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ESTIMATION OF PHENOLICS, FLAVONOIDS AND TERPENOIDS CONTENTS IN RHIZOMES OF *Curcuma zedoaria* ROSC. AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Curcuma zedoaria, often known as C. zedoaria Rosc is a botanical species recognized for its utilisation in traditional medicine. It possesses aromatic rhizomes that emit a pleasant fragrance and is reputed for its therapeutic attributes. The material presented herein provides a comprehensive study on the pharmacognostic research, chemical ingredient analysis, and antioxidant capacity of C. zedoaria rhizomes in a systematic manner. The pharmacognostic investigation was conducted in accordance with the quality control assessments outlined by the World Health Organisation (WHO). Both macroscopic and microscopic analyses were conducted on the crude drug and powdered rhizomes, respectively. Various analyses were conducted to determine the foreign matter content, ash values of different types, extractive value, volatile oil content and loss on the drying parameters. A phytochemical screening was conducted to assess the presence of total alkaloid, glycoside, terpenoid, flavonoid, and phenol compounds. The studied possess a significant abundance of terpenoids, as evidenced by quantitative chemical analysis. The assessment of the antioxidant capacity of the extract derived from the rhizomes of C. zedoaria was conducted using the DPPH, hydrogen peroxide and phosphomolybdenum methods. It has been determined that plants with a high concentration of terpenoids also possess antioxidant capabilities.

Keywords: *Curcuma zedoaria, Chemical analysis, rhizomes, Total antioxidant potential*.

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INTRODUCTION

Ayurveda is sometimes referred to be the progenitor of therapeutic practises, whilst the earth is recognised as the originator of several herbs and natural remedies [1,2]. Ayurveda encompasses a vast repository of information pertaining to numerous natural plants, shrubs, and trees [2]. The essential principle of Ayurveda is the harmonisation of the mental, physical, and spiritual aspects of persons. According to the complete requirements, Ayurvedic medications have a high level of effectiveness [3]. However, the text contains a specific reference to herbal medicaments and their quality and qualitative qualities. The quality of herbal products should be maintained as medications are intended for inhalation, ingestion or topical applications [4-6]. The World Health Organization (WHO) has provided recommendations about quality control parameters for the maintenance and evaluation of herbal medicaments to ensure their proper quality [6-7].

Curcuma zedoaria, a white turmeric and one of the important crude drugs belonging to Zingiberaceae family and genus Curcuma [8,9], is a herb in the Ayurvedic tradition. *Curcuma zedoaria* Rosc is a perennial plant with a reed-like appearance and a fragrant rhizome that possesses medicinal benefits. *Curcuma zedoaria* exhibits uncontrolled growth in the regions of Europe, North America, and South and East Asia [10,11]. The preceding studies conducted on this particular plant unequivocally demonstrate that it does not exhibit any adverse effects [11]. The rhizome of *Curcuma zedoaria* possesses several qualities such as antifungal, anti-helminthic, anti-bacterial and bug repellent [12,13]. The material presented in this study provides a comprehensive pharmacognostic evaluation and phytochemical examination of the rhizomes of *Curcuma zedoaria* Rosc. in a systematic and organized manner.

Materials and Methods:

The plant drug has undergone a pharmacognostic examination in accordance with the quality control requirements for herbs and herbal drugs established by the World Health Organization (WHO) [6]. Initially, the morphological characteristics involve the apparent properties of a substance which are visible to the naked eye, and are crucial in the initial identification of the pharmaceutical compound. The aforementioned attributes of herbal medicaments are sometimes referred to as organoleptic characteristics or macroscopic features. This test incorporates several characteristics such as colour, odour, taste, fracture, size, form, *etc*.

Microscopical analysis:

Microscopic identification entails the observation and examination of the test medication using a microscope. Microscopic analysis was conducted on both the rhizomes and powdered form. The transverse section of the rhizomes was cut and thereafter observed under a microscope. Glycerol, safranine, and phloroglucinol stains were employed to enhance the identification of the drugs [14].

Physicochemical evaluations

A. Foreign matter content: It is imperative that herbal materials are devoid of any visible impurities such as stones, mouldy substances or any other extraneous components. The analysis focused on particular characteristics and sensory characteristics [15]. The plant materials undergo a thorough process to ensure total removal of any kind foreign substances. The first step involves measuring a specific quantity of herbal material, which is then evenly distributed in a wide layer. Next, the foreign matter present in the herbal material is segregated into groups. This can be done through eye inspection, aided by a magnifying lens with a 6 × 10 magnification, or by using a suitable sieve. The method chosen depends on the specific requirements for the particular herbal material being examined. The remaining portion of the sample should be passed through a No. 250 sieve, as the dust present is considered a mineral admixture. Evaluate the quantity of these organised foreign particles within the prescribed limit of 0.05 g.

B. **Determination of loss on drying:** The loss on drying value provides valuable information regarding the volatile content of the aqueous component of the plant. A quantity of 2.0 g of pulverised raw material was carefully measured and placed in a pre-weighed tiny crucible. The crucible, together with its contents, was subjected to 105 ºC until a consistent weight was achieved. Each sample was replicated three times to ensure that it had achieved a stable weight.

C. **Ash value:** The ash value serves as an indicator of the overall concentration of inorganic constituents inside the pharmaceutical substance. To ascertain the weight, the silica crucibles were first measured, followed by the addition of 2-4 g of powdered substance into each crucible. The plant material was placed inside the silica crucible and heated to 500-600 ºC until a steady weight was measured. After ensuring that the temperature was maintained at the standard level, the sample was placed inside a desiccator to prevent moisture absorption. Subsequently, the total ash content of the sample was determined.

D. **Acid insoluble ash determination:** The addition of weighed ash was added to a 25 mL solution of dil. HCl followed by boiling for 5 min. Subsequently, the immiscible solution was filtrated with Whatman filter paper No. 1 followed by the addition of a quantity of hot water to eliminate any acidic impurities. The ashless paper was subjected to ignition at 450 ºC until a stable weight was achieved.

E. **Water soluble ash:** The whole ash was dissolved in distilled water and filtered containing the little ash content. Subsequently, the water-soluble fraction of the ash (filtrate) was determined.

F. **Extractive value:** The extraction procedure involves the separation of chemical elements from a crude product. The selection of solvents was based on their respective polarities. The extraction process was conducted using a Soxhlet device. A quantity of 4 g of powdered product was dissolved in methanol. The extraction process was conducted for 6 h, after which the remaining sample was collected. The solvent is further subjected to evaporation and subsequently, the extractive value of the sample is determined [16].

G. **Volatile oil determination:** Volatile oils, often known as aromatic oils were characterized by their distinctive aroma. The determination of volatile oil can be conducted using the steam distillation method, following the procedure outlined in the quality control recommendations provided by the World Health Organisation (WHO) [6,7].

Quantitative phytochemical screening

Total alkaloid content: A mixture of 2.5 g of powdered *C. zedoaria* Rosc. extract with 100 mL of ethanol and 40 mL of acetic acid in a solution containing 100 mL of ethanol and 40 mL of acetic acid was prepared. Utilizing the reflux condenser methodology, the reaction mixture was subjected for refluxation for 4 h. Subsequently, approximately 15 drops of conc. NH_4OH solution were added. The aforementioned solution was subjected to maceration for 3 h. During digestion, the precipitate was obtained. Using separating funnel and the resulting precipitates were carefully collected, subjected to drying and then weighed accurately weighed. The total alkaloid content was calculated using the following formula:

$$\text{Total alkaloid content } (\%) = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100$$

Total phenolic content

In their study, Senguttuvan *et al*. [17] employed the Folin-reagent Ciocalteu's method to quantify the total phenolic content of the medication. The absorbance of the sample and standard against a blank at a wavelength of 765 nm was measured using a spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian). In brief, the test sample, consisting of 0.2 mL with a concentration of 60 mg/mL, was mixed with 0.6 mL of water and 0.2 mL of Folin-phenol Ciocalteu's reagent in a 1:1 ratio. Following a time interval of 5 min, a 1 mL solution containing sodium bicarbonate at a concentration of 8% weight/volume in freshwater was introduced, and subsequently, the total volume was adjusted to 3 mL by the addition of refined water. The mixture was allowed to rest for 30 min prior to undergoing centrifugation. The measurement of absorbance at a wavelength of 765 nm was thereafter conducted to determine the presence of blue pigment in different samples.

Total glycosidic content

The glycosidic content was analyzed with the Folin-Ciocalteu's reagent assay. To assess the overall concentration of saponin glycosides, 5 g Folin-Ciocalteu's reagent were mixed with 100 mL of ethanol and then heated the solution at 55 ºC on a water bath. The sample should be subjected to vaporisation, followed by the addition of 20 mL of diethyl ether to a separating funnel. Subsequently, the mixture should be vigorously shaken, as described by Ezeonu *et al*. [18]. Next, the aqueous layer was collected and carefully transferred it into a crucible. Subsequently, ensure that the aqueous layer is well dried, and subsequently measure its weight.

$$\text{Total glycoside content } (\%) = \frac{\text{Weight of product}}{\text{Weight of sample}} \times 100$$

Total terpenoid content

The analysis of total terpenoid content was analyzed using the Folin-Ciocalteu's reagent, as outlined in the methodology of Ezeonu & Ejikeme [18]. The determination of the total terpenoid content involved the dissolution of 1 g of plant in 100 mL of ethanol. The solution was incubated for 3 h and then the solution was filtered and subjected to fractionation using petroleum ether. Subsequently, the fraction of petroleum ether was collected, subjected to a drying process, and subsequently weighed for further experimental studies. The overall percentage of terpenoid contents was evaluated using the following equation:

$$\text{Total terpenoid content } (\%) = \frac{\text{Final weight of sample} - \text{Initial weight of sample}}{\text{Initial weight of sample}} \times 100$$

Total Flavonoid content

The analysis of the total flavonoid content in the plant extract was conducted using the colorimetric method as reported by Chandra *et al*. [19]. Quercetin was employed as a standard reference compound for the purpose of quantifying the total flavonoid content. The stock solution of quercetin was prepared by dissolving 5.0 mg of quercetin in 1.0 mL of methanol, whereas the standard solutions of quercetin were prepared by sequentially diluting methanol (5–200 µg/mL). The experiment involved the combination of 0.6 mL of 2% $AlCl_3$ solution with 0.6 mL of dilute quercetin solution. The solution was combined gradually, then allowed to digest at room temperature for 60 min. The absorbance of the mixture was determined using a UV-VIS spectrophotometer at 420 nm. The absorbance values were measured relative to a blank using the Cary 50 Bio UV-VIS Spectrophotometer Varian instrument.

Total antioxidant potential

Total antioxidant potential is a term used to describe the process by which oxidative substances are degraded. The total antioxidant capacity of the powdered extract derived from *C. zedoaria* Rosc. was analyzed utilising the following methodologies.

DPPH scavenging method

The DPPH percentage scavenging method was conducted following the protocol reported by Valko *et al*. [20]. The DPPH solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. DPPH can be described as a solution containing oxidative moieties. Subsequently, 4 mL of DPPH solution was combined with 5 µL of sample extract. The combination was subjected to vigorous agitation for 10 min, followed by a further period of 30 min during which it was stored in dark environment. The absorbance of the aforementioned solutions was quantified through the utilisation of UV spectrophotometry at a wavelength of 550 nm.

Phosphomolybdenum method

The phosphomolybdenum reagent was synthesised by mixing 28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid within sealed test tubes. To initiate the sample preparation, 0.05 g was dissolved in 1 mL of methanol within a vial. Subsequently, the reaction mixture was subjected to incubation on a water bath maintained at 95 ºC for 100 min [21]. Following the incubation period, it is wise to allow the test

tubes to cool to ambient temperature. The concentrations were then measured by their absorbance at 695 nm with a UV spectrophotometer.

RESULTS AND DISCUSSION

Morphological and Microscopical examination Characteristics

Table-1 shows the morphological characteristics of *C. zedoaria*, whereas Fig. 1 displays the microscopic identification of the rhizomes of C. zedoaria Rosc. Circular, thin-walled epidermal cells were observed in the transverse section microscopy. These cells were followed by an aerenchymatous cortex with shapeless air chambers. Additionally, vascular strands have been found. The vascular strands had a broad morphology, characterised by thick cell walls and angular xylem elements. These structures were encompassed by phloem tissue and protected by sclerenchymatous cells. The researchers discovered that the core bundles exhibited a substantial size and possessed an amphivasal arrangement, characterised by a significant presence of phloem tissue that was enveloped by two or more layers of xylem elements. In contrast, the peripheral vascular bundles were connected to the endodermis. The phloem is enveloped by a layer of xylem components. Powder microscopy provides evidence of the presence of tracheids, fibres, stone cells, and calcium oxalate crystals. Hexagonal and pentagonal cells have been observed in the powdered microscopy analysis of *C. zedoaria* rhizomes.

Table 1: The morphological data of *C. zedoaria* rhizomes

S. No.	Morphological Characters	Results
1	Colour	Pale yellowish to brown
2	Odour	charasterstic volatile smell
3	Taste	Pungent
4	Fracture	Fibrous
5	Shape	Longitudinal irregular
6	Size	6-10 cm long and 1-1.5 cm width

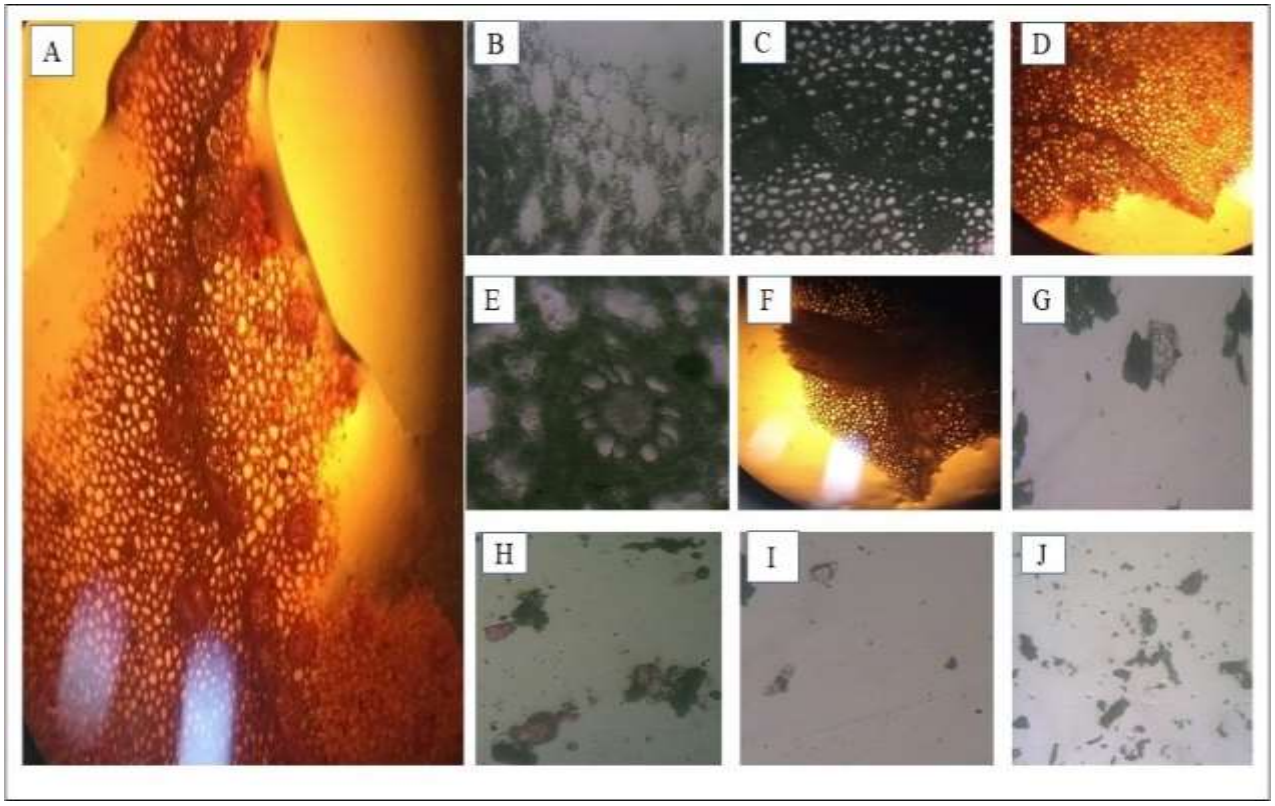

Fig. 1: Microscopical views of *C. zedoaria* rhizomes, whereas G-J shows the powdered microscopical views of *C. zedoaria* rhizomes

Physico-chemical evaluation

According to the results of the investigation (Table-2), there was a 1.2% concentration of foreign materials present. The measured loss on drying exhibited a value of 25.63%. The analysis revealed that the total ash content was determined to be 18%. The weighted average score (WSA) was determined to be 9.66% while the average individual score (AIA) was discovered to be 5.85%.The analysis revealed that the content exhibited volatility at a rate of 9-10%.

Table-2 Results of the Pharmacognostic Parameters

Parameters	Values
Foreign Organic Matter	1.12%
Extractive value	5.85 %
Aqueous	6.63%
Hydroalcoholic	18 %
Methanol	19 %
Chloroform	12%
Petroleum ether-CF	
Ash value	19%
Water soluble ash	9.85%
Acid insoluble ash	5.85%
Loss on Drying	25.35 %
Volatile oil content	9-10%

The results obtained from the morphology and microscopic analyses have provided confirmation of the presence of *C. zedoaria* rhizomes. The microscopic examination of the rhizome powder demonstrated the existence of parenchymatous cells, stone cells, and calcium oxalate crystals when observed under the aqueous, glycerol, and diluted safranine phases. The results obtained from the analysis of extractive values indicate that the *C. zedoaria* powder exhibited a maximum extractive value of 20% when dissolved in methanol, while a minimum extractive value of 6% was seen when dissolved in an aqueous solution. These findings suggest that the methanolic extract of *C. zedoaria* powder contains a higher concentration of phytoconstituents compared to the aqueous extract.

Quantitative phytochemical Screening

The quantification of total alkaloid content was performed using the formula given in the materials and methods section. The quantity of the precipitates was determined to be 0.02 g following the process of filtration and subsequent full drying. The formula provided in the materials and methods section was utilised and the total was calculated.

The quantification of phenolic content was conducted by measuring the amount of gallic acid equivalents (GAE) per gramme of dry plant material, using a standard curve of gallic acid. A standard solution of gallic acid was generated by diluting quantities ranging from 50 to 300 mg/mL. The standard calibration curve is depicted in Fig. 2, with the X-axis representing the concentration and the Y-axis representing the absorbance. The experimental results indicated an absorbance value of 0.309, corresponding to a concentration of 100 mg/L. The findings of this section indicate that the phenolic content in a 60 mg/ml solution of *C. zedoaria* extract is equivalent to that of 100 mg/ml solution of gallic acid.

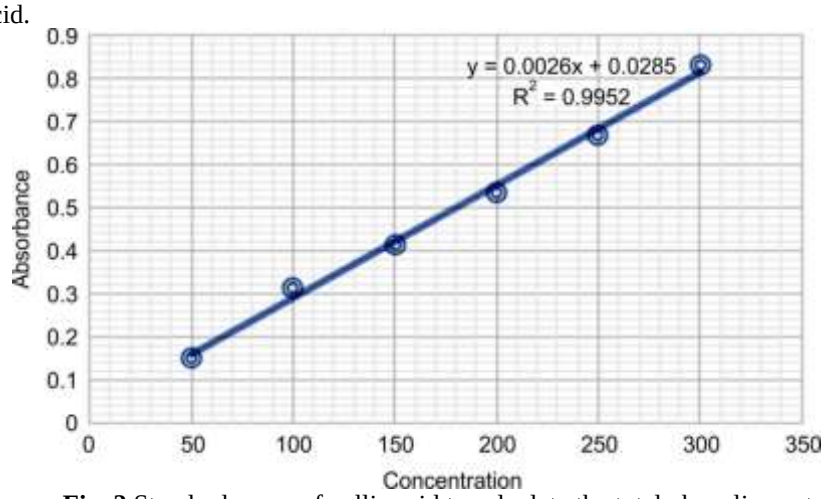

Fig. 2 Standard curve of gallic acid to calculate the total phenolic content

The total glycoside content was determined using the formula described in the materials and methods section. After proper drying, a residual mass of 0.039 g was observed for the precipitates. The concentration of *C. zedoaria* rhizomes was found to be 0.78%. Fig. 3 shows a graphic representation of the total amount of alkaloids, glycosides, and terpenes in the rhizomes of *C. zedoaria*.

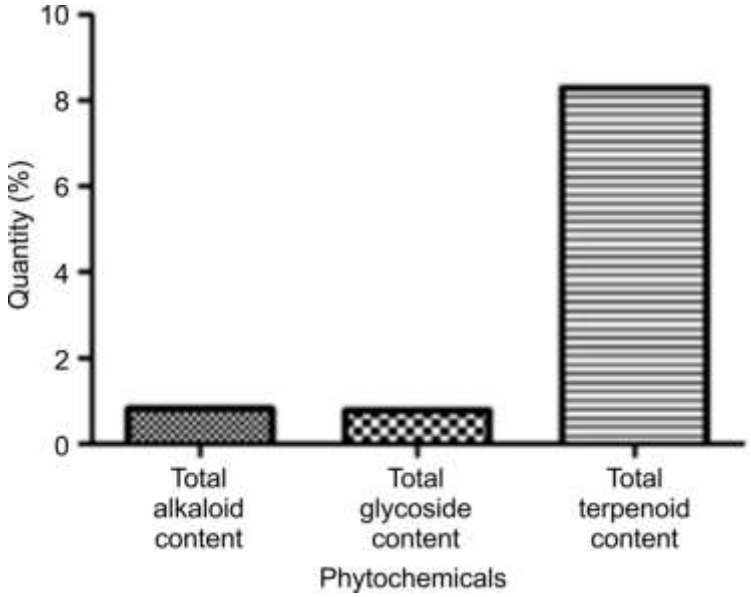

Fig. 3 The graphical representation of total alkaloid, glycoside and terpenoid contents found in *C. zedoaria* rhizome

The quantification of terpenoid content was determined to be 8.3%. The experiment revealed that the terpenoid content exhibited the highest value among all the quantified phytoconstituents. The graphical depiction of the combined alkaloid, glycoside and terpenoid levels in the rhizomes of *C. zedoaria* is presented in Fig. 3.

The quantification of flavonoid content in the test sample was accomplished by utilising a standard plot of quercetin. This plot encompassed a range of concentrations from 0-50 µg/mL, as depicted in Fig. 4. The X-axis of the plot represented the concentration, while the Y-axis represented the absorbance. The measured absorbance of the test moiety was determined to be 0.512. All the determinations were conducted in triplicate. The total flavonoid content of the *C. zedoaria* extract solution is reported as 10 µg/mL, which is equivalent to the total flavonoid content of quercetin solution at a concentration of 30 µg/mL.

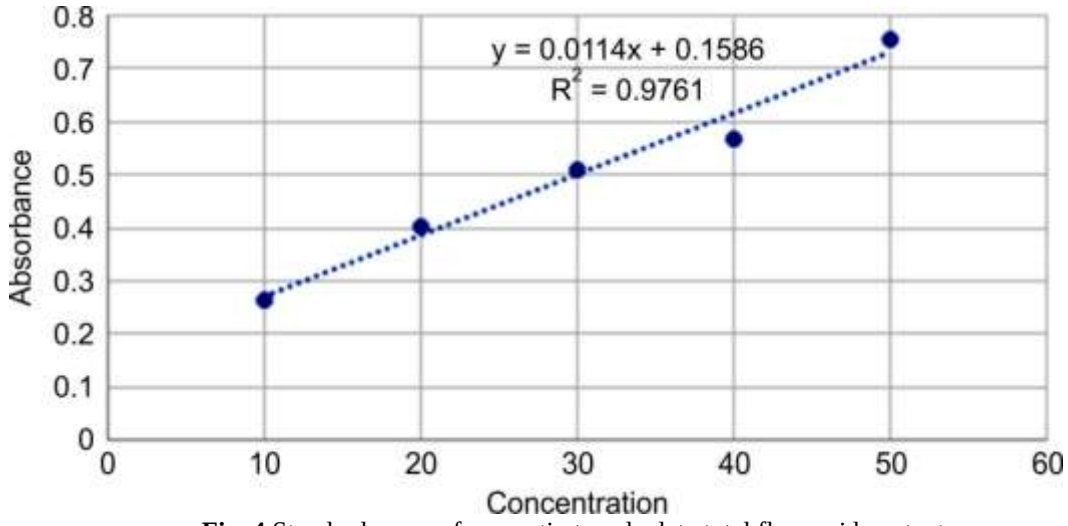

Fig. 4 Standard curve of quercetin to calculate total flavonoid content

Determination of antioxidant activity

The DPPH percentage scavenging activity of *C. zedoaria* Rosc. rhizome was investigated using quercetin as a standard. The DPPH radical scavenging capacity of *C. zedoaria* extract and quercetin exhibited similar levels of activity at a dose of 200 µg/mL. The 600 µg/mL solution of quercetin demonstrates a 57% inhibition rate against DPPH, whereas the *C. zedoaria* extract exhibits a 51% inhibition rate against DPPH. The results are presented in Fig. 5.

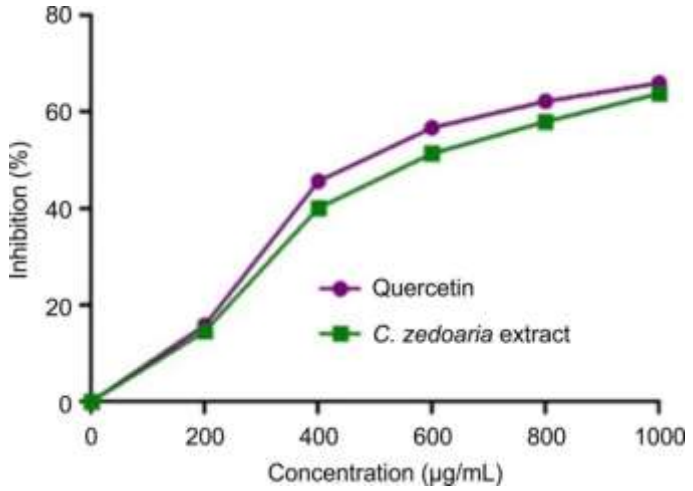

Fig. 5 The total antioxidant activity using %DPPH scavenging method

The findings pertaining to the percentage of H_2O_2 scavenging activity have been elucidated as the percentage of H_2O_2 scavenging activity in Fig. 6. Quercetin was employed as the reference compound. Initially, there was a discernible variation in the percentage inhibition of hydrogen peroxide at a concentration of 200 µg/mL. However, at a concentration of 600 µg/mL, the drug solution exhibited an equivalent percentage inhibition as the standard quercetin.

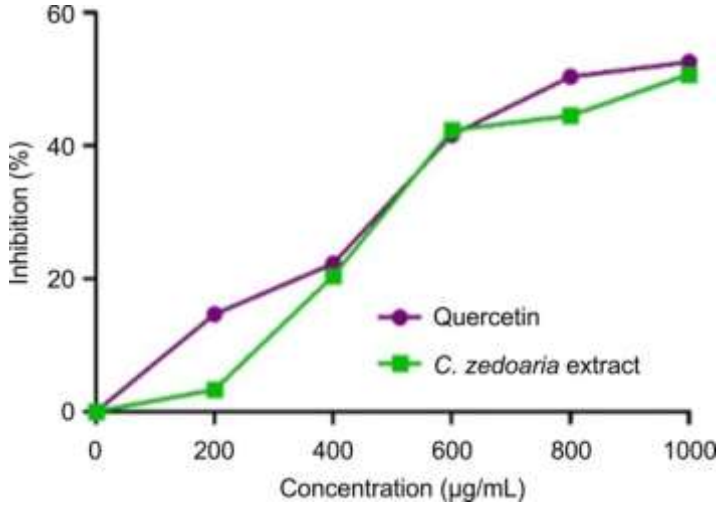

Fig. 6 Scavenging activity of *C. zedoaria* extract by H_2O_2 scavenging method

The findings of the phosphomolybdenum scavenging method have been presented in terms of the percentage of phosphomolybdenum scavenging activity as shown in Fig. 7. The total antioxidant capacity (TAC) was quantified and reported as quercetin equivalent (QE) in µg/mg. Quercetin served as the reference compound. In all five concentrations, quercetin has demonstrated a slightly higher percentage of inhibition compared to the *C. zedoaria* extract solution.

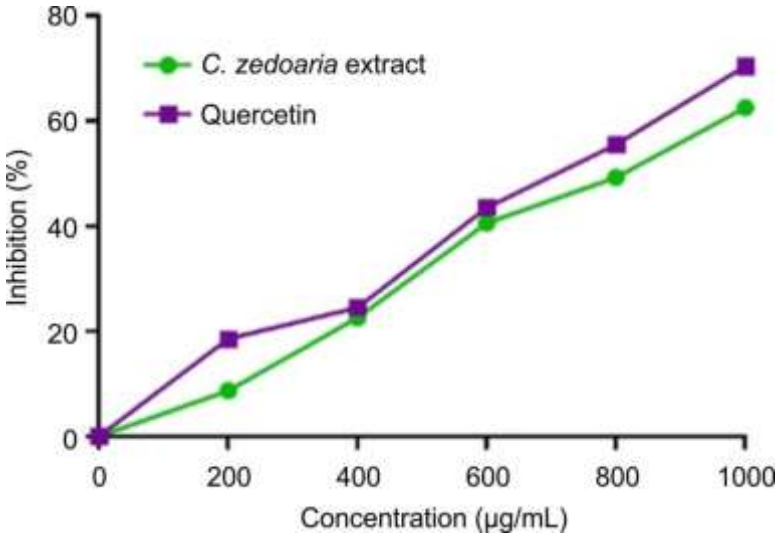

Fig. 7 Scavenging activity of *C. zedoaria* extract by phosphomolybdenum scavenging method

The quantitative chemical analysis revealed that the total terpenoidal content was found to be at its highest. It exhibited a higher level of potency compared to other phytochemicals. The antioxidant capacity of *C. zedoaria* Rosc. rhizome extract has been evaluated using three distinct methodologies. Upon conducting a comprehensive analysis of the results obtained from various methodologies, it can be assumed that *C. zedoaria* rhizomes have a marginally lesser antioxidant potential compared to quercetin, which served as the benchmark antioxidant agent. The enhanced antioxidant capacity effectively suppresses the activity of free radicals within the human body, so promoting overall health by reducing oxidative stress and facilitating improved digestive function.

CONCLUSION

The formulation of *Curcuma zedoaria* exhibits a substantial presence of inorganic components as well as a significant number of aqueous soluble substances. Furthermore, the formulation has higher antioxidant potency, hence contributing to its antioxidant impact. Based on a comprehensive analysis, it has been determined that *C. zedoaria* Rosc. exhibits a considerable presence of terpenes and terpenoids, while demonstrating commendable antioxidant properties. The standards reported in this work are applicable to various pharmaceutical industries and research laboratories engaged in the manufacturing and production of herbal formulations. These standards are designed to ensure the efficacy and quality control of herbal products, thereby facilitating consistent batch-to-batch maintenance and maximising the therapeutic effectiveness of the products.

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