SUMMARY

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Relevant to human existence and living is good nutrition. 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.

Nutrients e.g. oils and fats, carbohydrates, proteins etc. 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.

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.

In developing countries like ours, majority of people are devoid of their balanced diet. It is the reason, why, they suffer with hunger or starvation and consequently with different ailments. Hence to reduce the adverse effect of such problems, it was pertinent that some important plants of Bilaspur region of Chhattisgarh state should be investigated for their nutritive value for the need of humans.

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.

Plant seeds have been reported to contain oils and fats, carbohydrates, proteins and amino acids with good nutritive value.

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., specially of Bilaspur region of Chhattisgarh state.

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 using various chromatographic, spectrophotometric, GLC and HPLC techniques were done.

The work done has been incorporated in the thesis entitled "**Chemical Studies on Calorie and Nutrient Values of Some Seeds**" and is divided in five Chapters, supported by relevant references at the end of each chapter.

Chapter–I, provides an introduction to the subject and the problem undertaken. This chapter describes in brief about various types of nutrients present in the plant seeds e.g. oils and fats, proteins, amino acids, carbohydrates, Minerals/Trace elements etc.

Methods used for phytochemical studies e.g. Paper and Thin Layer chromatography, Gas liquid chromatography, High performance liquid chromatography, Extractive colorimetsic techniques and atomic absorption spectroscopy have been described in brief.

It also introduces in brief the problem taken and work done. At the end of this Chapter a brief of the available literature pertaining to the problem under investigation is also presented. The work published in various national and international journals by Badami and Daultabad, Pant and Tulsiani, Kimal and Laximinaraina, Sengupta et al., Siddiqui et al., Rao and Nigam, Singh and Bajpai, Ansari et al., Joshi and Shrivastava, Mishra et al., Sujatha et al., Jamal et al., Kittur et al., Jain and Banerjee, Anuja et al., Daultabad et al., Riaz and Choudhary, Mohan and Janardhanan, Udaishekhar

Rao, Fernandez et al., Sengupta and Basu, Zia-ul-Haque, Arora et al., Desai and Chavan, Chouaibi et al., Jindal and Mukherjee, Kapoor and Mukherjee, Pant, Vijay Laxmi and Chauhan, Varshney et al., Ozcan and Chalchat, Mokhtar, Ashraf et al., Kamel et al., Barminas et al., Chinyere et al., Embaby and Mokhtar, Sengupta and Chakraborty, Subramanian, Sinha and Gupta, Kapoor et al., Watson and Fowden, Gautam and Purohit, Bhatnagar et al., Joshi et al., Prakash and Mishra, Banerjee and Jain, Wood et al., Falk et al., Anhwange et al., Andreia, and Ingle and Shrivastava etc. related to oil and fats, fatty acids, Carbohydrates, minerals, protein and amino acid chemistry respectively are worth consideration with regard to the present problem taken.

Chapter–II, describes about Proximate and Ultimate analysis of four subjected plant seeds. This section of thesis includes a brief introduction of Annona Squamosa Linn., Citrullus Vulgaris Var. fistulosus (Stocks), Brassica Oleracea Linn. (Gongylodes group) and Moringa Oleifera Lam seeds. In this chapter, methods for analysis of Moisture content, Ash content, Fiber content present in the seeds have been described. Methods for oil extraction from the seeds, analysis of oil seed residues, Qualitative and Quantitative analysis of minerals present in the seeds have also been given. Various observation tables related to Proximate and Ultimate analysis and mineral analysis have been given here.

Maximum moisture and ash contents were recorded in A. Squamosa seeds, while maximum organic matter content was found in C. Vulgaris seeds. M. Oleifera seeds contained 38.2% oil. Oil contents in these seeds varied from 25.6% to 38.2%, protein contents varied from 21.64% to 36.76% (maximum in B. Oleracea seeds). A. Squamosa seeds recorded maximum carbohydrate contents i.e. 28.08%, while minimum carbohydrates contents were observed in M. Oleifera seeds (13.28%), calorific values of A. Squamosa, C. Vulgaris, B.

Oleracea and M. Oleifera seeds were 6548, 6785, 6324 and 6876 calories per gram of sample respectively. Qualitative paper chromatographic study indicated the presence of Fe^{+++}, Cu^{++}, Zn^{++}, Ca^{++}, Mg^{++}, P., Na^{+} and K^{+} ions in the ashes of these seeds. M. Oleifera seeds ash contained Co^{++} and Mn^{++} ions too. AAS method was used to estimate (in ppm) the quantity of these cations. Perusal of results revealed that ashes of all these seeds were rich source of potassium, sodium and calcium cations and phosphorous.

Chapter-III, deals with studies on oils, fats and sugars. In this chapter isolation of oils from plant seeds and their physico-chemical constants, saponification of isolated oils and isolation of mixed fatty acids, preparation of methyl esters of the fatty acids, preparation of potassium hydroxamate derivatives of methyl esters have been described.

Qualitative identification of free fatty acids using paper, cellulose thin layer and argentated thin layer chromatography have also been described. A separate table showing different free fatty acids present in the oils of subjected plant seeds has been given. Six free fatty acids in A. Squamosa, C. Vulgaris and seven in B. Oleracea and ten free fatty acids in M. Oleifera seed oils were recorded.

The methyl esters of fatty acids of seed oils were analysed for their fatty acid composition by Gas chromatography. The apparatus used for this purpose was Perkin-Elmer Auto System-XL equipped with flame ionization detector (FID). Most common fatty acids which were identified and determined in all these seeds using this method were Myristic, Palmitic, Palmitoleic, Stearic, Oleic, Linoleic and Linolenic acids. Palmitic and Stearic acids are present in good quantity in these seed oils. Presence of Oleic acid were recorded in all these seed oils. Good percentages of linoleic acid in A.

Squamosa, B. Oleracea have been recorded. Psresence of Arachidic and Behinic acids were also recorded in A. Squamosa and B. Oleracea seed oils. M. Oleifera seed oil contained Gadoleic (9-Eicosenoic) acid.

Paper and cellulose thin layer chromatographic qualitative techniques have been used to detect the sugars present in the ethanolic sugar extracts obtained from defatted seed meals. Glucose and fructose were common monosaccharids present in all the sugar extracts. Similarly Rhamnose and Raffinose in three, Galactose, Stachyose and Cellobiose in two sugar extracts were found present. Quantitative determination of the sugars was done by using paper chromatographic technique in association with extractive spectrophotometric method. Good percentages of glucose and fructose in all the seeds were recorded. Sucrose percentages ranged between 11.8% and 18.4% in these seeds. Presence of stachyose was also recorded in A. Squamosa and B. Oleracea seeds.

Chapter-IV, of the thesis deals with studies on proteins and amino acids. This chapter describes methods used for isolation of proteins from four seeds and preparation of their protein hydralysates. Unidimensional ascending and descending paper and cellulose thin layer chromatographic methods were used for examination of prepared protein hydrolysates and subsequently qualitative identification of amino acids present in these hydrolysates have been described in this chapter.

Two dimensional descending and ascending paper and cellulose thin layer chromatographic methods were also used for the detection of amino acids. These investigations indicated the presence of different essential and non-essential amino acids in all these four seed protein hydrolysates. A. Squamosa protein hydrolysate contained sixteen, C. Vulgaris seventeen, B.

Oleracea contained eighteen and M. Oleifera contained in total fourteen amino acids.

Two dimensional paper chromatography in combination with spectrophotometric determination of ninhydrin colours after elution with 75% aqueous ethanol and HPLC techniques were used for the quantition of amino acids present in the hydrolysates. The results of two dimensional chromatography, combined with spectrophotometeric determination of ninhydrin colours were comparable to those obtained from HPLC analyses. A. Squamosa seed hydrolysate contained lesser contents of various amino acids as compared to other three protein hydrolysates.

Results and discussion are given in **Chapter-V**. Perusal of Proximate and Ultimate analysis indicate that these seeds contain comparatively lower moisture contents and consequently these can be stored for a longer period. Ash contents ranged between 4.5% to 6.28%, which indicate presence of mineral contents in them. Organic matter contents of these seeds indicate that these possess high nutritional value. Perusal of analyses results indicate that these plant seeds contain good percentages of proteins, oils, carbohydrates and mineral contents. The calorific values from the yielding components from original seeds of A. Squamosa, C. Vulgaris, B. Oleracea and M. Oleifera were found to be 6548, 6785, 6324 and 6876 calorie/gram. Their seed ashes contained Fe^{+++}, Cu^{++}, Zn^{++}, Mg^{++}, Ca^{++}, Na^{+}, K^{+} and P. as common minerals. Rich contents of K^{+}, Na^{+} and Ca^{++} were recorded in all these seeds.

Physico-chemical properties of petroleum ether extracted oils of the plant seeds were determined, these were-Specific gravity, Refractive index, Iodine, Acid and Saponification values. Refractive indexes of the oils indicated the presence of long chain fatty acids. Iodine values of all the four

seed oils indicated the presence of lower unsaturated bond numbers in the fatty acids. Lower acid values of C. Vulgaris, B. Oleracea and M. Oleifera seed oils indicated that these oils may be used as edible. Comparatively higher saponification values of the oils indicated that oils are normal triglycerides and may be used for the production of liquid soaps and shampoo etc.

Free fatty acids isolated from the oils and their mixed methyl esters were subjected to paper and thin layer chromatographic examination. Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were the common acids detected in almost all the seed oils. M. Oleifera seed oil contained Myristic, lauric, pentadecanoic and Arachidic acids also.

Fatty acid composition was determined by using GLC. FAME were subjected to GLC examination. The identification of fatty acids was carried out on the basis of their retention times and quantified by measuring the peak areas of chromatograms obtained. Palmitic and Stearic acids are most abundant saturated fatty acids of these seed oils.

A. Squamosa seed oil contained a good percent i.e. 53.58% of linolenic acid. Presence of oleic acid in varying amounts was found in all the four seed oils. B. Oleracea seed oil was found richer in linoleic acid i.e. it contained 11.51% linoleic acid. The presence of unsaturated fatty acid components in all these four seed oils make them nutritionally good. Infact the total percentages of unsaturated fatty acids in C. vulgaris, B. oleracea and M. oleifera seed oils was found comparatively lesser, while A. squamosa seed oil contained, total unsaturated fatty acid component amounting to 62.46%. Moderate quantities i.e. 7.98%, 11.51% and 2.85% of linolenic acid in A. squamosa, B. oleracea and M. oleifera seed oils respectively were recorded, which make these oils indispensable for healthy growth of human skin. Molla

et al. have described that unsaturated fatty acids, if present in good amounts, in the oils, make them effective to reduce the risk of cardiovascular problems in human beings.

Qualitative and Quantitative examination of ethanolic extracts of subjected defatted seed meals indicated the presence of Glucose, Fructose, Galactose, Rhamnose, Sucrose, Raffinose and Stachyose in fairly good amounts in all the plant seeds. Present investigations have shown that these plant seeds possess nutritional qualities and hence may play important roles in the living organisms.

The protein contents in A. Squamosa, C. Vulgaris, B. Oleracea and M. Oleifera were 21.64, 33.6, 36.76 and 30.22% respectively, which indicate that these plant seeds are potential sources of proteins. Paper and Cellulose thin layer chromatographic examination of the said protein hydrolysates revealed the presence of 16 amino acids in A. Squamosa, 17 in C. Vulgaris, 18 in B. Oleracea and 14 in M. Oleifera seeds. All the four seed protein hydralysates contained essential as well non-essential amino acids. For quantitative study of amino acid profile in protein hydrolysates, reversed phase HPLC technique was used. The quantitative estimation of amino acids was also carried out using two dimensional chromatographic method combined with spectrophotometric determination of ninhydrin colours. This technique gave comparable results with those obtained from HPLC technique. Analyses of seed protein hydrolysates indicate that essential amino acids like lysine, leucine, isoleucine, phenyl alanine, cystine, threonine, methionine and histidine were present in varying amounts. Good percentages of various non-essential amino acids in all the four isolates have also been recorded.

Thus the results of present study indicated very clearly that the subjected plant seeds form potential sources of oils and fats, carbohydrates, proteins and amino acids and minerals. The physico-chemical properties of these seed oils revealed that these oils contain comparatively higher contents of saturated fatty acids. These fatty acid compositions of the seed oils make them desirable in terms of nutrition. However A. Squamosa oil does not satisfy the requirement as edible oil. The seed ashes contain quite good amount of potassium, calcium, magnesium and phosphorous. The higher contents of calcium make these seeds attractive as natural source of calcium supplementation for pregnant and lactating women, children and other elders.

The high protein contents of these seeds make them good sources of proteins which could be used as feeds in livestocks. Thus these four seeds are also good sources of essential and non-essential amino acids which make them a healthy food for human and animal nutrition.

In conclusion, above cited investigations reveal that these plant seeds contained oils and fats, carbohydrates, proteins and amino acids and minerals. These components express their high nutritional values. Thus it is evident that these seed oils and seed kernels (except A. Squamosa) could be used for edible purpose provided toxicants, if any, present in them are eliminated.

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