



Proximate and Elemental Analysis of *Careya Arborea* Roxb Plant's Root

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Abstract

Medicinal plants and herbal healing are the new emerging routes of the health care system. The minerals and nutritional values of many plants have been reported to vary, which depending on generic background, location, environmental condition, and cultivation methods. In this article, the phytochemical screening, proximate and elemental analysis have been carried out for the *Careya arborea* Roxb (CAR) plant's root, which was collected from Pamgarh Tehsil, District Janjgir-Champa, Chhattisgarh, India by using the standard methods. The proximate composition of CAR showed the percentage of the extract including moisture content (18.37%), ash content (17.23%), carbohydrate content (43.76%), nitrogen content (12.52%), crude protein (14.81%), crude fiber (19.43%), crude fat (6.93%) and total available energy (347.32%). The elemental analysis of the CAR root indicates the presence of various minerals, mainly including elements Na (11.93%), K (14.72%), Ca (12.41%) and Mg (9.76%); trace elements, Fe (22.82%), Cr (0.83%), Mn (1.67%), Co (1.31%), Cu (7.39%), Zn (8.68%) and Se (1.21%), and toxic heavy metals Pb (0.08%), Cd (0.06%), Hg (0.05%) and As (0.03%). The outcome is to validate the potential use of CAR as a plant with an appreciable medicinal agent.

Keywords: *Careya arborea* Roxb, root; Phytochemical screening; Proximate and elemental analysis.

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1. Introduction

Natural products have always exhibited a vibrant role in the Ayurveda, Allopathic, Homeopathic, Chinese, and Egyptian medicinal systems.^[1] In Vedic literature, various plants are reported with their medicinal uses in various indigenous medicinal systems.^[2] The World Health Organization (WHO) describes any type of plant containing therapeutically useful chemical substances as medicinal plants. Various parts, such as fruits, seeds, leaves, rhizomes, roots, barks, and seeds of this plant are used to control or treat diseases.^[3] Most parts of the world prefer natural herbal medicines to synthetic medicines. Herbal medicine is well established due to its therapeutic benefits, safety, low side effects, being inexpensive, effective, relatively low toxicity, and easy availability in the vicinity.^[4] Since herbs generally come

directly from natural sources, they can contain heavy metals, toxic substances, impurities, biological contaminants, and harmful foreign substances. Therefore, these parameters should be verified before consuming the herbal medicine, otherwise, it may cause serious health risks.^[5] The standardisation process can be achieved through gradual pharmacognosy research, phytochemical research, proximate and elemental analysis, and toxicity research.^[6] These studies help to improve the safety and authenticity of herbal plants in various diseases. Evaluation of the chemical properties of the original feed, such as the determination of the extraction value, moisture content, different types of ash value, content of impurities, trace elements, heavy metals, and active phytochemicals plays an important role in the standardisation of drugs.^[7] Phytochemicals are biologically active chemicals derived from plants and are considered secondary metabolites because the factories that manufacture them hardly need them. They are naturally synthesised from various parts of plants.^[8]

Careya arborea Roxb. (CAR), common name wild guava, is a member of the Lecythidaceae family. CAR is generally known as "Padmaka" in Ayurveda,^[9-10] Ka Li Yu Rui in Chinese medicine, Credon Oak in Thai,^[11] Katabhi in Sanskrit,^[12] Kalindi in Hindi and Kumhi in Chhattisgarhi. It is

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found in India and other parts of tropical climates around the world. In India, it is found in Jammu, Kashmir, Laddakh, West Bengal, Madhya Pradesh, Chhattisgarh, Tamil Nadu, and forest grasslands. CAR is a very attractive and handsome, medium-sized deciduous tree with a height of 20 m, like a spreading crown. The leaves are simple, alternate, glabrous, obovate or oblong, clustered at the ends of branches, marginal, and the leaves turn red in the winter season. The flowers are large, sessile, pink, yellowish-white, and the persistent calyx has crowns. The flowering period is from March to April.^[12-14] The fruit is a drupe, berry-shaped, large, green, fleshy, crowned with a calyx and globose.^[9] The bark is dark grey^[12] and usually peeled off in thin strips.^[15-18] The seeds are dark brown in colour, not cracked, hard, and wrinkled,^[9] and nest in large cotyledons of fleshy embryos. The root is reddish-brown, odourless, and wrinkled on the surface. The non-areal part (root) of CAR. is used in Ayurveda for tuberculosis and bone fractures, and it is also used in Vata and Kapha^[19] It has many pharmacological activities, such as anti-fertility, antibacterial, hyper-glycaemic, antidiarrheal, and antifungal.^[20-22] No researcher has been reported till date on the proximate and elemental analysis of any plant parts of CAR.

The proximate analysis of plant raw materials provides information about the quality and purity of the samples.^[23] The quality control issue has been covered by direct monitoring of competent professionals in almost all traditional systems of medicine. However, in terms of developing current procedures, it necessitates adjustments in their approach in terms of quality control. As a result, quality control is becoming a major focus for the evaluation of historically used medicinal plants and herbal preparations. To define the limit for the reference standards for quality control and quality assurance of herbal remedies, it is necessary to investigate the parameters connected to standardisation to be carried out in different batches. Proximate analysis is employed in natural material characterization as a split of a human consumable product into its primary elements. They are good estimations of the contents of packaged consumable items and serve as a simple and cost-effective technique of nutrition verification. Such herbal formulations must pass through standardization processes the ash content of the herbal sample specifies the existence of natural impurities such as carbonate, oxalate, and silicate *etc.* The moisture content provides information about drying loss and excess moisture in the raw material.^[24] Heavy metal contamination is also an important parameter and should be checked before using the crude drug. According to physiological functions, heavy metals are divided into two categories: essential heavy metals and non-essential heavy metals. Cr, Fe, Co, Cu, and Zn are essential and required for our enzymatic system, vitamin synthesis, and haemoglobin formation. Cd, As, Hg, and Pb are non-essential heavy metals that do not need to perform specific functions and can have harmful effects even at very low concentrations in the body.^[25] Plants absorb various elements from soil and water, and the absorption rates of different elements are different. More

importantly, heavy metals such as Cd and Zn have high transfer coefficients and are easily absorbed by plants, while heavy metals such as Pb, Cr, Co, and Cu have the least transfer capacity. That is, the transfer coefficient from soil to plants is low because they maintain a stable connection with the soil system. The goal of elemental analysis is to identify the amount of a certain element present in a molecule or substance. Proximate and nutrient analysis of herbal drugs plays a vital role in evaluating their nutritional importance. There is no report on the proximate, Fourier-transform infrared spectroscopy (FTIR), elemental, and nutritional analyses of CAR roots. Thus, the purpose of this study was to establish the proximate composition, FTIR, mineral content, and nutritional analyses of the root of CAR.

2. Materials and methods

2.1 Plant collection and identification

The fresh, healthy roots of CAR. were collected from Pamgarh, District Janjgir-Champa, Chhattisgarh, India. The plant materials were taxonomically identified and verified by the Botanical Survey of India, Allahabad (U.P.) India and a voucher specimen was deposited there.

2.2 Preparation of extracts

The root of the collected plant, CAR was washed, shade-dried at room temperature, and ground to a fine powder using a grinder machine. Then, 100 g of fine powder samples were placed in a thimble of Soxhlet apparatus. Successive solvent extraction was done with different solvents like, pet. ether, ethyl acetate, EtOH, MeOH, and H₂O as per solvent polarity, and the extracts were concentrated in a rota evaporator. The extracts were stored in a fresh and clean container for further experiments.

2.3 Qualitative screening for the phytochemicals

To analyse the different kinds of phyto-materials present in CAR Root, it was subjected to qualitative analysis with some known analytical procedures. Accordingly, we did Salkowski's test for terpenoids, ferric chloride test for tannins, Liebermann-Burchard test for sterol, Shinoda test for flavonoids, Molisch's test for carbohydrates, Wagner test for alkaloids, ninhydrin test for amino acids, Keller Killiani's test for glycosides, ferric chloride test for phenols, precipitate test for phlobatannin, acetic acid test for oxalate, Borntrager test for quinones, froth test for saponin *etc.*^[32-32] We got positive responses in most cases, except oxalates, quinones, and phlobatannin, which implies that CAR root contains a variety of phytoconstituents, which are shown in [Table 1](#).

2.4 Proximate analysis

As per Ayurveda, herbal drugs in the form of oral dosage are given to the patient as powdered materials mixed with water. For that, we performed proximate analysis to determine the physicochemical properties as well as solubility and nutrient content of herbal drug materials. Using a standard procedure

Table 1. Preliminary Phytochemical screening of CAR root.

S. N.	Phytochemicals	Pet. ether	Ethyl acetate	EtOH	MeOH	H ₂ O
1	Alkaloids	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Saponins	+	+	+	+	+
4	Sterol	+	+	+	+	+
5	Tannins	-	+	-	+	-
6	Terpenoids	+	-	+	+	-
7	Phenol	-	+	-	+	+
8	Carbohydrates	-	+	-	+	+
9	Cardiac glycoside	-	+	+	+	+
10	Steroids	+	-	+	+	+
11	Oxalates	-	-	-	-	-
12	Quinones	-	-	-	-	-
13	Phlobatanins	-	-	-	-	-
14	Amino acid	-	-	-	+	+

+: present, -: absent

and formula from the reported literature, the moisture content, ash content, carbohydrate content, nitrogen content, crude protein, crude fiber, crude fat, and total available energy of CAR root. were determined.^[26-28]

Ash content:

5 g powdered sample is taken in a crucible and burned in a hot air oven at 450 °C for 3 hrs. The sample is cooled to room temperature in a desiccator and then weighed to determine the ash content using the given formula.

$$\text{Ash content \%} = A/B \times 100$$

where, A = Weight of ash (g) and B = Weight of dry sample (g).

Moisture Content:

5 g sample was placed in a weighted petri dish. The petri dish is kept in a hot air oven at 100 °C. After cooling, the sample was placed in a desiccator and weighed it to determine the moisture content using the given formula:

$$\text{Moisture Content (\%)} = \frac{(W_1 + W_2) - W_3}{W_2} \times 100$$

where, W₁ = Petridish weight, W₂ = Sample weight, W₃ = Dried sample weight + Petridish weight.

Crude fiber content:

After extraction with petroleum ether, 5 g of the dry material was boiled with 2% H₂SO₄ and NaOH solution for 30 minutes, respectively. It was filtered and washed the residue with boiling water and dried for 2 hrs at 130 °C, cooled in a desiccator and weighed. Then the residue was ignited at 550 °C for 25 min. and cooled in a desiccator before reweighing.

$$\text{Crude fiber content \%} = \frac{\text{Loss in Weight on Ignition}}{\text{Weight of Sample}} \times 100$$

Determination of Crude fat:

5 g of powdered sample was placed in a 250 mL round-bottom flask and refluxed with petroleum ether for 6 hours at 40–60 °C in a soxhlet extractor. The extract was distilled, pet. ether was revived, and the extract in the RB flask was dried in a hot air oven at 105 °C, and the oil containing RB flask weighed. The percentage of crude fat content is enumerated as follows:

$$\text{Crude fat \%} = \frac{\text{Weight of Pet.ether extract}}{\text{Weight of Sample}} \times 100$$

Determination of % of Nitrogen and total Protein:

5 g of powdered sample was digested with 20 mL of conc. H₂SO₄ in a 250 mL Kjeldahl flask and permitted to cool, diluted to 250 ml before transferring to a 300 mL Kjeldahl flask having a buffer and 30 mL of 30% NaOH. By dipping the end of the condenser in the liquid to trap the discharged ammonia gas, the extract from the solution is shifted to an accumulating liquid containing 250 mL of 2% H₃BO₃ and a few drops of mixed indicator. Then, the liquid mixture was titrated with 0.01 M HCl until the endpoint was purple, and the % of nitrogen content was calculated as:

$$\% \text{ of Nitrogen} = \frac{14 \times A \times B \times C}{\text{Weight of Sample} \times D} \times 100$$

Percentage crude protein: % of Crude protein = % of Nitrogen × 6.25, where, A = Actual molarity of HCl, B = Total volume of the diluted digest, C = Titre value of HCl used, D = Aliquot volume distilled.

Total Carbohydrates content (TCC):

The TCC is calculated by the difference between the total dry

matter and the added percentage of ash, crude fat, crude protein, and crude fiber, using the formula:

$$\text{TT C \%} = 100 - (\text{Ash \%} + \text{Crude fat \%} + \text{Crude protein \%} + \text{Crude fiber})$$

Determination of Total available Energy:

The total available energy value in the sample is estimated in kilo joules per hundred grams, and calculated by using these factors to add up the values of carbohydrates, and crude protein; 16,736 KJ, 37,656 KJ, and 16,736 KJ, correspondingly, as given below:

$$\text{Energy content} = (\text{Crude protein \%} \times 16.736) + (\text{Crude fat \%} \times 37.656) + (\text{Carbohydrate \%} \times 16.736)$$

2.5 Elemental analysis

To find out the essential and trace elements, we did the elemental analysis following the reported procedure of CAR root and the elemental analysis, including major elements Na, K, Ca, and Mg; trace elements Fe, Cr, Co, Cu, Zn, Mn, and Se; toxic heavy metals Pb, Cd, Hg, and As, *etc.* were determined using the atomic absorption spectrophotometer (Systronic, SYS-WFX-320).^[28]

For this, 1 gm CAR root powdered sample was digested after adding 20 ml conc. HNO₃, 2 ml HClO₄ and HCl (10:1), left for 5 minutes, and digested at 80 °C on a hot plate. The solution was allowed to evaporate to dryness until all the tissue had been digested and raised the temperature 105 °C to reduce the volume to 1.0 ml and added 10 ml of distilled water, boiled the residues. The mixture was cooled and filtered through a whatman no. 541 filter paper into a 100 ml volumetric flask and made up to mark with deionized water and measured the elements in atomic absorption spectrophotometer (AAS).

2.6 FT-IR analysis

The chemical analysis of the MeOH extract from CAR root was completed using FTIR. The details of this method are mentioned as follows.

FT-IR chromatogram

The FTIR spectrum of the sample is measured by FT-IR (Perkin Elmer). Approximately 2 mg of the semi-solid MeOH extract of the CAR root sample was ground with 200 mg of dry KBr to form a very uniform fine powder, and then a 15-ton hydraulic press was used to press the powder into fine granules. Then use 16 scans to record the KBr pellet held in the KBr pellet holder for FTIR measurement in the wavenumber range of 4000 to 450 cm⁻¹.

3. Results and discussion

3.1 Phytochemical analysis

The preliminary phytochemical screening of the root of CAR in pet. ether, ethyl acetate, EtOH, MeOH, and H₂O extract is given in Table 1. Terpenoids, saponins, alkaloids, tannin, flavonoids, sterols, steroids, phenols, glycosides, carbohydrates, and amino acids were identified. Oxalate,

quinones, and phlobatannins are not found in these extracts. From the therapeutic view, biologically active components are new sources of valuable compounds that can lead to the investigation of new drugs. In this study, fourteen phytochemical tests were employed in pet. ether, ethyl acetate, EtOH, MeOH, and H₂O extract of the root of CAR. and their results are given in Table 1. Preliminary phytochemical analysis revealed that flavonoids, alkaloids, sterols, and saponin are present in all extracts, while cardiac glycosides are not present in petroleum ether, and steroids are only absent in ethyl acetate extracts. Tannin and phenols are present in ethyl acetate, MeOH, and H₂O extracts. Terpenoids are not present in ethyl acetate and H₂O extracts, while carbohydrates are not present in petroleum ether and ethyl acetate extracts, and amino acids are only present in MeOH and H₂O extracts, but not in other extracts.

3.2 Proximate analysis

The proximate analysis of the root of CAR is given in Table 2 and Fig. 1. The proximate analysis of the root of CAR revealed the moisture content of 18.37%, ash content of 17.23%, carbohydrate content of 43.76%, nitrogen content of 12.52%, crude protein 14.81%, crude fiber 19.43%, crude fat 6.93%, and total available energy of 347.32% in Kcal/100g. The moisture content of CAR (18.37%) was relatively high; therefore, microorganisms would grow and the sample's life span of storage would be low. The ash content (17.23%) recorded for the root of CAR belongs to the acceptance range. Ash content is a diagnostic purity index. It represents both physiological and non-physiological ash. It contains a similar amount of certain mineral nutrients. The ash content is a reflection of the amount of mineral elements present in the sample. Therefore, the plant contained a good amount of minerals. The higher occurrence of carbohydrate content makes CAR a good source of energy for the body and aids digestion and the assimilation of other nutrients.

Table 2. Proximate Composition results of CAR root [w/w, (%)].

S. N.	Proximate composition	Value
1	Moisture content	18.37
2	Ash content	17.23
3	Carbohydrate Content	43.76
4	Nitrogen Content	12.52
5	Crude Protein	14.81
6	Crude Fiber	19.43
7	Crude Fat	6.93
8	*Available Energy	347.32

*Kcal/100 g

Crude protein (14.81%) of the root of CAR is also higher, which implies that the root could be used as a potential source of protein. Crude fiber content (19.43%) of the root of CAR is higher. This has the nutritional implication that fiber prevents diverticulosis and aids absorption of trace elements in the gut as well as helps in the elimination of undigested food material

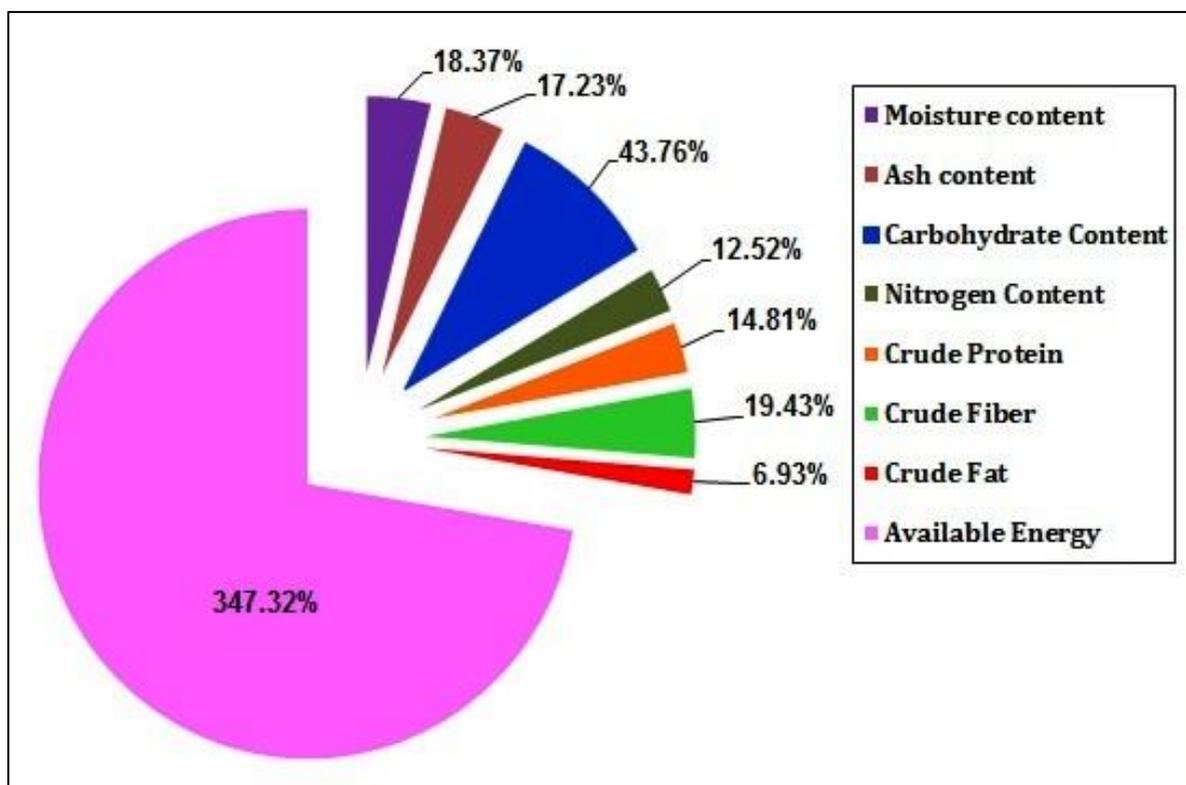


Fig. 1 Proximate analysis of CAR Root.

through the bowel. The crude fat (6.93%) was recorded at a low percentage, which implies that they could be used for weight monitoring through the reduction of the cholesterol level. Dietary fat increases the palatability of food by absorbing and retaining flavour. Diet fat provides much of its caloric energy as fat is said to be deficient for human beings as excess fat consumption is implicated in certain cardiovascular disorders. The available energy (347.32 %) of the root of CAR was high, thus making them good source of energy. The presence of moisture content, ash content, crude fiber, crude fat, and crude protein in CAR root suggests that it may be safe for body repairing, anti-ageing while the high percentage of crude fiber content will improve diarrheal problems.^[29]

3.3 Elemental analysis

The elemental analysis of the root of CAR is given in Table 3 and Fig. 2. The mineral composition analysis indicates the presence of major elements, Na (11.93%), K (14.72%), Ca (12.41%), and Mg (9.76%); trace elements, Fe (22.82%), Cr (0.83%), Mn (1.67%), Co (1.31%), Cu (7.39%), Zn (8.68%), and Se (1.21%), and toxic heavy metals, Pb (0.08%), Cd (0.06%), Hg (0.05%), and As (0.03%) etc. present in the plant sample (root). Na, K, Ca, and Mg all play important roles in the regulation of blood and heart health.^[30] They also improve the immune system and protect against malnutrition-related diseases. Mineral elements are essential for normal growth, muscle activity and bone growth, blood transportation, physiological action in the human body, neurotransmission, fluid and acid-base balance, anaemia prevention, and food

production.^[31-32] Therefore, their presence in the root gives a positive weight to the nutritional importance of the CAR plant. In the root, Pb, Cd, Hg, and As were identified in 0.08 %, 0.06 %, 0.05 %, and 0.03 %, respectively. The heavy metal concentration was determined to be within the permitted limit. Because the heavy metals measured are in such small quantities, they have no adverse consequences. According to the above study, the root part of CAR is not toxic or harmful to humans if it is consumed orally.

Table 3. Elemental Analysis Result of CAR root (mg/100g).

S. N.	Element	Value (%)
1	Na	11.93
2	K	14.72
3	Ca	12.41
4	Mg	9.76
5	Fe	22.82
6	Cr	0.83
7	Mn	1.67
8	Co	1.31
9	Cu	7.39
10	Zn	8.68
11	Se	1.21
12	Pb	0.08
13	Cd	0.06
14	Hg	0.05
15	As	0.03

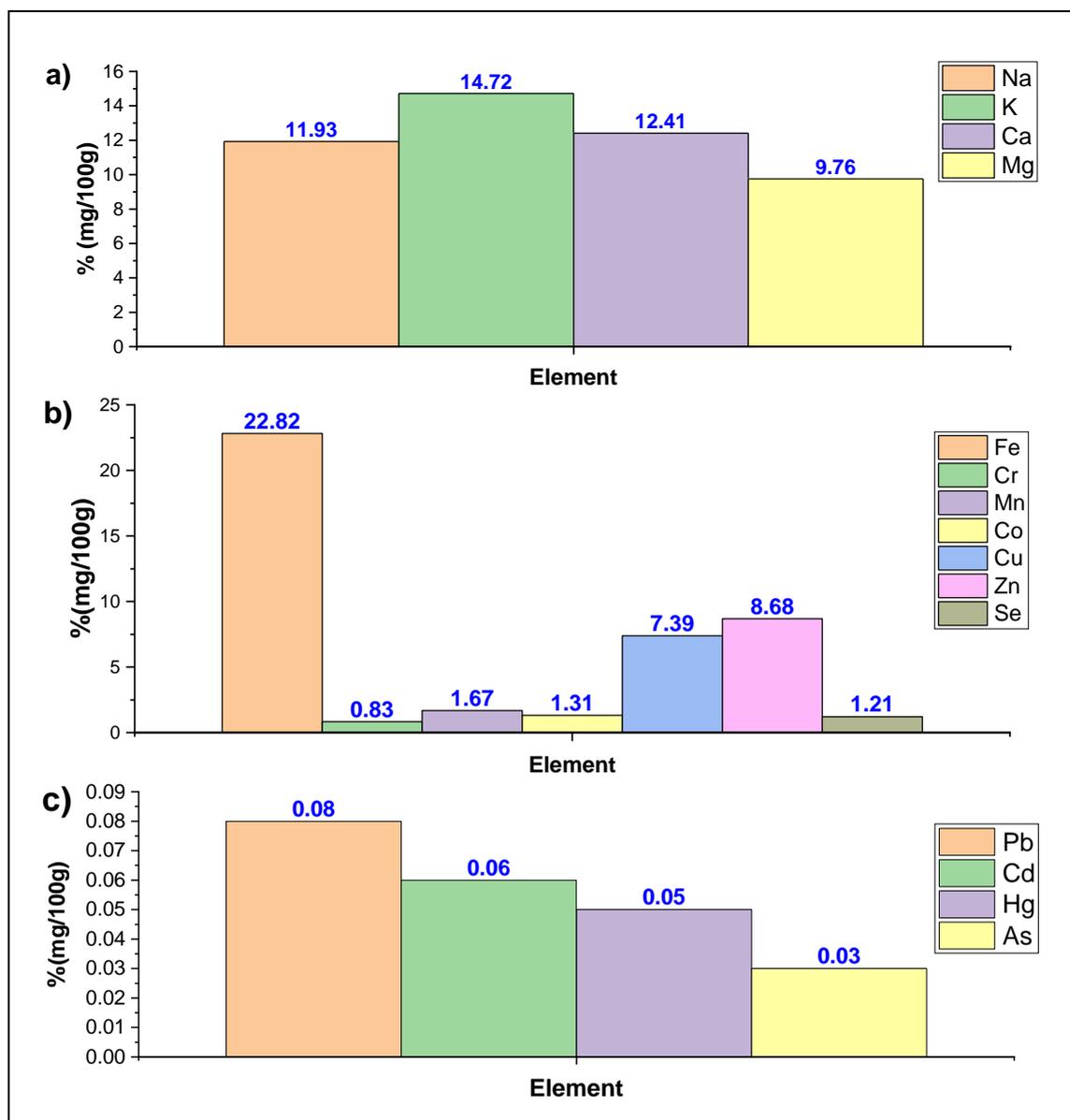


Fig. 2 Mineral analysis of root part of CAR a) Macro element b) Micro element and c) Toxic element.

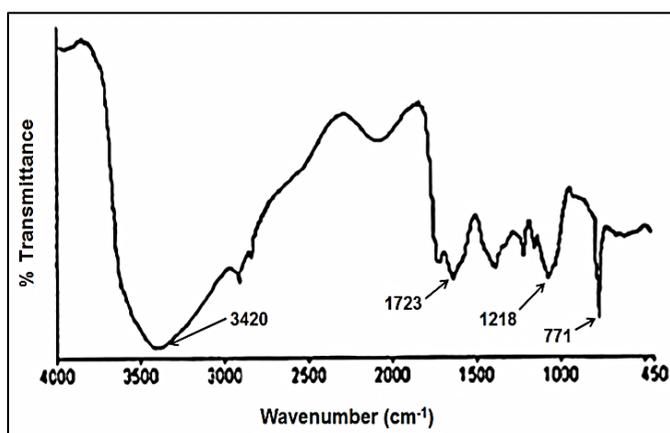


Fig. 3 FT-IR analysis of methanolic extract of CAR root.

This is due to the presence of alkaloids in root extract. The broad peak at 3420 cm^{-1} corresponds to the OH stretching of the phenolic hydroxyl group, which indicates the presence of phenolic compounds in methanolic root extract.

3.4 FTIR analysis

The FTIR analysis displayed (Fig. 3) a strong band near 1723 cm^{-1} , which indicates the C=O stretching of flavonoids, as demonstrated by the preliminary phytochemical screening of MeOH extract. The band at 771 cm^{-1} indicates the presence of secondary N-H bond elongation, while the peak at 1218 cm^{-1} indicates the C-N elongation of the aliphatic unit.

4. Conclusion

As per WHO, standardisation of herbal drugs highlights the necessity and relevance of evaluating proximate and micronutrient analysis. Proximate analysis of herbal drugs is an important issue in the quality control aspects of natural products. Proximate analysis is a critical step in monitoring chemically active metabolites in medicinal plants, which leads to the discovery of new medications and the evaluation of their efficacy. CAR was conducted for preliminary phytochemical screening, proximate analysis, and elemental profiling. CAR provides various health advantages depending on its

composition. The current study reveals that CAR root has a high concentration of secondary metabolites. This is the first comprehensive research on CAR roots' proximate analysis, mineral ion content, and elemental profile. According to this study, the root of CAR is high in elements, mineral nutrients, total accessible energy, and other essential factors that can aid with malnutrition. These studies show that CAR root contains a high content of mineral elements, secondary metabolites, and a significant amount of moisture content, ash content, carbohydrate content, nitrogen content, crude protein, crude fiber, crude fat, and total available energy, highlighting the plant's nutritional potential and illuminating the possibility of using CAR root to overcome malnutrition and as a source of herbal drugs. This can be a good source to balance the amount of potassium, calcium, and iron in an animal's body and is a good food supplement to combat hypocalcemia, and anaemia. And thus, this plant has proved its usefulness in ethnic herbal medicines as well as in the extraction of natural drug molecules, in formulation and development for the future.

Conflict of interest

There are no conflicts to declare.

Supporting information

Not applicable.

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