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COMPARATIVE STUDY OF PHARMACOGNOSTICAL, PHYTOCHEMICAL & BIOLOGICAL INVESTIGATION OF THE RHIZOME & LEAVES OF "*CURCUMA CAESIA*"

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ABSTRACT:
In the current work, Pharmocognostic standards are attempted to be established in order to assess the plant material of C. caesia Roxb (family: Zingiberaceae), also known as Kali haldi. Numerous factors were examined, including Morphology, Microscopy, Physicochemical constants, Phytochemical, and Antioxidant efficacy profiles of the plant's rhizome and leaf, and the key diagnostic characteristics were recorded. With the aid of alcoholic (ethanol), and hydroalcoholic (water + ethanol) solvents, the rhizome and leaves of Curcuma caesia were gradually extracted.

This work used the DPPH free radical scavenging assay to investigate the antioxidant activity of the ethanolic and hydroalcoholic extract of Curcuma caesia rhizomes and leaves. By calculating the inhibitory concentration IC50 (The concentration of sample needed to scavenge 50% of DPPH free radical), a graph between % Inhibition and Concentration was created. Ascorbic acid was employed as a conventional antioxidant instead of Curcuma caesia's ethanolic and hydroalcoholic extract. The Inhibitory concentration IC50 value ranges in the order of 272.09 µg/ml (Standard Ascorbic acid) > 551.43 µg/ml (EE of C.caesia Rhizome) > 936.84 µg/ml (HAE of C. Caesia Rhizome) > 1320 µg/ml (EE of C. Caesia leaves), > 2950.77 µg/ml (HAE of C. Caesia leaves) the lowest being the highest antioxidant activity. This suggests that ethanolic & hydroalcoholic extract of rhizomes of Curcuma caesia had moderate IC50 value as compared to standard Ascorbic acid. But higher antioxidant activity as compared to the ethanolic & hydroalcoholic extract of the leaves of Curcuma caesia.

Key words: Antioxidant Efficacy, Kali Haldi, Physicochemical Constants, Inhibitory concentration.

INTRODUCTION:

There are vast reserves of medicinally useful substances in nature. Since the Stone Age, plants have been a significant source of basic ingredients for both conventional and modern medicine. Remedial herbs are still commonly utilized in many underdeveloped countries to treat a variety of communicable diseases due to their simple and low-complication nature. Traditional tropical medicinal floras may serve as an excellent source of new, reliable, environmentally friendly, and long-lasting medications for the treatment of a variety of illnesses. The presence of certain phytochemicals is primarily responsible for plants' medicinal potential. They are basically plant metabolites that are created in each plant cell and serve specific purposes for animals. [1, 2]

The Zingiberaceae family includes Curcuma caesia Roxb. (Black Turmeric). It is a rhizomatous herb that can be found all across globe and other tropical regions of the world. Kali haldi is a botanical perennial herb with an upright to semi-erect herb posture. It is a rhizomatous, aromatic herb with a 30- to 60-cm-long leafy bunch. Large, long, petiolate, oblong-lanceolate in shape, tapering at both ends, acuminate at the leaf apex, hairless, and green on both sides are the characteristics of the leaves. a pale

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yellow lip flower with a long, semielliptic form. On the long, erect peduncle of Curcuma caesia, there is an inflorescence with five to six sheaths that is hidden by the sheathing bases of the leaves. The inflorescence is a spike at the foot of the peduncle, about 15 cm long and 30 cm high overall. The flowers are smaller than the bracts and are pale yellow with a crimson border. About the same length as the blade is the petiole and sheath. Before the leaves, there are spikes. Fruits ripen in September and October, while flowers bloom in June and July. [3-8]

The camphoraceous, tuberous rhizome has a diameter of 2 to 6 cm and is camphoraceous. Size and form might vary widely. It possesses adventitious roots and root scars in addition to being sessile, lateral flattened, wart- and scar-covered, and having longitudinal circular surface wrinkles that resemble nodal and internodal zones on the rhizome. It is blue black, or buff, on the inside, and dark brown on the outside. The fragments of scaly leaves are organized in a ring like arrangement, resembling growth rings. Branching is symmetrical in nature. In vitro, it has a wide range of pharmacological actions, such as anti-diabetic, antioxidant, antimicrobial, analgesic, anti-cancer, anti-ulcer, antiemetics, antiviral, antitumor, anti-inflammatory, anti-tubercular, anti-asthmatic, anti-hyperglycemic, astringent, anti-diarrheic, and antipyretic properties. In this study, Curcuma caesia's leaves and non-aerial component (rhizome) were extracted using a variety of solvents in order to identify the drug in dry form, prevent adulterants, and evaluate the medication's physicochemical characteristics and possible phytochemicals. Moreover, to evaluate the antioxidant capacity of various solvent extracts from the Caesia caesia rhizome and leaves. [3-10]

MATERIAL & METHODS:

Plant Material

1) Collection and Authentication

In the month of September, plant material was collected from Jaddi Butti Kendra in Kolhupani Premnagar, Dehradun, and validated by the Botanical Survey of India (BSI), in Dehradun. BSINRC Tech.Herb (Ident.)2022-23506 is the specimen authentication number.

2) Method of Extraction

To remove dust, the rhizome and leaves were rinsed under running water. Rhizome and leaves were dried in shade or dried in oven at 40–45°C for 2 hours, and then ground into a coarse powder using a mechanical grinder. By employing a Soxhlet apparatus and 50g of powdered rhizome and leaf in 250mL of the solvent for 10 hours, extraction was carried out. As extraction solvents, we employed double distilled water. After that, extracts were concentrated in a rotary evaporator at 40°C. For later use, dried concentrate was then kept in a refrigerator at 4°C.

The crude concentrate's % yield (w/w) was estimated using the formula below:

$$PY = \frac{\text{Wt of crude concentrate recovered}}{\text{Wt of powder taken}} * 100$$

(PY= is % yield of extract)

Pharmacognostical study:

1) Morphological studies: The morphological characteristics of the Curcuma caesia leaf and rhizome were examined, including dimensions, form, tip, border, base, surface, hue, aroma, and flavor.

2) Microscopic studies: To prepare the requisite samples of powdered Curcuma caesia leaf and rhizome, chloral hydrate was used to clean and strain the material. Phloroglucinol and strong hydrochloric acid were then used to further strain the material [11].

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3) Powder Microscopy: Phloroglucinol and strong hydrochloric acid were used to stain rhizome and leaf powder for microscopic analysis in order to analyze the lignified cells such as xylem and sclerenchymatous tissues. Additionally, they were dyed with iodine solution to look for starch grains. Using a little amount of powder solution mounted in chloral hydrate, calcium oxalate crystals were identified. [12, 13]

4) Preliminary Physicochemical studies:

For the quantitative analysis, such as LOD, total ash, acid insoluble ash, and alcohol soluble extractive values, air dried rhizomatous as well as leaf materials were used. These physicochemical analysis were carried out in accordance with Indian Pharmacopoeia standards and WHO recommendations. [14–18]

Loss on Drying: Loss on drying is the mass loss, given as a ratio of weight to weight (w/w). The loss on drying test identifies the volatile and water content of crude oil. The raw medicine will inevitably contain moisture, which must be eliminated as much as possible.

A tarred glass Petri plate was filled with a carefully weighed quantity of approximately 5 g of powdered drug. The powder was dispersed equally. A steady weight was recorded after the sample was dried in Hot air oven for 2 hrs. at a temperature "between" 100 to 105°C in a Petri dish that was held open under vacuum. It was subsequently cooled in desiccators to room temperature, weighed, and the percentage loss noted during drying was computed using the procedure below.

Loss on drying = (weight of moisture in sample/ Net weight of moist sample) * 100

Total Ash Value: The entire ash can be used to detect raw pharmaceuticals that have been combined with different mineral components to improve their look, such as sand, earth, calcium oxalate, and other drugs. A tarred silica crucible was filled with 2 grams of powdered drug. The temperature of the powder was gradually raised until the carbon was burned off, at which point it was kept for cooling. The ashes were weighed, stored in desiccators, and the percentage of whole ash was calculated.

Total Ash = (Ash weight/ sample weight) * 100

Acid Insoluble ash value: The whole ash obtained from the aforementioned process was heated for 5 minutes with 25 ml of diluted HCl. After filtering, the insoluble ashes were gathered on ash-free filter paper, rinsed in hot water, burnt in a tarred crucible, cooled, and then put in desiccators. The acid-insoluble plant ash was estimated after the residue was weighed.

$$\% \text{ Acid insoluble Ash} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of sample}} * 100$$

Water Soluble ash value: After completing the aforementioned process, the complete amount of ash was heated in 25 ml of water for 5 minutes. After filtering, the indissoluble ashes were gathered on ash-free filter paper, rinsed in warm water, burnt in a tarred crucible, cooled, and then put in desiccators. The water soluble plant ash was estimated after the residue was weighed.

$$\% \text{ Water soluble Ash} = \frac{\text{Total Ash wt. – water insoluble residue in total Ash}}{\text{Weight of the sample}} * 100$$

Extractive values: Is the quantity of extract that a medicine delivers on a dry weight basis to a specific solvent. A rough estimation of the amount of a specific chemical constituent or set of related chemicals that the medication contains is helpful.

Water soluble Extractive value: For medications containing water-soluble ingredients such tannins, sucrose, vegetable acids, and mucilage, the water-soluble extractive value is utilized.

A closed flask was used to macerate 5 g of precisely weighed powdered, air-dried medication for 24 hours with 100 ml of chloroform water. The first six hours of the maceration involved continuous shaking, while the latter 18 hours involved standing still. It was then swiftly filtered to stop solvent loss. 25 cc of the filtrate were evaporated to dryness, dried to a constant weight, and weighed in a shallow

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dish with a flat bottom that had been coated with tar. The proportion of extractive that is water soluble was calculated using the medication that had been air-dried as a reference.
The following formula was used to calculate the result:

% of Water soluble extractive value = Weight of the extract × 100 × 100/ 25 × weight of the sample taken

Alcohol soluble extractive value: For drugs containing alcohol-soluble ingredients including tannins, resins, and alkaloids, the alcohol soluble extractive value is utilized.

In a closed flask, 100 ml of alcohol was macerated for 24 hours with 5 g of carefully weighed powdered, air-dried medicines, with regular shaking during the first six hours and standing time of 18 hours. It was then swiftly filtered to stop solvent loss. 25 cc of the filtrate were evaporated to dryness, dried at 105°C to a consistent weight, and weighed in a shallow dish with a flat bottom that had been coated with tar. Using the air-dried medicines as a foundation, the amount of extractive that is soluble in alcohol was calculated.
The following formula was used to calculate the result:

% of Alcohol solvable extractive value = Weight of the extract × 100 × 100/ 25 × quantity of the sample taken

5) Non-quantitative Phytochemical estimation:

Using the prescribed procedures, the extracts were examined for the presence of bioactive components. [19-22]

Alkaloidal test

A smooth paste was generated by combining approximately 2 gram of the powdered substance with 1gram of slake lime, 5mL of water, and 5min. On a water bath, it was then evaporated to dryness. 20ml of chloroform was mixed to the residual, thoroughly mixed, and refluxed on a steam bath for 30 minutes. The chloroform was then evaporated after it had been filtered. 5mL of weak hydrochloric acid was poured to the leftovers. The color that emerged from adding two milliliters of each of the following chemicals to each of the four halves of the solution denotes the presence of alkaloids.

a) Mayer's Reagent test - Cream ppt.
b) Wagner's Reagent test - Reddish brown ppt.

Test for sugars

Moilsh test: The hydrous extract of the powdered leaf & rhizome when treated with an ethanolic solution of Alpha-naphthol while being exposed to sulfuric acid. Carbohydrates are present as indicated by the color purple.

Test for cardiac glycosides

Killer Killiani's test:

5 ml of each concentrate were placed in a test tube, which was then mixed with 2 ml of anhydrous acetic acid and a drop of Iron (iii) chloride solution. On top of that, one ml of mineral acid (pure sulfuric acid) was carefully administered. The presence of a amber colour ring at the junction indicated the presence of the cardenolide-specific deoxysugar. A lavender colour ring may develop below the ring and a greenish ring may form in the acetic acid layer.

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Test for flavonoid
Shinoda test:
The extract was heated for five minutes with some magnesium chips and a few droplet of strong HCl. Flavonoids are identified by red coloration.

Test for phenolic group
The presence of phenolic compounds was detected when a small amount of the powdered sample was examined using the following reagents.
a. A deep bluish-black colouration with ferric chloride (5%).
b. White precipitate from the lead acetate solution.
c. Bromine water addition produces Bromine water decoloration.
d. Red-color on addition of acetic acid solution.
f. A transient red color in a diluted iodine solution.
f. Precipitate produced with tannic acid.

Phlobatannins detection test
Precipitate test
When 2 ml of concentrate were heated with 1 ml of 1% hydrous HCL, a red precipitate formed.
Test for amino acids and proteins

Ninhydrin test
In order to detect the development of a purple color, 2 ml of concentrate was treated with 2–5 drops of ninhydrin reagent and placed in a warm steam bath for 1–2 min.

Saponin estimation
Froth test
In a test tube, 2 ml of extract received 6 ml of water. The existence of saponins might be verified by vigorously shaking the contents and watching for the emergence of persistent froth.
Test for sterol

Liebermann-Burchard test
Acetic anhydride, Trichloromethane, and conc. sulphuric acid were used to treat one ml of the extract, and the appearance of a dark pink or red color was monitored.

Ferric chloride test for tannins
Aqueous extract of the powdery drug was prepared in very small amounts. A few droplets of $fecl_3$ solution were mixed with the aqueous extract. The existence of tannins is indicated by a blue-black tint.

Test for terpenoid
Salkowski's test
2 ml of each concentrate were combined with 1 millitres of Trichloromethane and a few droplets of strong sulphuric acid was mixed. Terpenoids existed if a rust coloured precipitate appears.

Test for quinones
Concentrated HCl was used to treat a small quantity of concentrate, and the existence of a yellow precipitate (or coloration) was monitored.

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Test for oxalate
A few droplets of glacial ethanoic acid were mixed to a 3 millitre sample of the extracts. Oxalates are present if a greenish black colour develops.

6) In Vitro Antioxidant activity:
Method : Free Radical cleansing action using Diphenyl Picryl Hydrazyl free radical
Procedure [23- 26]
The C. caesia rhizome and leaf methanolic extract were produced as a stock solution at a concentration of 1 mg/ml. Three milliliters of methanol and one milliliter of DPPH were added to test sample concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mg/ml. Control was made in the same way as sample but without the sample. In the case of a blank, methanol was used instead of DPPH. For around 30 minutes, the reaction was allowed to run its course in utter darkness. The absorbance was then determined at 517 nm. The standard was vitamin C. Calculating the percentage scavenging required multiplying [(Control-Test)/Control] by 100. On a graph, the relationship between concentration and the degree of inhibition was shown, and an equation for linear regression was generated. Analyzing linear regression, the sample concentration needed to achieve a 50% drop in absorbance (IC50) was determined. The results obtained are presented in Tables and Figures under result & discussion section.

RESULT:
Percentage Yield
Studying the physical characteristics of Curcuma caesia's rhizome and leaf extracts, it was discovered that the rhizome concentrate were almost slippery to thick in its character and ranged in color from brown to yellowish. The percentage yield for rhizome extracts in hydroalcoholic solvents was also highest (19.12%), followed by ethanol (3.74%). The high percentage yield of the hydroalcoholic solvent may be explained by the simple fact that water is the solvent of choice that dissolves most compounds. The leaf extracts were virtually waxy to sticky in texture and ranged in color from dark green to brown. The hydroalcoholic solvents produced the highest percentage yield of the leaf extracts (17.2%), followed by ethanol (6.62%). The study mentioned above provides a clear illustration of the existence of various phytochemicals in various plant parts and their affinity for various solvents based on their polarities.

Table no 1: Material Characters & yield of C. caesia rhizome and leaves concentrate in several solvents.

Specifications	Percentage yield	Colour	Consistency
RHIZOME			
Ethanol	3.74%	Reddish Brown	Sticky
Hydro-alcoholic	19.12%	Yellowish	Less viscous
LEAVES			
Ethanol	6.62%	Greenish brown	Viscous
Hydro-alcoholic	17.2%	Dark Brown	Less Viscous

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Macroscopy of the plant

The plant is typically straight and grows to a height of 0.5 to 1.0 m; it is distinguished from the ground by a large, ovoid tuberous rhizome, also known as the rootstock, and an upright, floral and leafy aerial shoot.

Figure No 1: *Curcuma caesia* Whole plant Figure No 2: *Curcuma caesia* Fresh rhizomes Figure No 3: *Curcuma caesia* Flower

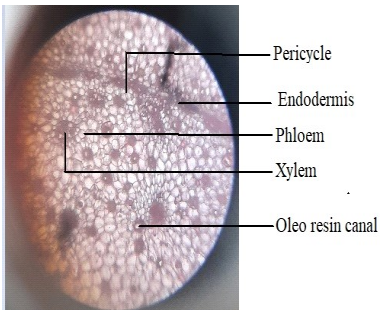
Microscopy

Rhizome: T.S of rhizome consists of [Figure no 4].

1. A single layer of cells with incredibly thick walls and a thick cuticle make up the outermost part called epidermis.
2. Collagenous cells with 3 to 5 layers and strong walls make up the cortex.
3. A poorly formed endodermis.
4. Pericycle - tightly packed, well-defined cells arranged radially.
5. Pith: The pith is a huge parenchymatous structure with many cells that are either packed with sphaeraphides or starch grains. The vascular traces that cross the surface of the pith may be leaf remnants .
6. Vascular tissue: Vascular bundles are joined together and scattered across the xylem, which is made up of vessels and xylem parenchyma. Phloem is made up of parenchymal sieve tubes.

Leaf – The plant's isobilateral leaf displays: [Figure no 5]

1. Epidermis: The top and lower epidermis are both cuticle-covered, single-layered, and stomata-punctured structures.
2. Mesophyll: The mesophyll, which is completely chlorophyllens with intermittent oil pores, combines the palisade with porous parenchyma. Epithelial cells make up the clearly defined wall of oil cavities.
3. The bundles of vascular tissue, which are intermingled with oil reservoirs and all of which has an arch of sclerenchyma covering the xylem, are conjoint and collateral.



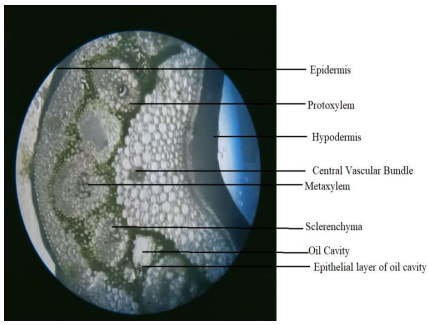


Figure No 4: T.S of *C.caesia* Rhizome Figure No5: T.S of *C.caesia* Leaf

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Powder Microscopy of Rhizome & Leaves

The powder has a camphoraceous odor and is dark brown in color. The flavor is harsh and contains fiber powder and tiny vessel granules. [Figure no 6].

a) The parenchyma is made up of clusters of spherical to angular cells. The grains are collections of starch-filled cells with parenchymatous structures that turn blue when exposed to an iodine solution.

b) Oligoresin crystals, which were initially impregnated in parenchyma, are observed in a dispersed state after being free in powder.

c) Vascular elements - numerous vessel components, either whole or in fragmented form. Tracheids are scarcely noticeable, and the majority of the components belong to the vessel group. They also have spiral and pitted thickenings.

The various leaf components, including stomata, trichomes, fibers, and calcium oxalate crystal, were identified by powder microscopy of the leaves, as shown in Figure no 7.

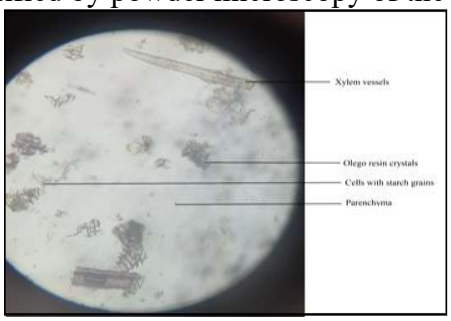

Figure No 6: Powder microscopy of Rhizome

Figure No 7: Powder microscopy of leaf

Physicochemical & Phytochemical studies

Numerous physicochemical parameters were measured in the rhizome and leaves of Curcuma caesia. The outcomes are displayed in Table no. 2. Alkaloids, steroids, phenols, and tannins were found to be the main components in the serial solvent extraction of the rhizome and leaves, according to preliminary phytochemical tests. [Table no. 3].

Table no 2: Physicochemical specifications of Black turmeric Rhizomes & leaves.

Specification	W/W Value (%) Rhizomes	W/W (%) Value Leaves
Ash level	5.5 %	12 %
Value of acid-soluble ash	1 %	4.5 %
Value of water-soluble ash	2 %	1.5 %
loss from dryness	8 %	5.8 %
Extractives soluble in water	6.4 %	10.4 %
Extractives soluble in alcohol	16 %	5.6 %

Table no 3: Phytochemical analysis of extracts from the rhizomes & leaves of Black turmeric.

S.No	Constituents	EE of rhizome	HAE of rhizome	EE of leaves	HAE of leaves
1.	**Alkaloids**				
	Mayer's Test	+	+	+	-
	Wagner's Test	+	+	+	+
2.	**Carbohydrates**	+	-	-	+

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	Molisch's Test Benedict's Test	+	-	-	-
3.	**Glycosides** Killer killani test Legal's Test	+ +	- -	- +	- -
4.	**Flavonoids** Shinoda test Alkali test Acid test	+ + +	- - +	- + -	- - -
5.	**Proteins** Millon test Biuret test Ninhydrin test	- + -	- + -	- + -	- + -
6.	**Phenol** $Fecl_3$ test Bromine water Tannic acid test	+ + +	+ - +	+ - +	+ - -
7.	**Saponins** Foam Test	-	+	+	+
8.	**Tannins**	+	+	+	+
9.	**Terpenoids** Salkowski test	+	+	+	+
10.	**Quinones**	-	-	-	-
11.	**Oxalates**	-	-	-	-

(+)

Indicates 'Presence'; (-) Indicates 'Absence', EE: Ethanolic extract, HAE: Hydroalcoholic extract.

In-Vitro Antioxidant capacity or Biological activity

By using a free radical cleansing technique, the plant's rhizome and leaves of Curcuma caesia were examined for their antioxidant activity under laborartory conditions. The Tables and Figures offer the graphical representations of the results achieved using this methodology.

Method : Radical cleansing action using Diphenyl Picryl hydrazyl (DPPH) radical.

Rhizome extract: The outcome of the C. Caesia rhizome's ethanolic & hydroalcoholic extract radical cleansing action towards the free radical (DPPH) is shown in Table No. 4 and graphically shown in Figure No.8 & 9.

Table no 4: % Inhibition of C. Caesia rhizome ethanolic & hydroalcoholic extract towards free radical at 517 nm

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S.No.	Concentration (µg/ ml)	Absorbance	% Suppression by EE of *C.caesia* rhizome	% Suppression by HAE of *C.caesia* rhizome
1.	100	1.671	25.60%	16.70%
2.	200	1.539	31.48%	23.82%
3.	400	1.398	37.76%	28.72%
4.	600	1.011	54.99%	39.67%
5.	800	0.797	64.51%	43.68%
	IC_{50}		**551.43 µg/ml**	**936.84 µg/ml**

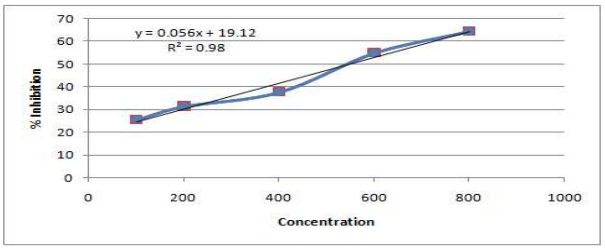

Figure No 8: Ethanolic concentrate of black turmeric rhizome

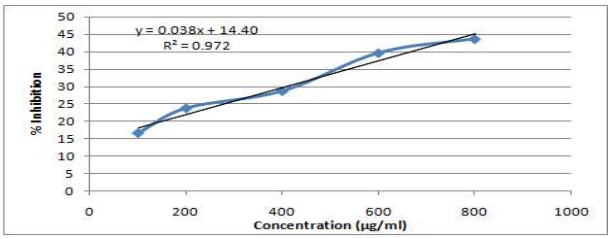

Figure No 9:

Hydroalcoholic concentrate of black turmeric

tested for its ability to scavenge free radicals against DPPHrhizome tested for its ability to scavenge free radical at 517nm. against DPPH at 517nm.

The EE & HAE extract of C. Caesia rhizome demonstrated a percentage suppression of 64.51% & 43.68 % at a dilution of 800 µg/ ml, as can be observed in Table No. 4. The IC50 value was determined to be 515.43 g/ ml & 936.84 µg/ ml using a linear regression analysis.

Leaf extract : The outcome of the C. Caesia leaves ethanolic & hydroalcoholic extract radical cleansing action towards the free radical (DPPH) is shown in Table No. 5 and graphically shown in Figure No. 10 & 11.

Table no 5: % Inhibition of C. Caesia leaves ethanolic &hydroalcoholic extract towards free radical at 517 nm.

S.No.	Concentration (µg/ ml)	Absorbance	% Suppression by EE of *C.caesia* leaves	% Suppression by HAE of *C.caesia* leaves
1.	100	1.919	14.56%	11.49%
2.	200	1.849	17.68%	15.36%
3.	400	1.745	22.31%	18.30%
4.	600	1.551	30.95%	20.35%
5.	800	1.481	34.06%	21.86%
	IC_{50}		**1320 µg/ml**	**2950.77 µg/ml**

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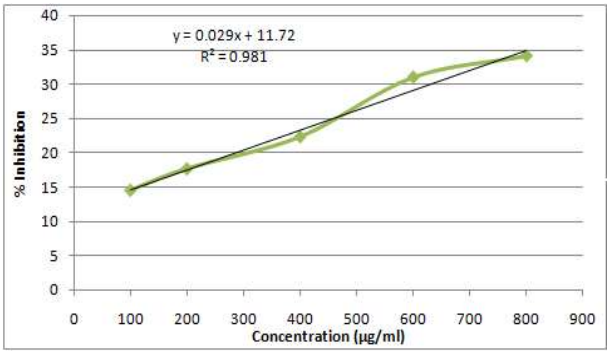


Figure No 10: Ethanolic extract of C. caesia leaf tested for its ability to scavenge free radical against DPPH at 517 nm.

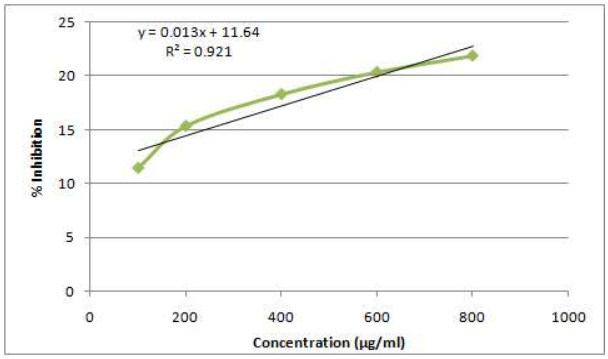


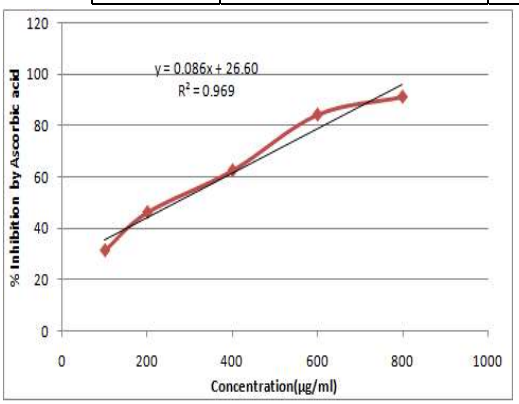
Figure No 11: Hydroalcoholic extract of C. caesia leaf tested for its ability to scavenge free radical against DPPH at 517 nm.

The EE & HAE extract of C. caesia leaves demonstrated a % suppression of 34.06 % & 21.86 % at a dilution of 800 µg/ ml, as can be observed in Table No. 12. The half maximal inhibitory concentration (IC50) value was found to be 1320 µg/ ml & 2950.77 µg/ ml using a linear regression analysis.

Standard (Ascorbic acid): The outcome of the standard ascorbic acid radical cleansing action towards the free radical (DPPH) is shown in Table No. 6 and graphically shown in Figure No. 12.

Table no 6: % of DPPH at 517 nm that Ascorbic acid (Vitamin C) suppress.

S.No.	Concentration (µg/ ml)	Absorbance	% Suppression by Vitamin C
1.	100	1.546	31.17%
2.	200	1.211	46.08%
3.	400	0.845	62.38%
4.	600	0.357	84.11%
5.	800	0.200	91.10%
	IC_{50}		**272.09 µg/ml**



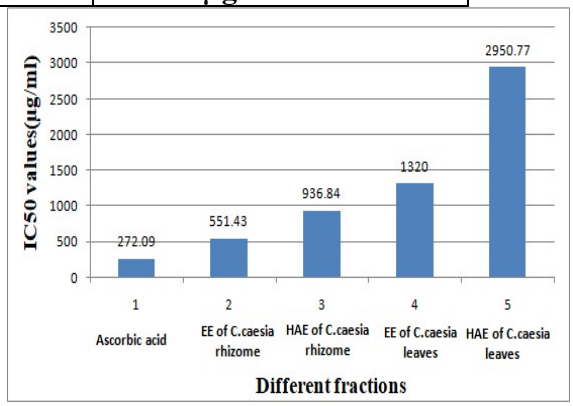


Figure No 12: Assay of Standard Ascorbic Acid's capacity to scavenge free radicals against DPPH at 517 nm.

Figure No 13: Comparison of IC_{50} value against different fractions

The standard vitamin C demonstrated a % suppression of 91.10 % at dilution of 800 µg/ ml, as can be observed in Table No. 6. The half maximal inhibitory concentration (IC50) value was found to be 272.09 µg/ ml using a linear regression analysis. The extract had effective anti-radical properties.

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DISCUSSION:

Physicochemical Studies: The physiochemical properties of the powdered leaves and rhizome extracts of Curcuma Caesia Roxb are shown in Tables 2. Plant matter degrades at a different rate depending on how much water is in it. If there is a high water content, the plant material may degrade quickly due to fungus. At 105°C, it was found that the LOD for rhizomes and leaves was 8% and 5.8%, respectively. The total ash value represented how many minerals and earthy elements were affixed to the plant material. The rhizome and leaves of C. caesia have total ash value contents of 5.5% and 12%, respectively, according to analytical results. There was discovered to be 1% and 4.5%, respectively, of acid-insoluble siliceous materials in the plant's rhizome and leaves. The water-soluble ash levels of the rhizome and leaves are 2% and 1.5 percent, respectively. The existence of polar elements including phenols, alkaloids, steroids, glycosides, flavonoids, etc. was revealed by the alcohol soluble extractive values.

Phytochemical Studies: All the extract samples lacked quinone and oxalates. Eight phytoconstituents including alkaloids, carbohydrate, cardiac glycoside, flavonoids, phenols, tannin, protein, and terpenoids, were detected in the EE of the C. caesia rhizome. This was followed by a hydroalcoholic extract, which detected seven secondary metabolites, including carbohydrate, phenols, saponins, tannin, alkaloids, amino acids, and terpenoids. Eight secondary metabolites, including flavonoids, glycosides, phenols, tannin, alkaloids, amino acids, saponins, and terpenoids, were detected in the ethanol extract of the C. caesia leaf. This was followed by a hydroalcoholic extract, which detected seven secondary metabolites, including carbohydrates, phenols, saponins, tannin, alkaloids, amino acids, and terpenoids. The outcome suggests that the rhizome & leaves of Curcuma caesia has the potential to be a source of important phytochemicals for the pharmaceutical industry. Rhizomes and other non-areal portions of plants contain flavonoids that have a role in metabolism and regulate growth in living systems. Plant flavonoids and phenolic compounds have been shown to have a wide range of biological advantages, such as anti-inflammatory, anti-carcinogenic properties, antioxidant and radical cleansing properties, astringent properties, anti-diabetic, anti-tubercular, and antipyretic activities. [27-29]

Pharmacological studies

The current study's analysis of antioxidant activity using a technique free radical neutralizing methodology revealed that Curcuma caesia has modest antioxidant activity in comparison to that of conventional ascorbic acid. The absorbing capacity of the control, calculated at 517 nm, was determined to be 2.246. Tables were used to display how concentration increased along with the decline in absorbance value and estimated% Inhibition. To gauge the strength of the antioxidants, the percentage of inhibition was calculated. The activity is better the higher the percentage of inhibition. From the above graph plot equations it has been calculated that: 551.43 µg/ml of EE of Rhizome of black turmeric scavenge 50% 1ml of 0.1mM concentration of DPPH solution, 1320 µg/ml of EE of leaves of black turmeric scavenge 50% 1 ml of 0.1mM concentration of DPPH solution, 936.84 µg/ml of HAE concentrate of Curcuma caesia rhizome scavenge 50% 1ml of 0.1mM concentration of DPPH solution, 2950.77 µg/ml of HAE of black turmeric leaves scavenge 50% 1ml of 0.1mM concentration of DPPH solution, 272.09 µg/ml of Ascorbic acid (Standard Antioxidant) scavenge 50% 1 ml of 0.1mM concentration of DPPH solution. In comparison to Curcuma caesia leaves, which had a 34.06% inhibition, the EE of Curcuma caesia rhizome was shown to be the most effective free radical scavenger (64.51% decrease at a dilution of 800 µg/ml). In comparison to the HAE of Curcuma caesia leaves, which had a 21.86% inhibition, the HAE of the rhizome of the herb was found to be an effective free radical scavenger (43.68% decrease at a dilution of 800 µg/ml). Standard vitamin C exhibits outstanding inhibition of 91.10% at a dilution of 800 µg/ml when compared to various alcoholic and hydroalcoholic

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extract of the rhizome and leaves of Curcuma caesia. The half maximal inhibitory concentration (IC50) value ranges in the arrangement of 272.09 µg/ml (Standard Ascorbic acid) > 551.43 µg/ml (EE of C.caesia rhizome) > 936.84 µg/ml (HAE of C. Caesia rhizome) > 1320 µg/ml (EE of C. Caesia leaves), > 2950.77 µg/ml (HAE of C. Caesia leaves) the lowest being the highest antioxidant activity.
There are many biological effects, including antioxidant activity, that have been connected to the existence of polyphenolic chemicals in plants, such as flavonoids and phenolic acids. Numerous antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, ascorbic acid, and tert-butylhydroquinone, have been used as food preservation agents for many years despite concerns about their long-term safety. Although the Rhizome of Curcuma caesia's ethanolic extract exhibits a little amount of antioxidant activity, the quest for new, potent natural antioxidants like ascorbic acid will go on. Organic extracts of C. caesia's rhizomes and leaves have shown possible antioxidant activity, indicating the plant has a preventive effect against oxidative damage and is a significant natural antioxidant. The extract can be used in the food and pharmaceutical industries due to its antioxidant act.

CONFLICT OF INTEREST:
The authors have no conflict of interest regarding this investigation

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