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Chemical Composition, Physico-chemical Properties and Nutritional Value of *Cleistanthus collinus* Roxb. Seeds and Seed Oil of Eastern Chhattisgarh.

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I. ABSTRACT

Cleistanthus collinus Roxb. commonly known as 'Garari' in Hindi and 'Karra' in Chhattisgarhi, is a small deciduous tree, 2-15 m tall, which belongs to the family Phyllanthaceae. *Cleistanthus collinus* Roxb. is a common suicidal poison. The remarkable plant possesses diuretic capabilities along with potent antiseptic, antibacterial, and antifungal attributes; hence recent research has focused on its therapeutic potential. These seeds are rich in lipid content and have high calorific value. Proximate analysis and Ultimate analysis of full-fat seed reveal Moisture – 14.01%, Ash – 2.85%, Volatile Matter – 61.59%, Fixed carbon – 21.55%, N – 1.35%, C – 54.52%, H – 8.367%, S – 0.19%. The *Cleistanthus collinus* Roxb. full-fat seeds are rich in mineral and nutrient content which are a rich source of Ca and K ions. Quantitatively, the primary fatty acids detected by GC-FID were nervonic acid – 28.23%, oleic acid – 21.93%, γ -linolenic acid – 16.16%, linoleic acid – 9.74%, behenic acid – 8.96%, palmitic acid – 6.64%, lignoceric acid – 4.13%, steric acid - 2.77%. The amino acid profile by HPLC result shows essential amino acids (EAA) at 26.18%. Between EAA, leucine exhibited the most elevated amino acid score among the group, with threonine, methionine, isoleucine, valine, and lysine, subsequently following in order of decreasing scores. In the complete array of amino acid composition, it is important to take note of the non-essential amino acids (NEAA) comprising 67.35%. Alanine exhibited the highest levels, trailed by glutamate, aspartate, serine, proline, tyrosine, glycine, cystine, and arginine, in descending order of abundance.

Key Words: Physico-chemical, Proximate-Ultimate Analysis, AAS, Fatty Acid Composition, GC-FID, Amino Acid, HPLC, *Cleistanthus collinus* Roxb. seeds.

II. INTRODUCTION:

The botanical name of the seed is *Cleistanthus collinus* (Roxb.) Benth. ex-Hook. f. after Bentham and Hooker, Roxburgh described a plant species from the Phyllanthaceae family that is typically called Garari (Hindi). The plant is indigenous to Sri Lanka and India, and the plant's primary habitat is within the deciduous (dry) forests of central and southern India. It is classified as vulnerable by the IUCN.[1] According to reports, leaves, roots, and the capsule's outer crust are highly toxic. In many rural areas of south India, the plant is mainly consumed as poison, which results in numerous fatalities.[2] The extract prepared from the leaf is also employed as poison for fish-catching and cattle as well as an abortifacient.[3] The primary phytochemicals that cause poisoning are glycosides, lignans, lactones, alkaloids, saponins, and tannins. The most noticeable active components are cleistanthins, oduvin, and diphyllin. Humans who suffer from severe poisoning often experience gastrointestinal issues, hypotension, renal impairment, hypokalemia, mixed metabolic, cardiac arrhythmias, hepatic effect, and

respiratory acidosis.[4] Inhibition of the vacuolar ATPase proton pump appears to be the poison's primary mechanism of action.

Classification

Kingdom- Plantae; Phylum- Tracheophyta; Division Angiosperms; Class- Eudicots; Sub-class Rosids; Order Malpighiales; Family- Phyllanthaceae (Euphorbiaceae); Genus- Cleistanthus Species- collinus

Synonyms

Amanoa collina (Roxb.) Baill., Andrachne cadishaco Benth., Andrachne orbiculata Roth., Bridelia collina (Roxb.) Hook. and Arn., Emblica palasis Buch. - Ham., Clutia collina Roxb., Lebidieropsis collina (Roxb.) Müll. Arg., Lebidieropsis orbiculata (Roth) Müll. Arg., Lebidieropsis orbiculata var. collina (Roxb.) Müll. Arg., Lebidieropsis orbiculata var. lambertii Müll. Arg. [5]

Vernacular names

Sanskrit- Indrayava, Kaudigam, Kutaja, Nandi; Hindi – Garrar, Garari, Oriya- Korodo, Karada; Kannada- Bodadaraga, Badedarige, Kadagargar; Telugu – Kandishe, Korsi, Vadise, Kadise, Malayalam- Odaku, Nilappala, Odugu; Marathi- Garari; West Bengal: Karlajuri; Tamil – Odishi, Odaichi, Nilaippalai, Odan, Oduvanthazhai, Other- Garari, Karra, Oddan thazhai, Odukkan thazha. [5]

Medicinal Properties

Recently research has focused on its therapeutic potential, and the plant is renowned for its diuretic, antibacterial, antifungal, and antiseptic. The active ingredients are tested on many cancer cell lines because they are cytotoxic and appear effective against HIV.[6] The plant extract has larvicidal properties that reduce mosquito populations that spread diseases like dengue and malaria. The plant is additionally employed in conventional medical practices like Ayurveda, Siddha, and homoeopathy. The plant has proven crucial in the agricultural area due to its natural insecticidal properties, which control agricultural pests like black moths and red flour beetles. Additionally, the plant concentrate is used by farmers to eliminate animal ticks and can be used as live fencing, fuel wood, and timber.[7] The paper summarises the plant *Cleistanthus collinus* Roxb. enormous potential and utility as a multifunctional tree.

III. METHODS AND MATERIALS REQUIRED

A. Seed selection and sampling

Cleistanthus collinus Roxb. seeds were collected from March to May from Eastern Chhattisgarh, removed the seeds from the seed coat. Dried the seeds in the shade at room temperature, crushed them using a mortar pestle and stored them in airtight bottles at 4°C. After the crushing of seeds, Extraction and Proximate – Ultimate analysis was performed from the original and defatted seed.

B. Extraction

Oil from the shade-dried seeds powder of *Cleistanthus collinus* Roxb. was extracted using Soxhlet Apparatus. *Cleistanthus collinus* Roxb. seeds were exhausted with petroleum ether (Boiling point 60-80°C). [8,9,10] The chemicals used are AR grade and of Sigma chemical company. A dark green colour oil is obtained in 6.85% yield.

C. Proximate and Ultimate Analysis

The proximate chemical composition of the seed samples and the standard methods were utilized to assess the physico-chemical traits of the extracted oil. Calorie contents were determined using Julius and Peter's Bomb calorimeter.

1) Proximate Analysis – Determines the approximate composition of a biological sample such as Moisture Ash content, Fix Carbon, Volatile Matter and Fibre etc. [11,12]

2) Ultimate Analysis – Determines all seed components elements like C, H, N, S. [11,12]

D. Mineral nutrient content of seed flour:

The seed flour's mineral nutrient composition was determined using a 5gm sample burned in a muffle furnace. Temperate of 500°C was maintained, and a 50.0 ML solution of 0.5 M nitric acid HNO_3 was used for dissolving the residues (Ash). The concentrations of minerals like Fe, Mg, Pb, Co, Cu, Ni, and Zn were identified by means of atomic absorption spectrophotometer (Make Thermo Scientific and iCE 3000 SERIES). The method of J. T. Ouilly et al. was followed [13].

E. Total fat and fatty acid analysis:

Analysis of seed oil fatty acids was conducted following the AOAC (2001.996.06) methods [14]. The process involved trans-esterifying the isolated fat; fatty acid methyl esters (FAME) were produced by utilizing 0.5 M methanolic KOH. Gas Chromatography (GC) was employed to analyze the fatty acids using an Agilent Technologies 7890B GC; with flame ionisation detector FID and attached the nitro terephthalic-acid-modified polyethylene glycol (PEG) column from Agilent, known as J&W DB-FFAP, possesses high polarity and is specially designed for the precise analysis of volatile fatty acids.

During the analysis, the column temperature was initially set at 100°C for 5 minutes, then increased gradually to 240°C at a rate of 4°C per minute. Nitrogen was used as the carrier gas at a 1.0 ml/min flow rate, and the detector temperature was maintained at 280°C. Standard 47885-U Supelco® 37 Component FAME Mix (10 mg/ml in methylene chloride) and individual trans-fatty acids standards, namely trans-11-Vaccenic methyl ester, trans-9, 12-Octadecadienoic (linoleliadic) methyl ester, and Supelco trans-9-Eliadic methyl ester (all at 10 mg/ml in heptane), were used.

To determine the lipid profile (fatty acid composition) of *Cleistanthus collinus* Roxb., sample fatty acids peak area percentages were normalized and compared to the standard fatty acid composition. The results of the analysis are presented in Table 4 and Fig. 2, depicting the fatty acid chromatograms obtained from the GC analysis.

F. Amino acid analysis:

Amino acid content was analysed by applying the method described by Wang et al. [15]. At 110 °C for 22 hours, the samples were hydrolysed by a standardised. The mixture was filtered and evaporated under a vacuum to dryness after hydrolysis. The hydrolysates were reconstituted with a mobile phase and filtered through a 0.50-µm pore-size membrane. The analysis was performed using an Agilent 1260 Infinity HPLC system equipped with G1379B - µ-degasser, G1316A - 1260 thermostatted column compartment, G1312B - 1260 binary pump, G1329B - 1260 standard autosampler, G1315C - 1260 diode array and multiple wavelength detector, and a Zorbax Eclipse-AAA column (250 mm x 4.6 mm, L x ID) particle size 5µm) (Agilent Technologies, Santa Clara, CA). The hydrolysed samples were automatically derivatised with OPA (o-phthalaldehyde for primary amino acids) and FMOC (9- fluorenyl methyl chloroformate (FMOC) for secondary amino acids) by programming the autosampler. After derivatisation, 0.5µl of the seed sample was injected at 55 °C into a Zorbax Eclipse-AAA column, with detection at λ1 = 338 nm and λ2 = 262 nm. The flow rate of 0.7ml/min was maintained while separation was performed. The amounts of individual amino acids were expressed as mg/100g protein in each sample. Amino acid chromatograms of the seed sample are given in Table 5 and Fig 3.

IV. RESULT AND DISCUSSION

A. *Physico-chemical properties.*

The physicochemical properties of *Cleistanthus collinus* Roxb. seeds oil in Table 1 show the qualitative property of seed oil.

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Table 1: Characteristics of Seed and Oil of *Cleistanthus collinus Roxb.* [16,17,18]

Sl No	Sample	Properties	Value
1	**Seed**	Moisture % in seeds	14.01
2		Oil %	6.85
3		Protein %	8.4
4		Ash %	2.85
5		VM %	61.59
6		FC %	21.55
7		Calorie content calorie/gm	3858.14 cal/gm
8	**Oil**	Colour	Dark Green
9		Total Fat	38 ml/ 600 gm of seed.
10		Refractive index	1.48
11		Specific gravity oil at $25^{O}C$	0.9112gm/ml
12		Acid Value %	3.5
13		Saponification Value	108.7
14		Ester Value	105.2
15		Free Fatty Acid %	1.7
16		Iodine Value	121
17		Unsaponifiable Matter %	1.91

The seed was found to contain a substantial amount of volatile matter-61.59%, protein – 8.4%, and Ash – 2.85%. Various factors, including the degree of unsaturation, fatty acid chain length, molecular weight, and degree of conjugation of oils, influence the refractive index of oil. The refractive index of *Cleistanthus collinus* Roxb. was 1.48. The refractive index and iodine value have a positive relationship, measuring the degree of unsaturation in oils and providing insight into their oxidative stability. The iodine value 121 of I2/100gm of oil is characteristic of unsaturation. *Cleistanthus collinus* Roxb. seeds oil exhibited a saponification value of 108.7, attributed to its high content of medium-chain fatty acids, such as C16 and C18. The concentration of free fatty acids was found to be 1.7, with an acid value of 3.3 mg of KOH/g of oil for *Cleistanthus collinus* Roxb. These relatively low values reveal lower hydrolysis of triglycerides, signifying that the oil may have an extended shelf life. The image of *Cleistanthus collinus* Roxb. is given in Fig.1

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Fig.1: Image of *Cleistanthus collinus* Roxb. Plant, Fruit, and Seeds.

B. Ultimate analysis of seed and seed ash

The Ultimate analysis used the standard method for original and defatted *Cleistanthus collinus* Roxb. seeds. The result of these analyses is tabulated in Table 2.

Table 2: Ultimate analysis of *Cleistanthus collinus* Roxb. seeds (%)

	Carbon	Hydrogen	Nitrogen	Sulphur
Original seeds	54.52	8.367	1.35	0.19
Defatted seeds	48.87	8.696	1.18	0.13

C. *Atomic Absorption Spectroscopy*

The Ash of the seeds was subjected to analysis [18] of mineral nutrients present in them using a flame photometer for Na, K, and Ca ions and atomic absorption spectrophotometer for other mineral nutrients. Evaluation of mineral nutrients of *Cleistanthus collinus* Roxb. seeds ash by using AAS. is tabulated in Table 3.

Table 3: Determination of mineral nutrients of *Cleistanthus collinus* Roxb. seeds ash by using AAS.

No.	Test Parameter	Measurement Unit	Test Method	Test Result
I.	**Chemical Testing**			
	Metals			
1	Sodium (as Na)	mg/kg	Flame Photometer	290.89
2	Potassium (as K)	mg/kg	Flame Photometer	10593.53
3	Calcium (as Ca)	mg/kg	Flame Photometer	1712.04
4	Magnesium (as Mg)	mg/kg	AAS	1333.98
5	Iron (as Fe)	mg/kg	AAS	47.01
6	Cobalt (as Co)	mg/kg	AAS	Absent
7	Nickel (as Ni)	mg/kg	AAS	7.83
8	Copper (as Cu)	mg/kg	AAS	12.73
9	Zinc (as Zn)	mg/kg	AAS	15.67
10	Lead (as Pb)	mg/kg	AAS	Absent

Cleistanthus collinus Roxb. seeds were found to possess substantial quantities of essential minerals. The most abundant was K, followed by Ca, Mg, Na, Fe, Zn, Cu, and Ni. By Ultimate analysis, C is in the highest % followed by H, N, S. These elements, except Pb and Ni, are required in minimal amounts (in micrograms) by the human body and animal. However, Pb and Ni cause toxic effects.

Table 4: Fatty acids content of *Cleistanthus collinus* Roxb. seeds oil.

Peak Number	Name	Retention Time	Area	Area %
1	Myristic Acid Methyl Ester (C14: 0)	20.559	301342	0.10
2	cis - 10 - Pentadecenoic Acid Methyl Ester (C15: 1)	23.667	233616	0.08
3	Palmitic Acid Methyl Ester (C16: 0)	25.478	20456214	6.64
4	Heptadecanoic Acid Methyl Ester (C17: 0)	27.579	213646	0.07
5	Stearic Acid Methyl Ester (C18: 0)	29.965	8521960	2.77
6	Oleic Acid Methyl Ester (C18: 1n9c)	30.421	67556309	21.93
7	Linoleic Acid Methyl Ester (C18: 2n6c)	31.3	30012549	9.74
8	Linolelaidic Acid Methyl Ester (C18: 2n6t)	31.447	239452	0.08
9	γ - Linolenic Acid Methyl Ester (C18: 3n6)	32.675	49793639	16.16
10	α - Linolenic Acid Methyl Ester (C18: 3n3)	33.801	911441	0.30
11	Arachidic Acid Methyl Ester (C20: 0)	34.185	913024	0.30
12	cis - 11 - Eicosenoic Acid Methyl Ester (C20: 1n9)	35.029	399181	0.13
13	cis - 5,8,11,14,17 - Eicosapentaenoic Acid Methyl Ester	37.651	348475	0.11
14	Behenic Acid Methyl Ester (C22: 0)	38.918	27600897	8.96
15	Erucic Acid Methyl Ester (C22: 1n9)	39.451	559178	0.18
16	cis - 13,16 - Docosadienoic Acid Methyl Ester (C22: 2)	41.343	199889	0.06
17	cis - 4,7,10,13,16,19 - Docosahexaenoic Acid Methyl Es	41.7	139444	0.05
18	Lignoceric Acid Methyl Ester (C24: 0)	42.883	12713141	4.13
19	Nervoinic Acid Methyl Ester (C24: 1n9)	43.755	86984717	28.23
Totals			**308098114**	**100**

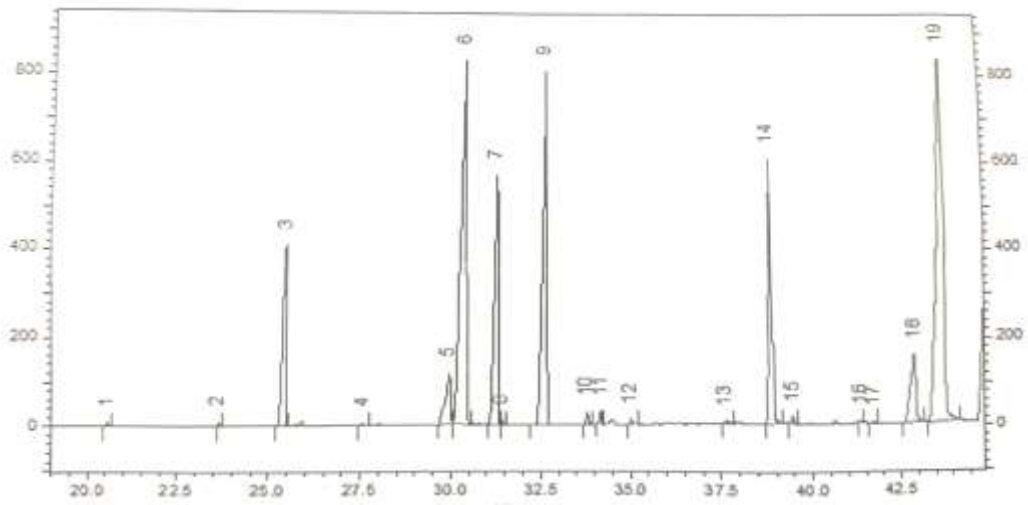

Fig.2: Chromatogram of fatty acid methyl ester of *Cleistanthus collinus* Roxb. seed oil.

Fatty acid composition of seed oil [19] of *Cleistanthus collinus* Roxb. by GC-FID reveals quantitatively presence of highest % of Nervoinic Acid – 28.23%, followed by oleic acid – 21.93%, γ-linolenic acid – 16.16%, linoleic acid – 9.74%, behenic acid – 8.96%, palmitic acid – 6.64%, lignoceric acid – 4.13%, steric acid - 2.77%. Nervoinic Acid - regulates the function of brain cell membranes and has a neuroprotective effect. Oleic Acid is

most used for precluding heart disease and reducing cholesterol. γ-Linolenic Acid (GLA) for eczema, asthma, arthritis, high blood pressure, and nerve pain related to diabetes. Linoleic Acid plays a particular part in support of heart health. Behenic Acid - to give hair conditioner and moisturisers for their soothing properties. Palmitic Acid is one of the most abundant saturated fatty acids in our human body because 20-30% of the phospholipid of each cell membrane are made of palmitic acid to enable its proper functioning. Stearic acid softens and smooths the skin's surface while also helping maintain the skin barrier. *Cleistanthus collinus* Roxb. seeds are rich in Saturated Fatty Acid Behenic acid, Palmitic acid, Stearic acid, Lignoceric acid (SFA- 22.5%), as well as Unsaturated Fatty acid, Nervonic acid, Oleic acid (MUFA-50.16%) and Linoleic acid, Omega fatty acids such as and γ-Linolenic acid (PUFA-25.9%).

Fig 3: Chromatogram of amino acid of *Cleistanthus collinus* Roxb. seeds.

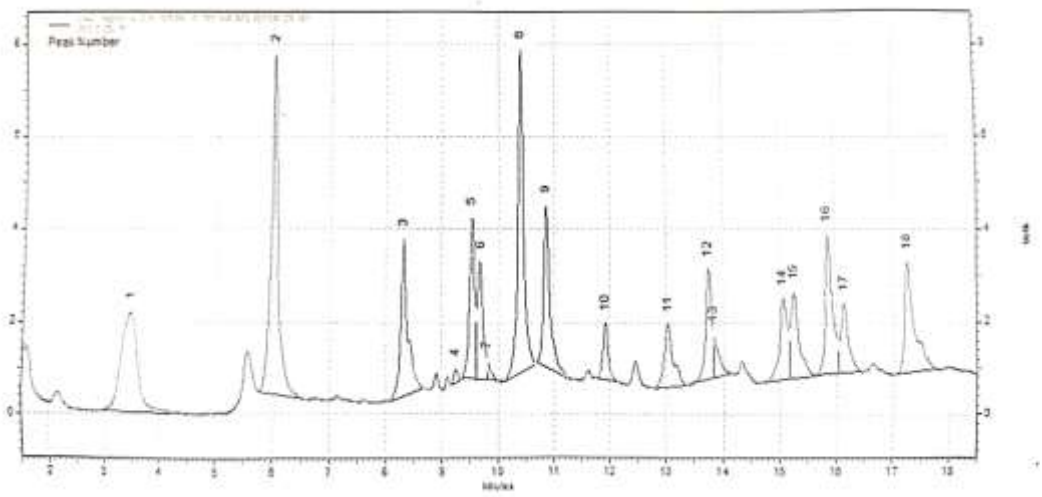

Table 5: Amino acids content of *Cleistanthus collinus* Roxb. seeds.

DAD: Signal A, 338nm/Bw:10 nm Ref 390, nm/Bw:20 nm Results				
Peak Number	**Name**	**Retention Time**	**Area**	**Area %**
1	Aspartate	3.480	94850	11.09
2	Glutamate	6.005	131490	15.37
3	Serine	8.318	60618	7.09
4	Histidine	9.250	3172	0.37
5	Glycine	9.538	46736	5.46
6	Threonine	9.682	36964	4.32
7	Arginine	9.817	2310	0.27
8	Alanine	10.405	105535	12.34
9	Tyrosine	10.862	57668	6,74
10	Cystine	11.917	17654	2.06
11	Valine	13.022	32551	3.80
12	Methionine	13.747	36555	4.27
13	Phenylalanine	13.828	11787	1.38
14	Isoleucine	15.062	34303	4.01
15	Leucine	15.248	39176	4.58
16	Unknown	15.860	55111	6.44
17	Lysine	16.127	29539	3.45
18	Proline	17.262	59471	6.95
Totals			**855490**	**100.00%**

Table 5 illustrates the total amino acid composition of the seed *Cleistanthus collinus* Roxb. The results demonstrated that essential amino acids (EAA) accounted for 26.18% of the total amino acid content, with most being present at levels higher than the requirements recommended by WHO/FAO/UNU [20]. In terms of amino acid score, leucine (4.58%) takes the lead, showcasing the highest value, closely trailed by threonine (4.32%), methionine (4.27%), isoleucine (4.01%), valine (3.80%), and lysine (3.45%). This indicates the significant presence and importance of these amino acids in the composition. Non-essential amino acids (NEAA) constituted 67.35% of the total amino acid content, with alanine being the most abundant, followed by glutamate, aspartate, serine, proline, tyrosine, glycine, cystine, and arginine.

Cleistanthus collinus Roxb. seed flour's overall amino acid composition closely resembles to that of Sunflower seeds [21]. The abundant presence of both essential and non-essential amino acids in this seed flour makes it a highly promising protein source, suitable for nourishing both livestock and human diets alike.

V. CONCLUSION

Based on the thorough phytochemical investigation encompassing physicochemical properties, chemical composition, and nutritional value, it is evident that *Cleistanthus collinus* Roxb. seeds hold immense promise as alternative sources of oil, protein, and essential micronutrients. These findings open up exciting possibilities for utilizing these seeds as valuable and sustainable resources in various applications. The oil is 6.85% highly rich in polyunsaturated fats, which can help improve heart health. The essential amino acid (26.18% of total amino acid) of *Cleistanthus collinus* Roxb. is very close to that of seeds of Sunflower seeds [21]. Further research and development efforts could explore their commercial applications in the food, pharmaceutical, and industrial sectors.

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VII. CONFLICT OF INTEREST STATEMENT

The authors declare that there was no conflict of interest regarding this paper.

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