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Proximate composition, minerals of seed and physicochemical composition of seed oil of Chinese rain tree (*Koelreuteria elegans*)

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Abstract
The objective of the work was to study the proximate composition, minerals of seeds and physico-chemical properties of seed oil of *Koelreuteria elegans* (*K. elegans*) in order to explore its nutritional valve in human and animal diets. The oil was extracted from the ground seed material using petroleum ether (60-80 ºC) in a soxhlet apparatus for 8h and physico-chemical properties were analysed by some standard methods. The results showed that seeds contained moisture content 7.1±0.2%, ash content 1.3±0.1%, crude fibre content 10.3±0.6%, crude protein content 19.5±0.6%, carbohydrates value 36.8±1.5%, energy value 627.3±6.6 KJ/100g and oil content, free fatty acids, peroxide value, saponification value, unsaponifiable matter, iodine value, total carotenoids, total tocoferol in the oil were, 25.0±0.2%, 1.2±0.02%, 1.8±0.1meqKg^{-1}, 198.1±0.2 mg/gKOH, 4.3±0.1%, 90.9±1.2 g/100 g, 15.1±0.3 mg/Kg, 34.7±1.8 mg/100g respectively. Fatty acid composition showed 61.0±0.5% of saturated fatty acids and 32.5±0.3% of unsaturated fatty acids. The mineral determination showed that *k. elegans* seeds contain 55.0±1.4 ppm of Zn, 175.0±3.5 ppm of Cu, 606.0±28.3 ppm of Mn and 519.0±16.5 ppm of Fe. The low moisture content high nutritional value indicates that it can be stored for a longer duration and can be a good source of feed however high proportion of long chain fatty acid and significant amount of heavy metals indicates that it is not suitable for edible purpose but it could be a good source of fuel for industrial purpose.

Keywords: *Koelreuteria elegans*, crude protein, free fatty acids, carotenoids, tocopherol, mineral content

1. Introduction
Vegetable oils are having great importance in our life due to having variety of uses besides edible applications. Oilseed cakes and meals find great importance in animal feed industry owing to rich protein and other nutritional values and most of them are used as valuable feedstuffs for all farm animals. Cakes and meals produced from soya bean, groundnut, cottonseed, rapeseed, sunflower, coconut, palm kernel, linseed and sesame seed have good nutritional values so are used as a feedstuff. Soya bean is not only a source of high-quality edible oil for humans, but also a high-quality vegetable protein in animal feed worldwide due to favourable attributes such as relatively high protein content and suitable amino acid profile except methionine, minimal variation in nutrient content, ready availability year-round (Dei, 2011) [5]. Groundnut cake is an alternate source of protein except some vital amino acids such as lysine and methionine (Davies and Ezenwa, 2010) [4]. Vegetable oils also find importance in various industrial applications such as soaps and detergents, cosmetics, lubricants, ink, varnish, paints etc.

Koelreuteria elegans (Chinese rain tree) belongs to family *Sapindaceae* is native tree of Taiwan. It is grown as an ornamental landscape tree due to its fast growing and tolerance to a wide range of environmental conditions. It is used as insecticidal, anti-fungal and anti-bacterial agent. Crude extract of this plant also possess anti-tumor properties. Its Leaves, bark and defatted seed cake extract have significant antioxidant properties (Kumari *et al*., 2018) [9]. The other species *K. paniculata*, *K. bipinnata*, *K. henryi* are widely distributed in Northern China. *Koelreuteria henryi* have been used for the treatment of diarrhea, malaria and urethritis in traditional folk medicine (Jeng, 1994) [8]. It also exhibits significant anti-proliferation activity against cancer cell lines (Song *et al*., 1994) [13]. Seeds of *K. bipinnata* have vitamins A, D_2, and E.

In the absence of any report on the proximate composition and physico-chemical composition of *Koelreuteria elegans* present study was conducted to explore its nutritional value and the possibilities of its industrial applications.

2. Materials and Methods

2.1 Seed material and chemicals

The seeds of *Koelreuteria elegans* were collected from the university campus CCS, Haryana Agricultural University, Hisar. After cleaning, the seeds were dried in shade and ground to fine powdered form. The commercially available chemicals from Sigma-Aldrich, Qualigens, Merk and Ranbaxy, of highest purity, were used for various experimental procedures.

Oil, iodine value, saponification value, unsaponifiable matter, peroxide value and free fatty acid were determined according to AOAC official methods [1-3].

2.2 Proximate nutrient determination

Moisture, ash, crude protein, crude fat and crude fibre were determined by standard methods

2.3 Estimation of moisture content

Two gram of powdered sample of seeds was taken in three replicates and dried initially at 80-90 ℃ and finally at 100-102℃ then weight of dried sample was noted until constant weight were obtained. The percentage of moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Wt of fresh seeds} - \text{wt of dry seeds}}{\text{wt of fresh seeds}} \times 100$$

2.4 Estimation of ash content

A cleaned crucible was placed in a muffle furnace at 600 ℃ for 1h then transferred to desiccators, cooled to room temperature. Empty crucible was weighed quickly to prevent moisture absorption. One gram of sample was taken in crucible and placed it in muffle furnace. Temperature was adjusted at 600 ℃ for 6h. Crucible was transferred to the desiccator and cooled to room temp. Then crucible was weighed quickly. The percentage of ash content was calculated as follows:

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Wt of sample}} \times 100$$

2.5 Estimation of crude fibre

Crude fibre was estimated by the method of Sadasivam and Manikam (1992) [12].

Five gram dried and defatted sample was digested with 200 mL of 1.25% H_2SO_4 for 30 min. using reflux apparatus. The acid solution was decanted and the residue was filtered through muslin cloth and washed with hot water. The residue was digested with 200 mL of boiling 1.25% NaOH, for 30 min. using reflux apparatus. The residue was filtered through sintered crucible and made free from alkali by repeated washing with hot water and then with alcohol and finally with diethylether. The residue was dried in an oven at 105℃ for 5h and weighed. The dried residue was ignited in a muffle furnace, at 500 ℃ for 6h, cooled and weighed. The difference in weight of residue and ash represented the crude fibre in samples used for analysis. The results are expressed as crude fibre per cent.

$$\text{\% crude fibre in ground sample} = \frac{\text{Loss in wt on ignition}}{\text{Wt of sample}} \times 100$$

2.6 Estimation of crude protein

Crude protein was calculated by multiplying % N with the factor 6.25. Five hundred mg of sample was weighed and transferred to 250 mL conical flask. After adding 15 mL of sulphuric acid and perchloric acid (9:1) the flask was heated continuously till the solution became clear. The contents were cooled, diluted and volume made to 100 ml with double distilled water. A suitable aliquot (5 mL) of the digest was transferred to conical flask. Ten mL of 40% NaOH was added to make it sufficiently alkaline and then steam distilled. The ammonia liberated by distillation was trapped in 10 mL of boric acid solution containing mixed indicator. The distillate was subsequently titrated against standard N/100 H_2SO_4. A blank was also run under identical conditions. Nitrogen content of the sample was calculated after taking into account suitable dilution.

2.7 Seed oil content

Dried and ground samples (100 g) of seed kernel were weighed in a thimble and set for soxhlet extraction using petroleum ether. The heating rate was adjusted to give a condensation rate of 2-3 drops/sec. and extracted for 16 h. removed the thimble and retained petroleum ether. The excess of petroleum ether was evaporated from the solvent flask on a hot water bath and dried the flasks in a desiccator and weighed.

$$\text{Oil content in sample (\% dry wt. basis)} = \frac{(b-a)\times 100}{\text{Wt.of sample (g)}}$$

2.8 Iodine value

Two gram of oil sample was taken in a 500 mL glass stoppered conical flask containing 10 mL of $CHCl_3$. The flask was swirled until the sample entirely dissolved. Iodine monochloride (25 mL) was added to it and again swirled the mixture. The flask was allowed to stand in dark place for one hour. After that, 15 mL of KI solution and 100 mL of water were added and shaken vigorously then titrated with standard sodium thiosulfate solution. Two blanks were made in the same manner by omitting the sample. If 'B' is volume of standard sodium thiosulphate solution (mL) used for blank and 'S' is volume of standard sodium thiosulphate solution (mL) used for sample, then Iodine number is calculated by:

$$\text{Iodine number } \left(\frac{g}{100}\right) = \frac{(B-S)(\text{Normality})(12.7)}{W}$$

2.9 Saponification value

The oil (2 g) was taken into flask (A) and added 25 ml of 0.5 N alcoholic KOH solution. Reflux condenser was attached and heated on boiling water for 60 min. Flask was swirled frequently during heating. After that 2-3 drop of 1% phenolphthalein indicator was added and titrated with 0.5 N HCl. Operations were performed with blank as well. Volume of 0.5 N HCl used for sample and blank were denoted by 'A' and 'B'.

$$\text{Saponification value} = \frac{(B-A)\times 28.05}{\text{Wt.of oil(g)}}$$

2.10 Saponification and removal of unsaponifiable material

Seed oil (5 g each) was refluxed separately with 50 ml of 2M potassium hydroxide in 95% ethanol for one hour. The mixture was allowed to cool, diluted with water (50 mL) and extracted with 50 mL diethyl ether three times. Sufficient quantities of water and ether were necessary for a good phase

separation. The combined diethylether extracts were washed thrice with 100 mL distilled water and allowed to stand overnight. The diethylether was removed under vacuum at 40ºC and the residue dried by evaporation with acetone to obtain the unsaponifiable matter.

2.11 Peroxide value
The oil (5 g) was taken into 500 mL conical flask. Acetic acid - $CHCl_3$ mixture (30 mL) was added to the flask. Saturated KI solution (0.5 mL) was added to it and allowed to stand for 1 min. About 450 mL of water was added to the flask and then titrated against standard 0.01 N sodium thiosulfate by using starch indicator to liberate all iodine free $CHCl_3$ layer until the blue colour just disappeared. Blank was titrated similarly in the absence of oil and final peroxide value was calculated by:

$$\text{Peroxide value} = \frac{A \times N \times 1000}{\text{Wt.of oil(g)}} \text{ meq / kg oil}$$

2.12 Fatty acid spectrum
A suitable amount of oil sample was taken in a test tube and 0.5 mL of 0.5 N sodium methoxide was added and covered with aluminium foil and then immersed in a water bath at 65ºC to a depth of half inch and was shaken vigorously for 2-3 min. The mixture became homogenous indicating the complete esterification of the oil sample. The test tube was removed from the water bath and cooled to room temperature. One mL of carbon disulphide was added and shaken for 1-2 min. Separated the lower layer and approximately 100 mg of activated charcoal was added mixed uniformly and filtered through Whatman No. 1 filter paper. The filtrate constituted all the methyl esters of fatty acids.

2.13 Fractionation of methyl esters by GLC
Methyl esters of fatty acids were separated using Chemito 8610 HT Gas chromatograph equipped with FID and a BPX70, 0.25mL fused silica column was used. The carrier gas was hydrogen and injection was operated in the split mode, the split ratio being approximately 50:1. Injector and detector temperature were 270 ºC and 280 ºC respectively. The oven temperature was held at 70 ºC for 1 min. and then programmed at 30 ºC/min. to 170 ºC followed by further programming at 30 ºC/ min. to 200 ºC and held at this temperature for 6 min. Data was analysed with, Chemito 5000 integrator (Toshniwal Instruments, India Ltd.)

2.14 Determination of free fatty acids
Free fatty acids were determined as per described by Rao *et al*. (1972) modified literature method [11]
Fifty ml of denatured alcohol was added to one g of oil sample in a 250 mL conical flask. The flasks were swirled and few drops of phenolphthalein were added and the contents were titrated against 0.1 N sodium hydroxide till a permanent light pink colour appeared which persisted for at least 1 min. The percentage of free fatty acids was calculated by using the following formula:

$$\text{Free fatty acids (in terms of oleic acid)} = \frac{100 \times 282 \times \text{Volume of NaOH used}}{\text{Wt.of oil} \times 10 \times 1000}$$

2.15 Determination of total tocopherol
Using aliquots of 10, 15, 20 and 25 mg of a solution of ∝−tocopherol, volume was made to 8mL with ethanol. Added 1mL of 2,2′ -dipyridyl reagent and mixed. 1mL the ferric chloride reagent was added and shook the mixture for 10 seconds. The absorbance of the mixture was read at 520 nm against ethanol as a blank. Then the standard curve was drawn. The above same procedure was followed by using 10, 20, 30, 40 mg sample solutions (Philip *et al*., 1954) [10]. The content of ∝−tocopherol in the extract was calculated by using regression equation of the standard curve. During color develop solutions were protected from sunlight.

2.16 Determination of total carotenoids
Oil (0.5 g) was taken in 100 ml conical flask. The oil sample was dissolved in cyclohexane (2.5% w/v) and the absorbance was read at 417 nm (Visconcellos *et al*., 1990) [14] and following equation was used to estimate total carotenoids.

$$\text{Mg carotene/kg oil} = \frac{(\text{absorbance at 417 nm}) \times \text{sample in volume in mL}}{0.204 \times (\text{sample weight in g})}$$

2.17 Determination of minerals contents
The sample was digested by wet oxidation. In a conical flask 0.2 g of extract was mixed with 5 mL nitric acid and 1mL of perchloric acid and kept it overnight at room temperature. After that, digestion was done on low temperature at 70-80 ºC and then at higher temperature. Digest the sample until the volume of the solution reduced to about 1mL. Made the final volume upto 10 mL using distilled water and analyzed by using an atomic absorption spectrometer [7].

3. Results and Discussion

Table 1: Proximate composition of seeds (%) of *K. elegans*.

Content	Composition (%)
Moisture	7.1±0.2
Ash	1.3±0.1
Crude fibre	10.3±0.6
Crude protein	19.5±0.6
Oil	25.0±0.2
Carbohydrates	36.8±1.5
Energy value (KJ/100 g)	627.3±6.6

Values are mean of three replicates ± standard error

Proximate composition of seeds indicates its nutritional values, Table1 shows the proximate composition of the *Koelreuteria elegans* seeds. The result shows that the seeds are having a good amount of crude protein 19.5±0.6%, crude fat 25.0±0.2%, crude fibre 10.3±0.6%, Carbohydrates content 36.8±1.5% and energy value 627.3±6.6 KJ/100g. The moisture content was found to be 7.1±0.2% and ash content was reported to be 1.3±0.1%. The low moisture content indicates that it can be stored for a longer duration and high value of nutrients indicates that it can be a good source of feed.

Table 2: Chemical characteristics of the seed oil.

Parameters	Composition
Peroxide value (meq/kg)	1.8±0.1
Iodine value (g of I_2/100g of oil)	90.9±1.2
Saponification value (mg/gKOH)	198.1±0.2
Unsaponifiable matter (%)	4.3±0.1
Free fatty acid (as % oleic acid)	1.2±0.02
Carotenoid content mg/kg	15.1±0.3
Total tocopherol mg/100g	34.7±1.8

Values are mean of three replicates ± standard error

On analysis by standard methods some physico-chemical properties were obtained given in Table 2. The mean peroxide value was 1.8±0.1 meq/kg. The low peroxide value is a good index for the stability of the oil and its susceptibility to rancidity during storage. The mean Iodine value was 90.9±1.2 g/100 g indicating a low degree of unsaturation when compared to most plant oils. The saponification value was 198.1±0.2 mg/g KOH. The unsaponifiable matter was 4.3±0.1%. The concentration of free fatty acid was 1.2±0.02 as % oleic acid. The content of carotenoids was 15.1±0.3 mg/kg. The content of total tocopherol was 34.7±1.8 mg/100 g.

Table 3: Fatty acids composition (%) of the seed oil.

Fatty acid	Composition (%)
Palmitic acid ($C_{16:0}$)	4.9±0.2
Oleic acid ($C_{18:1}$)	22.8±0.1
Linoleic acid ($C_{18:2}$)	9.7±0.2
Arachidic acid ($C_{20:0}$)	54.8±0.2
Behanic acid ($C_{22:0}$)	1.3±0.1
Unidentified	6.5±0.2
Total saturated fatty acids	61.0±0.5
Total Unsaturated fatty acids	32.5±0.3

Values are mean of three replicates ± standard error.

Fatty acid composition of an oil is very important from edible purpose, high content of unsaturated fatty acids in oil makes it suitable for human consumption, *K. elegans* seed oil fatty acid composition have been shown in Table 3. Oleic acid and linoleic acid were the major unsaturated fatty acid having 22.8±0.1% and 9.7±0.2% respectively. Total unsaturated fatty acid content was 32.5±0.3%. Arachidic acid was major saturated fatty acid 54.8±0.2% along with palmitic acid 4.9±0.2 and behanic acid %. Total saturated fatty acid was 61.0±0.05%. The high proportion of long chain fatty acid indicates that it can be used as a fuel but not suitable for edible purpose.

Table 4: Minerals (Zn, Cu, Mn, Fe) composition (ppm) of the seeds.

Element	Composition (ppm)
Zn	55.0±1.4
Cu	175.0±3.5
Mn	606.0±28.3
Fe	519.0±16.5

Values are mean of three replicates ± standard error

The mineral composition of *K. elegans* seeds shows that it contains significant amount of heavy metal content (Table 4). Zinc 55.0±1.4 ppm, Copper 175.0±3.5 ppm, Manganese 606.0±28.3ppm, and Iron 519.0±16.5 ppm. The mineral composition indicates towards heavy metals accumulation properties of *Koelreuteria elegans*. Some previous studies observed Mn-resistant behaviour of its sister species *Koelreuteria paniculata*. Huang *et al*. (2015) [6] observed that *Koelreuteria paniculata* is Mn-resistant species while studing the growth and heavy metals accumulation of *Koelreuteria paniculata* seedlings and their potential for restoring Manganese mine wasteland in Hunan, China.

4. Conclusion

The low moisture content indicates that it can be stored for a longer duration and high value of nutrients indicates that it can be a good source of feed however high proportion of long chain fatty acid and significant amount of heavy metals indicates that it is not suitable for edible purpose but it could be a good source of fuel for industrial purpose. The mineral composition also indicates towards its properties of heavy metals accumulation so there is a scope of further study on heavy metals accumulation and their potential for restoring heavy metals mine wastelands.

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