SECTION : 2

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

VITAMIN-A:

The existence of vitamin-A was first recognised through the research of McCollum and Davies,\(^5^9\) and of Osborne and Mendel\(^6^0\) on the basis of observation that a factor occurring in certain facts is essential for the growth of rats, this factor was subsequently named as vitamin-A. In 1919 Steenbock at the University of the Wisconsin demonstrated that the yellow pigments in plants, called CAROTENES, had 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. He found that in certain tissues isotropic fat droplets gave a fluorescence, which quickly faded with ultraviolet light. On the basis of observations he suggested that fluorescence was due to certain fat-soluble pigments called carotenoids. Later on Querner 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 needed for normal growth and development, and also promotes a smooth skin and healthy mucous membranes. It is necessary for good bones, nerve development and for healthy teeth. It builds resistance against infections and is important to the tissues of the eyes and for good vision. Dietary deficiency of vitamin-A may result in night blindness, poor growth, atrophy of epithelial tissues, and tissues of the eye. Excessive amount of vitamin-A have been shown to be toxic.

Vitamin-A is present in some animal foods like butter and ghee, whole milk, curds, egg-yolk, liver, etc. The liver oils of certain fish like cod, halibut, shark and saw fish are some of the richest known natural sources of the vitamin. Vitamin-A is not present as such in vegetable foods but these foods contain substances known as carotenes or carotenoids which are converted into vitamin-A in the body. Therefore, they are known as provitamins-A.

From the stand point 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 'invivo', usually in the intestinal track of animals by some, as yet unknown, mechanisms or 'in vitro' by careful oxidative degradation.

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. Among the other vegetables, carrots and yellow pumpkin are good sources. It can be said that in general the greener the plant material, the higher would be the carotene content, and consequently the outer dark green leaves of cabbage are richer in carotene than are in the inner white leaves.

It may be mentioned that the daily requirements of an adult are in the neighbourhood of 750 μg (about 2500 international units) of vitamin-A derived either from foods of animal or vegetable origin. The requirements are greater in pregnancy and lactation during growth. Animal foods rich in vitamin-A are more expensive, and therefore the easiest and cheapest way of ensuring a sufficiency of vitamin-A is plant materials.

As the medicinal plants under study are used extensively in some of the diseases, hence an assay of the carotene in them 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 A.D. 1700 it was observed that a lack of fresh fruits and vegetables resulted in scurvy and that this disease could be prevented and cured by the proper diet. Gradual recognition of the "antiscorbutic principle" was followed on.

Impressive strides were made towards the identification of this principle following the accidental discovery of induced vitamin-C deficiencies in the guinea pig. Preparation of successively more concentrated vitamin-C extract was followed by the isolation of pure compound and identification of its structure.

Vitamin-C is needed for growth, healthy bones, teeth and gum, also for development of strong blood vessels, resistance to infections, and especially for the healing of wounds. It facilitates the absorption of iron from intestinal tract. The conversion of folic acid to the metabolically active form, folic acid, requires ascorbic acid. On the basis of the
studies conducted on premature and young infants, vitamin-C also appears to be related to the metabolism of two aminoacids, tyrosine and phenylalanine. It appears that ascorbic acid is involved in the synthesis of steroid hormones from cholesterol.69

The results of a deficiency of vitamin-C in experiments with animals are lowered vitality, growth failure, capillary degeneration, spongy bleeding gums, anaemia, haemorrhage, dental defects, fragility of the bones, degeneration of various organs, secondary infections and scurvey.70 Many animals are able to synthesise ascorbic acid from simple sugars such as glucose; but men, guinea pigs and monkeys are dependent upon dietary sources.63

Almost all of the daily intake of ascorbic acid is obtained from the vegetable fruit group. Oranges, lemons and grape fruits are the richest common source of vitamin-C, while tomato juice contains approximately half. Strawberries, raspberries, cantaloupe and various other fresh berries and fruits as well as green peppers, cabbages, avocodes, fresh lime beans and liver are rich sources of this nutritional adjunct.

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. A warm environment exposure to air, heat, alkali and dehydration are detrimental to the retention of ascorbic acid in foods. A well balanced diet for school children and adults should contain some 30-50 mg of 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 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.

IRON:

Iron has been known since antiquity. The sources of iron are milk, gastric juice, ashes of plants and even the blood of animals. The modern concept of iron therapy began in the 17th century with Thomas Sydenham, who popularised the use of iron for the disease chlorosis. The studies of Wripple and Robscheit, Robbins and Halinand and their associates have now placed iron therapy on quantitative basis. The earlier studies are being expanded.

Iron may be administered to the patient orally,
intramuscularly or intravenously. Although knowledge concerning the absorption of iron when given orally is limited, it is generally well accepted that iron is absorbed largely 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 doubt upon this relationship of absorption to need and have emphasised the necessity for a more intensive study of the factors governing iron absorption.

In the moderate anaemias 15-20 mg of iron may be taken up per day in severe anaemias, perhaps as much as 50 mg per day. In man, iron given in the ferrous form is absorbed better than that in the ferric form. In fact, it is proved by some authorities that the gut is capable of absorbing only ferrous iron and that ferric iron must be reduced before it becomes available. The normal secretions of the duodenum and upper ileum maintain some of the iron in the ferrous state, a fact may be responsible for the better absorption in this segment of the gut. Iron should be given on an empty stomach or with fruit juice and should not be given in milk for maintaining a pH favourable to the ferrous iron or by acting as reducing agents or both.
Organic iron is as good as but not better than inorganic iron. The metal must, however, be easily dissociated from its organic complex. The action of iron in the blood is almost unique of its kind and improves the quality of blood. Iron accordingly is used as a haematinic in an endless variety of condition in which haemoglobin is deficient such as simple anaemia, scrofula, amenorrhoea, cardiac diseases, nephritis, syphilis, malarial cachexia, and convulsence from acute disease. The cautions already given respecting digestion must be faithfully respected, secure its haemantinic action over a length of time.

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
contra-indicate the use of iron; and the same has been said of the use of it except in mild forms or special combinations in tuberculosis.\textsuperscript{72}

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

\textbf{CALCIUM:}

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 in the form of 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 continuously replaced and the calcium is said to be in dynamic equilibrium.

Only 1\% of the body calcium is found in 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 the several factors in blood coagulation, in the normal response to nervous stimuli, in cell permeability and activation of some enzymes.°

Calcium is found abundantly in milk, cheese and green leafy vegetables. Among the root vegetables, tapioca is a good source.°

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 a 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.

Calcium enters the blood circulation in very small quantities only, and appears in the plasma as a phosphate. It is an essential factor in the process of coagulation of the blood, and chlorine is used as a haemostatic in haemophilia and haemorrhage. The greater part of calcium being expelled by the bowel, little remains to be excreted by the kidneys. An alkalinising effect on the urine can scarcely be
appreciated, but it is certainly diuretic in the form of the waters of bath which is valuable in gout rheumatism and gravel.

Children need relatively more calcium and other minerals than do adults to meet the need of the growing bones. Expectant and nursing mothers also require higher amount of calcium. Moreover man appears to be capable of adapting himself to low intakes of calcium without any deleterious effects. Calcium occurs in all parts of the plant and accumulated with age in certain parts, especially the foliage. Numerous calcium compounds have been isolated as crystals in certain cells.

COPPER:

It has been known for over a century that certain invertebrates utilize copper-containing pigments and proteins instead of haemoglobin for respiratory purposes. In more recent years copper has been shown to be an essential trace element for many higher species including rats, chicks, sheep, cattle and men.

Copper occurs fairly widely at low levels in most soils and finds its way into many food stuffs. However, there are some regions of the world, such as Western Australia, Florida
and Holland, where copper deficiency gives rise to diseases in animals. Copper from foods is fairly well absorbed by man and animals, but can be affected by many other dietary components such as high levels of other minerals. Some forms of copper such as cupric sulphide and cupric oxide are less well absorbed than others such as carbonates and sulphates. After absorption, copper is transported to the liver, the main organ for its storage, where it is metabolized into many of the copper-containing enzymes. Hepatic copper is secreted into bile and returns to the intestine, then being mainly excreted together with unabsorbed copper into the faeces. Of about 2.5 mg invested daily by man about 30% is absorbed and later excreted. Only small amounts appear in the urine perhaps 10 to 20 μg per day, but up to 0.4 mg per day may appear in human breast milk. A rare disease of man, hepatolenticular degeneration (Wilson's disease) is believed to be caused by a primary disturbance of copper metabolism, which results in excessive copper deposition in the tissues and greatly increased urinary excretion of copper.73

The adult human contains about 100 mg copper. Nearly 15% of this amount occurs in the liver and there is also a rather high concentration in the eye. Copper retention in the liver of sheep and cattle has been shown to be affected by the
intake of other minerals, especially molybdenum, zinc and iron. Copper is metabolized into many important enzymes and proteins. Most important is the blue protein, ceruloplasmin, an α-globulin, molecular weight 151000, which contains eight atoms of copper per molecule. This protein circulates in the plasma of man and other animals, and it functions as an oxidase for sulphates such as serotonin, epinephrine and perhaps vitamin-A. Some serum copper remains, in transport, bound to albumin and the total normal serum concentration of both forms in man is about 1.0 to 1.3 μg/ml. Copper is also found in red blood cells. The human erythrocyte contains 65 μg of copper. Several other copper-containing enzymes are found in tissues; including the important monoamine oxidases and cytochrome oxidases.

Copper deficiency in man apparently never arises from a primary dietary deficiency but can be caused by par-absorption, over-excretion, a metabolic defect in the synthesis or utilization of copper-containing enzymes or by copper loss caused by primary iron deficiency. Symptoms include anaemia, neutropenia and bone changes, all of which respond to copper therapy. In some parts of the world, copper deficiency occurs in cattle and lambs. It is typified by anaemia lack of growth, hair depigmentation, atatia and
impaired fertility. In sheep an impairment in wool keratinization is typical and is of considerable economic importance.  

The copper requirement of different species varies considerably. Normal diets supply adequate amounts for human needs, the richest source being shellfish, liver, kidney, legumes, nuts and cocoa (25 to 300 ppm). Dairy products and cereals are relatively poor in copper. Sheep and cattle although particularly prone in some areas to copper deficiency disease, only appear to require about 1 mg copper per day and this could be obtained from pastures containing as little as 1 ppm copper. It would seem therefore that deficiency disease in this species can be caused by faulty absorption or retention of copper, perhaps due to the presence of high levels of other elements particularly molybdenum or cobalt. Supplemental copper in such areas can be given by top dressing on the soil. Salt licks containing copper-sulphate drenching and dosing or by periodic injections of organocopper complexes.
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. 76

Of the several vitamin-A precursors found in nature, the better known ones are α-, β-, γ, and neo-β-carotenes contains two β-ionine rings and is capable of splitting into two molecules of vitamin-A, whereas the others possess only one β-ionine ring and therefore have less activity. The vitamin-A activity of most vegetables and fruits is due to their β-carotene content, the α-, γ-, and neo-β-isomers and cryptoxanthin appearing in much smaller quantities. The precise vitamin-A equivalent of each provitamins not definitely known, since utilization by the animal organism is dependent upon the vegetable sources, the species of the animal in question and the nutritional status of the animal at the time of feeding. An arbitrary provitamin value assigned by united states pharmacopoeia for β-carotene is 0.60 mg of β-carotene equal to 1 u.s.p. units of vitamin-A. 69
The provitamin-A content may be determined by bioassay, Solvent partition method, Chromatographic technique and U.V. absorption method. Nageswara Rao separated carotene extracts of vegetables on Calcium hydroxide columns. Wolff and Moore found ranges of liver reserves of vitamin-A in diabetes which were much above those in accidental death. 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, the increase of the provitamin in the blood was greater and more rapid than in normal subjects. Clausen and McCoord confirmed that the blood carotenoids were often high in diabetes. Brazer and Curtis found that diabetic patients had defective dark adaptation. Thus for diabetic subjects of both sexes they found an average of 291 μg per 100 ml. as compared with 206 μg for normal subjects.

In the present assay the carotenoids of the species were extracted, solvent removed and spectra were taken Ultraviolet spectrophotometer using isopropanol as solvent. Asoeva et al studied the dry herbaceous parts of Alhagi camelorum Fisch harvested during the flowering of the plant for vitamin-A composition. Dry roots were also subjected to vitamin-A analysis. The results showed 10% mg in
herbaceous parts and 6% mg vitamin-A in dry roots. Basu et al. studied several herbs and flowers for its vitamin-A content.

Our analysis showed Alhagi camelorum Fisch, 125.02; Crataeva religiosa Hook, 67.41; Asparagus racemosus Willd, 158.72; Swertia chirata Ham, 94.30; Vernonia anthelmintica Willd, 105.33; Holarrhena antidysentrica Wall, 234.66; Piper nigrum Linn, 224.48 and Vitex negundo Linn, 123.38 u.s.p. units vitamin-A per 100 gm of medicinal plant sample.

**VITAMIN-C:**

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

The vitamin-C content of foods may be determined by:

(i) Biological methods,

(ii) Chemical methods, and

(iii) Physical methods.

The chemical methods are based upon the great reducing
ability of vitamin-C. The reducing capacity is measured by treatment with a suitable oxidizing agents such as 2, 6-dichlorophenolindophenol, iodine, methylene Blue, ferricyanide, phosphotungstic acid, phosphomolybdc acid, p-sulphophenylhydrazine, uranium nitrate, Silicomolybllic acid, N-bromosuccinimide, etc.

Considerable effort has been expanded in attempt to find the most suitable extractant for vitamin-C. A number of extractants like trichloroacetic acid, metaphosphoric 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 vitamin-C of medicinal plants was extracted with 6% metaphosphoric acid and titrated with N-bromosuccinimide. Asoeva et.al studied the vitamin-C composition of dry herbaceous plant and roots and found 110 mg% vitamin-C in plant and 80 mg% vitamin-C in dry roots of Alhagi camelorum Fisch. Grebinskii and Yaroshin found 211 mg% of vitamin-C in Alhagi species plants. Basu et.al studied content of vitamin-C in different plants and concluded that vitamin-C content in Vitex negundo Linn increases very gently after frying in oil.

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Our analysis showed Alhagi camelorum Fisch, 9.0; Crataeva religiosa Hook, 17.2; Asparagus racemosus Willd, 13.3; Swertia chirata Ham, 9.6; Vernonia anthelmintica Willd, 7.6; Holarrhena antidysenterica Wall, 20.0; Piper nigrum Linn, 12.0; and Vitex negundo Linn, 7.9 mg percentage of vitamin-C per 100 gm of medicinal plant sample.

IRON:

Iron rarely found 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 ore, 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; reductimetric 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 in 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) and atomic absorption spectroscopy.

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

The causes of iron deficiency are haemorrhage, nutritional deficiency or inadequate supply at birth. Iron is not lost from the body via kidney and skin. 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 infants tissue. In men bleeding haemorrhoids, bleeding peptic ulcers are common causes of deficiency.

For the present work, we have used O-phenanthroline
reagent 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.

Jarmand et al. analysed the metal content of different condiments of Piper nigrum Linn. Iron was found in significant amount in white pepper.

Our analysis showed Alhagi camelorum Fisch, 26.1; Crataeva religiosa Hook, 19.7; Asparagus racemosus Willd, 15.0; Swertia chirata Ham, 52.0; Vernonia anthelmintica Willd, 11.2; Holarrhena antidysenterica Wall, 14.8; Piper nigrum Linn, 36.0; and Vitex negundo Linn, 16.8 mg percentage of iron per 100 gm medicinal plant sample.

**CALCIUM:**

Calcium is found in both organic and inorganic combination, it is absorbed probably in its inorganic form by the upper part of the small intestine. Absorption is favoured by the presence of an acid reaction and also 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 gm daily. During the growth period, pregnancy and period of lactation 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 gm of calcium must be taken daily with food to supply the adequate requirement. One litter of fresh cow's milk contains 1 gm of calcium. Calcium is probably utilised to the extent of 20 to 30 percent though it may be much higher in young infants. However, children require higher amount of calcium.100

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 approximately 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 colorimetric method using chloranilic acid,101 glyoxal-bis (2-hydroxyanil),102 turbidimetrically involving production of oxalate by Atomic Absorption Spectroscopy103 and Flame Photometry.53

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.

Our analysis showed Alhagi camelorum Fisch, 1.07 and 1.21; Crataeva religiosa Hook, 1.16 and 1.31; Asparagus racemosus Willd, 9.10 and 9.20; Swertia chirata Ham, 1.26 and 1.26; Vernonia anthelmintica Willd, 3.20 and 3.22; Holarrhena antidysenterica Wall, 0.67 and 0.69; Piper nigrum Linn, 0.50 and 0.53 and Vitex negundo Linn, 1.51 and 1.54 gm percentage per 100 gm of medicinal plant sample in potassium permanganate titration method and EDTA titration method respectively.

COPPER:

Copper although not in large doses but it is an element which is essential for growth. It is necessary for respiration in plants and in vertebrate animals. Traces of copper are essential for haemoglobin formation in the blood. When present in certain food, however it tends to act as a oxidation catalyst and as little as 2 ppm. causes a tallowry flavour to develop in milk.
Numerous colorimetric reagents have been employed for routine determination of copper. Serger and other earlier workers used a colorimetric method based on formation of deep blue cuprammonium. Maquenne and Demonssay and Guerithault increased the delicacy of above method. The more recent methods depend on color reaction with organic reagents which are as follows:


The present analysis was carried out by colorimetric method as copper diethyldithiocarbamate complex.

Copper deficiency in man apparently never arises from a primary dietary deficiency but can be caused by par absorption, over-excretion, a metabolic defect in the synthesis or utilization of copper containing enzymes or by copper loss caused by primary iron deficiency. Symptoms include anaemia, neutropenia and bone changes, all of which respond to copper therapy. In some parts of the world copper
deficiency occurs in cattle and lambs. It is typified by anaemia, lack of growth, hair depigmentation, ataxia and impaired fertility. In sheep an impairment in wool keratinization is typical and is of considerable economic importance.

The copper requirement of difficult species varies considerably. Normal diets supply adequate amounts for human needs, the richest sources being shellfish, liver, kidney, legumes, nuts and cocoa (25 to 30 ppm.).

Our analysis showed Alhagi camelorum Fisch, 2.66; Crataeva religiosa Hook, 2.40; Asparagus racemosus Willd, 3.00; Swertia chirata Ham, 1.12; Vernonia anthelmintica Willd, 2.52; Holarrhena antidysenterica Wall, 0.92; Piper nigrum Linn, 1.25; Vitex negundo Linn, 1.40 mg percentage per 100 gm of medicinal plant sample.
STANDARD GRAPH FOR IRON

OPTICAL DENSITY AT 515 nm

CONCENTRATION OF IRON IN μg
STANDARD GRAPH FOR COPPER

OPTICAL DENSITY AT 400 nm

CONCENTRATION OF COPPER IN µg
### TABLE-III

**Determination of VITAMIN-A, VITAMIN-C, IRON, CALCIUM AND COPPER OF SOME MEDICINAL PLANTS.**

<table>
<thead>
<tr>
<th>No</th>
<th>Name of Medicinal Plant (English name)</th>
<th>Vitamin-A u.s.p</th>
<th>Vitamin-C mg%</th>
<th>Iron mg%</th>
<th>Calcium gm% KMnO₄</th>
<th>Calcium gm% EDTA</th>
<th>Copper mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alhagi camelorum Fisch (camel thorn)</td>
<td>125.02</td>
<td>9.0</td>
<td>26.1</td>
<td>1.07</td>
<td>1.21</td>
<td>2.66</td>
</tr>
<tr>
<td>2</td>
<td>Crataeva religiosa Hook (Three leaved caper)</td>
<td>67.41</td>
<td>17.2</td>
<td>19.7</td>
<td>1.16</td>
<td>1.31</td>
<td>2.40</td>
</tr>
<tr>
<td>3</td>
<td>Asparagus racemosus Willd (Wild asparagus)</td>
<td>158.72</td>
<td>13.3</td>
<td>15.0</td>
<td>9.10</td>
<td>9.20</td>
<td>3.00</td>
</tr>
<tr>
<td>4</td>
<td>Swertia chirata Ham (Chiretta)</td>
<td>94.30</td>
<td>9.6</td>
<td>52.0</td>
<td>1.26</td>
<td>1.26</td>
<td>1.12</td>
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<tr>
<td>5</td>
<td>Vernonio aneth-mintica Willd (Purple flea bane)</td>
<td>105.33</td>
<td>7.6</td>
<td>11.2</td>
<td>3.20</td>
<td>3.22</td>
<td>2.52</td>
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<td>6</td>
<td>Holarrhena anti-dysentrica Wall (Easter Tree or Ivory Tree)</td>
<td>234.66</td>
<td>20.0</td>
<td>14.8</td>
<td>0.67</td>
<td>0.69</td>
<td>0.92</td>
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<tr>
<td>7</td>
<td>Piper nigrum Linn (White pepper)</td>
<td>224.48</td>
<td>12.0</td>
<td>36.0</td>
<td>0.50</td>
<td>0.53</td>
<td>1.25</td>
</tr>
<tr>
<td>8</td>
<td>Vitex negundo Linn (Five leaved chaste tree)</td>
<td>123.38</td>
<td>7.9</td>
<td>16.8</td>
<td>1.51</td>
<td>1.54</td>
<td>1.40</td>
</tr>
</tbody>
</table>
EXPERIMENTAL

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

**VITAMIN-A**: 62,112

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

**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, until the saponification was completed.

10 ml of distilled water was added for the washing, cooled, 50 ml of water was added and transferred to a separatory funnel. Then 80 ml ether was added and transferred 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 four 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 gram of anhydrous Na$_2$SO$_4$ distributed evenly on a filter paper in a glass funnel. The separatory funnel and Na$_2$SO$_4$ 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 stream of nitrogen until all the ether was removed. Immediately the residue was taken in propanol-2 and diluted to the concentration of 8-15
Spectrophotometric Measurements:

The absorbencies were determined at 310, 325 and 334 μm on Hitachi 101 Japan make U.V. Spectrometer. 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 absorbencies at specified wave length.

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

The corrected value was then substituted in the following formula.

\[ \frac{(A_{\text{corrected}})/L}{C} \times 5700 \times \frac{1}{0.3} = \text{u.s.p. units of vitamin-A per gm/ml} \]

The symbols used in the above equation are:

- \( A \) = corrected absorbency
- \( L \) = 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.

\textbf{VITAMIN-C}^{84}

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, shake and 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 an 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.

Finely powdered dried sample (2.0 gm) of the medicinal plant was ashed in a silica crucible at 550°-800° c, cooled and 0.5 to 1.0 ml of conc. HNO₃ was added, evaporated off on a sand-bath, and further heated for an hour in muffle-furnace at 600° c. Cooled and 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 up to 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 Bromopheol Blue was added to the conical flask to bring pH 3.5-4.0 by adding Sodium acetate (2 ml). pH of the volumetric flask was adjusted by adding some amount of Sodium acetate. 1.0 ml of hydroxylamine hydrochloride (10%), 1.0 ml of o-phenanthroline (0.5%) was added and made up to exact mark, and then allowed to stand for 30 minutes. Then absorption were read at 515 mM on 105-systronic made spectrophotometer.

The average absorbance of blank was subtracted from the
average absorbance of the samples, the iron content was computed for a curve previously established with standard iron solution.

**CALCIUM**:114,115,53

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 gm) of the medicinal plants was ashed in a silica crucible. The ash was digested with 50 ml of 50% H₂O 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 ammonium hydroxide boiled until the precipitate was granular and coarse. It was then cooled and
ammonium hydroxide (1:4) was added until colour of the solution was faint pink (pH 5.0). It was allowed to stand for at least four 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 without sulphuric acid (1:4) followed by hot water. Then 10 ml of sulphuric acid (1:4) was added. The solution was heated below boiling point 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.

**Method-II:**

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 up to the mark with water. 25 ml of the aliquot was pipetted out into a white porcelain dish and diluted to 50 ml. Neutralised with 1.0 N Sodium hydroxide and 5 ml excess of 1.0 N Sodium hydroxide was added to produced a pH of 12.
Then 0.2 gm murexide (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.

**Copper**

Accurately weighted 0.5 gm of plant sample was taken in a digestion flask. To it few glass beads, 1 ml of Sulphuric acid and 1 ml of nitric acid were added, and mixture was kept in fluid state. This fluid mixture was heated gently till the liquid became dark, excessive frothing was avoided. Then nitric acid was added in small proportions and heating was continued for 5 to 10 minutes until the colour became dark. When all the organic matter was oxidised, the solution was allowed to cool and little amount of water was added. The colour of the fluid was yellow or light brown; little amount of 30% analytical reagent grade hydrogen peroxide and nitric acid were added. After each addition of hydrogen peroxide the fluid was heated until the residue was colourless; i.e. the pale yellow colour of fluid was reduced. The solution was cooled, diluted with 10 ml of distilled water and evaporated to fuming. It was again diluted with 5 ml of distilled water and evaporated to fuming. Finally the solution was diluted to 250 ml with distilled water.
25 ml of wet digested solution was pipetted out into a 250 ml separating funnel. 10 ml of citrate EDTA reagent was added. Then, two drops of thymol blue indicator and 6 N ammonium hydroxide solution was added dropwise till colour became green. The solution was cooled, 1 ml carbamate and 15 ml carbon tetrachloride was added. The separating funnel was vigorously shaken for 2 minutes, the layers were allowed to separate and lower\(^\text{\textregistered}\) was drained off through cotton wad. The optical density was measured at 400 \(\text{m} \mu\).

**Calculation:**

\[
\text{Copper mg}\% = \frac{\mu g \text{ of Cu in a liquiot} \times \text{Total Volume} \times 100 \text{ of digest}}{\text{ml of digest taken for extraction} \times \text{Weight of sample} \times 100 \text{ taken}}
\]

(1) \(\mu g \text{ of Cu in the liquiot} = \text{From the optical density reading from graph.}\)

(2) Total Volume of digest = 250 ml.

(3) ml of digest taken for extraction = 25 ml.

(4) Weight of sample = 500 mg.