is used for bathing syphilitic sores and ulcers. The bark in warm lotions and infusions is used in Guinea as a febrifuge.

A yellow dye is obtained from the leaves, and is used for dyeing textiles.

- ***Bridellia ferruginea***

The bark of this plant yields black, orange, red and red – brown dyes for cotton and wool. It belongs to the Euphobiaceae family.

Some local names include:

Twi – Pam fufuo Ga – Flatso Baule – Sea

Its main habitat is in the Savannah forest and open coastal plains; sometimes on rocky soil. It is distributed from Guinea to Angola, British Congo, Belgium Congo, Sudan to East Africa and Rhodesia.

Description

It is a shrub or a small straggly tree up to 45 feet high and 5½ feet girth. The young stems are thorny and rather zig-zag. The bark is grey and scaly. Leaves are up to 4inches by 2inches and are broadly elliptic with undulate margins. The lower surfaces of the leaves are rusty pubescent, with 6-8 prominent nerves.

The flowers which appear between February and June are minute, greenish yellow or brownish white, in small sessile cluster. Male and female flowers are on the same tree. The fruits which mature around June are about 1/3 inches long; ovoid and usually turn black.

Medicinal Uses:

The root decoction, drunk in the Ivory Coast is diuretic and is also used for treating gonorrhea Portions of the bark with pickles attached are used in the preparation of a popular mouth wash and remedy for thrush in children.

The root bark is used in Togo for intestinal and bladder troubles, and externally for skin diseases and eruptions. The bark is used in Northern Nigeria as an antidote to arrow poison. In the Ivory Coast, the pulped bark, with water, is taken as a beverage or enema for dysentery and diarrhea. A leaf decoction, is used for fevers or as a local application for oedemas. The leaves, boiled with rusty iron and some shea butter, yields a black dye for dying “adinkra” cloths.

- ***Jatropha curcas***

The common name for this plant is physic Nut. Family: Euphobiaceae. Some local names are: Twi-Abortoto, Fante-Adaadze, Ga-Kplukatzo.

Habitat / Distribution

The plant is commonly grown for hedges and fences. It is commonly found in the Afram plains, Cape Coast, and Axim. It originated from America, but is now widely spread in the tropics. It was brought to Africa and cultivated by the Portuguese.

Description

Its is a short or a small tree, up to 20ft high. The branclets are thick and glabrous. The sap is viscid and sometimes becomes red and gummy. The leaves are 5-lobed or entire; margins undulating. The stipules are very small. Flowers, which appear in April / May are yellowish-green. The fruits are about 1inch long, and black. It is ellipsoid and scarcely lobed. The seeds, present in threes, is rich in oil.

Medicinal Actions and Uses

A root decoction cooked with flour is given for dysentery or with a leaf decoction and natron is used for gonorrhea. The dried and powdered root-bark is applied to sores, and with Guinea grains is rubbed on the gums to relieve spasms of infantile tetanus. The root decoction is used to stop incontinence.

The Anyis of Ivory Coast make suppositories with the root-pulp, adding Xylopia fruits. This forms a well-known remedy for dysentery.

The leaves give a grey dye for cloth. The bark gives a dark-blue dye also for cloth.

- ***Psidium guajava***

The common name is Guava, some local names are: Twi-Oguawa, Fante-Eguaba Ga. It belongs to the Myrtaceae family.

Habitat and Distribution

It is a native of Tropical America, now inter-tropical planted as a fruit tree in West Africa. It grows wild in Ghana.

Description

A small tree up to 30feet high-The bark peels off in stripes, leaving trunk and branches bare. The leaves are up to 4 x 1½ inches and opposite. The leaf veins are prominent on the lower surface: Flowers, which appear in May are white and singly on short stalks in leaf – axils. The sepals are green, in fours and persistent. The fruit is up to 4 inches long, fleshy, globose, ovoid

or pear-shaped. It is generally yellowish; the flesh varying from yellowish white to deep pink. The seeds are numerous, flattened, and kidney-shaped.

Cultivation

The tree may be propagated from seeds, cuttings and suckers, and bears fruit in 2-3 years. It grows in well-drained soil with a moderate rainfall, and can endure considerable drought.

Medicinal Actions and Uses

The pounded roots, with water, are used for diarrhoea and dysentery. The roots and bark are astringent. The strongly scented leaves are chewed to relieve toothache, and a leaf infusion is widely used for stomach complaints.

A leaf decoction, boiled with lemon grass, is taken for coughs. The ripe fruits are laxative. The bark has been found to contain 11-30 percent of tannin. The leaves have also been used for local tanning in India. A black dye obtained from the bark and leaves is used in dyeing silk.

COSMESTIC COLORANTS

- ***Lawsonia inermis***

This plant belongs to the Lythraceae family; its common name is Henna, or Egyptian privet. Dagbani name: Zabela, Hausa: - Lalle.

Description

A sweetly scented evergreen short or small tree crowing to 6m (20ft). It has narrow pointed leaves, clusters of small white or pink flowers and very sweet – scented and blue – black berries. The leaves are opposite, glabrous, up to 1½ inches by nearly linch.

Habitat and Cultivation

The plant is native to the Middle East, North Africa and the Indian sub-continent. It grows, naturally, but is cultivated in Ghana as a dye plant. It is distributed throughout Tropical and Northern Africa, Madagascar, Tropical Asia and Australia.

Parts used – Roots, leaves and bark – It is the leaves that yield a red dye for the hair, body and textiles. Constituents include coumarins, naphthaquinones (including lawsone), flavanoids, sterols and tannins.

History and Folkore

Henna has been used for thousands of years in North Africa and Asia as a red dye and as a scent. Mummies were wrapped in henna-dyed cloth in ancient Egypt. In Arabia and India, the leaves have traditionally been used to make a pigment for dyeing intricate linear patterns on the fingers, palms and feet. The leaves have also been used to dye not only human hair but the manes and tails of horses. Before meeting Antony, Cleopatra reputedly soaked the sails of her barge in heady henna flower oil. Wool, silk and even leather have been coloured with Henna.

Medical Actions and Uses

The fresh leaves with limejuice are used for inflamed bruises and swellings in man and animals, for skin diseases and leprosy. It may also be painted on the body for fever, especially in children. A root decoction, often with prepared indigo, is used as a powerful abortifacient by the Hausas. The root and leaves are said to be effective as emanogogues and anthelmintics. A bark decoction is used by Arabs for treating jaundice, urinary calculi and nervous symptoms attributed to the spinal cord.

- ***Daniellia ogea***

The common name for this plant is Gum Copal tree. Some local names are: Twi-Ehyedua, Fanti-Siadua; Nzema-Eyele; Ga-Ohe.

Habitat – The plant is found in evergreen and Deciduous Forest and in swampy areas.

Description

It is a large tree up to 180 feet high, with short rounded buttresses, usually evergreen but often deciduous in Ghana. The bark is grey and smooth with raised rings. The leaves are pinnate, leaftlets 7-8, and are large and leathery. They are gland-dotted, sharply acuminate, oblong-lanceolate. They are reddish-brown when young. The flowers (which appear between August and December) are purple or violet. The pods are flat, long, and obliquely, oblong. The seeds are flat and brown, about 1 x ½ inch in size.

Cosmetic Usage – The gum, burned and mixed with soot and oil, is used for tattooing. The gum, mixed with other; ingredients is rubbed on the skin as a perfume.

LEATHER COLORANTS

- ***Hibiscus rosa – sinensis***

The common name for this plant is Garden Hibiscus or shoe flower.

Description

It is a shrub, 6-12 feet high. The leaves are ovate, with toothed margins. The stipules are small. The flowers are large and red of 3inches in diameter. Seeds are seldom produced under cultivation except in very dry conditions.

It is a native of China, where horticultural races of it were cultivated before the arrival of Europeans. It is now found in all parts of the tropics, and is commonly grown in gardens for decorative purposes. The name Shoe-Flower arose because in India and China, the petals are used to blacken shoes. Eyebrows are also blackened with the juice. Certain, Northerners in Ghana eat the young leaves as spinach. In East Asia, this plant has many medicinal uses.

- ***Adansonia digitata***

The common name for this plant is Baobab or Monkey Bread Tree, or Cream of Tartar tree. Some local names are: Twi-Odadae or ototowaa; Ewe – Adido, Ashanti – Efoobrodedwo; Krobo – Saletso.

Habitat – It is found in dry coastal regions, in Savannah Forest and in open country, often near villages. It is distributed in the Tropics, and sub-tropical Africa.

Description

It is a large tree, up to about 60feet high. It is slow-growing, specimens being usually of great age. Girth is great, up to 60ft. the branches are short and stumpy with very few leaves. Its gouty appearance is characteristic. The bark is often purplish, exuding a gum. Its leaves are digitately 5-foliate leaflets, acuminate, up to 5 x 2 inches. Flowers which appear between May and July are large, white, leathery and pendulous on long stalks. The fruits are up to 9inches and covered with green – brown hairs. They are oblong – ellipsoid to globose, full of round black seeds in whit acid pulp. The bark contains tannin used in tanning leather.

Medicinal Uses:

The bark contains the active principles adansonin, and is known in French Antilles and in India as a quinine substitute in periodic fever. It has been used in South Africa medicinally. Adansonin, which is an alkaloid, is said to be an effective antidote to strophanthus arrow poison.

The dried leaf powder decoction is used internally or applied externally for inflammation, to prevent fever, for dysentery and genitor – urinary conditions.

POTTERY COLORANTS

- ***Bridelia micrantha***

Some local names for this plant are: Opam, Apakyisie, Ewe-Mible, Sissala:- Wallin jang or warrin jung.

Habitat: - It is usually found in Savannah, Fringing and Secondary Closed Forest, e.g. on the Akwapim and Kwahu hills.

Its distribution is from Senegal to Angola, and is widespread in Tropical Africa and South Africa.

Description: - It is a shrub or tree up to 50ft high, of rather crooked growth with dense, widely spreading – crown. Trunk and branchlets are occasionally spiny when young, becoming fibrous, glabrous or nearly so. The bark is grayish – brown, and smooth. The leaves are elliptic – obovate, shortly acuminate, glabrous or minutely pebulous.

The flowers which appear between March and June are yellowish green, in small axillary clusters. Male flowers are red. The fruits appear between June to October and are one – seeded with fleshy pulp, bluish – purple when ripe.

Colour

The bark contains tannin and is used as a red – brown dye for pottery and sometimes raffia.

Medicinal Uses

A root decoction is a febrifuge in Ashanti. An infusion of bark and leaves is considered effective in Guinea for respiratory tract infections. The powdered leaves are used as snuff in colds; or applied locally for various paints, e.g. headaches, rheumatic pains, and toothache; the leaves being crushed with lemon juice.

- ***Alchornea cordifolia***

The common name for this plant is Christmas bush. Local names include: Twi-Agyamma, Adangbe; Gboo, Gbloo, Ewe: - Ahame.

Habitat

The plant is common in Secondary Forests, its distribution is from Senegambia to Angola and widespread in Tropical Africa.

Description

A multi-stemmed, almost climbing shrub or small spreading tree up to 15ft high and of 1foot girth. The petioles are long, and the leaves broadly ovate and cordate. They are entire, wavy or slightly dentate.

The flowers which appear in November, and between February and March, are greenish – white, often deciduous. Fruits are greenish, 1/3 inches wide and 2-celled.

Colour

A black dye obtained from the plant is used for dyeing pottery and also calabashes and leather. It is also in ink ingredient.

Medicinal Uses

It is a widely used medicinal plant in West Africa. The roots are used in the Ivory Coast with other medicaments for jaundice, leprosy, and snake-bites. The pith of the roots, made into a lotion, or chewed, said to cure thrush and buccal ulceration, and placed hot in a carious tooth. An infusion or decoction of leafy, twigs with lime juice, is purgative and is given to women in Ghana. It is commonly used for venereal diseases e.g. as decoction used as a wash for urethral

discharges. It has a soothing effect and is used as an anti-spasmodic for headaches, coughs, colds and bronchial troubles. The powdered leaf and bark are applied in Sierra Leone to piles.

1.3.2 THE PLANT UNDER STUDY

Hibiscus sabdariffa

1.3.2.1 Description

Hibiscus sabdariffa, belongs to the Malvaceae family[6] and is an erect, slightly branched annual herb. It is variable in size, and slightly coloured red. The stem may be smooth or slightly hispid. The leaves are alternate, glabrous, long-petiolate and palmately divided into 3-7 lobes with serrate margins.

The lobes are between 6 to 15cm long, with the middle lobe longest. The flowers are large, short peduncled, red to yellow with purple centre. The calyces are fleshy at maturity, often coloured red, though sometimes pale yellow. They are edible. The root is a deep penetrating tap root.

Plate 1

***Hibiscus sabdariffa* plant**

1.3.2.2 Local and Foreign Names

Local Names [5]

Ga	:	Sakpa
Frafra	:	Nangana
Dagbani	:	Digbemne; Injamgbam
Guan (Krachi)	:	Riaripari
Konkomba	:	Tingyanbam
Moshi	:	Bito
Hausa	:	Siiro; Sooboro, Rarna
Ewe	:	Evema (meaning native spinach)

Foreign Names

Diverse foreign names of *Hibiscus sabdariffa* suggests the presence of the plant in several parts of the world, where they are either cultivated or imported from countries where they grow naturally. [10]

Arabia	:	Karkade
Egypt	:	Karkade
Guinea	:	Red Sorrel
India	:	Mesta
Iran	:	Sour Tea
Jamaica	:	Red Sorrel
Japan	:	Roselle
Malaysia	:	Roselle
Mexico	:	Jamaica tea
Nigeria	:	Zobo
Sierra Leone	:	Sour – sour
Sudan	:	furundu, Sudan Tea
Switzerland	:	Karkarde
Thailand	:	Thai Red
West Indies	:	Roselle

1.3.2.3 Uses

A. Food and Drink

The calyces of *Hibiscus sabdariffa* are used to prepare beverages especially as tea and coffee substitute for people who are sensitive to stimulants. [5]

In several parts of the world such as Switzerland, Arabia and West Indies, the fresh or dried calyces are used for making roselle wine, jelly, syrup, gelatin, pudding and cakes. It is used to colour and flavour rum in the West Indies. [11] In Ghana, a drink, made by boiling the calyces in water is sweetened with sugar and used as a refreshing drink or frozen into ice lollies.

In many West African countries such as Ghana, Guinea and Nigeria, the green leaves of the plant are eaten as a potherb. In this preparation, groundnut meal and "dawadawa" (ground, fermented seeds of *Parkia clappertoma*) are added and made into soup.

The green leaves may be dried and kept in pots, and used in soup during the dry season. When cooking, the water is changed many times to reduce the sliminess of the pot herb. This water maybe used in preparing porridge of millet or guinea-corn which keeps a few more days in good condition than that prepared with ordinary water. This suggests preservation properties. The fruit is cooked and used in soups. Groundnut, millet or corn meal is added to soften them up. The fruit may also be dried, stored and used during the dry season.

The seeds are used as groundnut substitute. They are roasted, ground, and the meal used in place of groundnuts in times of scarcity.

Oil is obtained from the seeds. This oil is used as a substitute for castor oil. The residue, after removal of the oil, is used as food, either in soup or mixed with beans and other ingredients into cakes. The fermented seeds are used as substitute for "dawadawa".

B. Medicinal Uses

The seeds of *Hibiscus sabdariffa* are used as laxative and aphrodisiac [5]. The leaves and seeds are used in the treatment of scurvy. This suggests the presence of vitamin C in the leaves, seeds and possibly the calyces. It may also have antioxidant properties. It is used as an antidote to arrow poison [11]. The powdered roots of *Cissus populnea* (Agyako in Twi) with the residue of *Hibiscus sabdariffa* seeds, after extracting the oil, is moistened and applied to wounds.

The plant is also a folk medicine for abscesses, bilious conditions, cancer, cough, debility, dyspepsia, dysuria, feverish conditions [14], heart ailments and hypertension.

The roots of *Hibiscus sabdarifa* is also used in treating syphilitic sores or chancre. An infusion of the calyces and leaves of the plant is used as a vehicle for various native medicines. Oil from the seeds is applied to camel sores. In Nigeria, the red calyces are soaked in water and the liquid, drunk as a tonic.

An infusion of the flower is the basis of an agreeable red beverage which is said to contain citric acid, and acts as a diuretic.

The flowers have also been found to contain gossypetin, anthocyanin and glycoside; hibiscin, which contributes to the diuretic and choleric effects, decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis. Phillipinos use the bitter root as an aperitive and tonic [15]. Taiwanese regard the seed as diuretic, laxative and tonic, Angolans use the mucilaginous leaves as emollient and as a soothing cough remedy; Central Africans poultice the leaves on abseesses.

Ingestion of the plant extract decreased the rate of absorption of alcohol, lessening the intensity of alcohol effects in chickens [16]. A strong bast fibre obtained is from the inner bark of the stem[5].

Source of Colour

The calyces of *Hibiscus sabdariffa* can be used as colouring matter. They contain red pigments known as anthocyanins [11]. Rovesti in 1936, isolated two colouring matters, hibiscin and gossypetin from the calyces. They are used to colour foods in East Africa [5]. In the West Indies, they are used to colour rum[16].

1.3.2.4 Distribution, Cultivation and Ecology [10]

Hibiscus sabdariffa is native to the West Indies, but is cultivated throughout the tropics. In Ghana, it is cultivated especially in the Northern, Upper East and Western Regions. In Southern Ghana, some settlers from Northern Region of Ghana do cultivate the plant. It is also available in several other countries of the world, either through cultivation or importation. This fact is evidenced by the foreign names of the plant, as listed above.

Hisbiscus sabdariffa is suitable for tropical climates with well-distributed rainfall of 1,500 – 2,000mm yearly, from sea level to about 600m altitude. It tolerates warm and humid climate, but is susceptible to damage from frost and fog.

It requires a permeable soil, a friable sandy loam with humus being preferable. It is able, however, to adapt to a variety of soils.

It is not shade tolerant and must be kept weed-freed. It will tolerate floods, heavy winds or stagnant water. The seed is planted into the bed, 18 inches apart in the rows and with 24 inches between the rows. Planting made in the early rain, gives the highest yield, but if insecticides are not used, these early plantings may suffer from insect attack.

The calyces can be harvested, three weeks after onset of flowering. For fibre, from planting to harvest is about 3-4 months, but ten months in Indonesia. Fibre quality is best if harvest just at flowering time.

On the beds of young *Hibiscus sabdariffa* plants will be found large numbers of small, lightly coloured beetles – usually bright red, but sometimes orange. These Geleracid beetles are most destructive on young foliage and can completely skeletonize leaves of the plant.

The plant may also be attacked by certain fungi, including *Aecidium garcheamum, Alternaria macrospore, Diplodia hibiscina* and *Sclerotium rolfsic*. Plants attacked by leaf-spot disease, caused by the above fungi are treated by spray applications of fungicides for control. *Hibiscus sabdariffa* plants are also attacked by several viruses, causing leaf curl, cotton leaf curl, and yellow vein mosaic disease. The plant may also be attacked by root-knot nematodes such as *Meloidogyne arenaria* and *Meloidogyne javanica.*

1.3.2.5 Constituents [14]

For each 100g, the fruit contains 49 calories, 84.5% water, 1.9g protein, 0.1g fat, 12.3g total carbohydrate, 2.3g fibre, 1.2g ash, 1.72mg calcium, 57mg phosphorus, 2.9mg Iron, 300ug B-carotene equivalent, and 14mg ascorbic acid.

For each 100g, the leaf is reported to contain 43calories, 85.6% H_2O, 3.3g protein, 0.3g fat, 9.2 total carbohydrate, 1.6g fibre, 1.6g ash, 213mg calcium, 93mg phosphorous, 4.8nmg Iron, 4135

ug B-carotene equivalet, 0.04mg thiamine, 0.6mg riboflavine, 0.5mg niacin and 14mg ascorbic acid[(2)].

The seeds contain 7.6% moisture, 24.0% crude protein 22.30% fat, 15.3% fibre, 7.5% ash, 0.3% calcium, 0.6% phosphorous and 0.4% sulphur. Seeds extracted with ether contained 0.7% fat, 29.0% protein, and 32-9% Nitrogen – free extract.
The dried flowers contain 13% of a mixture of citric acid and malic acid, two anthocyanins gossipetin (hydroxyflavone) and hibiscin and 0.005% ascorbic acid. The flowers also contain phytosterols and 15.3% hibiscic acid. The root contains saponins and tartaric acid.

The calyces contain 6.7% proteins by fresh weight and 7.9% by dry weight. Aspartic acid is the most common amino acid in them.

1.3.3 Work done by other researchers on *Hibiscus sabdariffa* [(23)]

- **Toxicological investigation of aqueous methanolic extract of the calyces of *Hibiscus sabdariffa.***

Results of this study showed that prolonged use of this extract at 250mg/kg body weight could cause liver injury; however effects were mild in small dose levels (1-10). It was concluded that the average consumption of 150-180mg / kg body weight is safe.

- **The effect of a water extract and anthocyanins of *Hibiscus sabdariffa* on paracetamol included hepatotoxicity in rats.**

Given for four weeks, the extract significantly improved some of the liver function tests evaluated, though it did not alter the histology of the paracetamol – induced sleeping time. At a dose of 200mg/kg, the hepatic histology and the biochemical indices of liver damage were restored to normal. It maybe concluded that the extract can potentially be used in mitigating paracetamol – induced hepatotoxicity.

- **Mechanisms of the blood pressure lowering effect of an extract of Hibiscus sabdariffa calyces in rats.**

Findings suggest that the anti-hypertensive effect of the extract of *Hibiscus sabdariffa* calyces is not mediated through inhibition of the sympathetic nervous system, but it could be mediated through acetylcholine – like and histamine like mechanisms, as well as via direct vaso – relaxant effects.

- **Changes in urinary chemical composition in healthy volunteers after consuming *Hibiscus sabdariffa* juice.**

The study, in which thirty-six healthy men participated showed that their urine, after consumption of *Hibiscus sabdariffa* juice showed a decrease of creatine, uric citrate, tartrate, calcium, sodium, potassium and phosphate, but not oxalate in urinary excretion.

In conclusion, a low dose of the extract (16g per day) caused more significant decrease in salt output in the urine than a high dose (24g/ day).

- **The chemical composition of the pigment of *Hibiscus sabdariffa***

The chemical composition of the pigment of Hibiscus sabdariffa was identified as delphinidin – 3-glycoside.

- **Effectiveness and tolerability of a standardized extract of *Hibiscus sabdariffa* in patients with mild to moderate hypertension:-**

The results showed that *Hibiscus sabdariffa* was able to decrease the systolic blood pressure from 139.05 to 123.73mmHg and the diastolic blood pressure from 90.81 to 79.52mmHg.

- **Polysacharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes.**

Raw polysaccharides and acidic sub-fractions isolated from the flowers of *Hibiscus sabdariffa* caused a strong induction of proliferation of human keratinocytes of up to 40%. They also induced early differentiation of primary natural keratinocytes.

- **Protective effect of Hibiscus anthocyanins against tert – buty1 hydroperoxide – induced hepatic toxicity in rats.**

The preliminary study showed that Hibiscus anthocyanins were able to quench the free radicals of 1,1 – dipheny1 – 2 – picry1hydrazy1. This antioxidant bioactivity was further evaluated using the model of tert-buty1 hydroperoxide – induced cytotoxicity in rat primary hepatocytes and hepatotoxicity in rats. The invivo investigation showed that the oral pretreatment of Hibiscus anthocyanins (100 and 200mg/kg) for 5 days before a single dose of tert – buty1 hydroxide significantly lowered the serum levels of hepatic enzyme markers (alanine and aspartate amino transferase) and reduced oxidative liver damage.

The histopathological evaluation of the liver revealed that Hibiscus pigments reduced the incidence of liver lesions including inflammatory, leucocyte infiltration, and necrosis in the rats.

Based on the above results, it is speculated that Hibiscus pigments may play a role in the prevention of oxidative damage in living systems.

- ***Hibiscus sabdariffa* inhibits the development of arteriosclerosis in cholesterol fed rabbits.**

The levels of triglyceride, cholesterol and low – density lipoprotein cholesterol were lower in the serum of rabbits fed *Hibiscus sabdariffa* extract (0.5% and 1%) than in the serum of rabbits fed only high cholesterol diet.

Feeding *Hibiscus sabdariffa* extract (0.5 and 1% in the diet) to rabbits significantly reduced severe atherosclerosis in the aorta. Histopathological examination showed that *Hibiscus sabdariffa* extract reduced foam cell formation and inhibited smooth muscle cell migration and calcification in the blood vessel of rabbits. These results suggest that *Hibiscus sabdariffa* extract (0.5% and 1%) inhibits serum lipids and shows an anti atherosclerotic activity.

- **The effect of *Hibiscus sabdariffa* extract on essential hypertension**

Statistical findings showed an 11.2% lowering of the systolic blood pressure and a 10.7% decrease of diastolic pressure in the experiment 12 days after the beginning of the treatment as compared with the first day.

Three days after stopping the treatment, systolic blood pressure was elevated by 7.9% and the diastolic pressure was elevated by 5.6% in the experimental and control groups. This study proves the public belief and the results of in vitro studies concerning the effects of *Hibiscus sabdariffa* extract on lowering blood pressure.

- **Inhibition of intestinal motility by methanol extracts of *Hibiscus sabdariffa* in rats.**

The methanolic extracts of *Hibiscus sabdariffa* showed significant dose dependent relaxant effect on rat ileal strip comparable to the effect shown by nifedipin and papaverine as reference compounds. Similarly, the extract when administered intra-peritoneally significantly reduced intestinal transit (13%-35%) in rats.

- **Alpha – amylase inhibitors from *Hibiscus sabdariffa* extract.**

Hibiscus sabdariffa tea extract was found to have high inhibitory activity against porcine pancreatic alpha – amylase.

Hibiscus acid and its 6-methy1 ester were respectively isolated as active principles from the 50% methanol and acetone extracts of *Hibiscus sabdariffa*. The activity of each isolate was compared to that of structurally related citric acid, a previously known inhibitor of fungal alpha-anylase.

- **Inhibitory effect of Hibiscus protecatechuic acid on tumour promotion in mouse skin.**

When Hibiscus protocatechuic acid (PCA), a phenolic acid isolated from *Hibiscus sabdariffa* was applied to the dorsal surface of the mine, before TPA (Tetradeconoylphorbol – 13 – acetate, a carcinogen) application, the former inhibited the induction of epidermal ornithine decarboxylase (ODC) activity by 6.5nmol. The same doses of PCA also reduced the formation of hydrogen peroxide in the mouse skin to an inhabition of 61,84, and 89% respectively, when compared with that of the TPA – treated group. These results indicate that PCA possesses potential as a cancer chemo-preventive against tumour promotion.

- **Protective effects of dried flower extracts of *Hibiscus sabdariffa* oxidative stress in rat primary hepatocytes.**

Dried flower extracts of *Hibiscus sabdariffa*, was found to possess anti-oxidant activity. The results indicated that the dried flower extracts (specifically the chloroform – soluble fraction and ethyl acetate – soluble fraction) protect rat hepatocytes from free radical toxicity and genotoxicity.

- **Investigation of the anti-spasmodic potential of *Hibiscus sabdariffa* calyces**

Addition of an aqueous extract of *Hibiscus sabdariffa* calyces (2.5ml / bath approximately 125mg of starting crude material) inhibited the tone of various isolated muscle preparations (rabbit aortic strip rhythmically contracting rat uterus, guinea pig tracheal chain and rat diaphragm). Other muscles were stimulated (quiescent rat uterus and frog rectus abdominis), intravenous injection of the extract to anaesthetized cats lowered the blood pressure in a dose-response manner.

The inhibitory effects were resistant to a number of standard receptor blockers but the hypotensive influence was practically blocked by atropine.

- **Hypocholesterolemic action of *Hibiscus sabdariffa***

A remarkable lowering effect in the level of different lipid fractions was noticed in spite of the continued cholesterol and cholic acid loading during the treatment.

1.3.4 Toxicity of some Synthetic colouring Agents

Matters of concern, particularly in the use of synthetic dyes such as amaranth, tetrazine, carmoisine and enythrosine have received considerable publicity in recent times[(3)]. This has resulted in restrictions on the extent to which these agents may be used. They are now very widely controlled.

Thus, the search for natural colouring agents has gained much importance and attention, due to their minimal side effects, if any at all. The ever increasing inclusion of natural sources of colour in the British Pharmacopoeia, Marntindale, United States Pharmacopoeia etc, bears witness to this fact. Examples include red poppy petals, tumeric, saffron, red rose petals, beetroot red; cherries and Raspberry.

In a double – blind placebo controlled study in children whose poor behaviour was attributed by their parents to the intake of food additives, were given oral challenges of synthetic food colours as mentioned above. The doses were far in excess of estimated normal daily intake. The study investigations could measure worsening of behavioural scores after intake of the food colours[3].

Tetrazine

Another study by Row KS and Row KJ 1994[3] of similar design, detected a dose – dependent association between tetrazine intake and behavioural changes, such as irritability, restlessness and sleep disturbances.

There have been numerous reports of reactions to tetrazine, and these cover angiodema, asthma, urtricaria and anaphylactic shock. Some of these reports have dealt with cross-sensitivity especially with aspirin, although the connection with aspirin has been questioned. Thus, the use of tetrazine in food and medicines appears to be diminishing. Much controversy has surrounded the hypothesis that certain synthetic food additives including artificial colours and flavour, are aetiologic agents in ADHD (Attention Deficit Hyperactivity Disorders) which is a heteregenous behaviour disorder which first becomes apparent during childhood and consists of developmentally inappropriate and socially disruptive behaviour characterized by varying degrees of hyperactivity, inattention and impulsiveness. Children with ADHD are easily distracted and have difficulty in completing tasks. Associated features include low frustration tolerance, mood liability and defiance.

Some children with ADHD continue to have symptoms throughout adolescence and into adulthood.

Sunset Yellow

There have been reports of an increased incidence of adrenal medullar adenomas in rats given sunset yellow for two years. [3]

Amaranth

A study carried out by Stenberk and Garinlerko (1970) [2] showed that amaranth decreased fertility; increased the number of still births, and produced deformities in the young of the rats

used. Survival of the young rats was also reduced. The percentage of dead fetuses increased in a dose – related manner.
At 200mg / kg body weight per day, there were foetotoxic effects. Resorptions increased from 15mg / kg upwards with total resorption of litters at 100 and 200mg / kg / day.

Amaranth has been shown to have mutagenic response in the host mediated assay for the two strains of *Salmonella typhirium* G46 and TA 1530, particularly when the compound was administered by multiple injection over five days[(3)]. Similar results were obtained in recombination tests with yeast.

Red 2G[(3)]

Concerns have been raised that Red 2G might produce heamolysis in subjects deficient in glucose -6- phosphate dehyrogenase. It has therefore been considered undesirable to use Red 2G in foods especially high in acidity, subjected to high temperatures during processing.

Paratoluenediamine [(3)]

Paratoluenediamine is a synthetic colouring agent in dyeing the hair. A report has been made of a woman who suffered aplastic anameia, after using a hair dye containing paratoleuenediamine.

Erythrosine [(3)]

This is a synthetic colouring agent for medicines and food. It is also used cosmetically as a disclosing agent for plaque on teeth.

It's use is currently being reviewed by the Food and Drugs Agency (FDA) because of carcinogenicity in rats. Thyroid studies indicated that evythrosine inhibits the de-ionization of thyronine to tri-iodothyronine and at high doses, activates secretary mechanisms for thryrotropine in the pituitary[(3)].

Allura – Red [3]

Allura Red is a synthetic colouring agent for foods, medicines and cosmetics. Concerns have been raised about para-cresidine, a potential carcinogen used in the production of Allura Red. While this compound could be detected in the free state in food grade Allura Red, down to a limit of 1ppm, reassurance was required that it was not formed as a metabolite.

Canthaxanthin [3]

Also known as Food Orange, canthaxanthin is used to colour the flesh of salmon, trout, biscuits, confectionary, pickles and sauces. It has been found to cause crystal deposition in the retina following ingestion of previously estimated acceptable daily intake. In some cases, impairment of vision has resulted.

Canthaxanthan is also used in cosmetics and taken orally to produce artificial suntan. There have been reports of deposits in the retina, known as gold speck maculopathy[3], a term used to describe deposits in the retina.

1.3.5 The Role of Colouring agents in Pharmaceuticals[1]

Pharmaceutical preparations are coloured for four main reasons

1. To increase their acceptability to patients

It is believed that brightly coloured tonics, cherry-red children's cough mixtures and flesh-tinted powders and ointments are more likely to be used because they are attractive.

2. To give warning

Legal acceptance of this principle in the instructions to use red warning labels for schedule I poisons, to include a violet dye in mineralized methylated spirit, and to distinguish by added colour, arsenical sheep dips and weed killers, provides powerful support for use of colouring agents to indicate preparations that should not be taken; example antiseptic solutions.

3. For identification

This is the chief reason for colouring medicated surgical dressings, non absorbable surgical sutures, medical gas cylinders, tablets and capsules. A specific example is the anaesthetic trichloroethylene, which may be coloured blue to distinguish it from chloroform, which it resembles in physical characteristics.

4. To produce standard preparations

Natural calamine, which is obsolete for pharmaceutical purposes is not constant in colour and has been replaced by artificially – prepared material tinted with a form of ferric oxide. Differences in the tint of green soft soap, caused by variation in the quality of oils used in its preparation are sometimes covered by a suitable dye.

When lactose is the diluent for powdered opium, it is coloured with caramel to give a uniform appearance to the product.

The elegance and eye appeal of a coloured product is valuable, especially for children whom it is often used to treat with syrups, tablets, or capsules, to avoid injections and allow treatment at home.

Colour can expedite recognition of a product and so allow more effective treatment of poisoning to begin earlier.

Colouring may help a doctor to recognize a previous treatment. Specific cloured products become known to doctors and pharmacists, and this can help sales.

Colour of non-injectable fluids

In 1956, the Codex Revision committee set up sub-committee of Hospital pharmacists to suggest a range of colours for non-injectable fluids. Their recommendations are given below

Preparation	**Colour**
1.3.6 Local analgesics for topical use	Green
1.3.7 Ampoule storage solution (0.4percent chlorocresol)	Red
1.3.8 Instrument storage solution	Red
1.3.9 Syringe storage solution	Red
1.3.10 Skin disinfection and cleaning preparations (1percent cetrimide)	Red

Physiological Aspects of Colouring

Red is considered stimulating and blue relaxing.

Goethe[(4)] in his Theory of Colour, described red and yellow as exiting and blue as restful and cold. More recent studies in the fields of advertising psychology and physiology have confirmed and extended these impressions. Schemes similar to the following are proposed:

Red (exciting) Orange (cheerful) Yellow (Tranquilizing) Green – Blue (subduing). It is interesting that there is only one well-known blue internal preparation (a tranquilizer), while red colours predominate in tablets, capsules and syrups.

Desirable Properties of a colouring Agent

1. It should be harmless to health and should have no physiological activity.
2. It should be a definite chemical compound because then its colouring power will be reliable, its assay practicable and it will be easier to ensure freedom from harmful impurities.
3. Ready solubility in water is desirable in most cases, but some oil – soluble colours are necessary.

4. It’s tinctorial power (colouring) should be high so that only small quantities are required.

5. It should not be affected by oxidizing or reducing agents.

6. It should not be affected by light, tropical temperatures, hydrolysis and by micro-organisms, and therefore be stable on storage.

1.3.6 Formulation of Liquid Pharmaceutical Dosage Forms [12]

Liquid pharmaceutical dosage forms include solutions, emulsions and suspensions. They may be prepared by dissolving the active ingredient(s) in an aqueous or non-aqueous solvent, by suspending the drug (if it is insoluble in pharmaceutically or therapeutically acceptable solvents) in an appropriate medium or by incorporating the medicinal agent into one of the two phases of an oil and water system. [13]

Solutions are homogeneous liquid preparations containing one or more dissolved ingredients and are used as a variety of dosage forms for internal and external use.

A pharmaceutical suspension is a type of disperse system in which one substance (the disperse phase) is distributed in particulate form throughout another (the continuous phase). Suspensions may be classified into coarse suspensions in which particles are larger than 1mm in diameter and colloidal suspensions in which the particles may be considerably less than 1mm in diameter.

An emulsion is a disperse system consisting of two immiscible liquids, one of which (the disperse phase) is finely divided and distributed through the other (the continuous phase).

Since this type of dispersion is inherently unstable, an emulsifying agent is usually required to maintain the dispersion. In pharmaceutical emulsions, one phase is usually aqueous and the other oily. When the continuous phase is aqueous, the system is described as oil-in-water (0/w), and when the continuous phase is oily the system is described as water-in-oil (w/0).

These pharmaceutical dosage forms are useful for a number of reasons. They can be formulated for different routes of administration including oral, introduction into body cavities or applied externally. The dose can easily be adjusted by dilution, and the oral liquid form can be readily administered to children or people unable to swallow tablets or capsules.

The preparation of these dosage forms involves several considerations on the part of the pharmacist. These are:- purpose of the drug, internal or external use, concentration of the drug, selection of the liquid vehicle, physical and chemical stability of the drug, preservation of the preparation and the use of appropriate excepients such as buffers, solubilizers, suspending agents, emulsifying agents, viscosity controlling agents, colours and flavours.

Buffers:-These are materials which when dissolved in a solvent will enable it to resist any change in pH should an acid or alkali be added.

The choice of a suitable buffer depends on the pH and buffering capacity required. It must be compatible with other excipients and have a low toxicity.

Most pharmaceutically acceptable buffering systems are based on carbonates, citrates, glyconates, lactates, phosphates or tartrates. Borates can be used for external application, but not to mucous membranes or abraded skin.

As the pH of most body fluids is 7.4, products such as injections, eye drops and nasal drops should in theory, be buffered at this value. Many body fluids themselves, however have a buffering capacity, and therefore when formulating low volume intravenous injections or eye drops, a wider pH range can be tolerated. This is potentially useful, should a compromise be necessary between pH which is physiologically acceptable and a pH of maximum stability, solubility and optimum bioavailability.

Flavours:-The use of sweetening agents many not be sufficient to render palatable, a product containing a drug with a particularly unpleasant taste. In many cases, therefore, a flavouring agent can be included. This is particularly useful in pediatric formulations to ensure patient compliance.

The inclusion of flavours has the additional advantage of making identification of liquid products much easier.

Flavouring agents can be obtained from either natural or synthetic sources. Natural products include fruit juices, aromatic oils, such as peppermint and lemon oils, herbs and spices.

They are available as concentrated extracts, alcohlolic or aqueous solutions or syrups and are particularly widely used in the manufacture of products for extemporaneous use.

Artificial flavors are of purely synthetic origin; often having no natural counterpart. They tend to be cheaper, more readily available, less variable in chemical composition and more stable than natural products. They are usually available as alcoholic or aqueous solutions or as powders.

The choice of a suitable flavor can only be made as a result of subjective assessment and, as consumer preferences vary considerably, this task is not easy.

In certain cases, there exists a strong association between the use of a product and its flavour content. For example, products intended for the relief of indigestion are often mint flavoured. This is because for many years, mint has been used in such products for its carminative effect.

Similarly, the odour of terpinol is often associated with antiseptic activity, and in a competitive market, it may be unwise to alter these flavours or perfumes. The fact that personal preferences for flavours often vary with age can also aid the formulator. Children in general prefer fruity tastes and smells, while adults choose flowery odours and acid flavours.

Other suitable materials for the masking of unpleasant tastes include menthol, peppermint oil and chloroform. In addition to their own particular tastes and odours they also act as desensitizing agents by the exertion of a mild anaesthetic effect on the sensory taste receptors.

Colours: - Once a suitable flavour has been chosen it is often useful to include a colour which is associated with that flavour to improve the attractiveness of the product.

Another reason for the inclusion of colour is to enable easy product identification. For example, several paediatric syrups are coloured red. Many types of antiseptic solutions used in hospitals are coloured differently, to differentiate between them.

It is essential to ensure that any colour chosen is acceptable in the country in which the product is sold.

As with flavours and perfumes, there is available a range of both natural and synthetic colours. The former, which tend to be more widely acceptable, can be classified into carotenes, chlorophyll, anthocyanins and a miscellaneous group which includes riboflavines, caramel and extracts of red beetroot. They can however exhibit the usual problems associated with natural products namely, variations in availability and in chemical composition, both of which may cause formulation difficulties. Synthetic dyes tend to give brighter colours and are generally more stable than natural materials.

Care must be taken to ensure that any dye used is not adversely affected by pH or by ultra – violet radiation, or by the inclusion of oxidizing or reducing agents.

Sweetening Agents

Low molecular weight carbohydrates and in particular sucrose are traditionally the most widely used sweetening agents. Sucrose has the advantage of being colourless, very soluble in water, stable over a pH range of about 4-8.

Also, by increasing the viscosity of fluid preparations, sucrose imparts to them, a pleasant texture in the mouth.

It masks the tastes of both salty and bitter drugs, and has a soothing effect on membranes of the throat. For this reason, sucrose is particularly useful as a vehicle for antitussive preparations. Polyhydric alcohols, such as sorbitol, mannitol and to a lesser extent glycerol, also possess sweetening power and can be included in preparations for diabetic use, where sucrose is undesirable.

Other less widely used sweeteners include hydrogenated glucose syrup, isomalt, fructose and xylitol.

Treacle, honey and liquorice are now very rarely used, having only a minor application in some extemporaneously prepared formulations.

Artificial sweeteners can be used both in conjunction with sugars and alcohols, to enhance the degree of sweeteners, or on their own in formulations for patients who must restrict their sugar intake. They are also termed intense sweeteners, because, weight for weight, they are hundreds of times sweeter than sucrose and are therefore rarely required at a concentration of greater than 0.2%.

The most common examples of artificial sweeteners are the sodium or calcium salts of saccharin. Less widely used is aspartane, acesulfane potassium and thaumatin.

The main disadvantage of all artificial sweeteners is their tendency to impart a bitter or metallic after-taste, and they are therefore often formulated with sugars to counteract this.

Preservatives: - When choosing a suitable preservative, it must be ensured that adsorption onto the container from the product does not occur; or that its efficiency is not impaired by the pH of the solution or by interactions with other ingredients.

For example, many of the widely used parahydroxy benzoic acid esters can be adsorbed into the micelles of some non-ionic surfactants. Although their presence can be detected by chemical analysis, they are in fact, unable to exert their antimicrotial activity.

Increasing attention has been focused on the use of mixtures of preservatives, and the addition of various potentiators to achieve better results.

The justification of using mixtures of antimicrobial compounds must reside in an increased spectrum of antimicrobial activity and a synergistic effect enabling, decreased levels of component preservatives to be used.

These lead to a decrease in toxicity and a reduction in the emergence of resistant microbes.

Reducing Agents and Anti-Oxidants:

The decomposition of pharmaceutical products by oxidation can be controlled by the addition of reducing agents or anti-oxidants.

Most anti-oxidants function by providing elections which will be accepted by any free radical, to terminate oxidation (a chain reaction).

A pre-requisite for effective anti-oxidant activity in any particular preparation is that, the antioxidant is more readily oxidized than the drug.

Liquid preparations may be handled by the pharmacist in one of three ways. The pharmacist may dispense the product in its original container, buy the product in bulk and repackage; or compound the solution, suspension or emulsion in the dispensary. (12)

The stability of the active ingredient in the final product is of prime concern to the formulator. In general, drug substances are less stable in aqueous media than in the solid dosage form and it is important, therefore, to properly buffer, stabilize or preserve, in particular those solutions, suspensions and emulsions that contain water.

Certain simple chemical reactions can occur in these products. These may involve an ingredient – ingredient interaction (which implies a poor formulation), a container – product interaction (which may alter product pH and thus, for pH – sensitive ingredients, be responsible for the subsequent formation of precipitates) or direct reaction with water (i.e. hydrolysis).

The more complicated reactions usually involve oxygen. Vitamins, essential oils and almost all fats and oils can be oxidized. Formulators usually use the word autoxidation when the ingredient(s) in the product react with oxygen, without drastic external interference. Such reactions must first be initiated by heat, light, peroxides or other liable compounds or heavy metals such as copper or iron. This initiation step results in the formation of a free radical which then reacts with oxygen. The free radical is thus regenerated and reacts with more oxygen.

The effect of trace metals can be minimized by using citric acid or Ethylene Diamine - Tetra-acetic acid. Antioxidants, on the other hand may retard or delay oxidation by reacting with free

radicals formed in the product. Examples of antioxidants are the alkyl gallates, butylated hydroxyanisole, nordihydroguaiaretic acid and the tocopherols or vitamin E.

Products may become contaminated with microbes for a number of reasons. The raw materials used in the manufacture of solutions, suspensions and emulsions are excellent growth media for bacteria. Water in particular must be handled with care. Substances such as gums, dispersing agents, surfactants, sugars and flavours can be the carriers of bacteria which ultimately contaminate the product.

Bacteria grow well in the nooks and crevices of pharmaceutical equipment, and in simple equipment used in the dispensary. Such equipment should be cleaned thoroughly prior to use. Environment and personel can contribute to product contamination. Hands and hair are the most important carriers of contaminants. Packaging should be selected so that it will not contaminate the product and will also protect it from the environment.
The formulator can add a preservative to the product and decrease the probability of product contamination. If the product contains water, it is almost mandatory to include a preservative in the formulation. This in no way replaces good in-plant control but merely provides further assurance that the product will retain its pharmaceutically acceptable characteristics until it is used by the patient.

The major criteria that should be considered in selecting a preservative are:- it should be effective against a wide spectrum of micro-organisms, stable for its shelf life, nontoxic, non sensitizing, compatible with the ingredients in the dosage form and relatively free of taste and odour. Preservatives maybe used alone or in combination to prevent the growth of micro-organisms. Ethanol is a highly effective preservative. It is used at the 15% level in acidic media and at the 18% level in neutral or slightly alkaline media.

Isoproyl alcohol is a fairly effective agent but it can be used only in topical preparations. Benzoic acid is effective only at pH 4 or less. Its solubility in certain aqueous preparations is poor, in those instances, sodium benzoate may be used. Sorbic acid has a broad range of

antimycotic activity but its antibacterial properties are more limited. It is effective only at a pH of less than 5.

The methyl and propyl esters of p-hydroxybenzoic acid (the parabens) are used widely in the pharmaceutical industry. They are effective over a wide pH range. (from about 3 to 8) and are employed up to about the 0.2% concentration level. The two esters often are used in combination in the same preparation. This achieves a higher total concentration and the mixture is active against a wide range of micro-organism. The hydroxybenzoates are effective against most micro-organisms; however, their activity may be reduced in the presence of nonionic surface-active agents because of binding.

In the formulation of liquid pharmaceutical dosage forms, therefore, the pharmacist has the responsibility, with the manufacturer, for the maintenance of product stability.

Products should be stored in the manner stipulated in the compendium, eg, in a cool place or a tight, light-resistant container.

Furthermore, products should be checked regularly for evidence of instability. With respect to solutions, elixirs and syrups, color change, precipitation and evidence of microbial or chemical gas formation are major signs of instability. Emulsions may cream, but if they break (i.e. separation of the oil phase) the product is considered to be unstable.

Sedimentation and caking are primary indications of instability in suspensions. The presence of large particles may mean excessive crystal growth has occurred. The pharmacist therefore, should use his knowledge to guard against instability and incompatibilities.

1.3.7 Pharmaceutical Extracts [18]

Extracts are defined as concentrated preparations of vegetable or animal drugs obtained by removal of the active constituents.

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures.

The products so obtained from plants are relatively impure liquids, semi-solids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, semi solid extracts and powdered extracts.

Extraction continues to be of considerable interest in order to obtain improved yield of drugs derived from plant and animal sources. For example, improved extraction of digitalis glycosides has been carried out using a pulsating, perforated bottom column. Other techniques include ultrasonic, rotary – film evaporators, liquid and supercritical carbon dioxide, hydrodistillation, liquid chromatography, multiple – solvent extraction, counter current extraction and gravitation dynamics.

Extraction differs from solution in that the presence of insoluble matter is implied in the extraction process.

The principal methods of extraction are maceration, percolation, digestion, infusion and decoction.

Maceration

In this process, the solid ingredient are placed in a stoppered container with the whole of the solvent and allowed to stand for a period of at least 3 days with frequent agitation, until soluble matter is dissolved. The mixture is then strained, the marc pressed and the combined liquids clarified by filtration or by decantation, after standing.

Percolation

This is the procedure used most frequently to extract the active ingredient in the preparations of tinctures and fluid extracts. The procedure involves the use of a percolator which is a narrow, cone-shaped vessel open at both ends. The solid ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately four hours in a well closed container, after which the drug mass is packed into the percolator. Sufficient menstrum is added to saturate the mass and the top of the percolator is closed. When the liquid is about to drip from the bottom of the percolator, the outlet is closed. Additional menstrum is added to give a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 hours. The outlet of the percolator is then opened and the liquid contained therein allowed to drip slowly, additional menstrum being added as required, until the percolate measures about three – quarters of the required volume of the finished product. The marc is pressed and the resulting liquid added to the percolate. Sufficient menstrum is added to

produce the required volume, and the mixed liquids clarified by filtration or by allowing it to stand and then decanting.

Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased thereby.

Infusion

An infusion is a dilute solution of the readily soluble constituents of crude drugs. Fresh infusions are prepared by macerating the drugs for a short period of time with either cold or boiling water.

Decoction

This method extracts water – soluble and heat – stable constituents from crude drugs by boiling in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume.

Extracts eliminate the need to isolate the drug in pure form, allow several ingredients to be administered from a single source (e.g. pancreatic extract) and permit the preliminary study of drugs from natural sources.

After a solution of the active constituents of a crude is obtained by maceration or percolation, it may be ready for use as a medicinal agent, as with certain tinctures or fluid extracts, or it may be processed further to produce a solid or semi-solid extract.

CHAPTER TWO
MATERIALS AND METHODS

2:1 MATERIALS

2.1.1 *Hibiscus sabdariffa* calyces

Dried samples of *Hibiscus sabdariffa* calyces were obtained from the Kumasi central market and authenticated at the Forestry Commission, Kumasi to be the dried calyces of *Hibiscus sabdariffa*.

Authenticity of the sample was further substantiated at the Faculty of Agriculture – Horticulture Department, KNUST, and then at the Crops Research Institute, Fumesua, Ashanti Region.

2.1.2 Materials Used in the Laboratory

- Ethanol 96% Merck, obtained from U.K Chemicals Ltd, Kumasi, Ashanti Region.
- Methanol, Analar, obtained from U.K Chemicals Ltd, Kumasi,
- Sodium metabisulphite BDH, obtained from the Faculty of Pharmacy, KNUST
- Disodium hydrogen orthophosphate BP, BDH, obtained from the Faculty of Pharmacy
- Sodium dihydrogen orthophosphate BP, BDH, obtained from the Faculty of Pharmacy
- Orthophosphoric acid BP, BDH, obtained from the Faculty of Pharmacy, KNUST
- Glacial acetic acid BDH, obtained from the Faculty of Pharmacy, KNUST
- Sodium Hydroxide BDH, obtained from the Faculty of Pharmacy, KNUST
- Potassium dihydrogen orthophosphate BDH, obtained from the Faculty of Pharmacy
- Sulphuric Acid, Merck, obtained from the Faculty of Pharmacy
- Hydrochloric Acid, Merck, obtained from the Faculty of Pharmacy
- Distilled water, obtained from the Department of Pharmaceutical Chemistry
- Ready made buffers of pH 4.01, 7.00 and 9.00 obtained from the Department of Pharmaceutical Chemistry, KNUST

2.1.3 Materials Used in the Formulations

Paracetamol powder BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti Region

Propylene glycol BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti, Region

Chloroquine phosphate powder BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti Region

Anise oil BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti Region

Citric Acid BP, obtained from U.K Chemicals Ltd, Kumasi Ashanti, Region

Diphenhydramine crystals BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti, Region

Ammonium Chloride BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti, Region

Sucrose (from Brazil),obtained from the open market

Sodium Benzoate BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti, Region

Compound Hydroxybenzoate solution, obtained from the Faculty of Pharmacy, KNUST

Sodium citrate BP, obtained from U.K Chemicals Ltd, Kumasi, Ashanti, Region

2.1.4 Microbiological Materials

Nutrient Agar, Oxoid, obtained from the Pharmaceutics Department, KNUST

Sterile water, obtained from the Pharmaceutics Department, KNUST

Micro-organisms, *Escherichia coli, Staphyloccocus aureus, Candida albicans, Bacillus subtilis and Pseudomonas aeruginosa*, obtained from the Pharmaceutics Department, KNUST

2.1.5 Equipment

Analytical Balance - - -	Mettler AE 160
Hot Air Ovens - - -	Gallenkamp Oven 300 plus series
Blender - - - -	Warring Commercial Heavy Duty
pH meter - - - -	Mettler Delta 350
Incubator - - - -	Gallenkamp Plus II
Magnetic Stirrer - - -	Stuart Scientific

Sherwood Colorimeter 257

2.1.6 Apparatus

Dessicator, petri dishes, syringes, porcelain motar and pestle, sieves of aperture sizes 1.701 and 1.181mm, Hot water bath, platinum crucible, general purpose glassware, filter paper, evaporating dish; Bunsen burner.

2.2 METHODS

2.2.1 Test for Foreign Matter in *Hibiscus sabdariffa* calyces[23]

Two hundred grammes of dried *Hibiscus sabdariffa* calyces were weighed out and spread out in a thin layer. The material was inspected with the naked eyes to detect any foreign matter such as moulds, insects and any undesirable plant parts. All foreign materials were separated and weighed. The percentage of foreign matter was calculated and recorded.

2.2.2 Macroscopic and organoleptic properties of *Hibiscus sabdariffa* calyces and Amaranth powder.

The macroscopic properties of *Hibiscus sabdariffa* calyces were determined by observing specified characteristics of the unpowdered sample. The specific characteristics that were observed on them were odour or aroma, colour, form or nature, and then taste. The same was done for Amaranth.

2.2.3 Microscopic evaluation of *Hibiscus sabdariffa* powdered calyces [23]

A small amount of the powdered calyces of *Hibiscus sabdariffa* in chloral hydrate solution was examined under a microscope

2.2.4 Preliminary Preparation of Crude Drug

After authentication, a 1kg sample of *Hibiscus sabdariffa* calyces was washed for ten seconds in water (to avoid losing colour). Excess water was blotted from the sample with a clean towel, and dried in a hot air oven for four hours at 30^0C. It was then blended into a moderately fine powder. The powder was allowed to cool and then stored in a black polythene bag to avoid deterioration by light.

2.2.5 Extraction by Cold Maceration[12]

Five hundred grammes of powdered *Hibiscus sabdariffa* calyces was weighed out and placed in a large beaker. One litre of 70% ethanol (730ml of 96% ethanol was diluted with 270ml distilled water to produce 70% ethanol) was added and stirred. It was covered and left to macerate for 5 days at room temperature, with occasional stirring. The liquid was decanted and filtered through a No. 1 Whatman filter paper. The marc was pressed, filtered and added to the first filtrate. The latter was placed in an evaporating dish, placed on a water – bath and evaporated to a thick syrupy mass.

This was placed in a desiccator for 5 days to dry up. The mass was still "sticky" and was therefore left in the desiccator for another five days. Afterwards, the mass became almost dry. It was weighed and placed in a beaker, and covered with a black polythene bag to avoid discoloration. The yield was calculated and recorded.

2.2.6 Aqueous Extraction[12]

Five different concentrations were prepared. These were: - 5g in 100ml (5% w/v), 8.5g in 100ml (8.5% w/v); 17g in 100ml (17% w/v) 25g in 100ml (25% w/v) and 50g in 150ml (33% w/v).

These represent weights of *Hibiscus sabdariffa* powdered calyces in their respective volumes of boiling water. Infusion was done for 30 minutes in each case, and then strained. The extracts were filtered using Whatman No. 1 filter paper. After cooling, 1% v/v compound hydroxybenzoate solution was added as preservative. Sodium metabisulphite (0.1% w/v) was also added as anti-oxidant, to help retain the bright red colour for a longer period. Each concentration was stored in 125ml amber bottles and labelled appropriately.

2.2.7 Preparation of Acid Infusion of *Hibiscus sabdariffa* calyces [22]

One litre of 33% w/v aqueous extract was prepared as described above, but this time, 10ml of dilute sulphuric acid was added and left to stand for one hour, before filtering into a Winchester bottle. It was labelled and stored.

2.2.8 Physico-chemical Properties of the solid extract and Amaranth powder

2.2.8.1 Identity Test for Amaranth powder [31]

To 50mg of amaranth powder was added 200mg of powdered sodium hydroxide. The mixture was transferred to a small test tube, and heated in a flame to fusion. Heating was continued for five seconds, cooled and 0.5ml water added. Ten millilitres of dilute hydrochloric acid was added and warmed. The result was observed and recorded.

2.2.8.2 Solubility Tests for the solid extract of *Hibiscus sabdariffa* calyces and Amaranth powder

To 20ml of cold water in a beaker, was added 0.5g of the solid extract of *Hibiscus sabdariffa* obtained by cold maceration. It was then stirred and allowed to stand for 20 minutes, according to BP specifications. This procedure was repeated using 96% ethanol, and then chloroform. The same procedure was done for amaranth powder. Solubilities in the three solvents were observed and recorded.

2.2.8.3 Test for Acidity and Alkalinity

To 20mls of water in a volumetric flask, 2.0g of the solid extract of *Hibiscus sabdariffa* was added. The mixture was then shaken for three minutes and then filtered, using Whatman filter paper. The filterate was then tested for acidity and alkalinity using a pH meter and litmus paper.

2.2.9.1 Test for water Insoluble Matter for Amaranth powder and solid extract of *Hibiscus sabdariffa*.

One gramme of each sample was dissolved in 100ml of boiling water and allowed to cool. The solutions were filtered and any residue, washed with water until the last washing was practically colourless. The residues were dried at 105^0C for two hours. The weights of each residue were recorded.

2.2.9.2 Test for Total Ash

For Hibiscus sabdariffa calyces, 3g of the powdered material was incenerated in a tarred silica dish. It was cooled and weighed. The percentage of ash was then calculated. For amaranth powder, 1g was used according to BP specifications.

2.2.9.3 Test for Acid Insoluble Ash

The ash for each sample was boiled for five minutes with 25ml of 2M hydrochloric acid. The insoluble matter was collected in a sintered glass crucible and washed with hot water. It was then ignited to a dull redness and weighed. The percentage of acid-insoluble ash was calculated with reference to the air – dried material.

2.2.9.4 Determination of Percentage Moisture

Two grammes of amaranth powder was dried at 120^0C to constant weight. The final weight was recorded and the percentage loss calculated.

Two grammes of *Hibiscus sabdariffa* powdered material was weighed out and dried to constant weight at 100^0C. The loss in weight was calculated in percentage.

2.2.9.5 Microbiological Test on the solid extract of *Hibiscus sabdariffa* calyces [24]

A 5% w/v methanolic solution of Hibiscus sabdariffa extract, as well as a 5% w/v aqueous solution (using sterile water) were prepared. Ten plates filled with nutrient agar were obtained; five for aqueous (sterile) extract tests, and five for methanolic. In each nutrient agar were cut single bores, using a sterile borer, into which were placed sufficient amount of 5% methanolic extract of Hibiscus sabdariffa or 5% sterile aqueous extract. Streaks of *Esherichia coli, Staphylococcus aureus. Bacillus subtilis, Candida albicans and Pseudomonas aeruginosa* were done separately on individual nutrient agar, streaking from the edges of the rectangular holes, towards the edges of the petri-dishes.

The same procedures were repeated, using amaranth powder. The plates were incubated for 24hours at 37°C, and the lengths of inhibition of growth of the various micro-organisms measured and recorded. To serve as control, the above procedure was repeated, using pure

methanol, to test whether the zones of inhibition obtained for the methanolic solution of *Hibiscus sabdariffa* extract was due entirely to *Hibiscus sadariffa* extract, or to both.

2.2.9.6 Test for Colour Value [22]

To 500mg of moderately – fine powder of *Hibiscus sabdariffa* calyces, 60ml of phosphate buffer, pH 8.0 was added and heated on a water bath for 30 minutes. (Phosphate buffer pH8.00 was prepared by mixing 50ml of 0.2M potassium dihydrogen orthophosphate with 46.8ml of 0.2 Msodium hydroxide, and adding sufficient distilled water to produce 500ml of solution)

It was cooled and sufficient phosphate buffer added to produce 100ml, and then filtered. Five millilitres of the filtrate was diluted to 100ml with phosphate buffer pH 8.0.
The same procedure was repeated, using amaranth powder. The absorbances of the resulting solutions at 540nm were taken and recorded. Tests for colour value were repeated on the *Hibiscus sabdariffa*, extract after three months, six months and nine months. The results were tabulated.

2.2.10 FORMULATIONS [21]

2.2.10.1 Syrup Paracetamol B.P

To prepare 2L of syrup paracetamol 1.334kg of sucrose was weighed out. Freshly boiled and cooled water was added to make the volume approximately IL. It was then boiled and cooled. Forty-eight grammes (48g) of paracetamol powder BP was dissolved in 200ml of 90% ethanol, and then 200ml of propylene glycol was added. The prepared syrup was added and stirred. Two milliliters (2ml) of compound hydroxybenzoate solution was added as preservative, sufficient quantity of orange flavour was added. The volume was adjusted to two litres (2l), and then filtered into a 2L Winchester bottle and labelled appropriately.

Each concentration of the prepared aqueous extracts, were tested in samples of the paracetamol syrup; 2ml of each type being added to 100ml of paracetamol syrup. It was observed that the 33% w/v aqueous extract produced the most desirable coloured product, as compared to a standard sample of paracetamol syrup coloured with amaranth. Thus, the 33% w/v aqueous extract was reproduced in bulk and used for colouring all the ensuing formulations.

For amaranth powder, a 1% w/v solution in purified water was prepared and used according to BP specifications.

2.2.10.2 Syrup Chloroquine BP

Two litres of Syrup chloroquine phosphate was prepared as follows: 1.334g of sucrose was weighed out, and boiled in enough purified water to give a volume of one litre.

Fifty grammes of chloroquine powder BP was weighed out and dissolved in a small amount of freshly – boiled and cooled water. 2ml of compound hydroxybenzoate solution was added as preservative. The syrup was added; and a small amount of orange flavour also added, stirred and filtered; into a 2L Winchester bottle. It was labelled appropriately.

2.2.10.3 Syrup Pediatric Cough Linctus B.P

To prepare 2L, 1.334g of sucrose was weighed out and boiled in sufficient amount of recently – boiled and cooled water to produce 1L of syrup. After cooling, 6ml of anise water was added. Also, 30ml of chloroform spirit; 12.5g citric acid and 2ml compound hydroxyl benzoate

solution were added and stirred. The product was filtered and the volume adjusted to give 2L of Pediatric cough linctus B.P It was appropriately labeled and stored for future use.

2.2.10.4 Syrup Diphenhydramine B.P (Paediatric)

To prepare 2L, 1.334g of sucrose was weighed out. Sufficient amount of recently – boiled and cooled water was added and boiled to produce 1L of syrup. To the syrup was added 2.8g of diphenhydramine; 13g of sodium citrate, and 10g of ammonium chloride, after they had been dissolved in a small amount of potable water. Also, 2ml of compound hydroxyl benzoate solution was added as preservative. It was stirred and the final volume made up to 2L.

2.2.11 Temperature Stability Tests [20, 22]

Temperature stability tests on four formulations, namely syrup paracetamol BP, Syrup Chloroquine Phosphate B.P, Syrup Paediatric cough linctus B.P. and Syrup Diphenhydramine Paediatric B.P were carried out as described below.

For each formulation, eight amber bottles were filled with 100ml each of the production. Four of these were coloured with 1ml 33% w/v *Hibiscus sabdariffa* aqueous extract, whereas the other four were coloured with 1ml of 1% w/v amaranth solution. Thus, in all, 32 samples were prepared; eight samples for each formulation.

Each bottle was appropriately labelled and exposed to the following temperatures as appropriate: - 26^{o}C (room temperature); 37^{o} C (body temperature); 52^{o}C and 65^{o}C (representing high temperatures). Thus, for each formulation, two samples one coloured with *Hibiscus sabdariffa* extract and the other coloured amaranth were exposed to the same temperature, in order to deduce any difference in temperature stability of the two colouring agents.

To serve as control, eight amber bottles were filled with 100ml distilled water four of them being coloured with 1ml 33%w/v of *Hibiscus sabdariffa* extract and other four with 1ml of 1% w/v amaranth. They were appropriately labeled. Two samples (one coloured with amaranth, the

other coloured with *Hibiscus sabdariffa* extract) were exposed to the same temperature level (i.e. to 26^{o}C, 37oC, 52^{o}C, and 65^{o}C)

Each sample was analyzed using a colorimeter at bi-weekly intervals, for a four-month period. Absorbances at 540nm were taken, recorded and tabulated.

Also, 100ml of pure 33% aqueous extract and 33% acid infusion were placed in amber bottles and subjected to similar temperature stability tests.

Each sample was labeled appropriately and placed in their respective temperature environments, using Hot Air ovens.

Samples were taken at bi-weekly intervals and analyzed using a colorimeter. Absorbances at 540nm were taken.

2.2.12 Light – stability Tests [20. 22]

The effect of light was tested by placing 100ml of each formulation in plain glass bottles (to admit light), and then in amber coloured bottles to serve as control.

The first group of formulations (in plain glass bottles), were coloured with 1ml of 33% w/v aqueous extract of *Hibiscus sabdariffa* calyces and the second group (in four plain glass bottles) coloured with 1ml of 1% w/v amaranth solution. They were observed at room temperature (26^{0}C) for a period of four months.

Samples of 33%w/v acid infusion and 33% w/v aqueous extract were also tested. A colorimeter was used to measure absorbances at 540nm fortnightly, for a period of four months

To serve as control, 100ml of each formulation were placed in amber bottles. The first group were coloured with 1ml, 33%w/v *Hibiscus sabdariffa* extract, whereas the second group were coloured with 1ml, 1% w/v amaranth. They were kept at room temperature (26^{o}C) and analyzed using a colorimeter at bi-weekly intervals for a four month period. The results were tabulated.

2.2.13 pH Stability Tests [20, 22]

The pH meter was calibrated at PH 4.01, 7.00, and 9.00 at temperature 26.5^0C, using ready – made buffers.

The pH change over time (4 months) was carried out on the four formulations, using 100ml of each product. The first group being coloured with 1ml of 33% w/v aqueous extract of *Hibiscus sabdariffa* calyces and the second group being coloured with 1ml of 1% w/v amaranth solution.

pH stability for the formulations was poor especially those coloured with *Hibiscus sabdariffa* extract. A series of tests, therefore, were carried out using various buffers to ascertain the pH at which the formulations were most stable.

Each formulation was prepared using buffers at various pH levels: - 2.5, 3.0, 4.0, 4.9, 5.4, 6.5 and 7.0

To prepare a buffer solution of pH 2.5 106g of potassium dihydrogen orthophosphate was dissolved in 800ml of water. The pH was checked with a pH meter adjusting when necessary with Hydrochloric acid. Sufficient water was added to produce 1,000ml of solution. To obtain a buffer solution of pH 3.0; 3.4g of potassium dihydrogen orthophosphate was dissolved in 250ml of distilled water. The pH of the solution was adjusted to 3.0, with orthophosphoric acid when necessary; checking with a pH meter.

For a buffer solution of pH 4.0, 5.04g of disodium hydrogen orthophosphate and 3.01g of potassium dihydrogen orthophosphate were dissolved in sufficient water to produce 1,000ml. The pH was checked, using a pH meter; adjusting when necessary with glacial acetic acid.

To produce a buffer solution of pH 4.9; 40g of sodium dihydrogen orthophosphate and 1.2g of sodium hydroxide were dissolved in sufficient distilled water to produce 100ml. The pH was checked using a pH meter.

Buffer solutions of pH 5.4, 6.5 and 7.0 were also prepared using formulae from the British Pharmacopoeia 2000.

Syrup Paracetamol, Syrup chloroquine phosphate Syrup Diphenhydramine (pediatric) and Cough linctus (pediatric) were formulated using the various buffers listed above. Their pH stabilities over a four-month period at bi-weekly intervals were tested. (Please see section 3.14.3). A plot of natural log of concentration against time in weeks was done for each formulation. A straight line was obtained in each case, indicating first order kinetics (please see figure 15).

By use of the equation

$$t\,½ = \frac{0.693}{K} \quad (18)$$

Where $t^{1/2}$ = half life of the solution and K = rate constant, a plot of rate constants (K) against the various pH levels used, was plotted. From the plot, a minimum pH of 5.0 was obtained (fig 16). This infers that breakdown was lowest for the various formulations at pH5.0.

Reformulation of syrups Paracetamol BP, Chloroquine phosphate BP, Syrup Diphenhydramine (Paediatric) BP and Syrup paediatric Cough linctus BP was done, using a buffer solution of pH 5.0. This buffer solution was prepared by dissolving 1.118kg of sodium citrate in sufficient distilled water to produce 5,000ml.

The pH of the resulting formulations was cross checked, using a pH meter. The stability of the formulations buffered at pH 5 was analyzed over a four-month period at bi-weekly intervals, using a colorimeter. The results were tabulated. (Please see table 29)

2:2:14 - Use of *Hibiscus sabdariffa* extract in colouring other dosage forms

Aqueous extracts of *Hibiscus sabdariffa* (1ml of 33% w/v), were successfully used in colouring 50g of simple cream and 100ml of castor oil emulsion, to prove that *Hibiscus sabdariffa* extract can be used in colouring these dosage forms, and not only paediatric syrups. Please refer to plate 6, on page 110. The use of 1ml, 25% w/v *Hibiscus sabdariffa* extract, did not give the desired colour intensity when compared with commercial products.

CHAPTER THREE

3.0 RESULTS, COMMENTS AND DISCUSSIONS

3.1 FOREIGN MATTER [22]

There were no yeasts or moulds present. Unwanted plant parts constituted 1.2%, calculated as follows:-

Weight of foreign matter = 2.4g
Weight of sample examined = 200g
That is percentage of foreign matter = $\frac{2.4}{200} \times 100\%$
= 1.2% w/w

The value for foreign matter is within the B.P standard for a similar natural red colouring agent. (Cochineal 2.0% w/w). Since *Hibiscus sabdariffa* is not found in the B.P, comparison were made with cochineal, which is a red colouring agent of natural origin.

Also, the value agrees with specification for dried *Hibiscus sabdariffa* calyces of 2.0% w/w, found on the internet [32]

3.2 Macroscopic / Organoleptic Properties

Table I: Macroscopic / Organoleptic Properties of *Hibiscus sabdariffa* calyces and Amaranth powder

	COLOUR	**TASTE**	**ODOUR**	**FORM / NATURE**
Hibiscus sabdariffa calyces (dried)	Purple-Red	Acidic-astringent	Floral, berry-like	Hard, not easily broken, curled up
Amaranth powder	Reddish–Brown	saline	Almost Odourless	Free flowing powder

3.3 Microscopic Evaluation of the Powdered calyces of *Hibiscus sabdariffa*

Examination under a microscope revealed the presence of vascular bundles, crystals of calcium oxalate and several broken parts of the dried calyces.

3.4 Percentage Yield from Cold Maceration extraction

The yield was calculated as follows

Weight of evaporating dish = 250g
Weight of evaporating dish + extract = 419.25g
Weight of extract = 419.25 – 250,00g
= 169.25g

% Yield = $\frac{169.25g}{500g}$ x 100 = 33.85% = 34% w/w

The yield of 34% w/w was quite encouraging; however, the initial bright-red colour kept changing till after three months, it had turned brown. This emphasizes the need to protect the extract from light, and probably the addition of an anti-oxidant to keep the red colour for a longer period.

3.5 Physico – Chemical Properties

3.5.1 Identity Test for Amaranth [31]

A sulphur – like odour was evolved, indicating the presence of sulphur dioxide.

This is an indication that the powder being used is amaranth, according to BP specifications.

3.5.2 Solubility Tests

Table 2: Solubility Characteristics of Amaranth powder and Solid extract of *Hibiscus sabdariffa*

SUBSTANCE	SOLVENT	RESULT
Solid extract of *Hibiscus sabdariffa* calyces	Cold water	Readily soluble
Amaranth powder	Cold water	Readily Soluble
Solid extract of *Hibiscus sabdariffa* calyces	96% alcohol	Soluble
Amaranth Powder	96% alcohol	Slightly Soluble
Solid extract of *Hibiscus sabdariffa* calyces	Chloroform	Slightly Soluble
Amaranth powder	Chloroform	Slightly Soluble

Both amaranth powder and extract of *Hibiscus sabdariffa* were readily soluble in cold water, thus they are suitable as colouring agents for water-based pharmaceuticals.

The solid extract of *Hibiscus sabdariffa* was soluble in alcohol, whilst amaranth powder was only partially soluble.

For chloroform as a solvent, solubility for both amaranth and solid extract of *Hibiscus sabdariffa* was only slight.

3.5.3 ACIDITY AND ALKALINITY TESTS

Table 3: Acidity and Alkalinity Tests

SUBSTANCE	OBSERVATION	INFERENCE
Solid extract of *Hibiscus sabdariffa* calyces	Blue litmus paper turned red after few minutes	Filtrate is acidic
Amaranth Powder	Red litmus paper turned blue after few minutes	Filtrate is alkaline
Solid extract of *Hibiscus sabdariffa* calyces	Mean pH = 4.5±0.1	Filtrate is acidic
Amaranth powder	mean pH = 10.5±0.1	Filtrate is alkaline

3.6 TEST FOR WATER INSOLUBLE MATTER

Table 4: Test for water Insoluble matter

SUBSTANCE	OBSERVATION	INFERENCE
Solid extract of *Hibiscus sabdariffa* calyces	0.4% w/w	conforms to limit of 0.5% on the net
Amaranth Powder	0.2% w/w	Conforms to B. P Limit of 0.5%
Weight of crucible = 20.05g Weight of Insoluble matter + crucible (from *Hibiscus sabdariffa* calyces) = 20.054g weight of water Insoluble matter = 0.004g Percentage of water Insoluble matter = $\frac{0.004g \times 100\%}{1g}$ = 0.4% w/w		

3.7 Test for Total Ash [22, 32]

Table 5: Test for Total Ash

MATERIAL	RESULT
Powdered calyces of *Hibiscus sabdariffa*	10% w/w
Amaranth Powder	6% w/w

The value of 10% for *Hibiscus sabdariffa* calyces agrees with the specifications for *Hibiscus sabdariffa* calyces [32]. That of Amaranth powder also falls within BP specification of not more than 7%.

3.8 Test for Acid Insoluble Ash

Table 6: Test for Acid – Insoluble Ash

MATERIAL	RESULT
Powdered calyces of *Hibiscus sabdariffa*	1.4% w/w
Amaranth Powder	1.0% w/w

Weight of glass crucible = 35.08g

Weight of acid insoluble ash + crucible = 35.0842g

Weight of acid insoluble ash = 0.0042g

Percentage acid Insoluble Ash = $\frac{0.0042g \times 100\%}{0.3g}$

= 1.4%

3.9 Determination of Percentage Moisture Content

Weight of empty crucible = 41.08g

Weight of powdered *Hibiscus sabdariffa* calyces = 2.00g

Weight of crucible + powdered calyces after drying = 42.86

Weight of Powdered calyces after drying = 42.86 – 41.08g

= 1.78g

Moisture content of Powdered calyces = 2.00g – 1.78g

= 0.22g

Percentage Moisture Content = $\frac{0.22 \times 100}{2.00}$

= 11% w/w

A similar procedure was done for Amaranth powder

The percentage moisture content was 1.0%. w/w This figure agrees with the BP value for amaranth powder, which is not more than 1.0% w/w. For the powdered calyces, it was 11% w/w which falls within the standard of not more than 12% w/w found on the internet [32]

3.10 Microbiological Tests

Table 7. Microbiological Tests on solid extract of *Hibiscus sabdariffa* calyces Amaranth powder and Methanol

MICRO - ORGANISM	MEAN INHIBITORY ZONES(mm)				
	AQUEOUS		METHANOLIC		PURE METHANOL
	PLANT EXTRACT	AMARANTH	PLANT EXTRACT	AMARANTH	
Escherichia coli	0.5	NIL	0.5	NIL	0
Candida albicans	NIL	NIL	0.2	NIL	0
Staphylococcus aureus	0.8	NIL	1.0	NIL	0
Bacillus subtilis	0.7	NIL	1.8	0.1	0
Pseudomonas spp	NIL	NIL	1.0	NIL	0

There was no zone of inhition for *Candida albicans* and a very small (0.2 mm) inhibitory zone using methananolic plant extract. This is an indication that *Hibiscus sabdariffa* extract is

generally ineffective against fungi. Extracts of *Hibiscus sabdariffa* especially aqueous ones, must be preserved against fungal attack.

There were appreciable zones of inhibition for all the bacterial specimens except *Pseudomonas* spp, using aqueous extract. This is probably due to the fact that *Pseudomonas* spp is resistant to the antibacterial activity of *Hibiscus sabdariffa* extract. This group of gram negative bacteria (especially *Pseudomonas aeuroginosa*); are known for being notorious for their ability to survive in the environment, particularly in moist conditions. It is a contaminant of medicines, surgical equipment, clothing, and dressing with the ability to cause serious infections in immuno compromised patients.
The intrinsic resistance of gram negative bacteria is especially apparent with *Pseudomonas aeruginosa.* Many disinfectants and preservatives possess insufficient activity against it to be of any use [(24).]

It can also be concluded that the methanolic plant extract was more potent against bacterial growth than aqueous extract. This may be explained by the fact that methanol was able to extract constituents from *Hibiscus sabdariffa* calyces with more potent anti-micobial activity than the aqueous.

For amaranth powder there was practically no demonstration of anti-microbial activity. The value 0.1mm obtained when methanolic solution of amaranth was used is not conclusive enough of any anti-microbial activity.

Methanol had no antimicrobial activity on all the micro-organisms used. It can therefore be concluded that the activity of the methanol plant extract was due entirely to the extracted constituents of the plant and not to the combined action of methanol and plant extract.

3.11 Tests for colour value

Table 8a: Test for colour value on fresh extract.

SUBSTANCE	**ABSORBANCES AT 540nm**					**MEAN ABSORBANCES**
Hibiscus sabdariffa	1	2	3	4	5	
extract	0.256	0.257	0.255	0.261	0.262	0.258±0.002
Amaranth powder	0.461	0.460	0.461	0.462	0.463	0.461±0.002

The BP standard for colour value is “not less than 0.25”. Both *Hibiscus sabdariffa* extract and Amaranth solution passed the test for colour value.

Amaranth powder was found to have a very high colour value, almost twice as high as that of *Hibiscus sabdariffa* extract.

Table 8b: Test for colour value on. *Hibiscus sabdariffa* extract and Amaranth after three months

SUBSTANCE	ABSORBANES AT 540nm					MEAN ABSORBANCES
Hibiscus sabdariffa	1	2	3	4	5	
EXTRACT	0.256	0.257	0.255	0.260	0.260	0.258 ± 0.002
AMARANTH POWDER	0.460	0.460	0.460	0.459	0.461	0.460 ± 0.001

Table 8c:- Test for colour value on *Hibiscus sabdariffa* extract and Amaranth after six months.

SUBSTANCE	ABSORBANCES AT 540nm					MEAN ABSORBRANCES
Hibiscus	1	2	3	4	5	
Sabdariffa extract	0.25	0.251	0.250	0.249	0.249	0.250 ± 0.001
Amaranth Powder	0.458	0.458	0.458	0.457	0.460	0.458 ± 0.001

Table 8d:- Test for colour value on hibiscus sabdariffa extract and Amaranth after nine months.

SUBSTANCE	ABSORBANCES AT 540nm					MEAN ABSORBRANCES
Hibiscus	1	2	3	4	5	
sabdariffa extract	0.200	0.201	0.200	0.201	0.198	0.200 + 0.001
Amaranth Powder	0.458	0.458	0.457	0.456	0.458	0.457 + 0.001

The B.P standard for colour value is “not less than 0.25”. Both *Hibiscus sabdaariffa* extract and Amaranth passed the test for colour value.

Amaranth was found to have a very high colour value, almost twice as high as that of *Hibiscus sadariffa* extract.

After three months, and six months *Hibiscus sabdariffa* kept its colour value within the B.P standard, but after nine months the colour value fell below the B.P standard.

It can therefore be concluded that extracts to be used in colouring pediatric syrups should not be kept for more than six months. Loss of colour over time may be due to oxidation. This can be prevented by the addition of 0.1% sodium metabisulphite, an antioxidant. Amaranth powder retained its colour value within BP standards even after nine months.

3:12 TEMPERATURE STABILITY TESTS

3:12:1 Temperature Stability Test on syrup Paracetamol

Table 9: Temperature Stability Test on syrup Paracetamol coloured with *Hibiscus sabdariffa* extract.

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^oc)	0.515	0.500	0.475	0.468	0.452	0.432	0.427	0.425	0.421
37^0C	0.515	0.485	0.480	0.443	0.387	0.361	0.348	0.340	0.340
52^0C	0.515	0.480	0.476	0.465	0.340	Discontinued	-	-	-
65^0C	0.515	0.470	0.450	Discontinued	-		-		
80^0C	0.515	0.407	Discontinued	-	-		-		

Table 10: Temperature Stability Test on Syrup Paracetamol coloured Amaranth powder

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^oc)	0.750	0.750	0.750	0.748	0.748	0.748	0.747	0.747	0.747
37^0C	0.740	0.740	0.740	0.740	0.740	0.740	0.737	0.737	0.737
52^0C	0.740	0.740	0.740	0.740	0.740	0.735	0.730	0.725	0.720
65^0C	0.740	0.740	0.740	0.735	0.735	0.730	0.725	0.700	0.695
80^0C	0.740	0.740	0.740	0.730	0.725	Discontinued	-	-	-

Comment/Discussion

Between room temperature (about 26^0C) and 52^0C, there was relative stability of syrup paracetamol, coloured with *Hibiscus sabdariffa* extract. Between 65^0 C to 80^0C however, syrup paracetamol coloured with *Hibiscus sabdariffa* extract was unstable.

These observations were deduced from the changes in mean absorbances over time. It can be concluded that stability was better at lower temperatures. At 80^0C, syrup paracetamol, coloured with *Hibiscus sabdariffa* extract, deteriorated completely after only one week of exposure; thus the experiments at that temperature had to be discontinued. Amaranth – coloured syrup Paracetamol, was heat-stable, judging from the fact that absorbances over a four months period at temperatures between 37^0C to 80^0C remained virtually the same.

3:12:2 Temperature stability Test on Syrup Chloroquine

Table 11: Temperature stability Test on Syrup chloroquine coloured with *Hibiscus sabdarriffa* extract

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^oc)	0.958	0.970	1.120	1.150	1.200	1.260	1.307	1.300	1.300
37^0C	1.020	1.015	1.019	1.049	1.051	1.059	1.062	1.064	1.064
52^0C	1.150	1.147	1.130	0.848	0.790	0.701	0.680	.650	-
65^0C	1.080	1.071	0.928	Discontinued	-	-	-		

Table 12: Temperature stability Test on Syrup Chloroquine coloured with Amaranth

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^oc)	0.800	0.800	0.800	0.800	0.790	0.780	0.760	0.740	0.740
37^0C	0.850	0.830	0.800	0.800	0.769	0.742	0.703	0.703	0.703
52^0C	0.718	0.700	0.680	0.650	0.604	0.542	0.500	0.480	-
65^0C	0.610	0.600	0.584	0.562	0.540	Discontinued	-		

Comments / Discussion

Syrup chloroquine, whether coloured with *Hibiscus sabdariffa* or with amaranth, was unstable at high temperatures. At room temperature and 37^0C however, absorbances tended to increase over time for syrup coloured with *Hibiscus sabdariffa* extract as can be seen in figure 3. The reason for this might be due to the fact that, chloroquine molecules have a high affinity for colour from *Hibiscus sabdarriffa* extract. Possible reasons for this trend are discussed on page 100, under the main discussion.

3.12.3 Temperature Stability Test on Syrup Diphenhydramine (Paediatric)

Table 13: Temperature Stability Test on Syrup Diphenhydramine (Paediatric) coloured with *Hibiscus sabdariffa* extract.

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^oc)	0.500	0.498	0.486	0.456	0.450	0.398	0.390	0.390	0.385
37^0C	0.390	0.382	0.374	0.370	0.361	0.358	0.350	0.348	0.346
52^0C	0.390	0.375	0.360	0.352	0.340	0.332	0.320	0.315	-
65^0C	0.390	0.365	0.350	0.330	Discontinued	-	-		

Table 14: Temperature Stability Test on Syrup Diphenhydramine (Paediatric) coloured with Amaranth.

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^{o}c)	0.600	0.590	0.585	0.575	0.575	0.575	0.570	0.565	0.565
37^{0}C	0.600	0.580	0.578	0.572	0.570	0.570	0.562	0.560	0.558
52^{0}C	0.600	0.570	0.570	0.563	0.560	0.557	0.550	0.545	-
65^{0}C	0.600	0.540	0.531	0.520	0.520	0.503	0.500		

Comment / Discussion

Syrup Diphenhydramine coloured with amaranth was very stable to heat, whereas that coloured with *Hibiscus sabdariffa* extract was unstable at high temperatures. The stability of amaranth in Syrup Diphenhydramine was demonstrated by the almost unchanging absorbance levels over the four months period no matter the intensity of heat applied. This can be seen figure 6.

Amaranth is a synthetic product and is therefore relatively more heat-stable than natural products.

3.12.4 Temperature stability Test on Syrup Paediatric cough linctus

Table 15: - Temperature stability Test on Syrup Paediatric cough linctus coloured with *Hibiscus sabdariffa* extract

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^{o}c)	1.820	1.804	1.749	1.670	1.551	1.450	1.440	1.435	1.430
37^{0}C	1.820	1.640	1.558	1.529	1.510	1.503	1.501	1.498	1.495
52^{0}C	1.820	1.249	1.175	0.965	0.845	0.740	Discontinued	-	-
65^{0}C	1.820	1.051	0.922	0.802	Discontinued	-	-		

Table 16:- Temperature stability Test on Syrup Paediatric cough linctus coloured with Amaranth.

Mean Absorbances at 540mm									
WEEK	0	2	4		8	10	12	14	16
Room Temp (26^{o}c)	0.680	0.675	0.673	0.670	0.660	0.655	0.655	0.650	0.650
37^{0}C	0.680	0.666	0.660	0.660	0.655	0.650	0.645	0.645	0.640
52^{0}C	0.680	0.660	0.653	0.650	0.600	0.600	0.600	0.595	-
65^{0}C	0.680	0.650	0.645	0.640	0.610	Disconti-nued	-		

Comments / Discussion

Syrup Pediatric cough linctus coloured with amaranth was stable to heat whilst that coloured with *Hibiscus sabdariffa* extract was unstable at high temperatures, i.e. 52^{0}C and above. This can be seen when figures 7 and 8 are compared. This means that Syrup Paediatric cough linctus coloured with the plant extract should be kept at low temperatures such as room temperature.

3.12.5 Temperature Stability Test on Aqueous Extract of *Hibiscus sabdariffa* calyces

Table 17: Temperature stability Test on Aqueous Extract of *Hibiscus sabdariffa* calyces

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^{o}c)	1.150	1.134	1.120	1.050	0.975	0.960	0.934	0.930	0.925
37^{0}C	1.150	1.102	0.980	0.916	0.901	0.817	0.777	0.770	0.770
52^{0}C	1.150	0.970	0.915	0.911	0.878	0.798	0.603	0.600	-
65^{0}C	1.150	0.903	0.783	0.581	0.470	0.315	Disconti-nued		

Table 18 : - Temperature Stability Test on Acid Infusion of *Hibiscus sabdariffa* calyces

Mean Absorbances at 540mm									
WEEK	0	2	4	6	8	10	12	14	16
Room Temp (26^oc)	1.495	0.490	0.480	0.395	0.390	0.381	0.363	0.355	0.350
37^0C	1.495	0.456	0.426	0.400	0.397	0.383	0.304	0.300	0.295
52^0C	1.495	0.401	0.360	0.350	0.342	0.331	0.300	0.298	-
65^0C	1.495	0.338	0.327	0.300	0.285	0.280	Discontinued		

Comments / Discussion

Both the aqueous and acid infusions of *Hibiscus sabdariffa* calyces showed deterioration on exposure to heat, especially at high temperatures i.e., from 52^0C. At low temperatures, such as at room temperature, deterioration was slow. This infers that the extracts should be stored at low temperatures, such as at room temperature.

Absorbances were generally lower for the acid Infusion, whereas that of the aqueous were high.

Table 19a: - Temperature stability Test on Blank formulations (as control), using *Hibiscus sabdariffa* as colouring agent

	MEAN ABSORBANCES AT 540nm								
WEEK	0	2	4	6	8	10	12	14	16
Room Temperature (26^oC)	0.514	0.501	0.484	0.456	0.441	0.432	0.430	0.428	0.424
37^oC	0.512	0.510	0.510	0.500	0.495	0.490	0.490	0.455	0.480
52^oC	0.512	0.485	0.480	0.476	0.464	Discont.	-	-	-
65^oC	0.511	0.408	0.400	Discon.	-	-	-	-	-

Table 19b: - Temperature stability Test on Blank formulations (as control), using Amaranth as colouring agent.

	MEAN ABSORTANCES AT 540nm								
WEEK	0	2	4	6	8	10	12	14	16
Room Temperature(26 ^{o}C)	0.740	0.740	0.740	0.740	0.740	0.740	0.740	0.740	0.740
37 ^{o}C	0.741	0.741	0.743	0.745	0.744	0.740	0.738	0.735	0.735
52 ^{o}C	0.740	0.740	0.735	0.730	0.725	0.720	0.715	0.715	0.705
65 ^{o}C	0.742	0.742	0.740	0.730	0.725	0.720	0.710	0.705	0.700

The temperature stability test on blank formulations (no drug present) revealed a similar pattern, as when a drug was present. This can be seen in the similar slopes in figure 1 and figure 12.

It can thus be concluded that exposure to high temperatures (i.e. 52 ^{o}C, and 65 ^{o}C) has a deteriorative effect on colour stability especially when the plant extract is used. Colour deterioration is therefore not due to the presence of drug but due to excessive temperature.

3:13 LIGHT STABILITY TESTS

Table 20a: - Light Stability Test on Formulations coloured with *Hibiscus sabdariffa* extract; and the two extract types (Aqueous and Acid Infusion), using plain glass bottles.

Mean Absorbances at 540mm									
Week	0	2	4	6	8	10	12	14	16
Syrup Paracetmol	1.050	1.022	1.007	1.000	0.990	0.980	0.970	0.960	0.950
Syrup Chloroquine	1.050	1.052	1.155	1.247	1.281	1.301	1.342	1.334	1.314
Syrup Diphenhydramine (Paediatric)	0.660	0.658	0.667	0.683	0.777	0.775	0.770	0.760	0.750
Syrup Paediatric Cough linctus	1.680	1.650	1.608	1.570	1.442	1.340	1.220	1.200	1.150
Aqueous Extract	1.150	1.122	1.023	0.950	0.806	0.712	0.680	0.650	0.620
Acid Infusion	0.500	0.495	0.480	0.472	0.457	0.424	0.401	0.380	0.360

Table 20b: Light stability Test on Formulations coloured with Amaranth, using plain glass bottles.

Mean Absorbances at 540mm									
Week	0	2	4	6	8	10	12	14	16
Syrup Paracetmol	0.745	0.740	0.740	0.740	0.735	0.730	0.730	0.725	0.720
Syrup Chloroquine	0.835	0.831	0.830	0.822	0.800	0.756	0.705	0.655	0.650
Syrup Diphenhydramine (Paediatric)	0.550	0.543	0.540	0.540	0.540	0.540	0.540	0.535	0.530
Syrup Paediatric Cough linctus	0.685	0.678	0.670	0.670	0.652	0.650	0.643	0.640	0.635

Comments / Discussion

All the pediatric syrups, coloured with amaranth were relatively stable, on exposure to light, whereas those coloured with *Hibiscus sabdariffa* extract showed much deterioration on exposure to light.

From the foregoing, it can be said that all formulations and extracts using *Hibiscus sabdariffa* should be stored in amber coloured bottles, to exclude light.

Syrup Diphenhydramine however showed an interesting behaviour in that absorbance values increased initially, and then stabilized on exposure to light when coloured with extract of *Hibiscus sabdariffa*. Absorbance values remained unchanged over the four month period when coloured with amaranth.

Table 20c: - Light stability Test on formulations coloured with *Hibiscus sabdariffa* extract using amber bottles as control.

	MEAN ABSORTANCES AT 540nm								
WEEK	0	2	4	6	8	10	12	14	16
Syrup Paracetamol	1.032	1.030	1.030	1.028	1.027	1.027	1.026	1.025	1.024
Syrup chloroquine	1.058	1.058	1.058	1.060	1.062	1.063	1.062	1.060	1.060
Syrup Diphenhyramine (Paediatric)	0.651	0.652	0.662	0.665	0.685	0.700	0.710	0.720	0.730
Syrup Cough linctus(Paediatric)	1.670	1.665	1.665	1.660	1.660	1.659	1.658	1.656	1.654

Table 20d: - Light stability Test on formulations coloured with Amaranth using amber bottles as control.

	MEAN ABSORTANCES AT 540nm								
WEEK	0	2	4	6	8	10	12	14	16
Syrup Paracetamol	0.745	0.745	0.744	0.744	0.742	0.742	0.741	0.741	0.740
Syrup chloroquine	0.824	0.824	0.824	0.823	0.822	0.821	0.819	0.819	1.818
Syrup Diphenhydramine (Paediatric)	0.550	0.550	0.550	0.549	0.549	0.548	0.548	0.547	0.547
Syrup Cough linctus(Paediatric)	0.684	0.684	0.683	0.683	0.682	0.682	0.681	0.681	0.680

In the control test (using amber bottles) all the formulations coloured with *Hibiscus sabdariffa* extract showed better stability as compared with those placed in plain glass bottles. This is revealed in figure 11. Absorbances over a period of time remained the same when amber bottles were used, whereas absorbances overtime kept reducing when plain glass bottles were used.

Those coloured with amaranth showed similar stability whether placed in plain or amber bottles.

The above observations goes to buttress the point that formulations coloured with *Hibiscus sabdariffa* extract are best stored in amber bottles since their stability is affected by light.

3:14. pH STABILITY TESTS

3.14.1 Table 21a: - pH Stability Test on the four un-buffered formulations coloured with *Hibiscus sabdariffa* extract.

Mean pH at fortnight intervals									
Week	0	2	4	6	8	10	12	14	16
Syrup Paracetmol	5.50	5.45	5.40	5.25	5.13	4.95	4.86	4.80	4.75
Syrup Chloroquine	5.00	4.90	4.85	4.70	4.64	4.53	4.47	4.40	4.32
Syrup Diphenhydramine (Paediatric)	5.50	5.25	5.15	5.10	5.06	4.83	4.75	4.70	4.65
Syrup Paediatric Cough linctus	3.50	3.28	3.20	3.13	3.00	2.95	2.84	2.80	2.73

3.14.2 Table 21b: - pH stability Test on the four un-buffered formulations coloured with Amaranth

Mean pH at fortnight - intervals									
Week	0	2	4	6	8	10	12	14	16
Syrup Paracetmol	6.85	6.80	6.60	6.49	6.38	6.30	6.16	6.06	6.00
Syrup Chloroquine	5.50	5.40	5.35	5.30	5.21	5.07	5.00	5.00	4.98
Syrup Diphenhydramine (Paediatric)	6.55	6.54	6.37	6.30	6.25	6.20	6.15	6.10	6.00
Syrup Paediatric Cough linctus	3.60	3.51	3.45	3.40	3.25	3.00	2.84	2.80	2.75

3.14.3 Search for minimum pH at which hydrolysis occurs

Table 22. Analysis of Breakdown of Formulations at pH 2.5

Formulation	Syr. Paracetamol						Syr. chloroquine					
Time (weeks)	1	2	3	4	5	6	1	2	3	4	5	6
Concentration (Absorbance at 540nm)	0.504	0.480	0.300	0.250	-	-	0.908	0.815	0.700	0.561	-	-
t ½(weeks^{-1})	2						2					

(Table 22, continuation)

Formulation	Syr. Diphenhydramine (pediatric)						Syr cough linctus (pediatric)					
Time (weeks)	1	2	3	4	5	6	1	2	3	4	5	6
Concentration (Absorbance at 540nm)	0.494	0.456	0.400	0.361	-	-	1.804	1.749	1.570	1.350	-	-
t ½(weeks^{-1})	2						2					

Table 23. Analysis of Breakdown of Formulations at pH 3.0

Formulation	Syr. Paracetamol						Syr. chloroquine					
Time (weeks)	1	2	3	4	5	6	1	2	3	4	5	6
Concentration (Absorbance at 540nm)	0.504	0.480	0.360	0.340	0.300	0.251	0.908	0.800	0.690	0.651	0.651	0.580
t ½(weeks^{-1})	3						3					

(Table 23, continuation)

Formulation	Syr. Diphenhydramine (pediatric)						Syr cough linctus (pediatric)					
Time (weeks)	1	2	3	4	5	6	1	2	3	4	5	6
Concentration (Absorbance at 540nm)	0.494	0.450	0.390	0.380	0.342	0.330	1.804	1.740	1.600	1.560	1.400	1.315
t ½(weeks^{-1})	3						3					

Table 24: - Analysis of Breakdown of Formulations at pH 4.0

Formulation	Syrup – Paracetamol										Syrup - Chloroquine									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.500	0.470	0.340	0.320	0.300	0.280	0.264	0.250	-	-	0.900	0.800	0.685	0.675	0.650	0.633	0.580	0.561	-	-
t ½	4										4									

(Table 24, continuation)

Formulation	Syrup – Diphenhydramine (Paediatric)										Syrup – Cough Linctus (Paediatric)									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.490	0.450	0.383	0.379	0.342	0.330	0.321	0.287	-	-	1.788	1.740	1.600	1.550	1.440	1.380	1.350	1.286	-	-
t ½	4										4									

Table 25: - Analysis of Breakdown of Formulations at pH 4.9

Formulation	Syrup – Paracetamol										Syrup - Chloroquine									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.500	0.470	0.430	0.370	0.300	0.264	0.260	0.256	0.250	0.228	0.900	0.800	0.750	0.727	0.700	0.650	0.640	0.582	0.575	0.560
t ½	5										5									

(Table 25 continuation)

Formulation	Syrup – Diphenhydramine (Paediatric)									Syrup – Cough Linctus (Paediatric)										
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.490	0.450	0.380	0.370	0.356	0.350	0.348	0.340	0.330	0.285	1.788	1.780	1.776	1.690	1.680	1.565	1.560	1.550	1.462	1.300
t ½	5										5									

Table 26: - Analysis of Breakdown of Formulations at pH 5.4

Formulation	Syrup – Paracetamol												Syrup - Chloroquine											
Time (weeks)	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Concentration (absorbance at 540mm)	0.480	0.464	0.452	0.450	0.441	0.437	0.430	0.400	0.380	0.350	0.300	0.248	0.888	0.880	0.860	0.850	0.830	0.810	0.800	0.791	0.785	0.772	0.701	0.651
t ½	6												6											

(Table 26 continuation)

Formulation	Syrup – Diphenhydramine (Paediatric)												Syrup – Cough Linctus (Paediatric)											
Time (weeks)	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Concentration (absorbance at 540mm)	0.498	0.495	0.490	0.485	0.480	0.476	0.470	0.465	0.460	0.455	0.450	0.422	1.800	1.791	1.782	1.766	1.756	1.736	1.706	1.651	1.623	1.600	1.550	1.500
t ½	6												6											

Table 27: - Analysis of Breakdown of Formulations at pH 6.5

Formulation	Syrup – Paracetamol										Syrup - Chloroquine									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.500	0.470	0.340	0.310	0.300	0.280	0.264	0.260	0.256	0.248	0.900	0.870	0.850	0.800	0.740	0.700	0.660	0.630	0.600	0.570
t ½	5										5									

(Table 27 continuation)

Formulation	Syrup – Diphenhydramine (Paediatric)										Syrup – Cough Linctus (Paediatric)									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.490	0.487	0.482	0.480	0.476	0.470	0.465	0.460	0.435	0.415	1.800	1.787	1.780	1.760	1.750	1.730	1.700	1.600	1.540	1.500
t ½	5										5									

Table 28: - Analysis of Breakdown of Formulations at pH 7.0

Formulation	Syrup – Paracetamol										Syrup - Chloroquine									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.500	0.470	0.340	0.320	0.300	0.280	0.264	0.250	-	-	0.900	0.800	0.685	0.675	0.650	0.633	0.580	0.561	-	-
t ½	4										4									

(Table 28, continuation)

Formulation	Syrup – Diphenhydramine (Paediatric)										Syrup – Cough Linctus (Paediatric)									
Time (weeks)	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Concentration (absorbance at 540mm)	0.492	0.481	0.470	0.464	0.452	0.450	0.443	0.400	-	-	1.800	1.780	1.740	1.680	1.600	1.540	1.500	1.480	-	-
t ½	4										4									

Comments / Discussions

pH values for the pediatric syrups coloured with *Hibiscus sabdariffa* extract were lower (acidic) than those coloured with amaranth. This is probably due to the fact that *Hibiscus sabdariffa* extract is acidic (refer table 3).

pH stability over the four month period, comparing Amaranth and *Hibiscus sabdariffa* as colourants for pediatric syrups showed a marked difference as seen in figure 13. The gradual slope for Amaranth shows better stability as compared with a steep slope for *Hibiscus sabdariffa* extract.

Generally, pH values reduced with time for both amaranth and *Hibiscus sabdariffa* extract formulations, though that for the latter was more marked. The reduction in pH over time is possibly due to hydrolysis of the preparations.

Drug degradation due to hydrolysis can be reduced by preparing the solution at the pH at which hydrolysis is minimized. This has been done in section 3.14.3. The minimum pH was found to be 5.0, on plotting the rate constants against pH (figure 15)
Buffer salts may be added to obtain this minimum pH, however, buffer salts may give rise to increased hydrolytic degradation due to the general acid-base catalysis.

Examples of buffers that could be used are phosphate buffer (sodium acid phosphate / sodium hydroxide) and citrate buffer (citric acid / sodium citrate). These buffers will help stabilize the pH of the pediatric syrups prepared especially using *Hibiscus sabdariffa* extract as colourant.

Antioxidants are useful in maintaining the stability of some drug formulations. For example sodium metabisulphite is employed in some eye drop formulation to act as reducing agents which are preferentially oxidized to maintain the drug in its active, reduced form [17].

Sodium metabisulphite in acidic preparations also have anti-microbial properties. This explains why it was used to preserve the colour of the *Hibiscus sabdariffa* extract.

3:14:4 pH stability tests on the formulations buffered at pH 5.00

Table 29: pH Stability Tests on the four buffered formulations (at pH 5.00), coloured with *Hibiscus sabdariffa* extract.

	Mean pH at bi-weekly intervals								
WEEK	0	2	4	6	8	10	12	14	16
Syrup Paracetamol	5.00	5.00	5.00	5.01	5.01	5.02	5.04	5.04	5.04
Syrup Chloroquine	5.00	4.98	4.98	4.98	4.98	4.98	4.98	4.98	4.98
Syrup(Paediatric) Diphenhydramine	5.00	5.00	5.00	5.00	5.00	5.01	5.01	5.01	5.01
Syrup Paediatric Coughlinctus	5.00	5.00	4.99	4.98	4.98	4.98	4.97	4.97	4.97

Table 30:- pH Stability Tests on the four buffered formulations (at pH 5.00), coloured with Amaranth

	Mean pH at bi-weekly intervals								
WEEK	0	2	4	6	8	10	12	14	16
Syrup Paracetamol	5.00	5.00	5.00	5.00	5.00	5.00	4.99	4.99	4.99
Syrup Chloroquine	5.00	5.00	5.00	5.00	4.99	4.99	4.98	4.98	4.98
Syrup(Paediatric) Diphenhydramine	5.00	5.00	5.00	5.00	5.00	5.00	4.99	4.99	4.99
Syrup Paediatric Coughlinctus	5.00	5.00	5.00	5.00	4.99	4.99	4.99	4.99	4.98

Comments

The minimum pH at which hydrolysis occurred was found to be pH 5.00 (See figure 15). The pediatric syrups buffered at pH 5.00 using sodium citrate buffer (See section 2.2.13), were found to have better stability over the four month test period. This can be seen from figure 14 which shows near-horizontal slopes, signifying good pH stability of the paediatric syrups over the test period.

Fig. 1: Plot of mean Absorbance at 540nm of Syrup Paracetamol against time (weeks) at various temperatures – using *Hibiscus sabdariffa* extract as colouring agent.

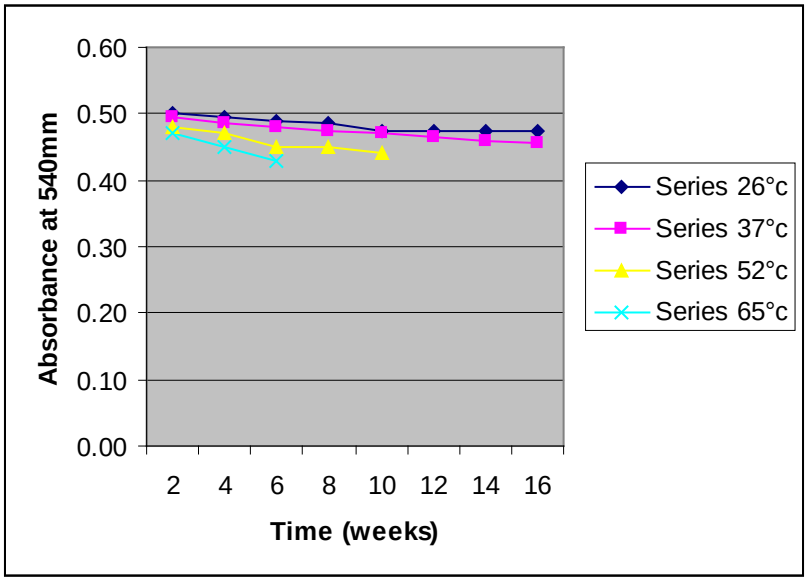


Fig. 2: Plot of mean Absorbance at 540nm of Syrup Paracetamol against time (weeks) at various temperatures – using Amaranth as colouring agent.

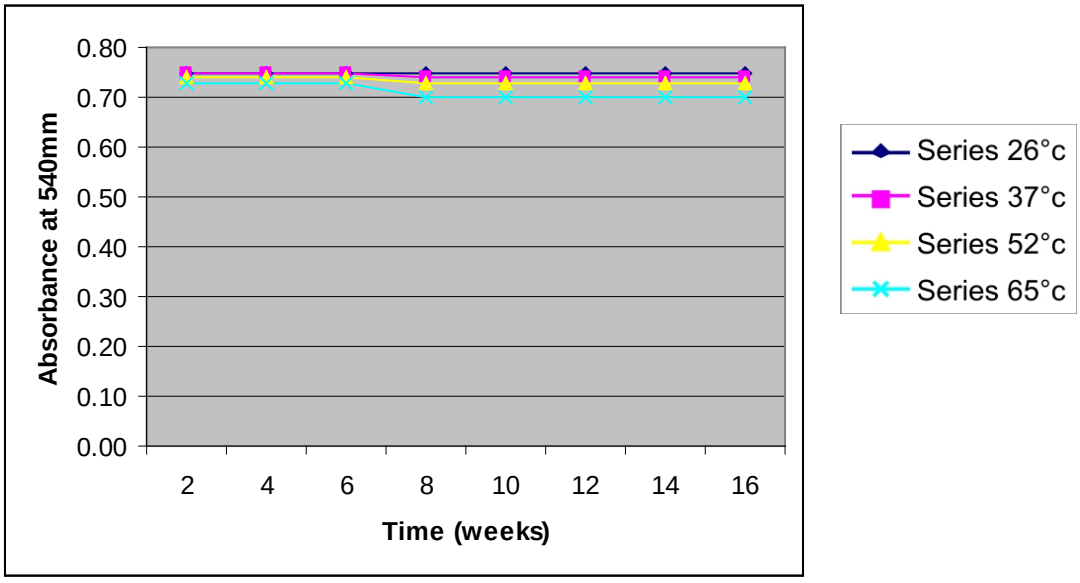


Fig. 3: Plot of mean Absorbance at 540nm of Syrup Chloroquine against time (weeks) at various temperatures using *Hibiscus sabdariffa* extract as colouring agent.

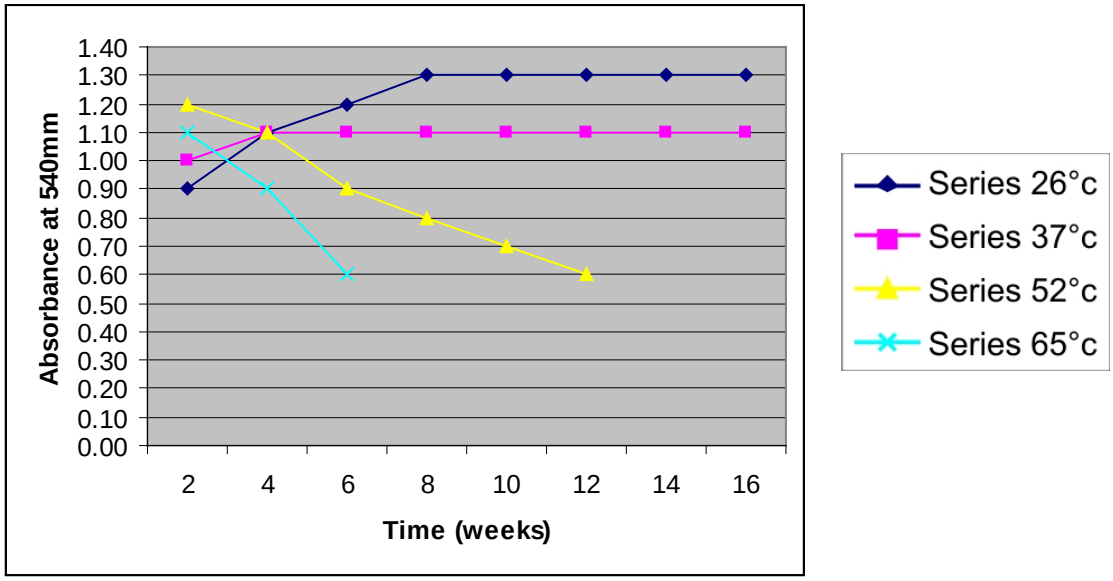


Fig. 4: Plot of mean Absorbance at 540nm of Syrup Chloroquine against time (weeks) at various temperatures, using Amaranth as colorant.

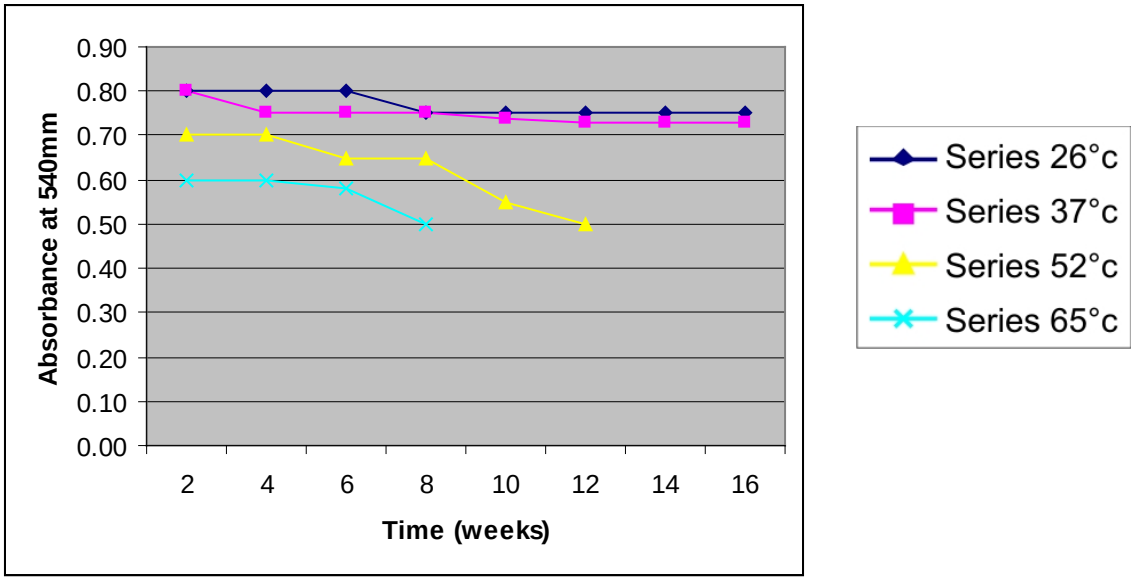


Fig 5: Plot of mean Absorbance at 540nm of Syrup Diphenhydramine (Paediatric) against time (weeks) at various temperatures – using *Hibiscus sabdariffa* as colouring agent.

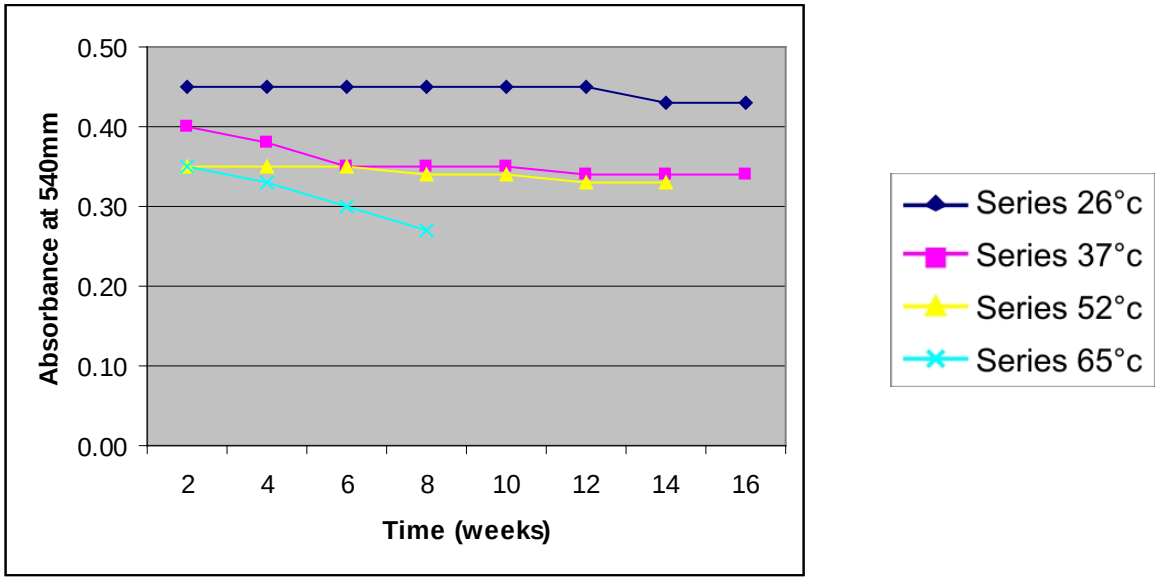


Fig. 6: Plot of mean Absorbance at 540nm of Syrup Diphenhydramine (Paediatric) against time (weeks), at various temperatures using Amaranth as colouring agent.

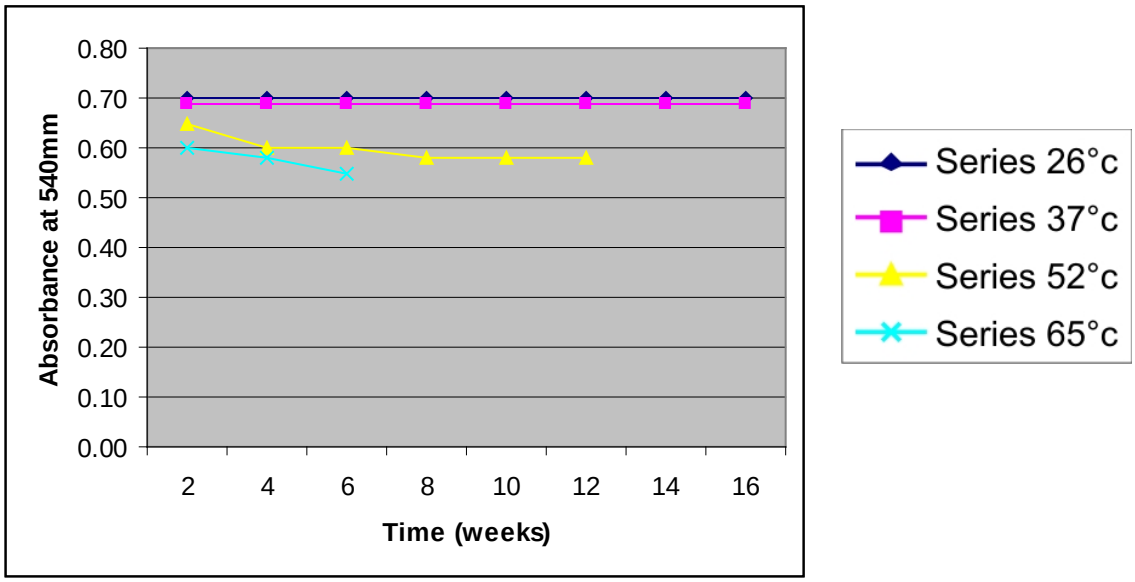


Fig. 7: Plot of mean Absorbance at 540nm of Syrup Paediatric cough linctus against time (weeks) at various temperatures, using *Hibiscus sabdariffa* as colouring agent.

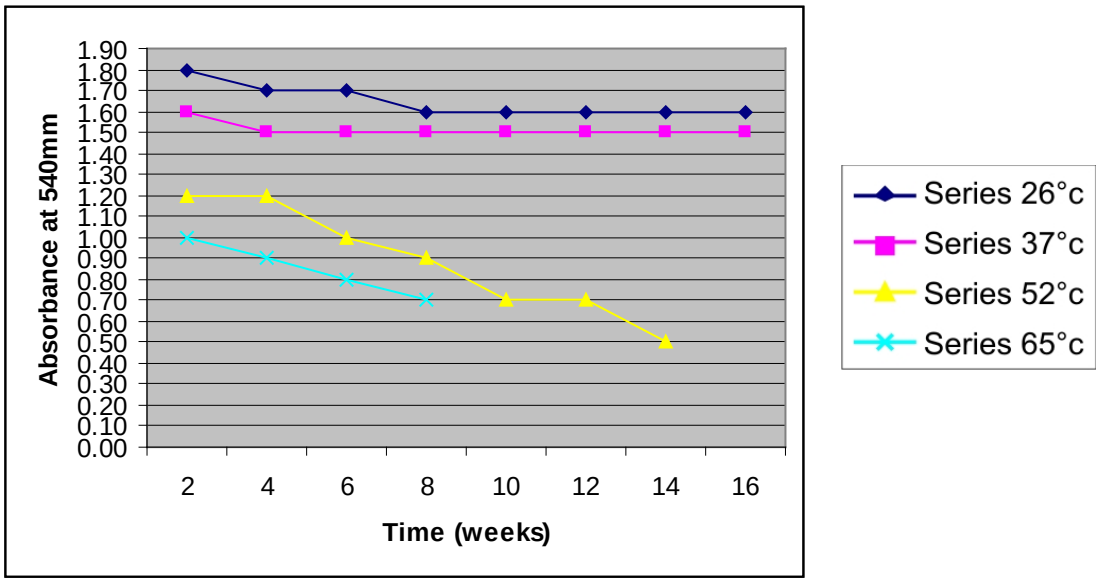


Fig. 8: Plot of mean Absorbance at 540nm of Syrup Paediatric Cough linctus against time (weeks), at various temperatures, using amaranth as colouring agent.

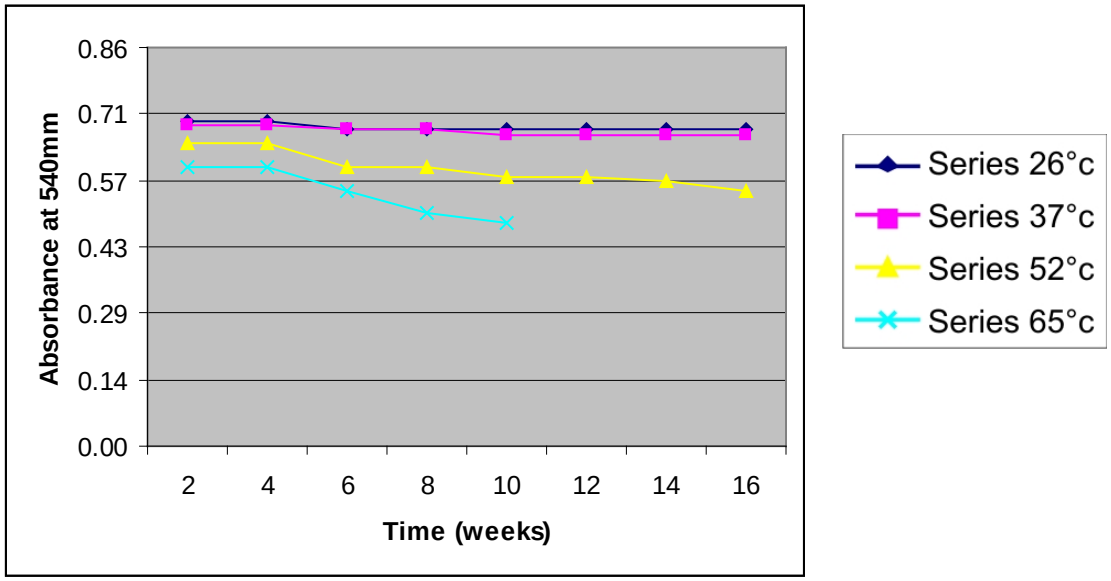


Fig. 9: Comparative plot of mean Absorbance at 540nm of Aqueous Extract (blue line) and Acid Infusion (red line) of *Hibiscus sabdariffa* against time (weeks) at Room temperature.

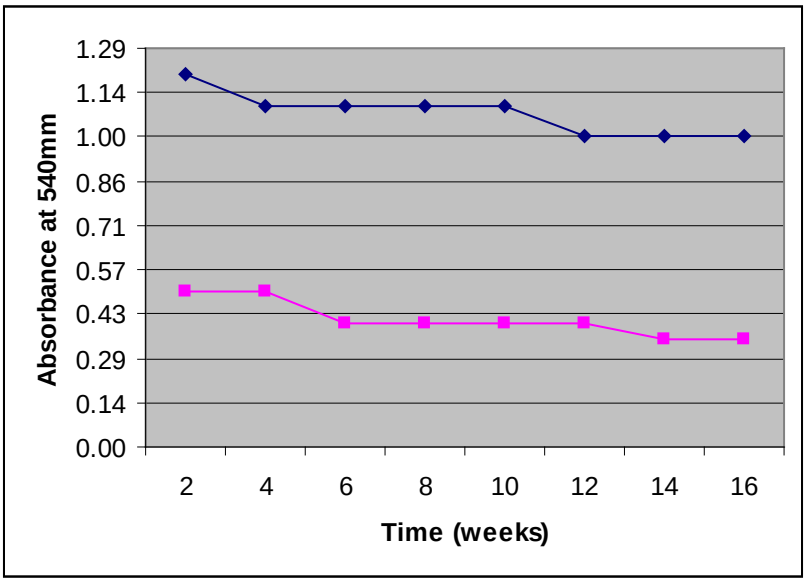


Fig. 10: Comparative plot of mean Absorbance at 540nm of Syrup Paracetamol's stability to light when coloured with extract of *Hibiscus sabdariffa* (Blue line) and with amaranth(red line) .

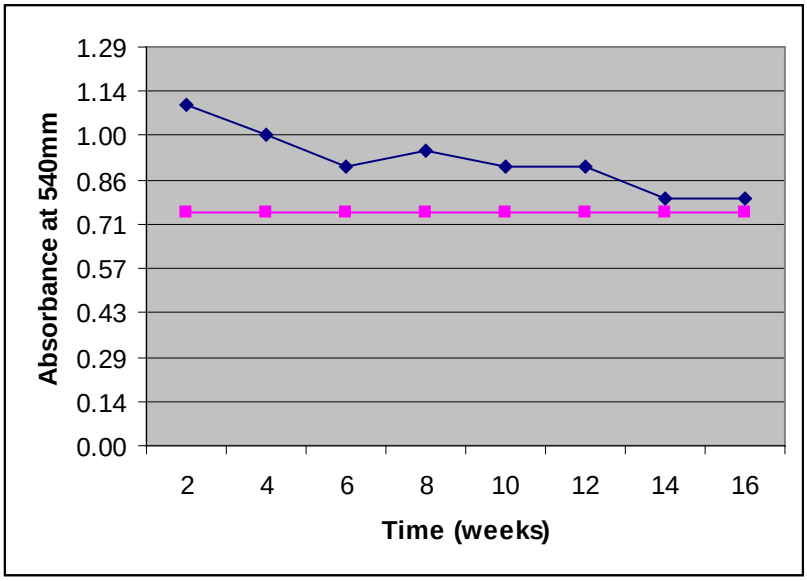


Fig. 11: Comparative plot of Mean Absorbance of syrup paracetamol coloured with *Hibiscus sabdariffa* when placed in amber bottles and when placed in plain glass bottles

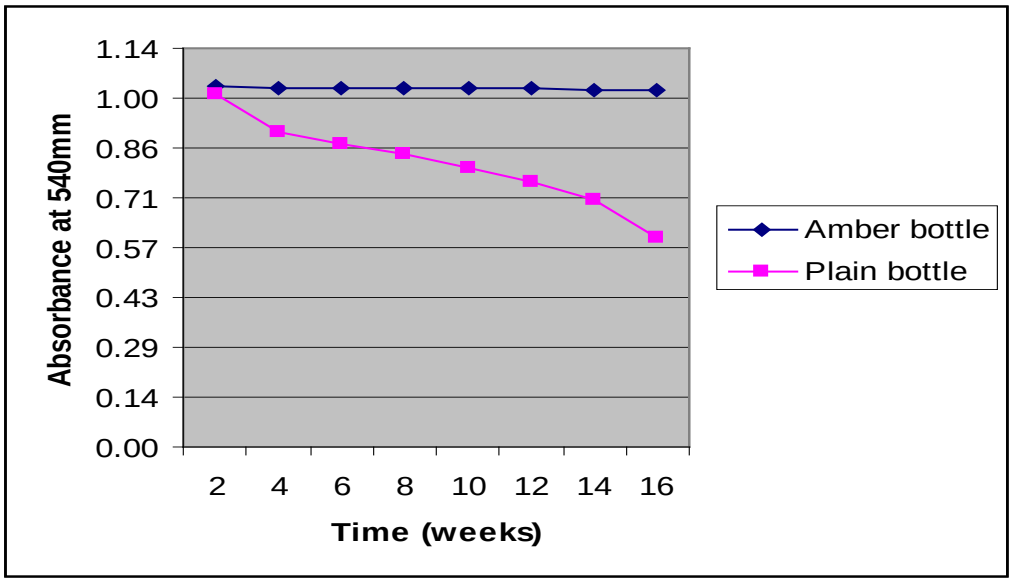


Fig. 12: Plot of Mean Absorbance of blank formulations against time at various temperatures

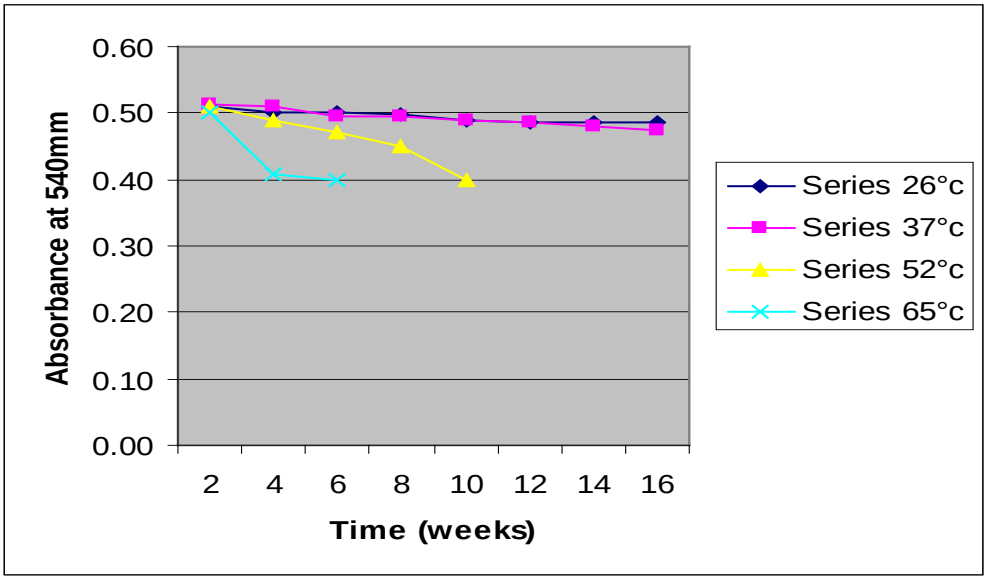


Fig. 13: Comparative plot of Mean pH values against time of Syrup Paracetamol, when coloured *Hibiscus sabdariffa* extract, and with Amaranth

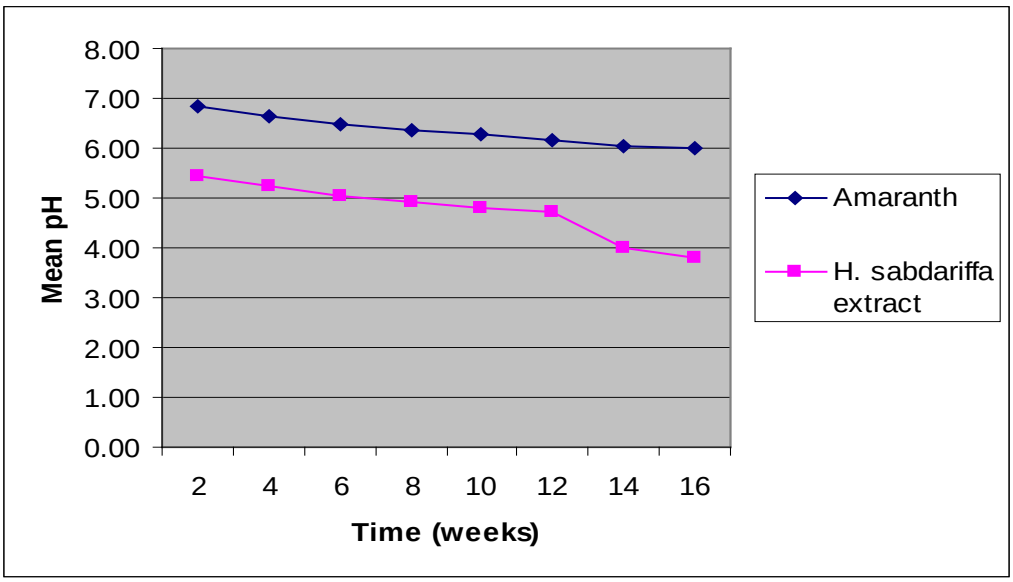


Fig. 14: Plot of Mean pH values of the buffered Formulations against time

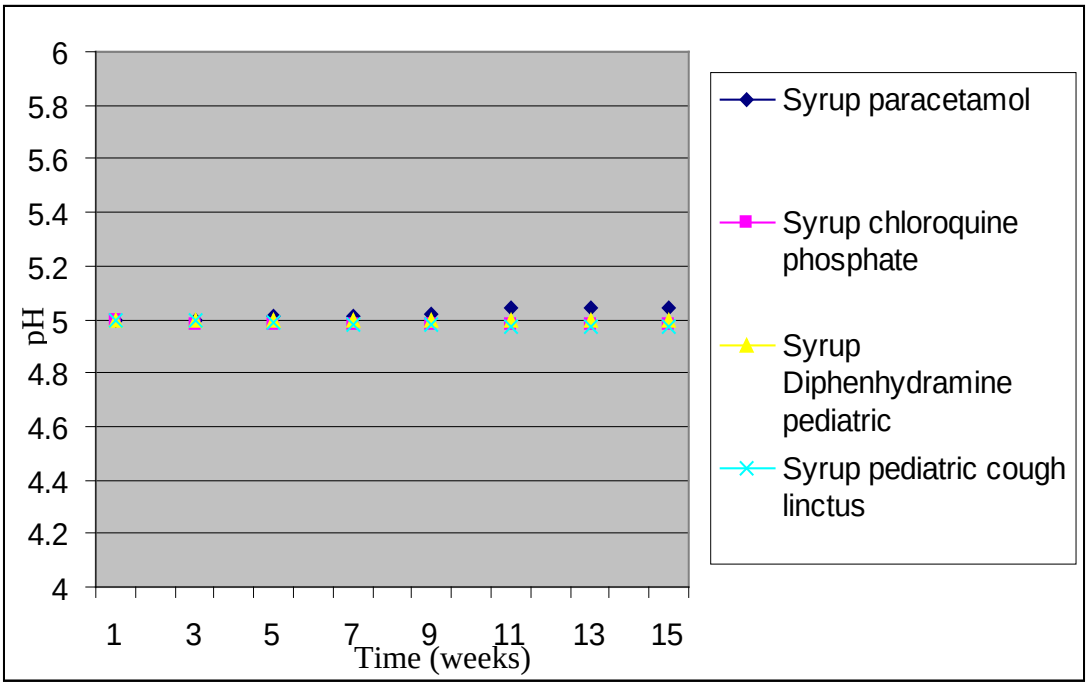


Fig. 15: Plot of ln concentration against time for Syrup Paracetamol coloured with *Hibiscus sabdariffa* extract.

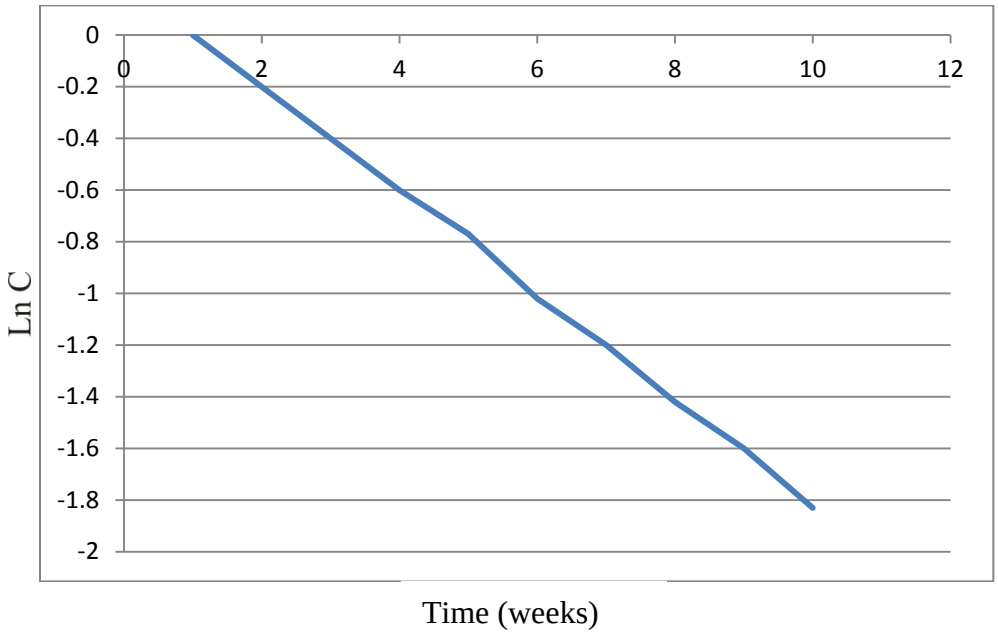
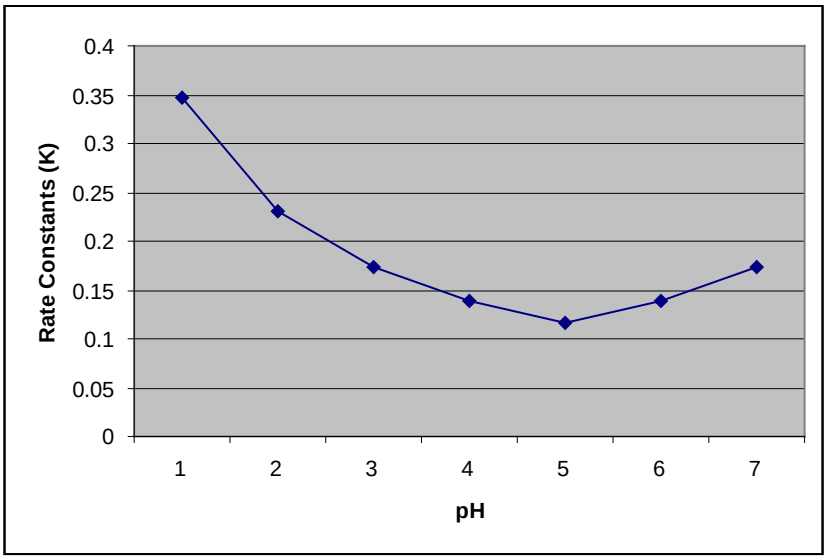


Fig. 16: Plot of Rate Constants against pH (for all formulations)



CHAPTER FOUR

COMPARATIVE TEMPERATURE, LIGHT AND pH STABILITY STUDIES ON THE FOUR FORMULATED PAEDIATRIC SYRUPS COLOURED WITH *HIBISCUS SABDARIFFA* EXTRACT OR AMARANTH USING CHI SQUARE TEST FOLOWED BY TUKEY'S COMPARISM TEST

Table 31: Comparison of temperature stabilities of the four formulated paediatric syrups, using *Hibiscus sabdariffa* extract or amaranth as colouring agent. [36, 38]

	Room Temp	**37^{0}C**	**52^{0}C**	**65^{0}C**
Syrup Paracetamol (HS) vrs Syrup Paracetamol (A)	ns	ns	*	*
Sryp Chloroquine (HS) vrs Syrup Chloroquine (A)	ns	ns	ns	ns
Syrup Paed. Cough Linctus (HS) vrs Syrup Paed. Cough Linctus (A)	ns	ns	*	*
Syrup Diphen (HS) vrs Syrup Diphen (A)	ns	ns	ns	*

Discussion:-

At room temperature and 37^{0}C temperature stabilities of all the formulated pediatric syrups showed comparable stability, whether coloured with *Hibiscus sabdariffa* extract or with amaranth. This infers that; paediatric syrups coloured with the natural product (which is more liable to temperature instability than the articfical product amaranth) is best stored at room temperature or at 37^{0}C. Chloroquine syrup was an exception, in that it showed good stability at all temperatures whether coloured with amaranth or extract of *Hibiscus sbadariffa*.

Please refer to Appendix II (p. 117) for statistical evaluations.

Table 32 Summarized statistical Comparison of light stabilities of the four formulated pediatric Syrups, using *Hibiscus sabdariffa* extract or Amaranth as colouring agent

Syrup Paracetamol (HS) vrs Syrup Paracetamol (A)	*
Sryp Chloroquine (HS) vrs Syrup Chloroquine (A)	ns
Syrup Paed. Cough Linctus (HS) vrs Syrup Paed. Cough Linctus (A)	*
Syrup Diphen (HS) vrs Syrup Diphen (A)	ns

Discussion

Syrups Diphenhydramine (Paediatric) and chloroquine phosphate whether coloured with *Hibiscus sabdariffa* extract or amaranth were stable on exposure to light for a 4 month period. Syrup Paracetamol and Pediatric cough linctus were unstable on exposure to light when coloured with *Hibiscus Sabdariffa* extract.

Table 33 Summerised statistical Comparison of pH stabilities of the four formulated paediatric syrups, using *Hibiscus sabdariffa* extract or Amaranth as colouring agent. .

Syrup Paracetamol (HS) vrs Syrup Paracetamol (A)	*	-
Sryp Chloroquine (HS) vrs Syrup Chloroquine (A)	*	-
Syrup Paed. Cough Linctus (HS) vrs Syrup Paed. Cough Linctus (A)	*	-
Syrup Diphenhydramine (Ped) (HS) vrs Syrup Diphenhydramine (Ped) (A)	*	-

Discussion

pH change over time using *Hibiscus sabdariffa* extract as colourant was more rapid than acidic with amaranth. Paediatric syrups coloured with *Hibiscus sabdariffa* extract ended towards acidity more rapidly than when coloured with amaranth.

Key:

ns - $p > 0.05$ (no significant change)

* - $p < 0.05$ (significant change)

HS - *Hibiscus sabdariffa* extract as colouring agent

A - Amaranth as colouring agent.

CHAPTER FIVE

5. 0 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

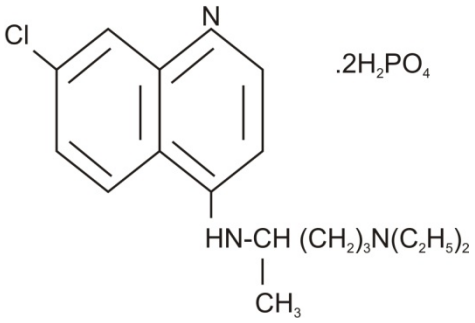
5.1 DISCUSSION

Temperature stability tests performed on the four formulations (Syrup Paracetamol, Syrup Chloroquine Phosphate, Syrup Diphenhydramine (Paediatric) and Syrup Cough linctus (paediatric), revealed that at room temperature, and at 37 ^{o}C, all the formulations were stable on storage for the four-month period, whether coloured with *Hibiscus sabdariffa* extract or with amaranth.

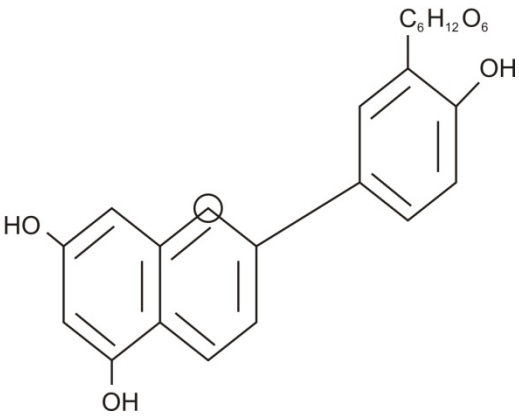
This can be seen from the gradual slopes for room temperature (26^{o}C) and 37 ^{o}C in figures 1 to 8, in contrast with the steep slopes for 52 ^{o}C and 65 ^{o}C.

For Syrup Chloroquine phosphate, coloured with *Hibiscus sabdariffa* extract absorbances increased, rather than decreased over the test period, at room temperature and at 37 ^{o}C (see figure three). This trend may be explained by the fact that Chloroquine molecules have a high affinity for *Hibiscus sabdariffa* extract. The pigment of *Hibiscus sabdariffa* has been found to be delphinidin 3-glucoside, an anthocyanin [23]. Comparing the chemical structures of Chloroquine phosphate and delphinidin 3-glucoside, it can be observed that both have two benzene rings attached to each other.

Chloroquine Phosphate



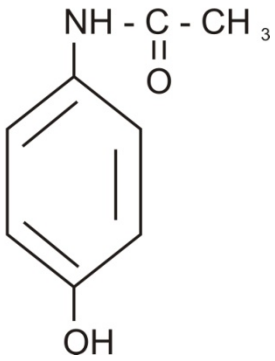
Delphinidin 3-glucoside



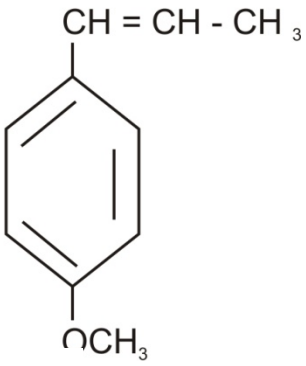
This similarity may explain the high affinity of *Hibiscus sabdariffa* pigment for chloroquine molecules.

Comparing the chemical structure of delphinidin 3-glucoside with those of paracetamol, anise oil and diphenhydramine, it can be observed that very little, if any similarities exist between them.

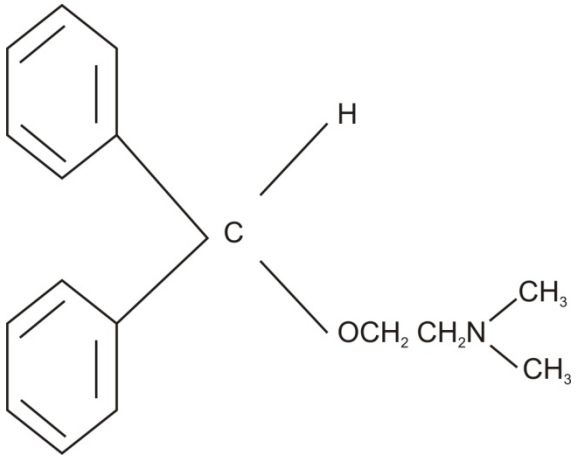
Paracetamol



Anise Oil (Anethole)

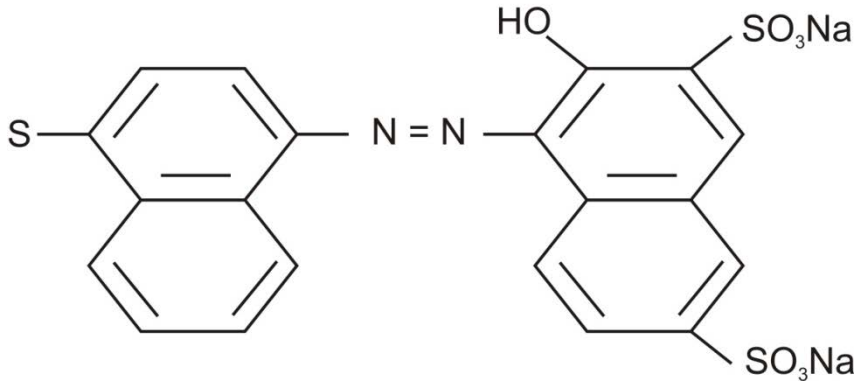


Diphenhydramine



Even though Diphenhydramine has two benzene rings, they are not attached to each other, hence affinity for *Hibiscus sabdariffa* pigment was not as high as with chloroquine.

Looking at the structure of Amaranth,



It can be observed that, there are four benzene rings, two attached to each other (in pairs of two). Comparing this structure to those of chloroquine and delphinidin 3-glucoside, it can be said that the chloroquine structure is closer to *Hibiscus sabdariffa* pigment, thus explaining the high affinity of chloroquine to *Hibiscus sabdariffa* pigment.

For Syrup Diphenhydramine (Pediatric), stability at the various temperatures were very similar, comparing those coloured with *Hibiscus sabdariffa* extract or amaranth (Compare figure 5 and 6). This may be explained by the fact that Syrup Diphenhydramine coloured with *Hibiscus sabdariffa* extract is as stable as those coloured with amaranth.

The statistical analyses using CHI-square test and Tukey's comparison test, revealed that at room temperature and 37 ^{o}C all the formulated syrups coloured with *Hibiscus sabdariffa* extract were as stable as those coloured with amaranth showing no significant change ($p > 0.05$)

For Syrup Chloroquine phosphate stability to high temperatures was demonstrated even at 52 ^{o}C and 65 ^{o}C (for both standard and test colouring a gents); showing no significant change: $p>0.5$.

Syrup Paracetamol and Syrup Cough linctus (paediatric) were the most unstable to high temperature showing significant change ($p < 0.05$) at 52 ^{o}C and extreme change ($p < 0.01$) at 65^{o}C.

Syrup Diphenhyramine (Pediatric), coloured with *Hibiscus sabdariffa* extract, was as stable as that coloured with amaranth and exposed to 52^{o}C temperature environment as no significant change ($p > 0.05$) was shown. Also comparing figure 5 and 6 it can be seen that slopes for temperatures 26 ^{o}C, 37 ^{o}C and 52 ^{o}C are similar indicating similar stabilities for both colouring agents.

At room temperature, it was observed that aqueous extract of *Hibiscus sabdariffa* had higher absorbance values than the acid infusion (see figure 9). Thus the aqueous extract rather than the acid infusion of *Hibiscus sabdariffa* calyces was chosen for colouring the formulations.

Tests for stability to light showed that all the four formulations coloured with *Hibiscus sabdariffa* were unstable on exposure to light, though syrup chloroquine and Diphenhyramine had better stability than syrups paracetamol and paediatric cough linctus. The control test in which amber bottles were used buttressed the point that formulations coloured with *Hibiscus sabdariffa* extract were best stored in amber bottles, since better stability was achieved when amber bottles were used as compared to when plain bottles were used. This is depicted in Figure 11. Formulations coloured with amaranth were stable to light whether placed in amber or plain bottles over the test period. This may be explained by the fact that Amaranth, being a synthetic product is stable to light.

The statistical analysis on light stability revealed that there was significant change between the test and standard coloured Syrups for Paracetamol and Paediatric Cough linctus (showing $p. < 0.05$).

For Syrups Chloroquine and Diphenhydramine (Paediatric), there was no significant change between test and standard formulations. This implies that Syrups Chloroquine and

Diphenhydramine (Paediatric) have better stability to light as compared with Syrups Paracetamol and Paediatric Cough linctus.

pH stability of the formulations coloured with *Hibiscus sabdariffa* extract was poor as pH declined over the test period. Those coloured with amaranth had better pH stability. This can be seen in figure 13.

Statistical analysis revealed that there was significant change ($p. < 0.05$) between pH stabilities of the four formulations when coloured with *Hibiscus sabdariffa* extract, and then with amaranth. This means that paediatric syrups coloured with *Hibiscus sabdariffa* extract needs to be buffered for better stability.

Tests to find out the minimum pH at which hydrolysis occurred for the four formulations put it at pH 5.00. This pH level is physiologically acceptable as it will not cause any gastro-intestinal problems when the formulations are buffered at that pH.
The formulations buffered at pH 5.00 showed good stability over the test period as can be seen in figure 14. There were only slight changes in pH over the four months test period, indicated by the gradual (almost horizontal) slopes in figure 14.

Hibiscus sabdariffa extract retained acceptable colour value (by BP standard of "not less than 0.25") after 3 months and 6 months. On testing after nine months the colour value had dropped to 0.200. The extract should therefore not be kept for more than six months for best results, as a colouring agent. Amaranth however retained its colour value within BP limits even after nine months. This can be explained by the fact that amaranth is a synthetic product and a dry powder thus hydrolysis hardly takes place to cause deterioration.

Hibiscus sabdariffa extract being a natural product is more susceptible to deterioration on storage. The natural product however has several other advantages over amaranth having many medicinal properties [(5).] Amaranth does not have any medicinal properties, and has been found to be carcinogenic [(3).]

Microbiological tests carried out revealed that *Hibiscus sabdariffa* aqueous extract has anti-microbial activity, but no anti fungal activity, thus the need to protect extracts and formulations prepared with *Hibiscus sabdariffa* against fungal attack. *Pseudomonas* spp. proved resistant to the anti-microbial activity of *Hibiscus sabdariffa* extract.

Methanolic extract of *Hibiscus sabdariffa* showed an enhanced antimicrobial activity and even some anti-fungal action. This can be explained by the fact that methanol was able to extract active ingredients from the plant material with better activity against microbes. Some activity against even the otherwise resistant *Pseudomonas* species was observed. Amaranth was found to have no anti-microbial actions.

Macroscopic and physico-chemical properties of *Hibiscus sabdariffa* calyces conformed to standards found on the internet [23]. *Hibiscus sabdariffa* calyces are not found in the BP hence resort to the internet. Those for amaranth powder conformed to BP standards [22].

Extraction by cold maceration of *Hibiscus sabdariffa* calyces yielded 34% w/w. This is quite encouraging, and makes the use of the plant extract as a colouring agent and as a medicinal product cost–effective.

Extraction by hot water infusion of the powdered calyces required 33% w/v to achieve the desired colour intensity (as compared to a standard coloured product), for colouring the formulations. Though this is on the high side, as compared to 1% for amaranth powder, it is still recommended for use because *Hibiscus sabdariffa* has many medicinal/health benefits, absent in amaranth. Amaranth powder is highly refined hence the lower percentage needed for colouring products, as compared with *Hibiscus sabdariffa* powdered calyces which is in the crude state.

5.2 CONCLUSIONS

The yield was very encouraging (34%), implying that the use of *Hibiscus sabdariffa* extract as a colouring agent is cost effective.
Colour, taste, odour and form of the *Hibiscus sabdariffa* calyces conform to general description of samples described on the internet [23].

Colour value obtained (0.258) conformed to the BP standard, and was retained within BP standards for up to six months.

Microbiological tests revealed that *Hibiscus sabdariffa* extract has antibacterial properties but very little artifungal properties; thus substantiating folklore medicine claims as to its use in healing syphilis, gonnorhea and other bacterial infections. Methanolic extracts had better antimicrobial activity.

Amaranth had no antibacterial nor antifungal properties; thus *Hibiscus sabdariffa* has a great advantage over amaranth in its use as a colouring agent.
Amaranth, being synthetic and highly concentrated into a powder form has an advantage of being used as a 1% or 2% solution; whereas a 33% solution of aqueous extract of *Hibiscus sabdariffa* achieved the same colouring effect. Nevertheless, the health benefits of the natural product out weighs this disadvantage; especially since amaranth has been found to be carcinogenic [23].

The pure extract and pediatric syrups formulated with the extract, are best stored at room temperature and also at 37^0C.

Generally, all pediatric syrups must be stored in amber bottles to avoid exposure to light which causes loss of colour and potency of the drugs.

pH was found to decrease with time, though Pediatric Syrups coloured with amaranth had a slower decrease than those coloured with extract of *Hibiscus sabdariffa.* Using citrate buffer to attain pH 5 provided good pH stability over the four month test period.

Syrup chloroquine and Syrup Diphenhydramine (Paediatric) were more stable to heat and light, than Syrups Paracetamol Syrups and Paediatric Cough Linctus.

The study results suggest that colour extract from *Hibiscus sabdariffa* calyces may be used as colouring agent for paediatric syrups.

5.3 RECOMMENDATIONS

- Since amaranth has been found to be carcinogenic, the use of *Hibiscus sabdariffa* colour extract is recommended for use in local industry.

- It is also recommended that a 33% aqueous infusion of the calyces be used in colouring paediatric syrups.

- Work needs to be done by way of refining the extract to reduce the concentration used in colouring paediatric syrups.

- Further investigation needs to be conducted on the anti-microbial properties of *Hibiscus sabdariffa* calyces, to ascertain the claim of folklore medicine as to the anti gonnorheal and anti-syphillitic use of the plant part; as well as other medicinal uses.

- *Hibiscus sabdariffa* colour extract is unstable in acid pH; thus it is recommended that the pure extract, as well as the paediatric syrups be buffered to maintain an alkaline pH (preferably 5.0) for better stability.

- It is also recommended that *Hibiscus sabdariffa* be included to the Ghana Herbal Pharmacopoea as a colouring agent for Paediatric syrups, as well as a medicinal herb.

Plate 2
Intact calyces of the *Hibiscus sabdrariffa* plant

Plate 3
Dried, separated calyces of *Hibiscus sabdrariffa*

Plate 4
Bottles holding the five different concentrations of aqueous extract of *Hibiscus sabdariffa* calyces.

Plate 5
Bottles containing syrup Paracetamol coloured with 1ml of various concentrations of *Hibiscus sabdariffa* extract

Plate 6
A jar of simple cream, coloured with 33% aqueous extract, castor oil emulsion and syrup chloroquine, all coloured, with 25% and 33% *Hibiscus sabdariffa* extract

Plate 7
Bottles of water, coloured with various concentrations of *Hibiscus sabdariffa* extract

REFERENCES

1. Carter S.J.(2000), Dispensing for Pharmaceutical Students, 12th Edition, Pitman Medical and Scientific Publishing Company Ltd, pp 47 – 51

2. www colouring agents.com. Clode S.A., Hooson J, Butler W. H.(2001) British Industrial Biological Research Association, Carshalton, Surrey, England

3. Martindale, W., (1999) The Extra Pharmacoepoeia, 29th Edition pp 857 – 861

4. Chevallier, A., (2001) Encyclopedia of Medicinal Plants. 2nd Edition; Dorling Kindersley pp 54-106; 299 – 310

5. Irvine, F. R., (1961) Woody plants of Ghana. Oxford University Press pp. 64; 193; 214 – 295; 741.

6. Dokosi, O. B., (1998) Herbs of Ghana. Ghana Universities Press, Accra, Ghana. pp 141 – 157.

7. Hostettman K, (1996) Chemistry, Biological and Pharmacological properties of African Medicinal plants, University of Zimbabwe Publications. pp 258 – 270.

8. Insel P. M., & Walton, T. R., (1998) Core Concepts in Health, McGraw Hill Higher Education (9th Edition). Pp. 326 328; 425 – 470.

9. Corry J. M., (1985) "Drugs – Facts, Alternatives, Decision" Wardsworth Inc, pp 14-28.

10. w.w.w hot.purdue.edu/newcrop/duke - Faraji M.H., Tarkhani A.H,(1999) Beheshti University of Medical Sciences, Tehran

11. Duke, J. A., Atchley A. A. (1984) The Handbook of Plant Science in Agriculture, CRS Press, pp 187 – 192.

12. Aulton, M. E. (1998). Pharmaceutics, The Science of Dosage Form Design, ELBS, Churchill Livingstone Publication, pp 256 – 267, 304, 649-660; 680

13. Abbiw, D. (1990) Useful Plants of Ghana; McGraw Hill Publishing pp-179 – 184; 198-218

14. Perry, L. M., (1980). Medicinal Plants of East and South East Asia. MIT Press, Cambridge pp 195 – 200.

15. Mehta, A. C. (1994) The Pharmaceutical Journal. Pp25; 84-86.

16. Chowhanz T., Li-Huachi (1986) Journal of Pharmaceutical Science 75; pp 542-545.

17. Banker, G. S., & Rhodes, C. T. (1990) Modern Pharmaceutics, 2nd Edition. Marcel Dekker Inc., pp 327, - 351.

18. Remington, W. (1990) Pharmaceutical Sciences, Philadelphia College of Pharmacy and Science Publications pp 1519 – 1520; 1527-1530, 1543 – 1544.

19. Lachman, L et al (1980) The Theory and Practice of Industrial Pharmacy. 2nd Edition; Lea and Febiger. pp 162-170; 680-685.

20. Beckett A. H. & Stenlake J.B. (1998); Practical Pharmaceutical Chemistry, 4th Edition, Part 2, University Press pp 35-37; 275 – 279.

21. The British National Formulary (1999) pp 651 – 653; 657 – 660

22. British Pharmacopoeia (BP) vol- I & II 2,000 BP CD – ROM

23. www. elsevier.com/food chemtox, - Akindahumsi A. A, & Olaleye M. T. (2002) Department of Biochemistry, Federal University of Technology, Akure Nigeria

24. Hugo, W. B., & Russel, A. D. (1990) Pharmaceutical Microbiology, 5th Edition Blackwell Scientific Publications pp 369-380; 487-492.

25. Altman, D. G. (1991) Practical Statistics for Medical Research. London. Chapman and Hall pp 295-298; 549-551.

26. Tufte, E. R. (1983) The Visual Display of Quantitative Information, Cheschire, Connectiant, Graphics Press pp 561-565.

27. Tyler, V. E., & Brady, C. R. (1988) Pharmacognosy. 9th Edition, Lea and Febiger Philadelphia pp 4-6; 459-461

28. Wallis, T. E. (1998) Textbook of Pharmacognosy. 5th Edition J & A Churchill Ltd, London pp 159-167

29. Aulton, C. (1986) Handbook of Pharmaceutical Excepients, American Pharmaceutical Association and Pharmaceutical Society of Great Britain pp 284-289

30. Samson J. A. (1992), Tropical Fruits, 2nd Edition. Longman Group U.K. Limited London pp 309-312

31. USP 1979, XVIII pp 31-33

32. http//www.hort.purdue.edu/newcrop/crops/roselle.ht.m.l Lafayette W. (2004) Center for New Crops and Plant Products, Purdue University

33. Gilbert, S.B. & Christopher, T. R. (1990), Modern Pharmaceutics, 2nd Edition. Marcel Dekker Inc., pp 372-376

34. Rawlins, E. A. (1992) Bentley's Textbook of Pharmaceutics, Bailliere Tindall – London pp269-312

35. Hocking, G. M. (1990) A Dictionary of Terms in Pharmacognosy Blackwell Scientific Publications, U.S.A pp 105-117.

36. Miller, J. C., & Miller J.N. (1994) Statistics for Analytical Chemistry. 3rd Edition. Ellis Horwood Ltd. pp 69-72; 171-172.

37. Abrahamson, J. H. (1999) Survey Methods in Community Medicine. 4th Edition, London: Churchill Livingstone pp. 302-306

38. Wernimont, G. T. (1995), Use of Statistics to Develop and Evaluate Analytical Methods. Associations of Official Analytical Chemists pp 162-164; 201 – 208.

39. Jawetz, E. et al, (1999) Review of Medical Microbiology (11th Edition), Lange Medical Publications pp 190-195, 207-228

40. Seeley, R. R. (1999), Essentials of Anatomy and Physiology. 2nd Edition, WmC Brown Publishers pp 29, 442-443

41. Gringanz, A. (1997) Introduction to Medicinal Chemistry, Wiley, VCH pp35-37,122.

42. Harbone, J.B. & Maby, T.J. (1982), The Flavanoids, Advances in Research. Chapman and Hall Ltd pp135, 163,146-148,302,649-650.

APPENDIX I: -Calculation of Rate constants

Formula: $t_{1/2} = \frac{0.693}{K}$

Where t ½ = half life of the formulation (in weeks)

K=Rate constant

e.g. For syrup Paracetamol at pH 2.5

$k = \frac{0.693}{t_{½}}$

$= \frac{0.693}{2} = 0.346$ …… Example I

(Refer Table 34)

For Syrup Diphenhydramine (Paediatric) at pH 5.4

$K = \frac{0.693}{t_{½}}$

$= \frac{0.693}{6} = 0.116$ …….. Example II

(Refer Table 36)

Table 34: - Indices for plot of pH against Rate constant for Syrup Paracetamol

pH	2.5	3.0	4.0	4.9	5.4	6.5	7.0
t ½(week)	2	3	4	5	6	5	4
K($week^{-1}$)	0.346	0.231	0.173	0.139	0.116	0.139	0.173

Table 35: - Indices for plot of pH against Rate constant for Syrup Chloroquine phosphate

pH	2.5	3.0	4.0	4.9	5.4	6.5	7.0
t ½(week)	2	3	4	5	6	5	4
K($week^{-1}$)	0.346	0.231	0.173	0.139	0.116	0.139	0.173

Table 36: - Indices for plot of pH against Rate constant for Syrup Diphenhydramine (Paediatric)

pH	2.5	3.0	4.0	4.9	5.4	6.5	7.0
t ½(week)	2	3	4	5	6	5	4
K($week^{-1}$)	0.346	0.231	0.173	0.139	0.116	0.139	0.173

Table 37: - Indices for plot of pH against Rate constant for Syrup Paediatric Cough linctus

pH	2.5	3.0	4.0	4.9	5.4	6.5	7.0
t ½(week)	2	3	4	5	6	5	4
K($week^{-1}$)	0.346	0.231	0.173	0.139	0.116	0.139	0.173

Table 37B: - Indices for plot of ln concentration against time for Syrup Paracetamol coloured with *Hibiscus sabdariffa* extract

Time (weeks)	0	2	4	6	8	10	12	14	16
Concentration (C) (Absorbances at 540 nm)	0.515	0.480	0.476	0.465	0.360	0.300	0.240	0.200	0.160
ln C	-0.66	-0.73	-0.74	-0.77	-1.02	-1.20	-1.42	-1.60	-1.83

APPENDIX II: Statistical Evaluation on Stability Studies, using Chi- Square Test and Tukey's comparison test

CHI – SQUARE TEST :

$X^2 = (O – E)^2 / E$

Where O = observed values

E = Expected values

X^2 = CHI – SQUARE

In this study, the observed values (O) are the absorbances recorded for formulations coloured with *Hibiscus sabdanriffa* (HS), whereas the expected values (E) are absorbances recorded for formulations coloured with amaranth (A)

The critical value used is 15.51 (taken from table A5) [36].

The mean differences in the table represent Chi-square values, minus the critical value, (X^2 – 15.51)

The null hypothesis is accepted ($p > 0.05$, i.e. no significant change, when X^2 value does not exceed 15.51.

The null hypothesis is rejected when X^2 exceeds 15. 51

For example: - mean X^2 value for the 2 sets:- syrup Paracetamol (HS) vrs , syrup Paracetamol (A)is 13.52.

Thus mean Difference = 13.52 – 15.51 = -2. 01

TUKEY'S COMPARISM TEST:

T values represent the number of values that are not within the range over which the two sets of samples overlap – the two sets in this study being absorbances of a particular formulation coloured with *Hibiscus saddariffa* (HS) extract , and then of those coloured with amaranth(A).

The null hypothesis is rejected if $T > 6$ (for $P = 0.05$)

Thus, if less than 6 values overlap in 2 sets that are being compared, the null hypothesis is accepted, and the conclusion of no "significant change "is adopted.

For Example: - for the 2 sets:
Syrup Paediatric cough linctus (HS) vrs. Syrup Paediatric Cough Linctus (A), the mean T value is 3. Since the number of values overlapping is less than 6 the null hypothesis of "no significant change" is accepted.

TABLE 38: CHI- Square testing on Temperature stabilities of the formulations coloured with *Hibiscus sadariffa* extract and then with Amaranth at 95%
Confidence interval

	ROOM TEMP(26 ^{o}C)		37 ^{o}C		52 ^{o}C		65 ^{o}C	
FORMULATIONS	Mean Difference	P Value	Mean Difference	P Value	Mean Difference	P Value	Mean Difference	P Value
Syr.Paracetamol(HS) Vrs Syr.Paracetamol (A)	-2.01	P>0.05	-3.02	P>0.05	3.05	P<0.05	4.07	P<0.05
Syr.Chloroquine(HS) Vrs Syr.Chloroquine (A)	-1.04	P>0.05	-2.05	P>0.05	-1.09	P>0.05	-3.03	P>0.05
Syr. Paediatric Cough Linctus(HS) Vrs Syr. Paediatric cough linctus (A)	-1.04	P>0.05	-2.02	P>0.05	2.01	P<0.05	4.05	P<0.05
Syr.Diphenhydramine paed (HS) Vrs Syr.Diphenhydramine paed (A)	-3.01	P>0.05	-3.01	P>0.05	-3.02	P>0.05	5.08	P<0.05

TABLE 39: - TUKEY'S COMPARISM TESTING ON TEMPERATURE STABLILITIES

	ROOM TEMP(26 ^{o}C)		37 ^{o}C		52 ^{o}C		65 ^{o}C	
FORMULATION	T Value	P Value	T Value	P Value	T Value	P Value	T Value	P Value
Syr.Paracetamol(HS) Vrs Syr.Paracetamol(A)	3	P>0.05	3	P>0.05	7	P<0.05	8	P<0.05
Syr.Chloroquine(HS) Vrs Syr.Chloroquine(A)	2	P>0.05	2	P>0.05	5	P>0.05	5	P<0.05
Syr. Paediatric Cough Linctus(HS) Vrs Syr. Paediatric cough linctus (A)	3	P>0.05	4	P>0.05	8	P<0.05	8	P<0.05
Syr.Diphenhydramine paed (HS) Vrs Syr.Diphenhydramine paed (A)	4	P>0.05	4	P>0.05	5	P>0.05	8	P<0.05

Table 40:- CHI -square. Testing on light stabilities of the formulations coloured with amaranth and *Hibiscus sabdariffa* extract.

FORMULATIONS	Mean Difference	P Value
Syrup Paracetamol(HS) Vrs Syrup Paracetamol(A)	2.37	P<0.05
Syrup Chloroquine(HS) Vrs Syrup Chloroquine(A)	-3.02	P>0.05
Syrup Paediatric Cough Linctus(HS) Vrs Syrup Pediatric cough linctus (A)	2.07	P<0.05
Syrup Diphenhydramine paed (HS) Vrs Syrup Diphenhydramine paed (A)	-3.01	P>0.05

Table 41:- Tukey's Comparison testing on light stabilities of the formulations

FORMULATIONS	T value	P Value
Syrup Paracetamol(HS) Vrs Syrup Paracetamol(A)	8	P<0.05
Syrup Chloroquine(HS) Vrs Syrup Chloroquine(A)	3	P>0.05
Syrup Paediatric Cough Linctus(HS) Vrs Syrup Paediatric cough linctus (A)	7	P<0.05
Syrup Diphenhydramine paed (HS) Vrs Syrup Diphenhydramine paed (A)	2	P>0.05

Table 42:- CHI -square. Testing on pH stabilities of the unbuffered formulations

FORMULATIONS	Mean Difference	P Value
Syrup .Paracetamol(HS) Vrs Syrup Paracetamol(A)	3.07	P<0.05
Syrup Chloroquine(HS) Vrs Syrup Chloroquine(A)	3.32	P>0.05
Syrup Paediatric Cough Linctus(HS) Vrs Syrup Paediatric cough linctus (A)	2.01	P<0.05
Syrup Diphenhydramine paed (HS) Vrs Syrup Diphenhydramine paed (A)	2.81	P<0.05

Table 43:- Tukey's Comparison Test on pH stabilities of unbuffered formulations

FORMULATIONS	T value	P Value
Syrup .Paracetamol(HS) Vrs Syrup Paracetamol(A)	7	P<0.05
Syrup Chloroquine(HS) Vrs Syrup .Chloroquine(A)	8	P<0.05
Syrup Paediatric Cough Linctus(HS) Vrs Syrup Paediatric cough linctus (A)	8	P<0.05
Syrup Diphenhydramine paed (HS) Vrs Syrup .Diphenhydramine paed (A)	7	P<0.05