SECTION : II

INTRODUCTION, DISCUSSION AND EXPERIMENTAL
TO
STUDIES ON VITAMIN A, VITAMIN C, CALCIUM AND IRON
OF
SOME MEDICINAL PLANTS
INTRODUCTION TO STUDIES OF VITAMIN A, VITAMIN C, CALCIUM AND IRON OF SOME MEDICINAL PLANTS:

VITAMIN A:

In 1913 McCollum and Davis of the University of Wisconsin\(^9\) and Osborne and Mendel of Yale University\(^8\) independently discovered that rats consuming purified diets with lard as the only source of fat failed to grow and developed soreness of the eyes. In 1919 Steenbock at the University of Wisconsin\(^9\) demonstrated that the yellow pigments in plants, the CAROTENES, has vitamin A activity.

This is one of the fat soluble vitamins which is found in food associated with animal fats, but not with lard and vegetable fats, and also with the green and yellow pigments of plants. The first intracellular localization of a vitamin was described by Querner in 1932\(^10\). He found in tissues that certain isotropic fat droplets gave a fluorescence, which quickly faded with ultra violet light. On the basis of observations that this fluorescence was due to certain fat-soluble pigments, e.g. carotenoids. Later Querner\(^11\) found this fluorescence to be present in liver, retina, adrenal cortex and pituitary gland of animals and said that it was due to vitamin A.
It has been demonstrated that all vertebrates require vitamin A. It is need for normal growth and development, and promotes a smooth skin and healthy mucous membranes. It is necessary for good bones, nerve development, and for healthy teeth. It builds resistance to infections and is important to the tissues of the eyes and for good vision\textsuperscript{102}. The work on experimental animals, it is apparent that normal reproduction and lactation can not take place without vitamin A\textsuperscript{103}.

Vitamin A deficiency has been characterized\textsuperscript{104} by three cardinal signs; xerophthalmia, dermatosis and night blindness and also poor growth and tissue of the eye\textsuperscript{105-106}. The severe manifestations of vitamin A deficiency are frequent even today. In India and other countries of the orient where grossly inadequate diets are the rule\textsuperscript{103}.

The recommended allowances of for vitamin A fully protect against the evidences of vitamin A deficiency when the absorption and metabolism are normal. Therapeutics doses of vitamin A in excess of 50,000 I.U. over prolonged periods may be toxic\textsuperscript{107-108}. The symptoms of toxicity included loss of appetite, failure to grow, drying and cracking of the skin, loss of hair, swelling and pain of the long bones, enlargement of the liver and spleen, and bone fragility.
Vitamin A is found in fats of animals origin like milk, cream, curd, butter, egg yolk and liver etc. The most important source is fish liver oil like cod, halibut, shark and saw fish, because the organism stores most of the excess of the Vitamin A in the liver. Vitamin A is not present in vegetable oil, e.g. linseed oil, olive oil, but these oils contains substances known as carotenes or carotenoids which are converted into Vitamin A in the body. The carotenes or carotenoids, therefore, are also known as provitamin A^109.

From the standpoint of human and animal nutrition the carotenoid pigments are of importance because of the conversion of some of them into vitamin A. The provitamin A are converted to vitamin A 'in vivo', usually in the intestinal tract of animals, by some as yet unknown mechanism, or 'in vitro' by careful oxidative degradation^114-115.

Since most of the vitamin A requirement of an Indian is met from vegetable sources. It is usual to recommend a larger allowance of vitamin A in such diets to account for the incomplete physiological availability of vitamin A from such diets. The ripe fruits such as mangoes, papaya and tomatoes are rich in carotene. Dark green leaves are rich in carotene, but the pale leaves, in lettuce and cabbage for examples, are significant courses.
It may be mentioned that the daily requirements of an adult are in the neighbourhood of 750 µg (about 2,500 I.U. units) of vitamin A derived either from foods of animal or of vegetable origin. Children and pregnant women require relatively more vitamin A than adults. According to accepted standards, an adult should receive 3000 I.U. daily; a child of one year 1200 I.U., adolescents 4000 to 5000 I.U., during the latter half of pregnancy and lactation the requirements is 4000 & 6000 I.U. per day respectively. Animals food rich in vitamin A are more expensive, and therefore easiest and cheapest way of ensuring a sufficiency of vitamin A.

As the medicinal plants under study are extensively used as medicine in India, an assay of the carotene in than was undertaken. The carotene content is expressed in terms of vitamin A in U.S.P. units.

**Vitamin C:**

Vitamin C has the distinction of being the first nutritional adjunct whose deficiency was recognised as a cause of disease. Probably as early as 1700 it was observed that a lack of fresh fruits and vegetables resulted in scurvey and that this disease could be prevented, and cured, by the proper diet. Vitamin C is called the 'antiscorbutic vitamin' because its absence results in a disease called 'scurvy'.
Impressive strides were made towards the identification of this principle following the accidental discovery of induced vitamin C deficiencies in the guinea pig\textsuperscript{116-118}. A British physician, Dr. Lind, in 1757 had shown the efficiency of citrus fruits in both curing and preventing scurvy. The new England pioneers recognized the value of potatoes and fruits in preventing this condition. Preparation of successively more concentrated vitamin C extracts\textsuperscript{119-120} was followed by the isolation of the pure compound\textsuperscript{121-125} and identification of its structure. Chemically vitamin C is known as ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$).

The most important function of ascorbic acid is the control which it exerts on the ability of cells to produce collagenous or intercellular material which holds the cells in proper relation to each other. Vitamin C is needed for growth, healthy bones, teeth and gums, also for the development of strong blood vessels, resistance to infections, and especially for the healing of wounds. It facilitates the absorption of iron from the intestinal tract. The conversion of folic acid to the metabolically active form, folinic acid, requires ascorbic acid. On the basis of studies conducted on premature and young infants, vitamin C also appears to be related to the metabolism of tyrosine and phenylalanine, two amino acids. It appears that ascorbic acid is involved in the synthesis of steroid hormones from cholesterol\textsuperscript{103}. 
The results of a deficiency of vitamin C in experiments with animals are due to lowered vitality, growth failure, capillary degeneration, spongy bleeding gums, anemia, hemorrhage, dental defects, fragility of the bones, degeneration of various organs, secondary infections, and scurvy.  

Many animals are able to synthesize ascorbic acid from simple sugars such as glucose; but man, guinea pigs and monkeys are dependent upon a dietary source.

Almost all of the daily intake of ascorbic acid is obtained from the vegetable fruit group. Raw, frozen, or canned citrus fruits such as oranges, grape fruits and lemons are excellent sources of vitamin, while tomato juice contains approximately half. Fresh strawberries, Raspberries, cantaloupe, pineapple, and guavas are also excellent sources. Other non acid fresh fruits such as peaches, pears, apples, bananas, and blue berries contribute small amounts of vitamin. Broccoli, Brussels sprouts, spinach, kale, green peppers, cabbage, and ceafy vegetables are rich sources of this nutritional adjunct. Raw liver contains about 30 mg per 100 g. of ascorbic acid, liquid milk contains 13 to 17 mg per liter, still more recently unripe walnuts content 0.6 to 1.0% ascorbic acid, and the west Indian cherry have been found to contain relatively large amounts of ascorbic acid (1.0 to 1.4%).
Vitamin C has been called the 'fresh food vitamin', since it is found in highest concentration just as the food is fresh from the plant. In general, the active parts of the plant contain appreciable amounts, while mature or resting seeds are devoid of vitamin. A warm environment, exposure to air, heat, alkali and dehydration are detrimental to the retention of ascorbic acid in foods.

An intake of 10 to 20 mg of ascorbic acid is sufficient to protect an adult from classical scurvy. The standards recommended by the Food and Nutrition Board provide 70 to 75 mg for the adult, 30 to 60 mg for infants and children, 75 to 100 mg for adolescents, 100 to 150 mg during pregnancy and lactation. Vitamin C is sensitive to heat, considerable loss occurs during cooking, especially if cooking is prolonged. Nevertheless, the inclusion of a few ounces of fresh fruits and leafy and other vegetables in a diet will ensure a satisfactory vitamin C intake.

The importance of vitamin C in human nutrition makes it necessary to study its content in foods. Hence, an assay of Vitamin C in the medicinal plants was taken up.
CALCIUM:

The chief compounds in bones and teeth are calcium, phosphate and calcium carbonate was well known as early as 1779. In 1843 by J.B. Boussingault, who performed calcium balance studies on animals.

Calcium is by far the most abundant mineral element in the body. In the body about 99% of this mineral occurs in bones and teeth (where it provides strength and rigidity) in the form of a complex salt composed of calcium phosphate and calcium carbonate embedded in an organic matrix of protein. Bones are also store house for calcium. The calcium is continuously removed from the bones to maintain the blood concentration; it is likewise being blood continuously replaced and the calcium is said to be in dynamic equilibrium.

Only 1% of the body calcium is found in the blood, other body fluids, and soft tissues. Its presence in proper proportions with sodium, potassium, and magnesium is necessary in the fluids which bathe the tissues and which are responsible for concentration of muscle fibres; the rhythm of the heart beat is dependent upon this fluid medium. Calcium is one of several factors in blood coagulation, in the normal response to nervous stimuli, in
cell permeability, and in the activation of some enzymes\textsuperscript{103}.

Calcium is found abundantly in milk (including skimmed milk and butter milk), cheese and green leafy vegetables. Among the leafy vegetables, amaranth, fenugreek and drumstick leaves are particularly rich in calcium and among the root vegetable, tapioca is a good source\textsuperscript{129}.

On the glands of the stomach the action of calcium appears to be depressant; it is not suited for administration before meals. Lime water is indeed a general gastric sedative, arresting some forms of vomiting, especially in the acid dyspepsia of infants and in pregnancy. The astringent effect in diarrhoea may be in part due to their control of acid fermentation; in part referable to an obscure sedative action on the intestinal glands which diminishes the excretion of water in the bowel.

Children need relatively more calcium and other minerals than do adults to meet the needs of the growing bones. Expectant and nursing mothers also require higher amounts of calcium. Calcium requirements in quantitative terms by man are not known with any degree of definiteness because there are no signs or symptoms ascribable directly to a deficiency of calcium. Calcium are the great importance for the development of the bones and teeth. Moreover men appears to be capable of adapting himself to low
intakes of calcium without any apparent deliterious effects.

Calcium is transported through out body by means of the blood circulation. When the blood calcium is reduced, calcium is mobilized from the bone; when the blood level is increased, calcium is excreted by the kidney. Kidney stone formation is one of the most important lesions of the body. Renal stone occurs as as a primary lesion or as a complication of many conditions.

Suboptimal intakes of calcium may result in retarded calcification of bones and teeth in the young. Acute deficiency of calcium is not usually seen unless there is a concurrent lack of phosphorus. Such deficiency leads to stunted growth and rickets, as evidenced by bowing of the legs, enlargement of the ankles and wrists, and a hollow chest.

It is a misconception that adults are not subject to calcium deficiency. The gradual drainage from the bones to replace calcium ions which are lost from the body daily may not be detected by X-rays until 10 to 40% of the calcium has been removed. Calcium deficiency leads to thin, fragile bones which break easily, and which heal with difficulty. Osteomalacia and osteoporosis may result from deficiencies of calcium.
IRON:

The ancient believed that iron was of heavenly origin. The Greek in fact, referred to it as 'sideros', a word which indicate its relation with stars. In 1744, iron deficiency anemia was first described by Johnnes Lange of Basle as 'De Morbo Virginea'. Iron was first used in the treatment of chlorosis in 17th century by Thomas Sydenham an english clinician. In 1838 Berzelius, a famous swedish chemist, concluded that the iron in hemoglobin made it possible for the blood to absorb much oxygen. Iron in its various forms had long been used as a therapeutic agent for anemia.

Iron may be administered to the patient orally, intramuscularly or intravenously. Although knowledge concerning the absorption of iron is given orally is limited, it is generally well accepted that iron is absorbed urgently in the duodenum and upper ileum. The amount is always small and in the part has been considered to be dependent primarily upon the need of the body for iron. Certain study with tracer iron, however, have cast double upon this relationship of absorption to need and have emphasised the necessity for a more intensive study of the factors governing iron absorption.
The amount of iron present in the adult body is about 3 to 5 gm. Of this a little more than half is in the circulating hemoglobin, and about 10% is in the myoglobin of the muscle. The rest is stored in the liver, bone marrow, spleen and kidneys.

Iron is essential for the oxidative processes in the body. It is a constituent of hemoglobin, a complex substance composed of the protein, globin, and an organic iron compound, heme. In fact, iron is present in all body cells. Several enzymes contain iron as a part of the molecule.

The rate of iron absorption is regulated by the body's need for iron. Approximately 10% of the dietary iron is normally absorbed, but Moore observed that absorption might range from 45 to 64% in anemic individuals. The average U.S. dietary intake is 12-18 mg/day; daily loss, except for bleeding, is about 0.4-0.6 mg/day, hence the healthy adult male requires no supplementary intake.

Iron improves the quality of blood. Iron and its preparations are generally given with certain selected vehicles. As haematinic tonic prepared iron is used in many disease like Anaemia and Chlorosis. Iron is of great value in both simple and secondary anaemias. The benefit is specially marked in case of chlorosis and in anaemia caused by malaria, Kala-azar, chronic
discharges or repeated passive haemorrhage. It is useful in anaemic dropsy and diseases of the spleen. In secondary anaemia from chronic intermittent fever, iron is very useful adjuvant to anti-pyretic drugs. In haemorrhagic diseases such as haemoptysis, haematuria, bleeding from piles, etc., iron is commonly given with good results. In leucorrhoea leading to anaemia, preparations containing iron are useful. Iron is a valuable remedy in Bright's disease and not only cure the anaemia but also lessens the albumin. It is useful also in chronic dyspepsia with anaemia, scrofula and tuberculosis and in anaemia due to intestinal worms. Iron is of great value when given internally in some skin diseases, i.e. erysipelas, carbuncles and farunculosis.

In diabetes and other urinary diseases, female complaints etc., pills called Vrihat Somantha rasa are recommended to be administered with honey. For diabetes, late Hakeem Ajmal Khan Saheb of Delhi prescribed 1 grain of reduced emerald and $\frac{1}{2}$ grain of reduced iron, mixed and made into one dose to be used with a Majoon (confection) suited to the disease.

Milk, Cheese and Ior cream are poor source of iron. All varieties of liver are excellent source of the blood regenerating factors. Other organ meats, lean meats, shellfish, and egg yolk are good meats,. The leafy vegetables are fair to good source
or iron. Fruit supply a number of minerals although the concentration is usually low. Fresh or dried apricots, raisins, Prunes, Peaches, and berries are good source of iron. Whole grain and enriched cereals and breads, legumes and dark molasses are also significant source.

Iron is stored in the liver cells and there synthesised into various albuminous compound. Its tonic effect appeared to be entirely referable to its action on the red corpuscles. Abundance of oxygen is essential for every bodily and mental function; and the feeling of 'tone', vigour and mental fitness varies with the degree of oxygenation of the blood, i.e. with the quality of the blood as regards haemoglobin. Nervous muscular and cardiac debility are thus removed by iron; and even digestion is restored by this gastric irritant, if it can be introduced successfully into the blood. The temperature is said to be slightly raised by iron, showing increased oxidation. Fever is generally held to contraindicate the use of iron; and the same has been said of the use of it except in mild forms or special combinations in tuberculosis.¹³³

The importance of Iron in human nutrition makes it necessary to study its content foods. Hence, an assay of Iron in the medicinal plants was taken up.
DISCUSSION

VITAMIN A:

The study of vitamin A and that of the closely related carotenoid pigments which act as the precursors of this vitamin has been the subject of numerous investigators during the past few decades. Much information of lasting value has been accumulated.

Of the several vitamin A precursors found in nature, the better known ones are α-, β-, γ-, and neo-β-carotenes and cryptoxanthin. β-carotene contains two β-ionone rings and is capable of splitting into two molecules of vitamin A, whereas the other possess only one β-ionone ring and therefore have less activity. The vitamin A activity of most vegetables and fruits is due to their β-carotene content, the α-, β-, γ- and neo-β-isomers and cryptoxanthin appearing in much smaller quantities.

The precise vitamin A equivalent of each provitamin is not definitely known, since utilization by the animal organism is dependent upon the vegetable sources, the species of animal in question and the nutritional status of the animal at the time of feeding. An arbitrary provitamin value assigned by United State Pharmacopeia for β-carotene is 0.60 mcg of β-carotene equal to 1 U.S.P. units of vitamin A.
The provitamin A content of foods may be determined by bioassay, by the solvent partition method, and by the chromatographic technique and U.V. absorption method. In the present assay the carotenoids of the species extracted, solvent removed and spectra, were taken on ultraviolet spectrophotometer using isopropanol as solvent.

Pandel1 determined carotene in the principal varieties of vegetables of Romania by Colorimetrically with axobenzene after chromatographic separation. Berger quantitatively determined \( \beta \) carotene (Provitamin A) and other carotenoids in fresh and dried plant materials by chromatographic technique. Nageswara Rao separated carotene extracts of vegetables on calcium hydroxide columns and reported that the true vitamin A value of a vegetables is based on its \( \beta \) carotene content only and is 50-97% of the vitamin A calculated from total carotene content.

Wolff and Moore both found ranges of liver reserves of vitamin A in diabetes which were much above those in accidental death. Apparently without knowledge of the evidence of high liver reserves of vitamin A. Ralli et al concluded that diabetes causes a defect in the conversion of carotene. In support of this view they found that when diabetic patients were given a large doses of carotene, either in oily solution or as carrots, the
increase of the provitamin in the blood was greater, and more rapid than in normal subjects. Clausen and McCoord\textsuperscript{141} confirmed that the blood carotenoids were often high in diabetes, and Brazer and Curtis\textsuperscript{142} found that diabetic patients had defective dark adaptation. Murrill et al\textsuperscript{143} agreed with previous workers about the high levels of carotenoids in the blood of diabetics. Thus for diabetic subjects of both sexes they found an average of 291 g per 100 ml, as compared with 206 g for normal subjects.

The edible pulp of the whole fruit of Eugenia Jambolana contained 80 I.U. vitamin A per 100 gm\textsuperscript{144}. From the results of analysis it can be seen that Eugenia Jambolana contains only 142.98 U.S.P. units of vitamin A per 100 gm.

Basu et al\textsuperscript{145} analysed 42 botanical materials. Among these the leaves of Momordica charantia contained 2400 mcg per 100 gm of vitamin A. The fruits of Momordica Charantia contains 210 I.U. vitamin A per 100 gm\textsuperscript{146}. From the results of present analysis it can be seen that Momordica Charantia fruit contain 92.44 U.S.P. units of vitamin A per 100 gm.

The analytical data for other species, show that Cassia sophera seed, 154.58; Embilica Officinalis seed, 20.14; Alpinia Galanga rhizomes, 93.77; Tephrosia Villosa Leaves, 82.46; Madhuca Indica bark, 144.30; Kickxia Ramosissima Plant, 63.18 U.S.P. units of vitamin A per 100 gm.
From the results recorded in Table III, it can be concluded that medicinal plants are poor in vitamin A. It has been already established that the daily requirement of vitamin A by an adult are in the neighbourhood of 750 μg or 2500 U.S.P. units. Since all of the medicinal plants contain 20 to 154 I.U. per 100 gm. Among the medicinal plants cassia sophora seed contains highest amount of vitamin A where as Emblica officinalis seed least.
VITAMIN C:

Since the first isolation of vitamin C in 1928, a great deal of work has been done in determining the ascorbic acid content of many foods, fruits, vegetables and plants. It is now apparent that ascorbic acid is widely distributed in the plant and animal kingdoms.

The vitamin C content of foods may be determined by (1) biological methods (2) Chemical methods and (3) Physical methods. Of these, chemical methods have received more attention because they are rapid, less expensive and more precise.

The chemical methods are based upon the great reducing ability of ascorbic acid. The reducing capacity is measured by treatment with a suitable oxidizing agent such as 2, 6-dichlorophenol indophenol, iodine, methylene blue, ferricyanide, phosphoric acid, phosphomolybdic acid, P-sulphophenylhydrazine, uranium nitrate, silicomolybdic acid, N-bromosuccinimide etc.

Considerable effort has been expended in attempt to find the most suitable extractant for vitamin C. A number of extractants like trichloroacetic acid, meta phosphoric acid, oxalic acid, acetic acid, a mixture of 10% acetic acid and oxalic acid and a mineral acids have been tried. The choice of extractant solely depends on the material to be extracted.
In the present study, the ascorbic acid of medicinal plants was extracted with 6% metaphosphoric acid and titrated with N-bromosuccinimide.\(^{148}\)

Hung-Kai Ho et al.\(^{149}\) analysed the seed of Cassia sophera, and found to contain total ascorbic acid 107.3 mg% and dehydro-ascorbic acid 93.1 mg%. From the results of present analysis it can be seen that Cassia sophera seed contain 60.0 mg vitamin C per 100 gm.

Barua\(^{150}\) analysed vitamin C content of thirty-six samples of fruits and vegetables grown in Assam. Of which Emblica Officinalis contains 7.2 mg vitamin C per 100 gm. Soman and Pillary\(^{151}\) isolated crystalline vitamin C from the Emblica Officinalis; 100 gm. of the fresh fruit pulp contain 280-720 mg; dry powdered pericarp contains 1561-2660 mg; and 100 ml of the fresh juice contains 900-940 mg of vitamin C. Mustard\(^{152}\) shown that Emblica officinalis fruit contains 1561 mg per 100 gm of vitamin C. Naik et al.\(^{153}\) analysed vitamin C content of 36 vegetables and 27 fruits of Gujarat region, of which Emblica officinalis fruit contains highest amount of ascorbic acid, 588.1 mg per 100 gm. The edible portion of the fruit of Emblica officinalis contain probably the highest amount of ascorbic acid amongst all the fruits and vegetables known in India.\(^{46}\) Sastry and Siddappa\(^{154}\) reported that ascorbic acid changes during the preparation of Emblica officinalis preserve. The fresh Emblica officinalis contained 964 mg of ascor-
bic acid per 100 gm fruit. Shah and Hamid\textsuperscript{155} shown that Emblica officinalis fruits, when softened by boiling in water and dried at 60\textdegree\textsuperscript{C}, lost 32.4\% of the vitamin C and 90\% vitamin lost by preserving. Emblica officinalis pickles and sirup contained 19-31 mg and 3.40 mg\% of ascorbic acid. The sirup lost 6\% of vitamin C when stored for 6 months. Ramasastri\textsuperscript{156} analysed effect of storage on the ascorbic acid content of dehydrated Emblica officinalis powder. About 25\%, 50\%, 60\% of vitamin was lost respectively at 20, 40, 60 of week of storage. In the pickles 50\% of ascorbic acid was lost during preparation and > 90\% was lost in 4 weeks. From the results of analysis it can be seen that Emblica Officinalis contains only 19.6 mg\% vitamin C.

Siddappa\textsuperscript{157} analysed the ascorbic acid in green Momordica charantia fruit during storage. Ascorbic acid was considerably lost after the ripening fruit storage. Slices of tender fruit lost 80\% of ascorbic acid on drying in the sun and 40\% lost during the cooking of the fresh Momordica charantia fruit. Bitter gourd is a good source of ascorbic acid and values up to 188 mg per 100 gm have been reported in fresh tender fruit; ripe yellow fruit contains about half as much ascorbic acid\textsuperscript{158}. From the results of analysis it can be seen that Momordica Charantia contains 32.4 mg\% vitamin C.
The analytical data for other species are Eugenia Jambolana seed, 26.8; Tephrosia Villosa leaves, 40.0; Madhuca Indica bark, 14.0; Kickxia Ramosissima plant, 11.9 mg % vitamin C.

The results of the present assay are given in Table III. From the results it can be concluded that medicinal Plants in general are greater in vitamin C. The daily requirement of vitamin C to maintain optimal health is 30 mg for adults, 35-75 mg for children, 75 mg for adult men, and 70 mg for women \(^{159}\). Increased allowances, 100-150 mg are indicated during pregnancy. Cassia sophera, Momordica Charantia and Tephrosia Villosa fulfil the above requirement.
CALCIUM:

Calcium is found in both organic and inorganic combination; it is absorbed probably in its inorganic form from the upper part of the small intestine. Absorption is favoured by the presence of an acid reaction and also by sugars which yield organic acids on decomposition in the intestine.

The requirement of calcium by an adult in quantitative term is about 0.5 to 1.0 gram daily. During the growing period, pregnancy and period of location the demand is greater. It is absorbed with difficulty and it has been estimated that only 50 percent of calcium of the food is absorbed. Therefore 1 gram of calcium must be taken daily with food to supply the adequate requirement. One liter of fresh cow's milk contains 1 gram of calcium. Calcium is probably utilised to the extent of 20 to 30 percent through it may be much higher in young infants. However, children require higher amount of calcium. Calcium content may be determined by both gravimetric and volumetric determination. The most common volumetric method for calcium in food can be determined by precipitation as the oxalate. The precipitate is dissolved in approximate 1.0 M sulphuric acid and the resultant solution titrated with permanganate. Many workers have described methods involving titration of calcium in
ash using EDTA as complexometric titration. Smaller amount can be determined by colorimetrically using chloranilic acid, glyoxal-bis (2-hydroxyanil), terbimetrically involving production of oxalate, by atomic absorption spectroscopy, and Flame photometry.

In the present study the calcium content was determined by two methods: (I) calcium was precipitated as oxalate, dissolved in 10% sulphuric acid and titrated with potassium permanganate solution, and (II) by titration of the calcium in ash with EDTA solution.

Brahamachari et al. analysed the fruits of Emblica Officinalis and found to contain 0.33% calcium. Emblica Officinalis fruit contains 0.05% calcium. From the results of present analysis it can be seen that Emblica Officinalis seed contains 7.4 - 7.7% calcium.

Kehar and Sahai showed that Eugenia Jambolana seed is fairly rich in calcium 0.41%. The edible pulp of Eugenia Jambolana fruit contains 15 mg per 100 gm calcium. From the results of analysis, it can be seen that Eugenia Jambolana contains 6.5 - 6.7% calcium.
Airan and Ghatge\textsuperscript{70} analysed the Momordica Charantia fruit ash and found to contain 0.005\% calcium. Vasistha and Antony\textsuperscript{165} reported that the fruit of Momordica Charantia contain 0.27\% calcium. The availability of calcium from Mormodica Charantia, albino rats of sprague and Dawley strain were used by Balance and Deposition methods respectively, 89 and 79\%\textsuperscript{166}. From the results of analysis it can be seen that Momordica Charantia contains 2.0 - 2.4\% calcium.

Sutaria and Magar\textsuperscript{87} analysed Madhuca Indica flowers which contain 0.25\% calcium, and flowers from various districts like Nasik, Nadiad, Himatnagar, Godhra, E.Khandesh and Prantij, were analysed and found to contain calcium 250.7, 268.4, 235.9, 226.8, 177.3 and 255.1 mg per 100 gm respectively. From the results of analysis Madhuca Indica contains 23.3 - 23.6\% calcium.

The analytical data of the other species found to contain Cassia Sophora seed, 6.4 - 6.8; Alpinia Galanga rhizomes, 3.4-3.6; Tephrosia Villosa leaves, 3.3 - 3.5; Kickxia Ramosissima plant, 10.7 - 10.9\% calcium. Among the all species Madhuca Indica bark are rich in calcium content where as Momordica Charantia fruit the poor.

The daily requirement of calcium is 0.5 to 1.0 gm for adult. During the growing period, pregnancy and period of lactation
the demand is greater. It can be seen that all medicinal plants fulfil the above requirement of calcium.
Iron rarely met with free in nature, though very widely distributed in both organic and inorganic kingdoms. They are found in soils and rocks, variously combined with oxygen as haematite, magnetic iron etc etc, with sulphur as iron-pyrites, and as carbonate of iron, in spathic iron; also in the milk, gastric juice, bile and urine. They are also widely distributed in the ashes of plant and animal kingdom.

The iron content may be determined volumetrically by oxidimetric titration with various reagents and then titration with potassium permanganate, potassium dichromate and ceric sulphate, F. Iodimetric titration using potassium iodide, stannous chloride and ascorbic acid, and complexometric titration with EDTA. The iron content in the medicinal plants are very minor and trace amount. They can be determined by colorimetric methods of their ashes. The most widely used organic reagents were thiocyanate, mercaptoacetate, 4:7-diphenyl-1,10-phenanthroline (bathophenanthroline), o-phenanthroline and atomic absorption spectroscopy.

For the present work, we have used o-phenanthroline, reagent for standard colorimetric determination of iron in traces.
It is adaptable due to the stable color and intense enough that very small quantities of iron can be readily determined, down to about 0.1 ppm.

Iron is used in medicine largely as a haematinic for the treatment of an anemia caused by a deficiency of this metal; the so-called iron-deficiency or hypochronic anemia. The anemia is a clear-cut entity\textsuperscript{179}, characterized by a decreased hemoglobin concentration per unit volume of whole blood (less than 15.5 gm/100 ml in the adult male and 14.5 gm/100 ml in adult female) and a decreased erythrocyte concentration, with the percentage decrease in former greater than that in the latter.

The causes of iron deficiency are haemorrhage, nutritional deficiency or inadequate supply at birth. Iron is not lost from the body via normal routes (kidney and perhaps skin) in amounts sufficient to cause iron deficiency. Excessive menstruation and childbirth are common causes in women. An average figure of 50 mg of iron per period has been given as menstrual loss, while at each normal delivery the mother loses about 300 mg iron for the infant's tissue stores and about 200 mg in haemorrhage. In men, bleeding haemorrhoids, bleeding peptic ulcers and tumors of the bowel are common causes.
Nutritional inadequacy of iron is almost wholly confined to infants and children. The rapid increase in size of the blood volume and the consequent great demand for haemoglobin in growing children necessitate a constant and large intake of iron, whereas many diets given to children, especially to young infants are in iron content. If, in addition to the above factors, the infants has not received his full share of iron during fetal life, as is the case in premature offspring, or in children whose mothers had an iron deficiency anemia and hence inadequate iron stores during gestation such an infant will almost certainly be affected with in a few months after birth by this type of anemia. 

A 70 kg adult male contain about 4 g, 70 % in the form of haemoglobin and about 26 % in storage compounds territin and haemosiderin. The femail must replace that lost in menstrual flow, and in pregnancy the 300 mg or so stored in the fetus. During growth the child must provide for the increasing quantity of blood. Iron deficiency anemia, therefore, is more common in women and children than in men. The toxic condition iron overloading, is race, but can arise from long usage of supplements, or in children from overdosing.
Brahamachari et al.\textsuperscript{46} analysed the fruits of Emblica officinalis sundried powder contain 0.079\% iron. The fruits of Emblica officinalis pulp contained 1.2 mg per 100 gm\textsuperscript{164}. From the results of analysis it can be seen that Emblica officinalis seed contain 78.5 mg \% iron.

The edible pulp of Eugenia Jambolana fruit contained 1.3 gm per 100 gm iron (ionisable iron, 0.1 gm per 100 gm)\textsuperscript{144}. From the results of analysis it can be seen that Eugenia Jambolana seed contains 38.1 mg \% iron.

Airan and Ghatge\textsuperscript{70} analysed the dried fruit ash of Momordica Charantia which contained 0.35\% iron. Shah and Patel\textsuperscript{183} analysed the fruit of Momordica Charantia contained total iron (available iron) 2.20 (0.98) mg per 100 gm. Dhalla et al.\textsuperscript{71} detected Fe, Na, K from the ash of Momordica Charantia fruits. It contained 9.4 mg per 100 gm iron\textsuperscript{164}. From the results of analysis Momordica Charantia contains 34.6 mg \% iron.

The other species contain cassia sophera seed, 24.5; Alpinia Galanga rhizomes, 26.6; Tephrosia Villosa Leaves, 42.5; Madhuca India bark, 42.3; Kickxia Ramosissima Plant, 29.6 mg \% iron. Among the all species Emblica officinalis seed contain highest amount of iron, where as cassia sophera seed the least.
A minimum of 10 to 15 mg of iron is required for adult males and 15 to 20 mg for adult females to maintain the equilibrium of diet. All most all species fulfil the above requirement of iron.
### TABLE III
**ANALYSIS OF VITAMIN A, VITAMIN C, CALCIUM AND IRON IN SOME MEDICINAL PLANTS**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Medicinal Plant (English name)</th>
<th>Vitamin A in U.S.P. units</th>
<th>Vitamin C in mg.</th>
<th>Calcium in g. Method I</th>
<th>Calcium in g. Method II</th>
<th>Trace Amount of Iron in mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cassia Sophera Linn. (Senna sophera)</td>
<td>154.58</td>
<td>60.0</td>
<td>6.4</td>
<td>6.8</td>
<td>24.5</td>
</tr>
<tr>
<td>2.</td>
<td>Emblica Officinalis Caerulescens Syn. (Indian gooseberry)</td>
<td>20.14</td>
<td>15.6</td>
<td>7.4</td>
<td>7.7</td>
<td>78.5</td>
</tr>
<tr>
<td>3.</td>
<td>Eugenia Jambolana Lam. OR Syzygium Camillii (Linn) Skeels Syn. (Black plum OR Indian blackberry)</td>
<td>142.98</td>
<td>26.3</td>
<td>6.5</td>
<td>6.7</td>
<td>38.1</td>
</tr>
<tr>
<td>4.</td>
<td>Alpinia Galanga Milld. (Greater galangal OR Java galangal)</td>
<td>93.77</td>
<td>26.4</td>
<td>3.4</td>
<td>3.6</td>
<td>26.6</td>
</tr>
<tr>
<td>5.</td>
<td>Momordica Charantia Linn. (Bitter gourd)</td>
<td>92.47</td>
<td>32.4</td>
<td>2.0</td>
<td>2.4</td>
<td>34.6</td>
</tr>
<tr>
<td>6.</td>
<td>Tephreria Villosa Pers. Syn. (Purple Tephreria)</td>
<td>82.46</td>
<td>40.0</td>
<td>3.3</td>
<td>3.5</td>
<td>42.5</td>
</tr>
<tr>
<td>7.</td>
<td>Madhuca Indica J.F. Gmel.Syn. OR Bassia Latifolia Roxb. (Indian butter tree)</td>
<td>144.30</td>
<td>14.0</td>
<td>23.3</td>
<td>23.6</td>
<td>42.3</td>
</tr>
<tr>
<td>8.</td>
<td>Kickxia Ranosissima Wall. 63.18 CR Linaria Ranosissima Wall. (Todd flex)</td>
<td>63.18</td>
<td>11.9</td>
<td>10.7</td>
<td>10.8</td>
<td>26.6</td>
</tr>
</tbody>
</table>
OPTICAL DENSITY AT 515 mµ

STANDARD GRAPH FOR IRON ESTIMATION

CONCENTRATION OF IRON IN µg

10 20 30 40 50 60 70 80 90 100
EXPERIMENTAL

STUDIES ON VITAMIN A, VITAMIN C, CALCIUM AND IRON
OF SOME MEDICINAL PLANTS

The medicinal plants studied for proximate analysis were taken up for studying Vitamin A, Vitamin C, Calcium and Iron.

VITAMIN A:

The carotene content of the medicinal plants was estimated by Ultraviolet Absorption method.105;184

SAPONIFICATION AND EXTRACTION OF CAROTENE:

Finely powdered dried sample (5.0 gm) of the medicinal plant was taken with 5 ml KOH (50% W/W) and 50 ml ethanol, into a saponification flask and refluxed for 30 minutes, untill the saponification was completed.

10 ml of distilled water was added for the washing, cooled, 50 ml of water was added and transfered to a separatory funnel. Then, 80 ml ether was added and transfered to the separatory funnel. Shaked continuously and released the pressure
and allow the layers to be separated. The lower layer was separated into a second separatory funnel and ether extract was collected. 50 ml of ether was added for the flask rinsings and transferred to the second separatory funnel. Shaked well continuously and ether extract was collected. This process was repeated for 3 to 4 times by adding 50 ml portions of ether. Last aqueous layer was removed.

50 ml of distilled water was added to the combined ether extract swirled gently, lower layer was separated out, and the process was repeated 4 times till the washings were free from alkali and gave no pink colour with phenolphthalein indicator.

**SOLVENT REMOVAL:**

The ether extract was filtered into a 250 ml flask through several gm of anhydrous Na₂SO₄ distributed evenly on a filter paper in a glass funnel. The separatory funnel and Na₂SO₄ was rinsed with 25 ml of ether and rinsings were added to the flask. The glass head was placed in the flask and the total ether extract evaporated to dryness on a water bath in a hood, the flask was removed from the source of heat during evaporation of the last few ml of solution. The ether removal was accomplished.
by heating the solution on a steam bath with the concurrent introduction of a steam of nitrogen until all the ether was removed. Immediately the residue was taken in propanol-2 (sp. quality), and diluted to the concentration of 8-15 U.S.P. units per ml.

SPECTROPHOTOMETRIC MEASUREMENTS:

The absorbency were determined at 310, 325, μ on Hitachi 101 Japan make U.V. spectrophotometer. Isopropanol was placed in the reference cell from the same container as that used to dissolve the sample.

CALCULATION:

The following formula was used for the correction of the absorbancies at specified wave length.

\[ A(\text{corrected}) = 7(A_{325}) - 2.625 (A_{310}) - 4.375 (A_{334}) \]

The corrected value was then substituted in the following formula:

\[ \frac{A(\text{corrected}) \times 5700 \times \frac{1}{0.3}}{LC} = \text{U.S.P. units} \]

Vitamin A per gm, ml or dosage units.
The symbols used in the above equation were:

\[ A = \text{corrected absorbancy} \]

\[ L = \text{length of light path in cm.} \]

\[ C = \text{concentration of sample in terms of gm per 100 ml of propanol-2.} \]

\[ 5700 \] a factor of conversion from spectrophotometric to gravimetric units.

\[ 0.3 \] a factor to convert gm to U.S.P. units.
**VITAMIN C**: 

Dried sample of the medicinal plants (10.0 gm) was ground up with sterilized sand and extracted with 50 ml of 6% metaphosphoric acid. The extract was centrifuged. The supernatant was diluted to 100 ml with 1% V/V acetic acid. 5 ml of diluted solution was transferred to a 6 inch x 1 inch test tube, 1.0 ml of glacial acetic acid was added, mixed, 5 ml of 4% W/V KI solution was added and mixed again. Then 3 ml of ether was added and the resulting mixture was titrated with N-bromosuccinimide solution. (1 ml of N-bromosuccinimide solution is equivalent to 0.2 mg of vitamin C). The end point was indicated by the first appearance of the brown colour of the liberated iodine in the upper ether layer. Comparison against on untitrated 'dummy' mixture permitted easy establishment of the end point. The average reading of the blank was subtracted from the average reading of samples. Standardisation of N-bromosuccinimide was done with standard ascorbic acid solution.
CALCIUM:

The calcium content of the medicinal plants was determined by two different methods:

(I) titration with KMnO₄ solution
(II) titration with EDTA solution

**METHOD I**

Finely powdered dried sample (5.0 g) of the medicinal plants was ashed in a silica crucible. The ash was digested with 50 ml of 50% HCl, and diluted to 100 ml with water in a 100 ml volumetric flask.

25 ml of aliquot was transferred to a 250 ml beaker and diluted to approximately 50 ml. The solution was brought to boil and 10 ml of hot saturated ammonium oxalate solution and a drop of methyl red indicator were added. It was almost neutralised with NH₄OH and boiled until the precipitate was granular and coarse. It was cooled.

Then ammonium hydroxide (1 : 4) was added until the solution was faint pink (pH 5.0). It was allowed to stand for at least 4 hours, filtered through Whatman No. 42 Paper and
the precipitate was washed with water until the filtrate was oxalate free. The point of the filter paper was cut and precipitate was washed into beaker, in which the calcium was precipitated with hot sulphuric acid (1 : 4) followed by hot water. Then 10 ml of sulfuric acid (1 : 4) was added. The solution was heated below B.P. and titrated with 0.05 N KMnO₄ solution. Finally the filter paper was added to solution and titration was completed.

1 ml of 0.05 N KMnO₄ = 1 mg calcium.
Finely powdered dried sample (5.0 gm) of the medicinal plant was ashed with the assistant of nitric acid, water and Perchloric acid. The ash was dissolved in slight excess of dilute nitric acid, transferred to a 100 ml volumetric flask and made upto the mark with water. 25 ml of the aliquot was pipetted out into a white procelin dish, diluted to 50 ml. Neutralised with 1.0 N NaOH and 5 ml excess of 1.0 N NaOH was added to produce a PH of 12. Then 0.2 gm muroxide (ammonium purpurate) was added and the mixture was titrated with the standard EDTA solution. The colour changed at the end point was from red to violet red.
Finely powdered dried sample (2.0 gm) of the medicinal plant was ashed in a silica crucible at 550-600°, cooled 0.5 to 1.0 ml of con. HNO₃ was added, evaporated off on a sand bath, and further heated for an hour in muffle furnace at 600° C. Cooled 10 ml of dil. HCl (1:9) was added and heated for 15 minutes on water bath, filtered through a Whatman No. 44 filter paper into 100 ml volumetric flask. Washings were collected and made upto the mark.

Suitable ml of aliquot was transferred into 50 ml volumetric flask and the same volume into a small conical flask. 5 drops of Bromphenol Blue was added to the conical flask to bring PH 3.5 - 4.0 by adding sodium acetate (2 m). PH of the volumetric flask was adjusted by adding some amount of sodium acetate. 1.0 ml of hydroxyl amine hydrochloride (10%), 1.0 ml of o-phenanthroline (0.5%) was added and made upto exact mark and then allowed to stand for 30 minutes. Then absorbances were read at 515 mp on 105 - systronic made spectrophotometer.

The average absorbance of blank was subtracted from the average absorbance of the samples, then the iron content was computed for a curve previously established with standard iron solution.
The analytical data is recorded in Table III.