CHAPTER-I

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Calories are a way of providing source of energy to our body. The amount of calories in a diet refers to how much energy the diet can provide for the body. A well balanced diet is one that delivers an adequate amount of calories while providing the maximum amount of nutrients. Calories are required for the maintenance of specific body temperature. Continuous supply of heat energy or calorie to the living beings is provided by slow and controlled oxidation of food materials, particularly carbohydrates, fats, vitamins and minerals.

Nutrients are substances derived from food during the process of digestion. A nutrient is a chemical that an organism needs to live and grow or a substance used in organism metabolism which must be taken in from vegetable seeds. Carbohydrates, fats and proteins are usually called macronutrients. The vitamins and minerals are equally important to our well being although they are needed in very small quantity.[1]

Recently, the analysis of nutritional value of various plant materials attracted attention due to the fact that they contain significant amount of essential nutrients that can be used for both human consumption and in the formulation of animal feeds.

A number of plants growing in newly formed Chhattisgarh state have been reported as rich sources for macro- and micro nutrients. They have also been reported to have medicinal potential for treating various ailments of human and animal. Unfortunately none of the systematic record is available in the literature regarding the different constituents of these plants from phyto-

chemical point of view. Hence macro/micro nutrients of some selected plant/tree seeds growing in Chhattisgarh State were studied and determined.

Under-nutrition is often a major problem in most of the developing countries of the world. Consequently the cases of under-nutrition are more rife in these countries. To reduce the adverse effect of hunger or starvation, it is pertinent that some plants and seeds having nutritional and medicinal applications, should be investigated for their nutritive value etc for human. Different parts of the plants have been identified to contain various classes of compounds. Fats and oils along with proteins and sugars occur in the seeds of the various plants. Apart from animal sources, the plants and their seeds also provide rich sources of proteins, fats and carbohydrates. Growing demand of oils and fats, proteins have placed these plants as important source to meet out the same.

Since long, the plant seeds have provided rich source of oils and fats. Oils and fats are composed of glycerides of fatty acids, mostly triglycerides and phytosterols. These vegetable oils and fats have very important place in human life. These are superior to proteins in terms of energy contents. The fats provide 9 calories per gram of energy as compared to 4 calories per gram from proteins. [2,3]

Essential nutrients, which are of fundamental importance of both, animal and vegetable worlds are oils and fats, proteins, carbohydrates and minerals, these are described in brief below,

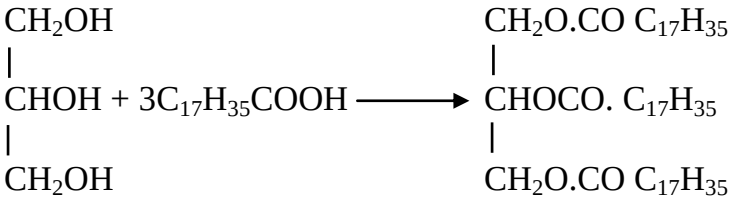
1.1 OILS AND FATS :

Oils and fats are mainly present in spores, seeds, fruits and in small amounts they are also present in leaves and roots of various plants. In spores, seeds and some tubers, oils function as food reserves to be

utilised during germination and in the early life of the plants. The oils from plants of temperate climate, contain more of unsaturation and are either of drying or semidrying type. On the other hand, oils of non drying type predominate in the plants of tropical region, whereby fats being rich in saturated fatty acids.

Vegetable fats and oils are lipid materials derived from plants. Physically, oils are liquid at room temperature and fats are solid. Chemically, both fats and oils are composed of triglycerides of fatty acids. As mentioned above, many plant parts may yield oil, in commercial practice oil is extracted primarily from seeds.

Generally fats belong to a class of substances known as esters of trihydric alcohol "glycerol". A simple triglyceride results when three similar acid molecules are involved in the combination with glycerol e.g. tristearin molecule.



Glycerol Stearic acid Tristearin

Natural oils are almost always mixed triglycerides and each molecule contains more than one type of fatty acid. Most natural oils contain small quantities of a variety of combined fatty acids, although usually two or three are predominant.

Qualitative and Quantitative analysis of fatty acids present in various oils and fats can be performed using techniques of reversed phase paper[4,5], thin layer[6,7], Gas liquid[8,9,10] chromatography.

1.2 PROTEINS :

Protein is essential for growth and development of living organisms and it constitutes 80-90% of all organic substances in animal body. The proteins are the most important components of all plants. As nucleoproteins in the genes, they control cell division and heredity in both single and multi-celled plants. In the form of enzymes, they catalyse various biochemical reactions essential for growth and maintenance of cell life. Proteins mediate several specific activities like cell interaction, cell motility, gene repression, netrogenesis and intra-cellular transport in plants. Proteins consist of single or several polypeptide chains with specific sequence of amino acids, resulting in the formation of particular three - dimensional structure possessing highly specific activity.

Hundreds of different proteins have been isolated in pure crystalline forms. Proteins are complex organic macro molecules which contain C,H,N,O and S. In addition to these elements many proteins also contain P, Fe, I and Co. Proteins are composed of 18-20 amino acids. Amino acids undergo condensation in presence of guanine triphosphate and form proteins.

Protein quality is measured by the type of amino acids present in them. There are twenty different types of amino acids, eight of which are essential, because they are not manufactured by the human or animal's body. This include Lysine, Leucine, Isoleucine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine[11]. The amino acid histidine is essential for the growth and development of children but it is only synthesised by adults. Other non-essential amino acids that are required to maintain health can be synthesised by the body if supplied with

necessary nitrogen. These non essential amino acids include alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, proline, serine and tyrosine[12]. Dietary protein with all the essential amino acids in the proportion required by the body is said to be a high quality protein. The amino acid that is in short supply is called **limiting amino acid**.

Proteins are the most abundant organic molecules in the living cells, making up more than half the mass of a living cell as measured by dry weight. All the hereditary information encoded in a cell's genetic material is transmitted initially to protein molecules. The amino acid sequence laid down during protein synthesis is the expression of this information and is the primary determinant of any protein molecule.

1.3 AMINO ACIDS :[13-17]

Microbes, higher plants and bacteria form amino acids required for their growth from carbohydrates in presence of simple nitrogeneous substances like ammonium salts, nitrates, nitrites and urea etc. Carbohydrates on biochemical oxidation via glycolysis and citric acid cycle, yield oxalacetic acid, pyruvie acid and α-keto glutaric acid as intermediates. Ammonia or hydroxylamine obtained from nitrogeneous substances convert oxalacetic acid and α-keto glutaric acid into aspartic and glutaric acids respectively. These two amino acids further react with other α-keto carboxylic acids eg. pyruvic acid and other suitable precursors in presence of enzymes called "Transaminases" and form other α-amino acids except lysine and threonine. This biochemical reaction involves an exchange of functional groups of the reacting molecules, is called "transamination". Pyridoxal (Vitamin B_6) acts as a Co-enzyme for

various transaminase enzymes and it functions through chelate formation. Umberger[17] demonstrated the formation of 17 different amino acids through transamination of glutarate.

1.4 BIOSYNTHESIS OF PROTEINS :

Protein synthesis from amino acids, involves three steps, these are (i) activation of amino acid, (ii) attachment of activated amino acid to t-RNA and iii) formation of polypeptide. In first step, the selection of specific amino acid from a heterogeneous pool in the cytoplasm takes place and it is accomplished through a highly specific enzyme. Each amino acid has at least the activating enzyme. In presence of ATP, the formation of energy rich enzyme bound amino acid-adenylate (E-AA-AMP) takes place and followed by a release of pyrophosphate. In second step attachment of t-RNA takes place. Once formed, m-RNA becomes associated with ribosomes in the cytoplasm forming polysome. Amino acids are conveyed to the polysome by t-RNA. One end of t-RNA is bound to the amino acid, while at the other end a triplet of nucleotides or anticodons is bound, which is a complement of the m-codons for the amino acid. The codons and anticodons are locked rapidly by complemetary attraction. When the anticodons of required number of t-RNA molecules are locked in place, the amino acids at their opposite ends are linked up in the sequence for peptide formation. The actual joining of the amino acids is controlled by protein synthesizing enzyme.

1.5 DIGESTION OF PROTEINS : [18]

During the process of digestion, the proteins are hydrolysed by enzymes producing amino acids, which are absorbed by blood and reach to the various tissues.

$$\text{Proteins} \xrightarrow[\text{Proteases}]{\text{Pepsin}} \text{Polypeptides} \xrightarrow[\text{Chemotrpysin}]{\text{Trypsin}} \text{Amino acids}$$

The degradation of ingested proteins into their constituent amino acids occurs in the gestro intestinal tract. Proteolytic enzymes like pepsin, trypsin, chemotrysin and erypsin play vital role during this degradation. The liberated amino acids, then enter blood stream, which carries them to different parts of the body and are utilised there.

1.6 CARBOHYDRATES :

Carbohydrates are class of compounds that include polyhydric aldehydes or polyhydric ketones and large polymeric compounds that can be broken down (hydrolysed) into polyhydric aldehydes or ketones.

Carbohydrates are derived mainly from plants, where they make up about 70% of the solid plant material. Their main functions are (i) to support the plant structure (cellulose) and (ii) to store chemical energy (Sugar and Starch).

Sugar is stored in body as glycogen $(C_6H_{10}O_5)_n$ which corresponds to starch in plants. During physical exertion, glycogen is converted into lactic acid and energy is released. Fatigue is due to an accumulation of lactic acid in the body tissues.

Starch and sugars are the most important carbohydrates. Starch is an important food material, but cellulose can not be digested by human beings. However, animals have enzymes which can digest cellulose.

1.7 MINERALS / TRACE ELEMENTS :

Minerals of inorganic elements required by man are, sodium, potassium, magnesium, calcium, phosphorous, iron and iodine. Some

elements like copper, zinc, cobalt, manganese, molybdenum and fluorine are required in small amounts and are called as trace elements.

Inorganic minerals are involved in various metabolic reactions of the cell and thus help in the growth and development of the cells. Ions play an important role in maintaining osmotic pressure and acid-base balance in the cells.

The importance and biological role of some minerals is given below -

(i) Calcium :

It gives strength and rigidity to bones and teeth along with phosphate by forming calcium phosphate. Calcium ions are also present in the cells and in the blood. It is involved in clotting of the blood.

(ii) Sodium and Potassium :

These two are responsible for maintenance of fluid balance in the cells. In muscles and nerve cells, these two ions maintain membrane potential. These ions are also responsible for the transmission of electrical impulses in the nerve cells.

(iii) Iron :

It is needed to synthesize a respiratory pigment, haemoglobin. Another strong pigment myoglobin is also formed from iron. Electron transport chain, enzymes, cytochromes, also require iron for their normal biological functions.

(iv) Copper :

Copper is essential in human metabolism, however, intake of excessive doses by man leads to severe mucosal irritation and

corrosion with wide spread capillary damage, hepatic and renal damage and central nervous system irritation followed by depression.

(v) Zinc :

Zinc is essential and beneficial element for growth of body.

(vi) Phosphorus :

It is an important element involved in the energy transfer reactions of the cell. It is an important constituent of ATP, nucleic acids, NAD, NADP. It also gives strength to bone and teeth.

1.8 METHODS OF ANALYSIS OF PLANT SEEDS :

Development of chemistry of Natural products was because of the application of Physico-chemical and Bio-chemical techniques used in their investigations. Sophisticated techniques like spectroscopy Viz., Ultraviolet, Infrared, NMR etc. and chromatographic methods starting from classical Paper and Thin layer chromatography to highly sophisticated Gas chromatography and High performance liquid chromatography have helped to a great extent in the study of various constituents present in various plant materials including seeds. Techniques of chromatography, colorimetry, Gas liquid chromatography, High performance liquid chromatography, Atomic absorption spectroscopy used during the present investigation are briefly reviewed below,

(a) Chromatography :

Technique of chromatography has now become in modern days a common and efficient method of isolation, purification and characterisation of constituents of plants, animals and of synthetic origin. During phytochemical examination, this technique is of

immense use during resolution of mixtures of amino acids, fatty acids and their methyl esters, vitamins, hormones, sugars and other mixture of natural products. Various aspects and applications of this method have been described in several reviews. [19-25]

(b) Paper and Thin layer Chromatography :

Both paper and thin layer chromatography provide remarkably simple and inexpensive means for separating and identifying the components of small samples of complex inorganic, organic and bio-chemical substances. Furthermore, the methods, particularly thin-layer chromatography, permit reasonably accurate quantitative determination of the concentrations of the components of such mixtures. Both of these techniques serve as important tools for the phytochemical studies.

Michael and Schweppe, H.[26], Hirayema and Neda[27], Fink and Fink[28], Joshi and Shrivastava[29] etc. have made use of reversed phase paper chromatography for the separation of fatty acid derivatives. Kaufmann[30] and Buchmann[31] have also achieved separation of fatty acids using paper chromatographic technique. Kaufmann et al. [32-37] and Roomi et al.[38] have used reversed phase thin layer chromatography for separation of fatty acid derivatives. These workers had also used argentated silica gel as adsorbent for the separation of fatty acid derivatives.

Dent[39], Giri et al.[40], Levy and Chung[41] have separated and isolated amino acids successfully from protein hydrolysates by using paper and thin layer chromatographic techniques. Paterson and Butler[42], Knight[43], Smith and Stocken[44] have used special ion exchange resin papers for the separation of amino acids from protein

hydrolysates. Atfield and Morris[45], Von Arx and Neher[46], Rokkones[47], Joshi et al. [25] have used thin layer chromatography for the separation of amino acids from their mixture.

Giri[48], Wilson[49], Deferrari[50], Hay et al.[51], Wolfrom et al.[52], Menzis[53] and Vajpai[54] have resolved sugars on paper strips and silica gel plates.

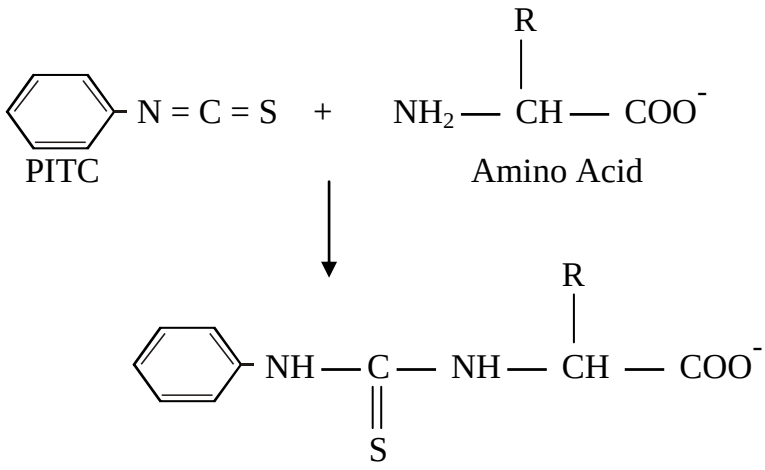
(c) Gas Liquid Chromatography :

Gas liquid chromatography is a separation and quantitative technique based upon the partitioning of volatile components of a mixture between a mobile aqueous and a stationary liquid or solid phase. The technique can be extended to the investigations of non volatile compounds after preparing their suitable volatile derivatives. GLC has been most significant tool for phytochemical studies in recent past. Quantitative separation of complex mixtures of fatty acid esters can be achieved, even when present in traces, using this technique. Though, gas liquid chromatography is a separation technique, but with due calibration, it permits the quantitative estimation of separated components as well. Estimation of individual fatty acid present in a mixture can be done by recording gas liquid chromatograms of their methyl derivatives. This technique has been used for separation and identification of various plant products by Brueckner and Hausch[55], Seewald and Eichinger[56], Pertiatkowiez and Wojtaszek[57], Hazai[58] etc.

(d) High Performance liquid Chromatography :

It is a powerful tool for the separation of closely related substances. It is improved version of liquid column chromatography,

in which high pressure arrangements are used (HPLC). This is highly sensitive technique, which is evident from the fact that samples can be analysed by this techniques in amount as low as one picomole. This is most sophisticated modern technique for separation of amino acid mixtures, peptides and proteins. Free amino acid mixture is analysed by reverse phase, HPLC using, method of pre-column derivatisation with phenyl isothiocyanate (PITC). Phenyl isothiocyanate reacts with an amino acid as below:



(Derivative of PITC with an amino acid)

This PITC - amino acid mixture is dried, dissolved in mobile phase and is injected in the HPLC column. HPLC technique for the study of amino acids present in protein hydrolysates in plant materials has many advantages over usual amino acid analyser and other conventional separation methods due to smaller sorbent column, smaller quantity of samples required and much shorter working time. It is preferred to, automatic amino acid analyser, because of high precision of apparatus, coupled integrator and computerised evaluation. Speed and accuracy of HPLC makes it, beyond doubt, one of the fundamental chromatographic method of the future. Various

reviews[59-61] have appeared in recent times, on the modern and highly sensitive technique of HPLC, for fractionation of amino acids from their protein hydrolysates.

(e) Extractive Colorimetric Technique :

This method involves extraction of colour of the individual spot from the stained chromatogram in certain solvent e.g. water, alcohol or aqueous butanol etc. and subsequent determination of its optical density etc. at suitable wave length with either photoelectric colorimeter or a spectrophotometer. This technique has been used by Wolwood[62], Souchen[63], Levy and Chung[41], Bode[64], Gerok[65], Longe[66], Adewuyi etal[67] etc.

(f) Atomic Absorption Spectroscopy :

This technique involves the study of absorption of radiations, most often from UV-vis region of spectrum, by atoms in gaseous state. The main advantage of this technique, apart from its high sensitivity is that, the determination can be carried out in presence of other metals and the separation of the element to be tested, from other present, is not necessary. AAS is essentially a method for determine quantitatively, the metal ions present in trace amounts [68]. AAS can be used to determine nearly sixty metal ions and the only limitation is that, the sample must be capable of giving solution of the metal ion concerned in either in aqueous or in organic solvent. Atomic absorption spectroscopy permits the determination of metals in quantities as low as 1 ppm level.

Chinyere et al.[69] have used AAS technique to determine various trace metals present in the ash of Lagenaria sphaerica seeds.

Mohan and Janardhanan[70], Chavan et al.[71], Glew et al.[72] have also determined trace metals present in the ashes of various plant seeds.

1.9 PROBLEM TAKEN AND WORK DONE :

Chemical composition of a particular plant is greatly influenced by factors like climate, soil and fertilizer conditions, cultural operation such as growth, variety and maturity etc. Variation in compositional data of a plant may also depend upon techniques adopted for extraction of plant constituents and their subsequent qualitative and quantative investigation.

Phytochemical examination of various parts of large number of plants have been carried out by various workers, who found these plants to be rich sources of fats, carbohydrates and proteins. Seed oils of many plants have been reported to contain high percentages of polyunsaturated fatty acids with good nutritive value. Many of the seeds of wild and other variety of plants have been reported to possess antiphysiological properties. Literature survey revealed very little published work on calorie and nutrient composition of some edible variety of seeds viz. Annona Squamosa Linn, Citrullus. Vulgaris Var. fistulosus (Stocks), Brassica Oleracea Linn (Gongylodes Group) and Moringa Oleifera Lam especially of Bilaspur region of Chhattisgarh State. No work is reported in literature, particularly by recent Chromatographic and Spectrophotometric techniques on oils, carbohydrates, amino acid composition of proteins and mineral etc of above mentioned seeds.

Therefore, detailed investigations involving determination of calorie contents, fatty acid composition of their oils, Carbohydrate

makeup and amino acid profile of proteins of these seeds by various chromatographic techniques were studied.

The seed ashes were also subjected to qualitative and quantitative determination of the cationic composition present in them. Many of the cations e.g. Cu^{++}, Co^{++}, Ni^{++}, Fe^{+++}, Zn^{++}, Na^{+} and K^{+} were found to be present in the ashes of these seeds.

These seeds were found to be rich in oil contents. Fatty acid composition of these oils were studied both qualitatively and quantitatively using chromatographic techniques like reverse phase paper, cellulose, argentated Silica gel-G thin layer chromatography and Gas liquid chromatography was used for quantitative study of the fatty acids present in the oils. Oleic, linoleic and linolenic acids were the important constituent among the usual fatty acids, which were found to be present in these seed oils. Quantitative gas liquid chromatographic study revealed fairly good percentage of linoleic acid, linolenic acid in the oils of seeds of Annona Squamosa oil. Linoleic acid was found in all the four seed oils, an essential fatty acid, which reduces cholesterol level in blood, hence these seeds can be regarded as having good nutritive value.

Defatted seed meals were treated with 80% ethanol solution and ethanolic extracts so obtained were studied for the presence of various sugars using different chromatographic methods and solvent systems. These studies revealed that the seeds contained monosaccharides and some of the oligosaccharides eg. glucose, fructose, galactose, rhamnose, sucrose, cellobinose, raffinose and Stachyose. p-Anisidine-o-phosphoric acid method was used for the quantitation of these sugars.

For the study of proteins and their amino acid profile, the defatted seeds were hydrolysed by using mixture of 6N-hydrochloric acid and 80% formic acid in the ratio of 1:1 (v/v). The use of formic acid reduces the time required for the hydrolysis. It also helps to check the decomposition of lysine. threonine and methionine. Qualitative paper and thin layer chromatography of all the protein hydrolysates of all the seeds revealed the presence of usual amino acids. The hydrolysates of Citrullus vulgaris and Brassica oleracea contained almost all the common amino acids which are usually found in the seed proteins. However, the hydrolysates of Annona Squamose and Moringa oleifera contained lesser number of amino acids i.e. 17 and 14 respectively. Tryptophan in low concentration was detected in Citrullus vulgaris and Brassica oleracea seeds only.

For quantitative analysis of these protein hydrolysates, two dimensional chromatography in combination with spectrophotometric determination of ninhydrin colour was used. Reverse phase high pressure liquid chromatography (HPLC) was also used for the quantitation of amino acids present in these protein hydrolysate samples. Comparative results of these two methods have been reported.

1.10 SURVEY OF LITERATURE :

Various workers have investigated nutritional and medicinal aspects of different plants and their components in recent past. These investigations included studies on proximate composition, proteins, fats, sugars and also studies on micronutrients found in the respective plant seeds or the other plant parts.

Fatty acid profile of different plant seeds were studied by several workers employing various conventional methods. Literature survey shows that reverse phase paper and thin layer chromatographic methods in conjunction with gas chromatography and HPLC were most used techniques for the study of fatty acid composition of seed oils. Some workers have also supported their results by ultraviolet and infrared spectroscopy. Some important, contributions made by different workers in this field are as below :

Crombie and Comber[73], Badami and Daultabad[74], Pant and Tulsiani[75], Kapoor et al.[76], Tandon et al.[77], Bhakare and Rao[78], Arora[79], Gelpi et al.[80], Endo et al.[81], Kimal and Laxminaraina[82], Salam et al.[83], Umarov et al.[84], Sengupta et al.[85], Dorrell[86], Rankov et al.[87], Raikar and Magar[88], Gray[89], Siddiqui et al.[90], Rao and Nigam[91], Singh and Bajpai[92], Ansari et al.[93], Ahmed et al.[94], Joshi and Shrivastava[95], Joshi et al.[96], Salma[97], Pillet[98], Kaul et al.[99], Mishra et al.[100], Rao and Laxminaraina[101], Habib[102], Sujatha et al.[103], Ahmed et al.[104], Jamal et al.[105], Malik et al.[106], Dave et al.[107], Nalesco et al.[108], Brow et al.[109], Kittur et al.[110], Mukarram et al.[111], Hallobo et al.[112], Ahuja et al.[113], Jain and Banerjee[114], Akhtar et al.[115], Ukhun and Ifebigh[116], Kanya et al.[117], Mirralis et al.[118], Ahuja et al.[119], Daultabad et al.[120], Riaz and Choudhary[121], Rudrappa and Revadi[122], Rais et al.[123], Ali et al.[124], Sagrero[125], Shreedhar and Laxminaraina[126], Mohan and Janardhanan[70], Lee and Kim[127], Udaishekhar Rao[128], Barminas et al.[129], EL-Adawy et al.[130], Fernandez et al.[131], Farooq and Bhanger[132], Bagci and Sahin[133], Dhan Prakash and Pal[134], Sengupta and Basu[135], Glew et al.[72], Rouhou et al.[136], Stevenson et al.[137], Zia-Ul-Haqu[138], Xiuzhu Yu et al.[139], Ozcan and Chalchat[140], Arslan[141], Anwar and Rashid[142], Akinhanmi et al.[143], Akbar et al.[144],

Chinyere et al.[69], Ariffin et al.[145], Arora et al.[146], Nehdi et al.[147], Oladije et al.[148], Amza et al.[149], Desai and Chavan[150], Ardabili et al.[151], Asharaf et al.[152], Chouaibi et al.[153].

Ethanolic extracts of defatted seed meals of a large number of plant/tree seeds were investigated for their sugar constituents, both qualitatively and quantitatively by Jindal and Mukherjee[154], Kapoor and Mukherjee[155], Saunders[156], Rao et al.[157], Sequeira and Lev[158], Bourne et al.[159], Pant[160], Jakimov[161], Vijaylaxmi and Chauhan[162], Rao and Nigam[163], Joshi and Nigam[164], Varshney et al.[165], Koto et al.[166], Li Jian et al.[167], Longe[66], Barminas et al.[129], Chavan et al.[71], Achu et al.[168], Rouhou et al.[136], Ozcan and Chalchat[140], Adewuyi et al.[169], Zubr Olaposi et al.[171], Nehdi et al.[147], Embaby and Mokhtar[172] and Ashraf et al.[152].

Studies on ashes of a large number of plant seeds for their minerals/trace metals composition were carried out qualitatively and quantitatively by large number of phytochemists. They used various methods available to them from classical methods to most sophisticated modern instrumentation methods e.g. AAS and AES. Some of them are Kamel et al.[173], Nwokolo and Sim[174], Mohan and Janardhanan[70], Barminas et al.[129], Chavan et al.[71], EL-Adawy[130], Fernandez et al.[131], Odoemelam[175], Glew et al.[72], Cheikh-Rouhou et al.[136], Borges et al.[176], Xiuzhu Yu et al.[139], Ozcan and Chalchat[140], Ekop[177], Frota et al.[178], Akinhanmi[143], Chinyere et al.[69], Mariod et al.[179], Lohlum et al.[180], Nehdi et al.[147], Amza et al.[149], Martinez et al.[181], Sowemimo et al.[182], Embaby and Mokhtar[172], Asharaf et al.[152], and Chouaibi et al.[153]

A large number of researchers have studied & investigated protein hydrolysates of various edible and non-edible plant seeds for their

amino acid composition. They have used qualitative and quantitative chromatographic methods. They had also used modern methods like automatic amino acid analyser, high performance liquid chromatography and also UV / IR methods for the quantitation of amino acids present in the protein hydrolysates of the seeds. Some important contributions of these researchers in this field are enlisted here. They are Kelkar et al.[183], Sengupta and Chakraborty[184], Ramkrishna and Shankara[185], Ramkrishna and Subramanian[186], Sinha and Gupta[187], Garcha and Chopra[188], Nahid and Zaidi[189], Kawtara et al[190], Kapoor et al.[191], Sodek and Wilson[192], Dardemi et al.[193], Collinus and Kalinius[194], Watson and Fowden[195], Kapoor et al.[196], Pant and Bishnoi[197], Gautam and Purohit[198], Gupta et al.[199], Sharma et al.[200], Bhatnagar et al.[201], Joshi and Nigam[202], Bakxi and Thakur[203], Joshi et al.[204], Iqbal and Karzi[205], Vasi and Kalintha[206], Ikediobi[207], Joshi et al.[208], Rivett et al.[209], Low and Rogers[210], Lashkar et al.[211], Marfo et al.[212], Nwokolo and Sim[174], Ali and Quadri[213], Dhan Prakash and Mishra[214], Banerjee and Jain[215], Khatta et al.[216], Ukhun and Ifebigh[217], Gallop[218], Aremu[219], Hagop et al.[220], Jain et al.[221], Oomah and Mazza[222], Wood et al.[223], Mohan and Janardhanan[70], Kinketa and Bezard[224], Mohan and Janardhanan[225], Tsevegsuren et al.[226], Udaishekhar Rao[128], Falk et al.[227], Oshodi[228], Chavan et al.[71], Prakash et al.[229], Gomes and Rose[230], El-Edway et al.[130], Fernandez et al.[131], Farooq et al.[132], Anhwange et al.[231], Achu et al.[168], Glew et al.[72], Cheikh-Rouhou et al.[136], Borges et al.[176], Xiuzhu Yu et al.[139], Andreia P.[232], Frota et al.[178], Akinhanmi et al.[143], Chinyere et al.[69], Mariod et al.[179], Olaposi et al.[171], Lohlum et al.[180], Ojiako et al.[233], Nehdi et al.[147], Mariod et al.[234], Martinez et al.[181], Kanu[235], Desai and Chavan[150], Ardabili et al.[151], Chouaibi

et al.[153], Ashraf et al.[152], Embaby and Mokhtar[172] and Ingale and Shrivastava[236].

Bobita Sharma et al.[237] have investigated fatty acid composition of seed oil obtained from fifteen domesticated accessions of four wild rose species. Bachheti et al.[238] have determined proximate composition of the seeds of wild Prunus armeniaca L (wild apricot) from Garhwal region. They have also determined the fatty acids and various elements present in the seed oil. Salimon and Ahmed[239], have studied the physico-chemical characteristics and fatty acid compositions of oils obtained from Jatropha curcas seeds collected from Malaysia, Indonesia and India. Balogun and Olatidoye[240] have studied chemical composition and Nutritional properties of Velvet Beed seeds (Mucuna utilis) collected from Ibadan (Nigeria). Narsing Rao et al.[241] have studied chemical, amino acid and fatty acid composition of Sterculia Urens L seeds. They used GC-FID method to study FA. They have reported stearic, linoleic, palmitic, eicosadienoic and eicosatrienoic acids present in its oil.

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