

CHAPTER-II

PROXIMATE AND ULTIMATE ANALYSES OF SEEDS

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No work is reported in literature on oils, fatty acids, sugars, minerals and protein bound amino acid composition of the seeds of the plants of Bilaspur and its suburbs. Hence the following plants of Bilaspur region have been selected for their detailed chemical investigations. Their brief description is as follows.

2.1 *Annona Squamosa* Linn : ^{1,2}

Annona Squamosa L. (Annonaceae), Commonly known as Sitaphal or Sharifa in India. It is widely grown throughout the tropics in India. Its seed is well known for killing head lice but there has been no report on the active component. The plant is deciduous and small; reaching a maximum of 6 meters in height with many lateral braches, it grows well in regions of medium humidity. It is found in semi wild condition in the vicinity of villages. Its seeds comprise 30% of its fruit's weight, which is edible. Its roots are purgative. Its seeds, fruits and leaves are insecticidal and powdered seeds are used as fish poison. If powdered seeds applied to uterus, it induces abortion. These powdered seeds are highly irritant and if exposed to eyes can cause even blindness. In India its crushed leaves are sniffed to overcome hysteria and fainting spells; they are also applied on ulcers and wounds and a leaf decoction is taken in cases of dysentery. The crushed (India) ripe fruit, mixed with salt, is applied on tumours. Its bark and roots are both highly astringent. The bark decoction is given as a tonic and to halt diarrhoea.

2.2 *Citrullus Vulgaris* Var. *Fistulosus* (Stocks) : ^{1,3}

A climbing or trailing herb, leaves lobed, stems and petioles fistular. It is commonly known as 'Tinda' (Sanskrit name). This name

'Tinda' is commonly used in other parts of the World. It belongs to family Cucurbitaceae. It is also called Indian **round gourd**. This Squash-like gourd is native to India. It is cultivated in India, Sri Lanka, Indonesia and China. In India, It is grown in U.P., Punjab, Rajasthan, Bihar, M.P. and Chhattisgarh states. Its cooked fruits are used as vegetable. Its seeds are roasted and consumed in the same way as that of water melon. In India tinda is used as fodder and in medicine. Its seeds are helpful to break and remove the stones present in kidney, urinary bladder and gall bladder. The small pieces of stone in latter stage pass through urine.

2.3 Brassica Oleracea Linn (Cultivar group - Gongylodes group):^{1,4}

It is also known as **Kohlrabi** (German Turnip) (Cruciferae family). It is a low, stout cultivar of the Cabbage that can grow anywhere. The taste and texture of Kohlrabi are similar to those of a broccoli stem or cabbage heart, but milder and sweeter with a higher ratio of flesh to skin. It has swollen stem.

It provides high amounts of vitamin-C and soluble fiber and contains multiple nutrients with potent anti cancer properties. 3,3'-diindolylmethane, sulphoraphane and selenium. Its leaves are bitter, stomach and cardiogenic. These are used as medicine in gout and rheumatism. Its seeds are diuretics laxative and stomach. It is cultivated all over India.

Because it has a high vitamin-C content, it is good for the skeletal, digestive and lymphatic systems.

2.4 Moringa Oleifera Lam :^{1,5-7}

Moringa Oleifera Lam belongs to the family Moringaceae which is a single genus family of shrubs and trees. Cultivated across the whole of the tropical belt and used for a variety of purposes. (Jahn, 1986). It is called Moringa in the Dravidian language (India), which means

"generic root". Other regional names are Moonga, Marango, Mulangay etc. In English it is called **Horse radish tree**, Drumstick tree or Never Die tree (Fahey, 2005). It is a small or medium sized tree, about 10 m height, cultivated throughout India. It is a multipurpose tree known as nature's medicine cabinet, as almost every part of the tree can be used for food, or has some other beneficial property. In the tropics it is used as foliage for livestock. It is exceptionally nutritious vegetable tree with a variety of potential uses. It is also a natural energy booster. Different parts of this plant are being employed for the treatment of various ailments in the indigenous system of medicine. The leaves, fruit, seeds, roots and even the flowers, each possess their own particular medicinal virtues.

Moringa Oleifera is considered an effective treatment for anaemia, loss of appetite and it increases lactation in women. It also combats gastric discomfort, stomach ulcers, diarrhoea, dysentery, colitis and can be used as a laxative, purgative and a diuretic - to fight colds, bronchial infections, fever and head pain, rheumatic discomfort, muscular cramp, and bruises skin infections, scabies, ringworms and insect bites. As it possesses high value of nutrition for both humans and livestock, it is used for cattle fodder (cows, sheep, goats etc) and fish food (Carp).

Its dry seed suspension is known to be a natural coagulant (Sani, 1990; Kaser et al., 1990).

2.5 EXPERIMENTAL :

Sample Collection and Preparation :

The seeds were collected from villages around Bilaspur city. The seeds were oven dried at 60° C in an air circulated oven, ground with

porcelain mortar and pestle to fine particles and stored in screw capped plastic containers. Chemical analyses were carried out on the ground samples.

(i) Moisture Content : ^{8,9}

A clean crucible was dried to a constant weight in air-circulated oven at 105° C. A known weight of the seed sample was placed in the crucible and dried in the oven at 105° C to constant weight for 2 hrs. The crucible and its content was cooled in a desiccator and weighed. The moisture content was calculated and expressed in percentage.

(ii) Ash and Organic matter contents : ^{8,10}

A crucible was pre-heated in a muffle furnace at 500° C, cooled in desiccator and weighed. 2 gms of the seed sample was transferred into the crucible and weighed. The crucible and its content was kept in the muffle furnace at 525° C until white ash was obtained after twelve hours. Ash content was determined by weighing the crucible and difference was determined. The organic matter content in the seed was calculated by subtracting the percent ash content from 100.

(iii) Fiber : ¹¹

Fiber content in the seeds was determined according to the ISO method 5983 (Anon, 1981). A finely ground 2.5 gm sample of meal was weighed and freed from fat by extraction with 15 ml of n-hexane. The test portion was boiled with a 100 ml sulfuric acid solution (0.255 mol/L) followed by separation and washing of the

insoluble residue. The residue was then boiled with 100 ml sodium hydroxide (0.313 mol/L), followed by separation, washing and drying. The dried residue was weighed and ashed in a muffle furnace (EYEL, TMF-2100) at 600° C and the loss of mass was determined. The loss in weight of the dry residue upon ignition was taken as the amount of crude fiber.

(iv) Oil extraction :

Samples of dry seeds from mature fruits were harvested and were crushed and then fed (350 g) into a Soxhlet extractor fitted with a 1-L round bottom flask and a condenser. The extraction was executed on a water bath or heating mantle for 5-6 hrs, with 0.6L of Petroleum ether (B.Pt. 60-80°), the recovered oil from different seeds were further degummed and stored in a cool and dry place.

(v) Analysis of oil seed residues : ¹¹

The oil seed residues (meal) remaining after the extraction of oil from the seeds were analysed for protein and carbohydrate contents. Protein contents were determined according to the association of official Analytical chemists (AOAC) standard method 976.06 (Anon 1990)⁹. The carbohydrate contents were calculated by subtracting the sum of all other contents from 100.

The results of proximate chemical composition (Viz- percentage of moisture, ash, fiber, fats, proteins, Carbohydrates and minerals have been reported in Tables 2.1 and 2.2.

The ultimate analysis i.e. the composition of sample in terms of percentages of carbon, hydrogen, nitrogen and sulphur was

done for both, original seeds and defatted seed meals using standard methods^{9,12}. The results of these analyses are given in Tables **2.3** and **2.4**.

The Calorific values of seeds and defatted seeds were determined using "Julius and Peters Bomb Calorimeter" and the results are tabulated in Table No 2.5. These calorific values of the seed samples were determined on the basis of the energy yielding components i.e. fats, Proteins of Carbohydrates etc.

(vi) Mineral Analysis : ¹³⁻¹⁴

The ash obtained from various seeds was analysed qualitatively by uni-dimensional ascending paper co-chromatography for the presence of various cations. Spotting solutions of ash were prepared in moderately strong hydrochloric acid. Spotted sheets of chromatographic filter paper were irrigated with

- (i) Methanol, 40% HCl, Water (8:1:1, v/v)
- (ii) Acetone, 40% HCl, Water (8:1:1,v/v)
- (iii) Methanol, Ethanol, Isopentanol, 5 N HCl (20:50:20:10, v/v)
and
- (iv) Ethanol, Isopropanol, Isobutanol, 5 N HCl (50:20:20:10, v/v)

The cations were detected by using different specific staining reagents.

R_f values of various cations were measured and the same are given in Table No. 2.6.

The results of qualitative paper co-chromatographic analysis for the presence of various cations in different ashes are shown in Table 2.7.

(vii) Quantitative Analysis of Cations present in the Seed Ashes :

The ash residue of seed sample was dissolved in 10 ml of 50% of nitric acid solution and made upto final volume of 25 ml of distilled water. After that the minerals (Zn, Fe, Cu, Mn, Co, Mg, Ca, Na and K) were analysed separately, using ELICO make double beam Atomic absorption spectrophotometer SL-194. Phosphorus was determined by the phospho-vanado molybdate method of AOAC (1995).¹⁵

Working conditions for determination of cations by Atomic absorption spectrophotometer and the results of cationic analysis of seed ashes are given in tables 2.8 and 2.9 respectively.

Table - 2.1

Analyses of Moisture, Ash and Organic matter content of various seeds (Percentages)

S. No.	Name of Seeds	Moisture	Ash	Organic matter contents
1	Annona Squamosa Linn.	7.42	6.28	93.72
2	Citrullus Vulgaris Var. Fistulosus (Stocks)	7.3	4.5	95.5
3	Brassica Oleracea Linn. (Gongylodes-Group)	6.20	4.8	95.2
4	Moringa Oleifera Lam.	5.4	5.3	94.7

Table - 2.2
Protein, Fat, Fiber and Carbohydrate composition of various seeds
(Percentages)

S. No.	Name of Seeds	Protein	Fats	Fibers	Carbohydrates
1	Annona Squamosa Linn.	21.64	28.2	8.38	28.08
2	Citrullus Vulgaris Var. Fistulosus (Stocks)	33.6	32.2	5.6	16.8
3	Brassica Oleracea Linn. (Gongylodes-Group)	36.76	25.6	7.5	19.14
4	Moringa Oleifera Lam.	30.22	38.2	7.6	13.28

Crude Protein = %N x 6.25

Table - 2.3
Ultimate Analysis of Original Seeds (Percentages)

S.No.	Name of Seeds	Carbon	Hydrogen	Nitrogen	Sulphur
1	Annona Squamosa Linn.	58.4	6.44	3.4624	0.1986
2	Citrullus Vulgaris Var. Fistulosus (Stocks)	59.92	8.68	5.376	0.1854
3	Brassica Oleracea Linn. (Gongylodes-Group)	56.76	8.76	5.882	0.1688
4	Moringa Oleifera Lam.	60.4	9.34	4.835	1.624

Table - 2.4**Ultimate Analysis of defatted seed meals (Percentages)**

S. No.	Name of Seeds	Carbon	Hydrogen	Nitrogen	Sulphur
1	Annona Squamosa Linn.	46.88	7.66	6.86	0.2544
2	Citrullus Vulgaris Var. Fistulosus (Stocks)	45.48	9.82	8.62	0.3620
3	Brassica Oleracea Linn. (Gongylodes Group)	43.69	9.88	9.4	0.2786
4	Moringa Oleifera Lam.	44.80	7.88	7.36	2.120

Table - 2.5**Calorific Values of the Seeds**

S. No.	Name of Seeds	C.V. (Calorie Per gram sample)	
		Original Seeds	Defatted Seeds
1	Annona Squamosa Linn.	6548	4569
2	Citrullus Vulgaris Var. Fistulosus (Stocks)	6785	5077
3	Brassica Oleracea Linn. (Gongylodes Group)	6324	4112
4	Moringa Oleifera Lam.	6876	5158

Table 2.6 **R_f values of some cations in various solvents At $27 \pm 1^\circ\text{C}$**

S.No.	Solvent Systems	R_f values of Cations									
		Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cu ⁺⁺	Ni ⁺⁺	Mn ⁺⁺	Co ⁺⁺	Zn ⁺⁺	Fe ⁺⁺⁺
1.	Methanol, 40% HCl : Water (8:1:1)	0.36	0.17	0.55	-	-	-	-	-	-	0.66
2.	Acetone, 40% HCl : Water (8:1:1)	-	-	-	0.17	-	-	0.34	-	-	0.93
3.	Methanol, ethanol : Isopentano 1:5 N HCl (20:50:20:10)	-	-	0.22	0.36	0.42	0.32	0.35	0.34	0.94	0.68
4.	Ethanol : Isopropanol : iso-butanol : 5N HCl (50:20:20:10)	-	-	0.14	-	0.36	0.18	0.30	0.37	0.94	0.66

Table 2.8**Optimum Parameters used/fixed for determination of various cations present in different seed Ash samples**

Cation	Wave Length (nm)	Flame Gases	Slit Width(nm)	Detection Limit mg/lit.	Optimum Working range µg/ml	Lamp Current mA
Fe ⁺⁺⁺	248.3	A-AC	0.2	0.02	2-9	7.0
Cu ⁺⁺	324.7	A-AC	0.5	0.01	1-5	3.5
Co ⁺⁺	240.7	A-AC	0.2	0.02	1-5	7.0
Ni ⁺⁺	232.0	A-AC	0.2	0.02	1.8-8	4.0
Mn ⁺⁺	279.48	A-AC	0.2	0.02	1.4-5	4.0
Zn ⁺⁺	213.9	A-AC	0.5	0.005	0.4-1.5	5-0
Ca ⁺⁺	422.67	A-AC	0.2	0.07	0.4-1.5	5.0
Mg ⁺⁺	285.21	A-AC	0.5	0.02	0.4-1.5	3.5
Na ⁺	589.6	A-AC	0.5	0.002	0.4-1.5	5.0
K ⁺	766.5	A-AC	0.5	0.005	0.4-0.5	6.0

A-Ac = Air-Acetylene

Table 2.9**Cationic concentrations present in various seed Ash samples (in ppm)**

S.No	Name of Seed	Fe⁺⁺⁺	Cu⁺⁺	Co⁺⁺	Mn⁺⁺	Zn⁺⁺	Ca⁺⁺	Mg⁺⁺	P	Na⁺	K⁺
1.	Annona Squamosa Linn	134	63	-	-	92	847	710	1670	119	4768
2.	Cetrullus Vulgaris Var. fistulosus (stocks)	88	-	-	-	103	954	1067	1354	87	5840
3.	Brassica Oleracea Linn (Gongylodes Group)	154	66	-	-	144	844	945	1667	84	4740
4.	Moringa Oleifera Lam	178	71	23	12	137	1230	488	1360	113	3766

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