Journal of Natural Remedies
DOI: 10.18311/jnr/2026/54551

INFORMATICS JOURNALS

Short Communication

eISSN: 2320-3358 | Vol 26(3), 2026 | pp. 781-796

A Scientific Approach towards Phyto-pharmacognostical Profiling of *Coptis teeta* Wall. Rhizome with Prominent Antioxidant Activity

Vaidehi Vadhvana[1,2], K. Laya Shanu[3], C. R. Harisha[4], Pankajkumar Nariya[5], Kokkuvayil Vasu Radhakrishnan[2,3] and Mukeshkumar Nariya[1*]

[1]Department of Pharmacology, Institute of Teaching and Research in Ayurveda (Institute of National Importance), Jamnagar - 361 008, Gujarat, India; mukeshnariya@itra.edu.in

[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad - 201 002, Uttar Pradesh, India

[3]Chemical Sciences and Technology Division, CSIR-NIIST, Trivandrum - 695019, Kerala, India

[4]Department of Pharmacognosy, Institute of Teaching and Research in Ayurveda (Institute of National Importance), Jamnagar - 361008, India

[5]Department of Chemistry, Atmiya University, Rajkot -360 001, Gujarat, India

Abstract

Background: *Coptis teeta* Wall. belonging to *Ranunculaceae* family, is a renowned ethnomedicinal species of Northeast India with intense bitter, alkaloid-rich rhizome. *Ayurveda* and Traditional Chinese Medicine systems recognised its promising antioxidant efficacy to manage chronic oxidative stress-related diseases. **Aim:** The present research study is aimed to efficiently fill the research gap through detailed phyto-pharmacognostical studies while, being first to report heavy metal analysis, and correlating *in vitro* antioxidant assays of aqueous and methanol extract with its rich phytoconstituents. **Methods:** The present study was systemically designed to generate relevant data by following well-established standard protocols and assays obtained from *Ayurveda* Pharmacopoeia of India, related texts and online references. **Results:** Morphologically, dried rhizome were irregularly branched, dark brown externally and pale-yellow internally with characteristic bitter taste. Microscopical characters showed distinct appearance of cork, cortex, vascular bundles, and starch grains. Preliminary studies revealed presence of alkaloids, glycosides, carbohydrates, flavonoids, phenols, tannins, steroids, starch, lignans, while absence of proteins and amino acids. Notably, heavy metals *i.e.* lead, cadmium, mercury and arsenic were within the permissible limits defined by national and international pharmacopoeias. *In vitro* antioxidant assays resulted into significant effect of methanol extract in DPPH, LPO, SOD, NO and TPC except FRAP when compared with aqueous extract, due to predominance of protoberberine alkaloids among others. **Conclusion:** This first ever integrated research work successfully linked pharmacopoeial quality standards that eventually contributes to the richness of its bioactive phytoconstituents, established safety profile that further validates its therapeutic utility. Furthermore, the correlation of key alkaloidal composition with vivid biological actions exerted through antioxidant efficacy, bridges ethnomedicinal claims with modern science.

Major Findings: First-ever integrated approach towards pharmacognostical authentication, diverse phytoconstituents, antioxidant potential, heavy metal safety, complying with the pharmacopoeial standards to validate the ethnomedicinal claims of *C. teeta* Wall. rhizome.

Keywords: Antioxidant, Berberine, *C. teeta*, Heavy Metals, *In Vitro* Assays

(Article Chronicle: Article Received on: 30.01.2026; Revised on: 24.02.2026; Accepted on: 04.03.2026)

**Author for correspondence*

1. Introduction

Coptis teeta Wall. (Indian Goldthread), is a renowned perennial herb of *Ranunculaceae* (buttercup) family. In Traditional Chinese Medicine, its rhizome is a part of *Rhizoma coptidis* including *Coptis chinensis* Franch., and *Coptis deltoidea* C.Y. Cheng and P. K. Hsiao, widely used therapeutically since ancient time[1]. In *Ayurvedic* system of medicine, *C. teeta* is well documented as *Tiktamoola* and *Mamira* in later classical lexicons. Geographically, the plant is widely distributed across India, Bhutan, Nepal and Yunan province of China, with its natural profusion being reported in the Mishmi hills of Arunachal Pradesh of Northeast India. Ethnic Mishmi tribes, traditionally utilise the rhizome to treat malarial fever, gastrointestinal problems and other ailments in different external and internal dosage forms[2]. From a phytochemical perspective, *C. teeta* rhizome is a rich source of protoberberine alkaloids mainly berberine, along with palmatine, coptisine, magnoflorine, jatrorrhizine[3,4]. Comprehensive phytochemical characterisation of medicinal plants necessitates an integrated analytical approach, ranging from pharmacognostical evaluation to analytical techniques[5]. Notably, medicinal plant parts not only consist of secondary metabolites, particularly roots and rhizomes but are also susceptible to accumulate heavy metals from contaminated soil and water, which can significantly compromise safety and therapeutic quality. *Ayurvedic* Pharmacopoeia of India, prescribe permissible limits of toxic heavy metals to ensure the safe uses of medicinal plants, as trace amount can be toxic for human consumption[6].

Further, phytoconstituents derived from medicinal plants act as natural antioxidants, aids in counteracting the cellular damage caused by oxidative stress by neutralising free radicals[7]. Therefore, validated *in vitro* anti-oxidant assays are widely employed for corelating phytochemical composition with biological activity[8]. Despite the extensive ethnomedicinal uses and reported phytochemical richness of *C. teeta* Wall. rhizome, systematic scientific validation integrating standardisation to antioxidant potential has not been effectively studied. The present research work is systemically designed with objectives to study detailed pharmacognostical characterisation, standardisation aspects, presence of heavy metals and *in vitro* antioxidant potential of *C. teeta* using multiple validated assays.

2. Materials and Methods

2.1 Plant Material and Extraction Workflow

The rhizomes of *C. teeta* Wall. were collected from Mishmi hills of Arunachal Pradesh, Northeast India. The collected rhizomes were cleaned from extraneous substances and shade dried.

The rhizome was authenticated by a competent taxonomist at the Department of Pharmacognosy, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar. A voucher specimen (Ph.M. 6383/2023-24) was kept in the Pharmacognosy museum, Department of Pharmacognosy, ITRA, Jamnagar for future reference.

For detailed analytical studies, aqueous and methanol extracts of rhizomes of *C. teeta* were prepared.

The coarsely powdered rhizome (500 g) was subjected to successive solvent extraction for obtaining aqueous extract. The dried powder was extracted sequentially first using n-hexane, second with acetone, third with ethanol, and at last with distilled water in a Soxhlet apparatus. Each extraction cycle was continued until the siphon tube become colourless.

Separately, the methanolic extract was prepared by extracting 500 g of n-hexane-defatted, coarsely powdered rhizome with methanol to obtain moderately polar and polar phytoconstituents. The aqueous and methanol extracts were lyophilised to obtain a dry extract. The analytical grade reagents and chemicals, along with scientific grade glassware's were utilised throughout the research work.

2.2 Statistical Analysis

In the present study, all the experimental analyses were carried out in triplicate, and results were expressed as mean ± Standard Error of Mean (SEM). IC_{50} values were calculated by nonlinear regression analysis of log concentration-response curves.

2.3 Pharmacognostical Studies

Detailed macroscopy, microscopy and powder microscopy of dried rhizome were performed at Department of Pharmacognosy, ITRA, Jamnagar. The study was carried out into 3 parts;

2.3.1 Macroscopic Characters

The gross morphology and texture were observed through naked eye and with magnifying lens, followed by examining characteristic odour and taste. Rhizome powder was also subjected to organoleptic tests[9].

2.3.2 Microscopic Characters

For the transverse sections, rhizome was immersed in water for 72-96 hours. After proper softening, thin sections were prepared to analyse under Carl-Zeiss trinocular microscope. The distinct characters were noted[10].

2.3.3 Powder Microscopy

The admixture of coarse and fine powder obtained after pulverization, were observed under Carl-Zeiss trinocular microscope. The powder characters were noted without staining as well as with staining (phloroglucinol and HCl)[11].

2.4 Analytical Studies and Heavy Metal Analysis

The detailed analytical profiling and heavy metal analysis was carried out in 4 divided segments as follows;

2.4.1 Physicochemical Parameters

The standard protocols mentioned in *Ayurvedic* Pharmacopoeia of India (API) was followed, where aqueous and methanol extracts were subjected to selected parameters *i.e.* foreign matter, moisture loss, ash content, acid-insoluble ash, water-soluble ash, pH levels, and water soluble and methanol soluble extractive values[12]. All the readings were taken in triplicate, and results were expressed in mean ± SEM.

2.4.2 Preliminary Analysis

The key phytoconstituents extracted in aqueous and methanol extracts were analysed using established protocols. The preliminary tests cover different classes of phytoconstituents *i.e.* alkaloids, glycosides, carbohydrates, flavonoids, steroids, proteins, amino acids, phenolic compounds, tannins, quinones, saponins, terpenoids, starch, lignans, and phlobatannins[10,13-15].

2.4.3 Fluorescence Analysis

The fine powder of dried rhizome was evaluated for its characteristic natural fluorescence through documented method with slight modifications[16]. For the analysis, approximately 5 mg of powder was admixed with specific solvents mentioned, mixed thoroughly and observed under daylight and ultraviolet light at 254 and 366 nm wavelengths.

2.4.4 Heavy Metals Analysis

The quantification of heavy metals was carried out in Shimadzu AA-7000 Atomic Absorption Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The certified standard stock solutions for Lead (Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (As) heavy metals were procured from Sigma-Aldrich Production GmbH, Switzerland. The stock solutions of Pb and Cd were prepared at a concentration of 1 ppm, whereas those of Hg and As were maintained at 1 ppb. Working calibration standards were freshly prepared immediately prior to analysis by serial dilution of the respective stock solutions with deionised water. For Pb, calibration concentrations of 1, 2, 5, 10, and 20 ppm, for Cd standards 0.1, 0.2, 0.5, 1, and 5 ppm, for Hg at 5, 10, 20, 50, and 100 ppb, and arsenic standards at 10, 50, 100, and 200 ppb.

The treatment of *C. teeta* rhizome powder was conducted by following standardised sample preparation method with slight modifications[17]. Accurately weighed 2 gm of finely powdered rhizome was digested with 10 ml concentrated nitric acid on a hot plate maintained at 95°C until a clear solution with red fumes was obtained. Subsequently, an additional 10 ml of nitric acid was added, followed by 2 ml of hydrogen peroxide to ensure complete oxidation of organic matter. After cooling to room temperature, the digest was filtered, and final volume was made up to 50 ml with deionised water, and used for estimation. A reagent blank was prepared similarly without the sample.

The Atomic Absorption Spectroscopy is facilitated with Flame Atomic Absorption Spectroscopy (FAAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) methods, in order to detect even traces of heavy metals from composite matrices. The appropriate modifications were made in terms of optical (mA) and wavelength (nm) settings as per the heavy metal under analysis. For Pb 283.3 nm wavelength (10 mA), 228.8 nm (8 mA) for Cd, 253.7 nm (4 mA) for Hg, 193.7 nm (12 mA) for as were set for the assessment. Under BGC-D2 lamp operational mode, slit width was set at 0.7 nm for

all the heavy metals. The uniform support gas flow rate was maintained at 15.0 L/min, burner lateral position at 0 pulse and burner angle at 0°. For Cd, fuel gas flow rate was set at 1.8 L/min while 2.0 L/min for remaining heavy metals. Air-C_2H_2 flame composition was considered for Pb, Cd, and As analysis, while no flame was required for Hg analysis. For optimal atomisation, the burner height was set at 7.0 mm for Pb and Cd, 16.0 mm for As, and 18.0 mm for Hg. Moreover, standard calibration curve was obtained by aspiration of each standard working solutions in triplicates at duration and pre-spray interval of 5 and 3 seconds respectively. Reagent blank run was ensured between analyses for accuracy. The heavy metal concentrations in *C. teeta* rhizome were reported in ppb (Pd and Cd) and ppm (Hg and As) and compared with pharmacopoeial standards.

For the result interpretation, calibration curves were constructed using five concentration levels of each metal by plotting absorbance against concentration. Correlation coefficients (R^2) greater than 0.9 further confirmed the linearity. Reagent blanks and quality-control samples were analysed intermittently to ensure instrument stability and analytical accuracy.

2.5 *In Vitro* Antioxidant Assays

In the present study, the *in vitro* antioxidant potential of aqueous and methanol extract of *C. teeta* Wall., was investigated through six well-established antioxidant assays. Stock solution of both extracts was prepared at a concentration of 1000 µg/ml in their respective solvents. For each assay, from the stock solution, five different working solutions at 25, 50, 100, 200, and 400 µg/ml concentration ranges were freshly prepared.

The data reliability was ensured by performing all the antioxidant assays in triplicate under identical experimental conditions. To validate assay performance, standard antioxidants were analysed along with test samples. The results of DPPH, FRAP, LPO, SOD and NO assays were expressed in percentage inhibition calculated with the formula:

$$A_{\text{Blank}} - A_{\text{Test}}/A_{\text{Blank}} \times 100.$$

The IC_{50}value derived from regression analysis was defined as the concentration of the extract required to produce 50% inhibition, determined from log concentration-inhibition curves.

For all antioxidant assays, calibration curves were constructed exclusively using respective reference standards at defined concentration ranges. Regression equations and correlation coefficients (R^2) were derived solely from standard data points. The antioxidant activities of aqueous and methanol extracts of *C. teeta* rhizome were calculated by interpolating sample absorbance values into the corresponding standard calibration equations. Sample data points were not included in regression analyses to avoid analytical bias.

2.5.1 DPPH Free Radical Scavenging (DPPH) Assay

A DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) at concentration of 0.1 mM in methanol was admixed with 3 ml of each extract concentrations. The reaction mixture was incubated for 30 minutes in dark, followed by measurement of absorbance at 517 nm in UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Ascorbic acid standard dissolved in distilled water was employed as standard[18].

2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Different concentrations of extracts and standard ascorbic acid solution, was added with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and potassium hexacyanoferrate (1% w/v), incubated for 20 minutes at 50°C. After addition of 2.5 ml of trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant was collected in another set of test tubes, and mixed with 2.5 ml of distilled water followed by 0.5 ml of ferric chloride solution (0.1% w/v). The absorbance was noted at 700 nm in UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). Ascorbic acid was used as standard[19].

2.5.3 Lipid Peroxidation (LPO) Assay

A mixture each containing 400 µl of extracts concentrations, potassium chloride, ferrous ammonium sulfate, was incubated for 60 minutes in an incubator set at 37°C after brief vortexing. After that, 200 µl of 8% sodium lauryl sulfate and 1.5 ml of 2% thiobarbituric acid was added to each test tubes and placed in water bath (100°C) for 60 minutes. On cooling, note the absorbance at 560 nm in UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Curcumin standard was treated with same manner[20].

2.5.4 Superoxide Radical Scavenging (SOD) Assay

To 0.5 ml of each extract's concentration solutions, 0.5 ml of 0.1 mM EDTA, 0.0015% sodium cyanide solution, 0.12 mM riboflavin, 0.15 mM nitro BT and phosphate buffer (pH 7.4). After recording the initial absorbance of each concentration, the reaction mixtures were exposed to an incandescent lamp for 15 minutes and again the absorbance was measured at 530 nm using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Ascorbic acid was used as the reference standard[21].

2.5.5 Nitric Oxide (NO) Scavenging Assay

The reaction mixture encompassing sodium nitroprusside (10 mM) formulated in phosphate-buffered saline and extract solutions were incubated at ambient temperature for 150 minutes, followed by the incorporation of 0.5 ml of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride). The emergence of the chromophoric azo dye was quantified spectrophotometrically (Shimadzu, Kyoto, Japan) at 546 nm. Sodium nitrite functioned as the reference standard[22].

2.5.6 Total Phenolic Content (TPC)

For every 0.5 ml of extract solutions, 2 ml of Folin-Ciocalteu reagent (10 % v/v) was integrated, followed by the addition of 4 ml of saturated sodium carbonate solution (7.5% w/v) post a 5-minute period and incubated at room temperature for 30 minutes with periodic stirring. Absorbance was quantified at 765 nm utilizing a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) against methanol as a blank. Gallic acid was employed as the reference standard. The outcomes were reported in mg Gallic Acid Equivalent (GAE)/g[23].



1.1. Raw dried rhizome 1.2. Dried rhizome powder

Figure 1. Macroscopic characters of *C. teeta* Wall. dried rhizome.

3. Results and Discussion

The present study was systemically designed for comprehensive evaluation of pharmacognostical characterisation, phytochemical composition, heavy metal analysis and antioxidant potential of *C. teeta* Wall. rhizome following standardised procedures and assays. The study helps to integrate the key role of diversified phytoconstituents with plant-derived antioxidants for effective management of oxidative stress related diseases compared to synthetic alternatives. The combination studies including all the phyto-pharmacognostical, and antioxidant potential is limited. Further, medicinal plant safety in terms of heavy metal contamination establishes the quality standard as per the *Ayurvedic* pharmacopoeia, particularly for rhizomatous drugs. Notably, this unexplored aspect is successfully fulfilled in the present study, through systemic quantification of toxic heavy metals for *C. teeta* rhizome. This integrated approach further strengthens the baseline data for pharmacopoeial standardization with relevant information.

3.1 Pharmacognostical Studies

3.1.1 Macroscopy Study

Morphologically, irregularly branched 3 to 10 cm long cylindrical rhizome, appears dark brown to blackish coloured with tough, woody, rough and wrinkled texture externally, while pale yellow internally (Figure 1a and 1b). The rhizome gives characteristic intense bitter taste, as its distinct feature, while produces faint earthy odour. This morphological character helps for authentication and quality assessment.

3.1.2 Microscopy Study

Upon microscopic examination of rhizome transverse section (Figure 2a and 2b), discrete layers were observed, with outermost layer being cork, followed by cortex, vascular bundle, and central pith. The cork layer was formed by compressed, elongated cork cells in 10 to 15 layers, along with brown content in between. Isolated stone cells were found in the cortical region below cork

layer. This cortical layer consists of parenchymatous cells filled with simple starch grains and microcrystals. Scattered throughout the cortical region, one can find small pockets of pericyclic fibres. The endodermis is a single layer of tangentially elongated cells, followed by the phloem, which includes the phloem parenchyma and accompanying fibres. The xylem is arranged radially, with xylem vessels composed of xylem parenchyma and fibres occupying the remaining space. Air spaces are also present between vascular bundles. These bundles are separated by multi-serrate medullary rays. Finally, at the core, the central pith is composed of parenchyma cells containing simple starch grains. This structural arrangement and characteristic microscopical features confirm the species as *C. teeta* Wall.

3.1.3 Powder Microscopy Study

Powdered rhizome of *C. teeta* (Figure 3a and 3b) exhibited distinct diagnostic features under microscopic examination *i.e.* presence of groups of stone cells containing brown contents, fragments of annular vessels, and simple as well as compound starch grains. Stone cells with distinct lumina, pitted stone cells, and simple fibres were also prominently observed. Further, staining with phloroglucinol and conc. HCl highlighted the lignified tissues *i.e.* lignified groups of fibres, cork cells, and stone cells, as well as stone cells associated with cork cells in tangential view.

In previous studies on *C. teeta* Wall. rhizome conducted by Payum[16] and Namboothiri[24], the macroscopic characters *i.e.* presence of knotty rhizome, bitter taste, internal yellowish colour corroborate with the macroscopic characters obtained in the present study. Additionally, microscopic and powder microscopy features of present study are in close agreement with reported pharmacognostical characters. Along with it, this study details the presence of specific microscopic characters like phloem fibres, pericyclic fibres, compound starch grains and brown content. The powder microscopy of present study highlights the presence of annular vessels, pitted stone cells and simple fibres for characteristic identification of *C. teeta* rhizomes.

3.2 Analytical Studies and Heavy Metal Analysis

Medicinal plants are rich repositories of primary and bioactive secondary metabolites specifically responsible for treatment as well as management of multiple human ailments. For pharmacopoeial standardisation

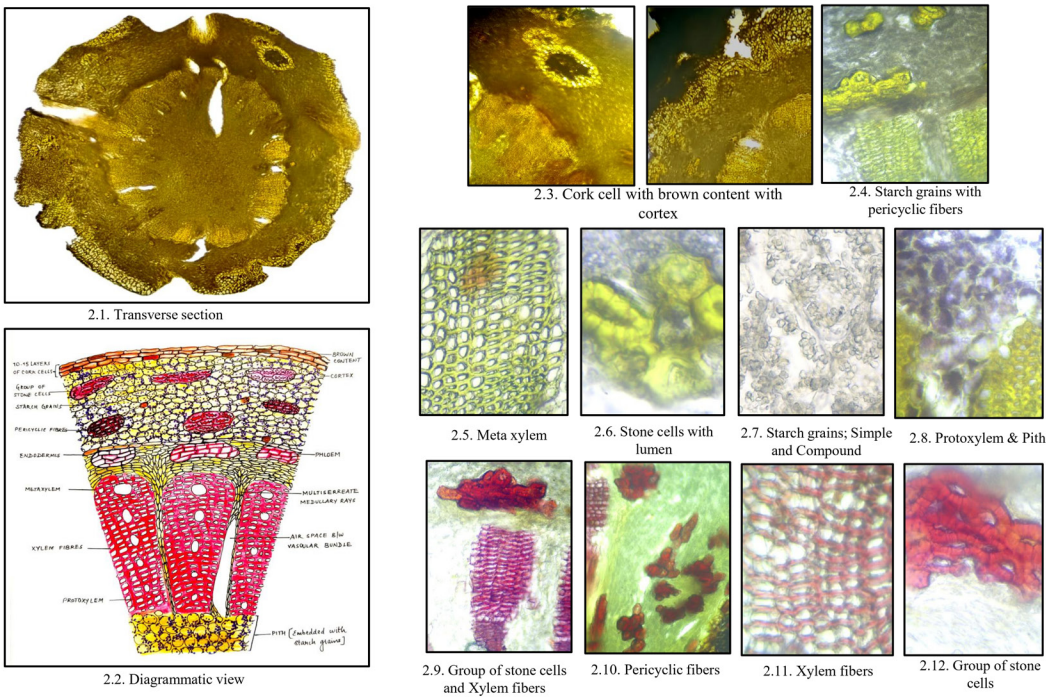


Figure 2. Transverse section and microscopic characters of *C. teeta* Wall. dried rhizome.

of medicinal plants, complete physicochemical and phytoconstituent profiling is thus essential[25]. The detailed analytical evaluation and heavy metal analysis undertaken in the present study establishes quality of *C. teeta* Wall. rhizome required for further standardisation and advanced phytochemical characterisation. Importantly, the inclusion of heavy metal analysis provides critical safety validation of rhizome provides required baseline dataset.

3.2.1 Physicochemical Parameters

The physicochemical parameters of *C. teeta* rhizome outlined in Table 1, shows compliance with *Ayurveda* Pharmacopoeia of India standards, thereby confirming quality and authenticity of raw material. Together, absence of foreign matter, lower percentage of moisture content and inorganic matters indicates appropriate post-harvest handling and storage. The higher alcohol-soluble extractive values highlighted the predominance of polar bioactive constituents, mainly alkaloids and phenolic compounds, characteristic of *C. teeta* rhizome. The slightly acidic pH value reflects the typical nature of herbal materials. Importantly, these findings align with the earlier reported works[16,24], thereby validating the reliability of present study in this context.

3.2.2 Preliminary Phytochemical Analysis

Preliminary phytochemical screening serves as an important criterion for identifying major classes of bioactive phytoconstituents that furthers aids in advanced phytochemical characterisation studies. In present study (Table 2), alkaloids were detected exclusively in methanol extract, whereas glycosides, carbohydrates, flavonoids, steroids, phenols, tannins, quinones, saponins, terpenoids, starch, lignin tests were present (+) in both aqueous and methanol extracts. Further, proteins, amino acids, and phlobatannins were absent (-) in both the extracts, highlighting the predominance of secondary metabolites rather than primary nitrogenous compounds.

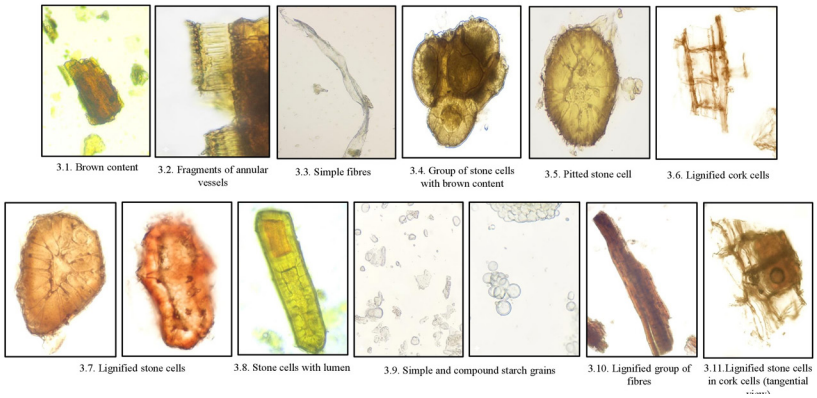


Figure 3. Powder microscopic characters of *C. teeta* Wall. dried rhizome.

Table 1. Physicochemical and quality-control parameters of *C. teeta* rhizome as per API standards (Data: mean ± *SEM, n=3)*

Physicochemical Parameter	Observed Value (mean ±SEM, n=3)	API Prescribed Limits
Foreign matter (%w/w)	Absent	Not more than 2%
Loss on drying (%w/w)	7.68±0.24	Not more than 10%
Total ash (% w/w)	3.66±0.55	Not more than 5%
Acid insoluble Ash (% w/w)	0.43±0.03	Not more than 1%
Water soluble ash (% w/w)	7.27±0.13	Not less than 5%
Water soluble extractive (%w/w)	12.39±0.18	Not less than 10%
Alcohol soluble extractive (%w/w)	22.18 ± 0.12	Not less than 10%
pH of 10% w/v aqueous solution	6.038	-

Table 2. Phytochemical evaluation of *C. teeta* rhizome: aqueous and methanol extracts

Phytochemical test	Detection Method	Findings	
		Aqueous	Methanol
Alkaloids			
Dragondroffs reagent test	2 ml of extract + 1 mL of Dragondorff's reagent - orange-red precipitate formed	-	+
Mayer's reagent test	2 ml of extract + few drops of Mayer's reagent - yellowish-white precipitate formed	-	+
Wagner's reagent test	2 ml of extract + few drops of Wagners' reagent - brown or reddish precipitate formed	-	+
Hagner's reagent test	2 ml of extract + few drops of Hagner's reagent - yellow precipitate formed	-	+
Glycosides			
Keller-Killiani test	2 ml of the extract + 0.5 mL of glacial acetic acid containing 2-3 drops of ferric chloride solution + 1 ml of concentrated H_2SO_4 - deep blue coloration at the interface	+	+
Coumarin glycosides	2 ml of the extract + 1M NaOH solution - blue-green fluorescence	+	+
Carbohydrates			
Fehling's test	2 ml of the extract + equal volume of Fehling's solutions A and B + heated for 5 minutes - red or dark red precipitate	+	+
Flavonoids			
Lead acetate test	2 ml of the extract + 2 ml of 10% lead acetate solution - light yellowish-green precipitate formed	+	+
Alkaline reagent test	2 ml of the extract + 2-3 drops of NaOH solution - deep yellow colour disappeared by adding few drops of dilute HCl	+	+
Steroids			
Salkowski test	2 ml of the extract + chloroform, shaken and addition of conc. H_2SO_4 - red colour appeared	+	+
Phenolic Compounds and Tannins			
Ferric chloride test	2 ml of the extract + a few drops of 5% ferric chloride solution - dark green or bluish-black color appeared	+	+
Quinones			
Conc. HCl test	2 ml of the extract + few drops of conc. HCl test - green color appeared	+	+
Saponins	5 ml of the extract + drop of Na_2CO_3 solution, vigorously shaken and allowed to stand for 5 minutes - formation of stable foam	+	+
Terpenoids	5 ml of extract + 2 ml of chloroform, mixture evaporated on a water bath + 3 ml conc. H_2SO_4 - grey-colored solution appeared	+	+
Starch			
Iodine test	2 ml of extract + 2 ml of iodine solution - blue colour appeared	+	+
Lignans			
Phloroglucinol test	2 ml of extract + few drops of phloroglucinol solution in ethanol + conc. HCl - red or pink color appeared	+	+
Phlobatannins	2 ml of extract + 2 ml of 1% HCl and boiled	-	-
Proteins			
Biuret test	1 ml of the extract + 2 drops of 3% copper sulfate solution + few drops of 10% sodium hydroxide solution	-	-
Xanthoproteic test	1 ml of the extract + few drops of concentrated nitric acid	-	-
Amino Acids			
Ninhydrin Test	2 ml of the extract + 2 drops of ninhydrin solution	-	-

These findings align with previous reported works on *C. teeta* Wall. rhizome. Bora *et al.*, conducted a preliminary phytochemical evaluation in water, methanol, acetone, chloroform, and *n*-hexane extracts, reporting presence of alkaloids, phenolics, flavonoids, and glycosides in aqueous and methanol extracts[25]. While Lone *et al.*, reported the presence of alkaloids, tannins, phenolic compounds and flavonoids in ethanol extract[26]. The harmony of the present results with these studies, specifically for aqueous and methanol extract, validates the suitability of polar solvents for efficient bioactive constituent's extraction from the rhizome.

3.2.3 Fluorescence Analysis

Fluorescence analysis of medicinal plants provides a characteristic chemical fingerprint, indicated by the sign of chromophore on treatment with various chemical reagents. The fluorescence behaviour is due to conjugated systems within phenolic and alkaloidal compounds[27,28].

In present study, *C. teeta* rhizome powder showed distinct colour variations under daylight and UV light at 366 nm (Table 3). The characteristic fluorescence in untreated rhizome powder is shown by a yellow hue in daylight. Notably, UV absorbance at 254 nm was absent. The fluorescence behaviour ranged from dark brown to dark green under daylight and from bright yellow to dark yellowish-green under UV illumination. Previously, Payum reported the fluorescence analysis of *C. teeta* rhizome powder under daylight and UV light[17]. The present study not only corroborated with earlier findings but also provided additional reagent-specific fluorescence pattern and further confirms the presence of conjugated phenolic and alkaloidal compounds in *C. teeta* rhizome.

3.2.4 Heavy Metals Analysis

The medicinal plant's rhizome is highly capable to bioaccumulate toxic elements from the contaminated soil that further leads to public health concern when utilised for various therapeutic purposes, due to poor elimination of these elements on chronic systemic exposure. Thus, pharmacopoeial authorities worldwide, set the permissible limits of heavy metals in herbal raw materials to ensure its safety and quality. The quantity of heavy metals is expressed in ppm (parts per million) ranges (Table 4)[29,30].

In the present study, the heavy metal quantification of *C. teeta* Wall. rhizome powder was performed using element-specific standard calibration curves for Pb, Cd, Hg and As (Figure 4a and 4b), ensuring analytical accuracy and reliability of method employed for the analysis.

The results demonstrated that Pb and Cd were below detectable limits as denoted by negative values, indicating negligible contamination from these highly toxic metals. However, Hg and As were detected at trace levels at 0.0969 ppm and 0.1955 ppm respectively, by converting ppb (parts per billion) to ppm (parts per million) as

Table 3. Characteristic fluorescence analysis of *C. teeta* rhizome powder

Sr. No	Chemical Reagent Applied	Characteristic Fluorescence	
		Under Daylight	Under UV Light (366 nm)
1	Dried powder (without chemical)	Yellow	Light yellow
2	NaOH	Dark brown	Dark green
3	Hydrochloric acid	Turmeric colour	Dark green
4	Nitric acid	Light brown	Dark brownish yellow
5	Sulphuric acid	Dark chocolate brown	Dark yellowish green
6	50% Sulphuric acid	Dark brownish yellow	Bright yellow
7	Chloroform	Light brown	Bright yellow
8	Dil. FeCl3	Dark yellowish brown	Dark yellowish brown
9	1N NaOH	Light reddish yellow	Dark green
10	Ammonia	Light reddish yellow	Dark yellowish brown
11	0.1 N NaOH	Light reddish yellow	Dark yellow

Table 4. Permissible limits of toxic heavy metals in medicinal plants as per Pharmacopoeia and regulatory standards

Pharmacopoeia / Regulatory Standard	Permissible Limit (ppm)			
	Pb	Cd	Hg	As
Ayurvedic Pharmacopoeia of India (API)	10.0	0.3	1.0	3.0
World Health Organization (WHO)	10.0	0.3	–	1.0
Chinese Pharmacopoeia (PRC)	5.0	0.3	0.2	2.0
ISO / Traditional Chinese Medicine	10.0	2.0	3.0	4.0
European Pharmacopoeia	5.0	1.0	0.1	–
U.S. Pharmacopoeia (USP)	5.0	0.5	1.0 (total), 0.2 (methyl Hg)	2.0 (non-organic)

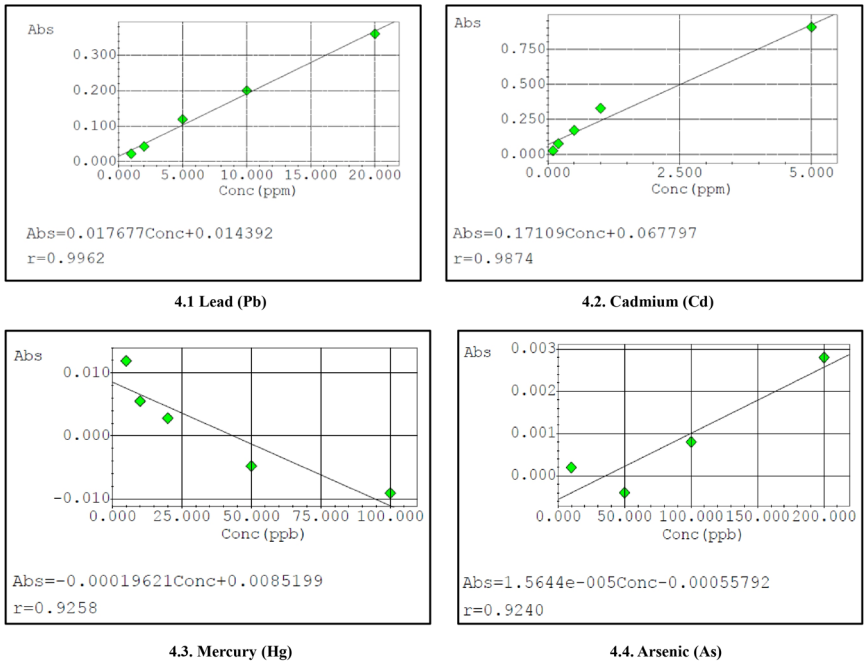


Figure 4. Standard calibration curves of Pb, Cd, Hg and As heavy metals.

presented in Table 5. Importantly, the concentrations of all the four heavy metals remained well within the permissible limits prescribed by pharmacopoeial and regulatory authorities (Table 4). The present data thus substantiate the safety profile of *C. teeta* rhizome. Moreover, as heavy metal profiling of this species has not been reported earlier, the findings establish the first baseline reference values for its future quality control, standardisation and regulatory assessment, along with pharmacopoeial quality compliance.

3.3 *In Vitro* Antioxidant Assays

Oxidative stress, caused by excessive generation of Reactive Oxygen Species (ROS), plays crucial role in the pathogenesis of several chronic diseases like metabolic syndrome, cardiovascular diseases, and age-mediated degenerative conditions by causing cellular damage. In order to absorb free radicals, body system could not be enough, thus external source of antioxidants are essential either natural or synthetic. As synthetic antioxidants prove harmful, medicinal plant-derived antioxidants have gained increasing scientific and therapeutic relevance, attributed to their multi-target action[31].

In present study, the antioxidant potential of *C. teeta* Wall. rhizome was evaluated using multiple *in vitro* assays to recognise different antioxidant mechanisms. Aqueous Extract (AE) and Methanol Extract (ME) were selected as the rhizome exhibited presence of polar

phytoconstituents *i.e.* alkaloids, flavonoids, glycosides, phenolics and terpenoids, that are well-documented for their redox-modulating properties. Overall, the antioxidant findings gave mechanistic support to the ethnomedicinal use of *C. teeta* rhizome for effective management of oxidative stress related disorders.

3.3.1 DPPH Assay

DPPH scavenging assay is a most common and popular to evaluate free radicals scavenging activity of medicinal plants, as conversion of intense and stable violet colour of DPPH radical into reduced, pale-yellow hydrazine (DPPH-H) occurs on reaction with hydrogen atom-donating substance, reflected by a decrease in absorbance[32]. In present study, both aqueous and methanol extracts of *C. teeta* rhizome showed concentration-dependent increase in DPPH radical scavenging activity (Figure 5.1). On comparing the IC_{50} values, methanol extract exhibited stronger scavenging potential (IC_{50} = 226 µg/ml) than aqueous extract (IC_{50} = 318 µg/ml), while ascorbic acid confirmed higher antioxidant potency with IC_{50} value of 100.1 µg/ml (R^2 = 0.9589) as mentioned in Table 6. The methanol extract gave enhanced activity as methanol efficiently extracted the protoberberine alkaloids and phenolic compounds. Thus, *C. teeta* rhizome exhibits moderate free radical scavenging potential.

3.3.2 FRAP Assay

Electron-donating ability of a compound is referred to as reducing power, reflecting its redox potential. The FRAP assay functions on the basis of reduction of colourless Fe^{3+}-TPTZ complex into a deep blue Fe^{2+}-TPTZ complex, where absorbance is directly proportional to the reducing capacity of potent antioxidants when interacted[33]. In the present study (Figure 5.2), the aqueous extract of *C. teeta* rhizome showed slightly stronger reducing activity (IC_{50} = 118 µg/ml), in comparison with methanol extract (IC_{50} = 126 µg/ml). The standard ascorbic acid showed the lowest IC_{50} value of 112.1 µg/ml (R^2 = 0.9714) as mentioned in Table 7. The aqueous extract is rich in water-soluble such as phenolic acids and glycosides reducing agents. As methanol extract is efficient in extracting more diverse phytoconstituents, aqueous extraction may favour compounds with strong electron-transfer properties.

3.3.3 LPO Assay

Due to formation of free radicals, lipid peroxidation chain reaction gets initiated that further forms malondialdehyde that damages cellular functions. The lipid peroxidation assay measures the ability of antioxidant activity of medicinal plants for inhibition of oxidative degradation of lipids and membrane damage caused by oxidative stress[34]. In present study, both aqueous and methanol extract of *C. teeta* rhizome showed dose-dependent lipid peroxidation inhibition (Figure 5.3). The curcumin standard showed lowest IC_{50} value of 180.2 µg/ml (R^2 = 0.9169). Comparatively, methanol extract exhibited comparatively better inhibitory activity (IC_{50} = 592.3 µg/ml) than the aqueous extract (IC_{50} = 636.2 µg/ml) as mentioned in Table 8. The result indicates potent membrane-protective potential of methanol extract. Although, both the extracts showed weak activity than that of well-established lipid peroxidation inhibitor, curcumin. The link of observed activity may be due to presence of alkaloids and phenolic compounds capable of intercepting peroxyl radicals and terminating lipid oxidation chains.

3.3.4 SOD Assay

Superoxide anion is a reactive oxygen species that further accelerate the formation of various forms of oxygen radicals. Superoxide dismutase enzyme helps to decrease superoxide anions thereby protecting cells and tissues against oxidative damage[35]. In the present study (Figure 5.4), methanol extract exhibited superior superoxide scavenging activity (IC_{50} =271.3 µg/ml) compared to the aqueous extract (IC_{50} =301.6 µg/ml). Ascorbic acid standard potent activity than both extracts with lowest IC_{50} value of 110.5 µg/ml (R^2 = 0.9205) as shown in Table 9. The enhanced efficacy of *C. teeta* rhizome may be due to methanol-extractable flavonoids and alkaloids known to interact

Table 5. Concentration of heavy metals in *C. teeta* rhizome assessed by AAS (Data: mean ± SEM, n=3)

Concentration Readings	Pb (ppm)	Cd (ppm)	Hg (ppb)	As (ppb)
Mean ± SD	-0.4351 ± 0.0179	-0.3308±0.0002	96.9347± 0.0006	195.4747± 0.0005

with superoxide radicals, thus showing moderate superoxide-scavenging potential.

3.3.5 NO Assay

Nitric oxide consists of single unpaired electron, and thus plays a central role as a signalling molecule for oxidative stress. In nitric oxide scavenging assay, the measurement of reduced nitrite formation occurred by competitive inhibition of nitric oxide with oxygen is carried out as an indicator of antioxidant activity[36]. In present study, both extracts of *C. teeta* rhizome showed concentration-dependent nitric oxide scavenging activity (Figure 5.5). Comparatively, methanol extract showed better activity (IC_{50} =75.3 µg/ml) than aqueous extract (IC_{50} =96.7 µg/ml), exhibiting increased interaction with reactive nitrogen species. Sodium nitrate standard showed higher scavenging activity *i.e.* IC_{50} value of 73.0 µg/ml (R^2=9477) as mentioned in Table 10. The antioxidant potential of methanol extract may be linked with nitrogen-reactive alkaloids and phenolic constituents present in *C. teeta* rhizome.

3.3.6 Total Phenolic Content

As medicinal plants act as a natural antioxidant, its capacity to reduce reactive oxygen species is mainly attributed to its wide array of phenolic compounds. In order to measure its quantity, total phenolic content serves as an important indicator due to its ability to donate hydrogen atom[37]. In the present study, total phenolic content was calculated as Gallic Acid Equivalents of phenols (mgGAE/g). Both extracts showed concentration-dependent increases in phenolic content (Figure 5.6). The methanol extract of *C. teeta* rhizome exhibited higher phenolic content (23.94 mg GAE/g) in comparison with aqueous extract (18.97 mg GAE/g) at 400 µg/ml, while gallic acid standard also followed dose-dependent increase in concentrations (R^2= 0.9754) as mentioned in Table 11. Thus, the result

Table 6. DPPH radical scavenging activity of *C. teeta* rhizome extracts compared to ascorbic acid standard

Samples	Concentrations (µg/ml)					IC_{50} (µg/ml)
	25	50	100	200	400	
	% Inhibition					
STD	16.84	32.14	43.98	59.79	89.05	100.1
AE	23.67	29.25	36.46	44.49	54.15	318
ME	26.53	33.33	41.84	52.28	63.20	226

Table 7. FRAP scavenging activity of *C. teeta* rhizome extracts compared to ascorbic acid standard

Samples	Concentrations (µg/ml)					IC_{50} (µg/ml)
	25	50	100	200	400	
	% Inhibition					
STD	16.84	32.14	43.98	59.79	89.05	112.1
AE	23.67	29.25	36.46	44.49	54.15	118
ME	26.53	33.33	41.84	52.28	63.19	126

Table 8. Lipid peroxidation scavenging activity of *C. teeta* rhizome extracts compared to curcumin standard

Samples	Concentrations (µg/ml)					IC_{50} (µg/ml)
	25	50	100	200	400	
	% Inhibition					
STD	18.68	21.46	41.26	52.15	69.94	180.2
AE	5.19	13.84	22.63	37.59	43.28	636.2
ME	7.35	18.50	28.58	36.56	43.41	592.3

Table 9. Superoxide anion scavenging activity of *C. teeta* rhizome extracts compared to ascorbic acid standard

Samples	Concentrations (µg/ml)					IC_{50} (µg/ml)
	25	50	100	200	400	
	% Inhibition					
STD	26.71	41.83	55.93	68.95	91.42	110.5
AE	17.49	25.68	34.81	45.73	56.06	301.6
ME	22.01	30.16	40.13	52.75	68.41	271.3

Table 10. Nitric oxide scavenging activity of *C. teeta* rhizome extracts compared to sodium nitrite standard

Samples	Concentrations (µg/ml)					IC_{50} (µg/ml)
	25	50	100	200	400	
	% Inhibition					
STD	36.77	47.60	58.73	70.98	91.42	73.0
AE	28.04	38.14	50.83	63.43	73.33	96.7
ME	32.21	43.63	56.23	68.92	78.33	75.3

Table 11. Total phenolic content of *C. teeta* rhizome extracts in comparison with gallic acid standard

Samples	Concentrations (µg/ml)				
	25	50	100	200	400
	mg Gallic Acid Equivalent (GAE)/g				
STD	2.75	4.78	9.85	20.04	-
AE	2.32	3.58	6.29	11.16	18.97
ME	2.53	4.42	8.09	15.84	23.94

indicates superior extraction efficiency of phenolic compounds in methanol compared with the aqueous solvent.

The findings of the present antioxidant study are in close agreement with earlier reports. A study conducted by Bora *et al.*, 2022, using different polar and non-solvents, where acetone extract showed highest DPPH radical scavenging activity, in ABTS assay, acetone and water extract showed potent antioxidant efficacy, while methanol extract showed highest antioxidant activity in FRAP assay. While, Lone investigated ethanol extract of *C. teeta* rhizome through DPPH and NO assays, and reported its significant free radical scavenging activity. Thus, these studies consistently highlighted the link of polar solvent extraction for obtaining superior antioxidant activities.

In present study results aligns with the previous work, as depicted by comparatively higher efficacy of the methanol extract, enriched with protoberberine alkaloids and phenolic compounds, across DPPH, SOD, LPO, NO assays and total phenolic content. The synergistic effect may be attributed through H^+ donation, electron transfer and pathway inhibition of lipid peroxidation. However, aqueous extract shown relatively strong reducing power in the FRAP assay, preferentially due to presence of glycosides and phenolic compounds that is capable of Fe^{3+} reduction. Collectively, the present study clarifies the selection of polar solvents as it highly efficient in extracting key bioactive phytoconstituents responsible for its antioxidant potential. This finding aligns with the significant role of solvent selection and method of extraction to extract out primary active alkaloid *i.e.* berberine and phenolics using microwave (AE) and Ultrasound Assisted Extraction (UAE) of *C. teeta* rhizome[4]. Consequently, phytoconstituent richness of *C. teeta* Wall. rhizome is well documented in previous literatures. Its characteristic bitter taste is linked with presence of vivid naturally occurring bioactive protoberberine alkaloids *i.e.* berberine, jatrorrhizine,

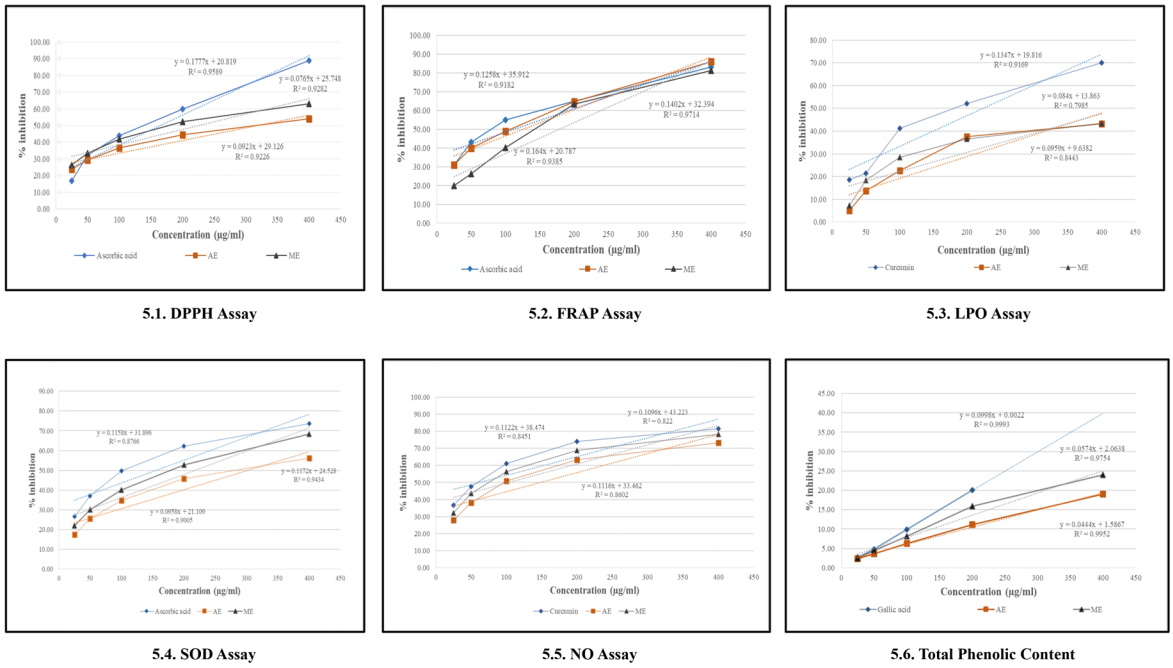


Figure 5. Standard calibration curves of *in-vitro* antioxidant assays with concentrations (µg/ml) v/s percentage inhibition.

palmatine and coptisine. Also, several non-alkaloidal components such as organic acids, quinones, coumarins and its derivatives and phenylpropanoids are present[38]. Among all, berberine is predominantly found in the rhizome[39]. This diversified classes of phytoconstituents gives promising pharmacological actions[40].

The convergence of phytochemistry reported in literatures with multi-assay antioxidant potential provides clear mechanistic insights into its ethnomedicinal relevance. The earlier research works on *C. teeta* rhizome is in fragmented pattern, the present study represents first systematically integrated evaluation study combining phyto-pharmacognostical, heavy metal analysis and antioxidant efficacy, thereby addressing the critical research gap.

2. Conclusion

The present work contributes to identify distinct pharmacognostical characters, provides validated physicochemical evaluation data that aligns with standards of *Ayurveda* Pharmacopoeia of India. This study establishes the safety profile of *C. teeta* Wall. rhizome, as heavy metals remained below permissible limits of pharmacopoeias. Thereby, the link of broad spectrum of bioactive phytoconstituents with potent antioxidant activity may be established that helps to bridge ethnomedicinal privileges with contemporary science. This approach provides integrated basis for its safe consumption, quality and therapeutic relevance.

3. Acknowledgments

The author sincerely acknowledges personnel's and faculties of Department of Pharmacognosy and Pharmacology, ITRA, Jamnagar, and the Chemical Sciences and Technology Division, CSIR-NIIST, Thiruvananthapuram, for efficient completion of research work by providing laboratory facilities and technical support.

4. References

1. Ran Q, Huang QW, Wang J, Wang L, *et al.* Rhizoma coptidis as a potential treatment agent for Type 2 diabetes mellitus and the underlying mechanisms: A review. Front Pharmacol. 2019; 10:434868. https://doi.org/10.3389/fphar.2019.00805 PMid:31396083 PMCid:PMC6661542
2. Mishra MK. An insight into *Coptis teeta* Wall., an endangered medicinal plant and its conservation strategies. Plants for

Immunity and Conservation Strategies. Singapore: Springer Nature. 2023. pp. 45-56. https://doi.org/10.1007/978-981-99-2824-8_3
3. Hung TM, Lee JP, Min BS, Choi JS, Na M, Zhang X, Ngoc TM, Lee I, Bae K. Magnoflorine from *Coptidis Rhizoma* protects high density lipoprotein during oxidant stress. Biol Pharm Bull. 2007; 30(6):1157-1160. https://doi.org/10.1248/bpb.30.1157 PMid:17541173
4. Sarma L, Patra F, Borah PK, Meena S, Duary RK. Comparative modeling of microwave and ultrasound assisted extraction of phenolics and berberine from *Coptis teeta* Wall. rhizomes. Sustain Food Technol. 2025; 3(2):570-81. https://doi.org/10.1039/D4FB00382A/v2/review1 PMid:39359621
5. Ghosh D. Quality issues of herbal medicines: Internal and external factors. Int J Complement Altern Med. 2018; 11(1):67-69. https://doi.org/10.15406/ijcam.2018.11.00350
6. Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J. The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. Int J Biochem Cell Biol. 2009; 41(8-9):1665-1677.https://doi.org/10.1016/j.biocel.2009.03.005 PMid:19433308
7. Abdel-Lateif KS, Eldeab HA, Maghrabi IA. The plant natural products: Their antioxidants, free radical scavengers, DNA protection and antimicrobial activities. J Bioprocess Biotechniq. 2016; 6(9):293. https://doi.org/10.4172/2155-9821.1000293
8. Nariya PB, Bhalodia NR, Shukla VJ, Acharya R, Nariya MB. *In vitro* evaluation of antioxidant activity of *Cordia dichotoma* (Forst f.) bark. AYU. 2013; 34(1):124-128. https://doi.org/10.4103/0974-8520.115451 PMid:24049418 PMCid:PMC3764870
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 47th Edition. Pune: Nirali Prakshan. 2012. pp. 6-22.
10. Khandelwal KR. Practical pharmacognosy: Techniques and experiments. 19th Edition. Pune: Nirali Prakashan. 2008. pp. 149-166.
11. Kay LA. The microscopical studies of drugs. 1st Edition. London: Bailliere, Tindall and Cox. 1938. pp. 1821.
12. Ayurvedic Pharmacopoeia of India. Part II, Vol I. Appendix 2: Physicochemical standards. New Delhi: Ministry of AYUSH, Govt. of India. 2008.
13. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of *Cayratia auriculata* (*in vitro*). Maedica (Bucur). 2019; 14(4):350-356. https://doi.org/10.26574/maedica.2019.14.4.350 PMid:32153665 PMCid:PMC7035446
14. Tambrey SK. Preliminary phytochemical evaluation for glycosides in bark of selected local trees of Korba and Janjgir-Champa district border region. Int J Pharm Res Appl. 2023; 8(3):3132-3137.
15. Silva GOD, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. Am J Essent Oils Nat Prod. 2017; 5(2):29-32.
16. Payum T. Distribution, ethnobotany, pharmacognosy and phytoconstituents of *Coptis teeta* Wall.: A highly valued and threatened medicinal plant of eastern himalayas. Pharmacogn J. 2017; 9(6):s28-s34.https://doi.org/10.5530/pj.2017.6s.154
17. Fatima Grace X, Sowmya KV, Rahul Raj S, Shanmughanathan S, Chamundeeswari D. Preliminary phytochemical screening and heavy metal analysis by atomic absorption spectroscopy of a marketed polyherbal Churna. Am J Drug Deliv Ther. 2014; 1-2:77-81.
18. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal. 2002; 13(1):8-17. https://doi.org/10.1002/pca.611 PMid: 11899609.
19. Oyaizu M. Studies on products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986; 44:307-315. https://doi.org/10.5264/eiyogakuzashi.44.307
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358. https://doi.org/10.1016/0003-2697(79)90738-3 PMid: 36810. PMCid: PMC8374294.
21. McCord JM, Fridovich I. Superoxide dismutase: An enzymatic function of erythrocuprein. J Biol Chem. 1969; 244(22):6049-6055. https://doi.org/10.1016/S0021-9258(18)63504-5
22. Sreejayan N, Rao MNA. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 1997; 49(1):105-107. https://doi.org/10.1111/j.2042-7158.1997.tb06761.x PMid: 9120760.
23. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999; 299:152-178. https://doi.org/10.1016/s0076-6879(99)99017-1
24. Namboothiri DG, Remya P, Bijeshmon PP. Pharmacognostic evaluation of the rhizome of *Coptis teeta* Wall. Int J Res Ayurveda Pharm. 2014; 5(4):402-405. https://doi.org/10.7897/2277-4343.05483
25. Bora H, Kamle M, Chopra S, Kumar P. Evaluation of phytochemical components, antioxidant, and antibacterial activities of *Coptis teeta* Wall. Int J Pharm Res Allied Sci. 2022; 11(4):140-150. https://doi.org/10.51847/1jgpbykz1k
26. Lone TA, Rahul M, Lone RA. *In vitro* anti-oxidant studies by using different methods and evaluation of anti-microbial potential of *Coptis teeta* Wall. Glob J Biotechnol Biochem. 2014; 9(4):99-104. https://doi.org/10.5829/idosi.gjbb.2014.9.4.91138

27. Kamble V, Gaikwad N. Fluorescence analysis, phytochemical and antioxidant activities in leaves and stem of *Embelia ribes* Burm. F. Asian J Pharm Clin Res. 2019; 12(4):225-229. https://doi.org/10.22159/ajpcr.2019.v12i4.30782
28. Chung YL, Hong RD, Wu HW, Hung WH, Lai LJ, Wang CM. Fluorescence enhancement for alkaloids by anions: Spectroscopic and electrochemical characterizations. J Electroanal Chem. 2007; 610(1):85-89. https://doi.org/10.1016/j.jelechem.2007.07.003
29. Dghaim R, Al Khatib S, Rasool H, Khan MA. Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. J Environ Public Health 2015; 2015(1):973878. https://doi.org/10.1155/2015/973878 PMid: 26000023. PMCid: PMC4426653.
30. Vinogradova N, Glukhov A, Chaplygin V, Kumar P, Mandzhieva S, Minkina T, Rajput VD. The content of heavy metals in medicinal plants in various environmental conditions: A review. Horticulturae. 2023; 9(2):239. https://doi.org/10.3390/horticulturae9020239
31. Tumilaar SG, Hardianto A, Dohi H, Kurnia D. A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. J Chem. 2024; 2024(1):5594386. https://doi.org/10.1155/2024/5594386
32. Gulcin I, Alwasel SH. DPPH radical scavenging assay. Processes. 2023; 11(8):2248. https://doi.org/10.3390/pr11082248
33. Spiegel M, Kapusta K, Kołodziejczyk W, Saloni J, Zbikowska B, Hill GA, Sroka Z. Antioxidant activity of selected phenolic acids-ferric reducing antioxidant power assay and QSAR analysis of the structural features. Molecules. 2020; 25(13):3088. https://doi.org/10.3390/molecules25133088 PMid: 32645868. PMCid: PMC7412039.
34. Rungsang T, Srivilai J, Rakasawapokin P, Rakasawapokin P, Mungmai L, Saesue K, Aoonboontum P, Plukham N, Siriwipanan P, Chaichanathawikit P, Khorana N. Assessment of antioxidant, anti-lipid peroxidation, antiglycation, anti-inflammatory and anti-tyrosinase properties of Dendrobium sulcatum Lindl. Cosmetics. 2023; 10(2):43. https://doi.org/10.3390/cosmetics10020043
35. Choudhary D, Ghosal M, Das AP, Mandal P. *In vitro* antioxidant activity of methanolic leaves and barks extract of Litsea plants. J Plant Sci Res. 2013; 3:99107.
36. Rout OP, Acharya R, Mishra SK. *In-vitro* antioxidant potentials in leaves of *Coleus aromaticus* Benth and rhizome of *Zingiber zerumbet* (L.) SM. J Appl Pharm Sci. 2011; 1(8):194-198.
37. Hatami T, Emami SA, Miraghaee SS, Mojarrab M. Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of *Artemisia biennis* willd. Iran J Pharm Res. 2014; 13(2):551-559. PMCID: PMC4157030. PMID: 25237350.
38. Bajpay A, Nainwal RC, Singh D. *Coptis teeta*: A potential endemic and endangered medicinal plant of Eastern Himalayas. J Pharmacogn Phytochem. 2019; 8(4):245-248.
39. Wang N, Tan HY, Li L, Yuen MF, Feng Y. Berberine and Coptidis Rhizoma as potential anticancer agents: Recent updates and future perspectives. J Ethnopharmacol. 2015; 176:35-48. https://doi.org/10.1016/j.jep.2015.10.028 PMid: 26494507.
40. Chelleng N, Sonia H, Tamuly C. *Coptis teeta* Wall.: A comprehensive overview of its traditional uses, pharmacological uses, phytochemicals and conservation. Future Integr Med. 2024; 3(1):21-34. https://doi.org/10.14218/fim.2023.00082

Author Contribution

Conceptualization, methodology, data acquisition, data interpretation, initial manuscript drafting, V.V.; methodology, L.S.K.; methodology, supervision, reviewing and editing, H.C.R.; provided guidance in analytical methodologies, K.V.R.; resource coordination, P.N.; conceptualization, resource coordination, data interpretation, manuscript drafting, supervision, essential revisions, M.N. All authors have read and agreed to the published version of the manuscript.