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Harnessing the phytochemical potential of *Curcuma angustifolia* rhizomes: A comprehensive evaluation of antibacterial and antioxidant properties

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Abstract

Herbaceous perennial *Curcuma angustifolia* Roxb. has been traditionally utilized in folk medicine for a variety of purposes. The objective of the current study was to assess the phytochemical, antimicrobial, and antioxidant qualities of *Curcuma angustifolia* plant rhizomes. Based on preliminary phytochemical investigations, the chemical classes found in the extracts included flavonoids, alkaloids, steroids, phenols, saponins, terpenoids, cardiac glycosides, and tannins. The findings suggested that the plant phytochemical qualities could be used to treat a variety of illnesses. With the agar-well diffusion method, the antimicrobial effectiveness of the plant extracts was evaluated against both clinical isolates and standard strains of certain bacteria. The extracts demonstrated an inhibitory impact on both gram-positive and gram-negative bacteria. Gram-positive bacteria like *Staphylococcus aureus* had the highest zone of inhibition, whereas gram-negative bacteria like *Klebsiella pneumoniae* had the lowest. The DPPH radical scavenging assay was used to assess the extracts antioxidant properties. Antioxidant activity increased with extract concentration. Since it has antibacterial and antioxidant qualities, it is useful for treating a range of illnesses, enhancing immunity, and combating harmful bacteria that infect humans.

Keywords: *Curcuma angustifolia*, Phytochemical, Antibacterial, Antioxidant, DPPH

Introduction

Medicinal and aromatic plants are those species that contain a high concentration of secondary metabolites, sometimes referred to as medical herbs. These plants are utilized for a variety of purposes, primarily medicinal, aromatic, and culinary ones, and have been used for thousands of years (Rodino and Butu, 2019). With the advancement of knowledge, they were later utilized as raw materials in the production

of natural foods, pharmaceuticals, cosmetics, and other health-related items. Additionally, they serve as the raw material for processed natural goods such dietary supplements, pharmaceutical intermediates, oleoresins chemical entities for synthetic medications, dry and liquid extracts, nutraceuticals, and essential oils for traditional remedies (Bindu *et al.,* 2018). It has been difficult for researchers to obtain these bioactive compounds, despite their contributions to the production of natural goods and the growing understanding of the relationship between chemical structure of a compound and the biological characteristics of a plant (Atanasov *et al*., 2015). Since many plant species are known to have antibiotic qualities, traditional medicine globally has made extensive use of them. It's thought that nature has provided treatment for every single illness (Dias *et al*., 2012).

Biologically active substances called phytochemicals are produced in plants naturally and provide advantages for human health over macro- and micronutrients (Hasler *et al*., 1999). Plant materials contain phytochemicals, which are chemical compounds that are biologically active and essential for regular metabolic functions. Terpenes and derivatives, phenylpropanoids, isothiocyanates, sulfur compounds, and glycosides are the main families of phytochemicals that are byproducts of plants secondary metabolism and are commonly found in plants (Murali *et al.,* 2021). Phytochemicals fall into two categories: primary or secondary ingredients, depending on their contribution to plant metabolism. Examples of basic components include common sugars, proteins, amino acids, and the pyrimidines and purines present in nucleic acids and chlorophyll, among others. Lignans, alkaloids, terpenes, flavonoids, curcumines, saponins, phenolics, flavonoids, and glucosides are among the remaining plant chemicals, sometimes referred to as secondary constituents (Hahn, 1998; Saxena *et al*., 2013).

Curcuma angustifolia Roxb., a member of the Zingiberaceae family, is referred to as koova (Malayalam), yaipan (Manipuri), tikhur (Hindi), and East Indian arrowroot is characterized by the presence of narrow, green, glabrous leaves with small inflorescences bearing yellow flowers with pink coma bracts distributed throughout India and Asian countries (Likhitkar, 2023; Jadhao and Bhuktar, 2017). *Curcuma angustifolia,* popularly known as Indian arrowroot, has a rich history of use in traditional medicine and as a dietary staple. The plants nutritional profile and promising pharmaceutical potential have made it a subject of interest in both food science and pharmacology (Allabaksh and Senthilraj, 2024). The starch from the plant has been rich source of food product used ethnobotanically. The species is very nutritious, especially when used as a source of carbohydrates for Indian cooking and medications. Local healers claim that *Curcuma angustifolia* is also used therapeutically to treat worms and stomach aches (Warrier and Badole, 2022). The plant is widely distributed, but primarily found in cool, damp conditions at elevations of roughly 450 meters.

The leaves and rhizomes of *Curcuma angustifolia* are utilized as a treatment for constipation by various ethnic groups in the Koraput area of Odisha (Dash and Mishra 2002). *Brassica campestris* oil combined with crushed *Allium sativum* bulb and the rhizome of *Curcuma angustifolia* has been confirmed to be effective as an antiseptic for wounds and healing by the Mache people of Jhapa district, Eastern Nepal (Rai 2004). According to research, rhizome juice can effectively treat jaundice (Abhyankar and Upadhyay 2011). The methanol rhizome extract of *C. angustifolia* shows potential antibacterial, anti-tumor, and antioxidant effects when it is given to HeLa cells from human cervical cancer (Sanghamitra *et al*., 2013). Rhizomes are used by the Madhya Pradeshi and Chhattisgarhi tribes to cure inflammation, digestive disorders, and bone fractures (Tiwari and Patel 2013). The essential oil isolated from various sectors of the spices have antifungal and antibacterial properties (Bhavana *et al*., 2010).

The various parts of the *Curcuma angustifolia* plant, including the leaves, flowers, and rhizomes, have been traditionally used for their medicinal properties. These parts have been shown to exhibit anticancer, antioxidant, anti-inflammatory, antimicrobial, and anti-ulcer activities. Additionally, the starch obtained from the plant is a valuable food source. Paul *et al.* (2024) highlighted that the leaves of *C. angustifolia* are typically discarded as waste following rhizome harvesting. However, these leaves possess significant potential as a source of essential oils with bio pesticide properties. Extracted essential oils from leaf waste have demonstrated efficacy against stored grain pests, exhibiting repellent, fumigant, and contact activity, while posing no risk of phytotoxicity to plants.

While several phytochemicals have been identified in *Curcuma angustifolia*, there are likely many more to be discovered. Further research is needed to understand the specific mechanisms through which the extracts of these phytochemicals produce their therapeutic effects. The objective of the present study was to evaluate its antibacterial and antioxidant properties.

Materials and methods

Sample collection and processing

Curcuma angustifolia fresh rhizome was collected from Manipur, Northeast India, and the plant sample was confirmed by Department of Botany, Annamalai University. The rhizome was carefully cleaned with tap water, cut into small pieces, and then allowed to dry in the shade at room temperature for a period of 15-30 days. The chopped rhizome was ground into a fine powder using an electric blender and then stored for later use in clean, airtight containers with the appropriate labeling.

Preparation of crude extracts

The Soxhlet method was used to extract the powdered dry rhizome. Petroleum ether, hexane, and methanol has been chosen for extraction due to their respective abilities to selectively extract non-polar and polar compounds from the plant material. To obtain crude extract, 100 g of plant material and 500 ml of each solvent were used, and the mixture was heated continuously for 18

to 24 hours. The solvent was drawn out of the crude extract using a rotating vacuum evaporator set at 60^0C until all of the solvent had evaporated. The crude extract was stored in refrigerator 4^0C for further analysis.

Percentage yield of extract

The weight of the plant powder weighed prior to extraction was divided by the weight of the purified extract produced after extraction, and the resulting number was multiplied by 100 to get the percentage of extraction yield (%). After extraction, the resultant extract was weighed to determine each solvent's extractive value.

Yield (%) = weight of the extract/weight of dry plant powder x 100

Phytochemical analysis

The phytochemical composition of rhizome extracts from *Curcuma angustifolia* was investigated. Using established procedures, phytochemical analysis was performed on each solvent crude extract (Hegde and Joshi, 2010; Harborne, 1998). The following protocol was used to conduct the tests in order to determine whether or not chemical elements such as alkaloids, flavonoids, steroids, proteins, saponin, phenols, tannins, coumarins, and cardiac glycosides were present. For each extract, a phytochemical study was performed using accepted techniques. The experiments relied on observing changes in color or the formation of precipitates when specific reagents were added to the samples.

Alkaloids (Hager's test): The presence of alkaloids was verified by the yellow-colored precipitate after 1 ml of the extract was treated with Hager's reagent.

Flavonoid (Alkaline reagent test): After treating the extract with a 10% NaOH solution, the presence of flavonoids is shown by the production of a strong yellow color.

Steroid: An equal amount of concentrated H_2SO_4 acid was added after 1 ml of the extract had been dissolved in 10 ml of chloroform. Its uppermost layer turns red, while the H_2SO_4 layer fluoresces green and turns yellow. The implication here is the presence of steroids.

Proteins (Xanthoproteic test): When a few drops of conc. HNO_3 were added to the extract, formation of yellow precipitate indicates the presence of proteins.

Saponins (Frothing test): To 5 ml of distilled water, 0.5 ml of plant filtrate was added, foaming persistence indicates the presence of saponins.

Phenols: To detect the presence of phenols in the plant extract, a few drops of 10% aqueous $FeCl_3$ was added. Formation of blue or green color will indicate the presence of phenols.

Tannins: A precipitate that appeared when a few milliliters of potassium dichromate were added to the plant extract suggested the presence of tannins and phenolics.

Coumarins: A yellow color production indicates formation of coumarins when 2 milliliters of aqueous extract is mixed with 3 milliliters of 10% NaOH.

Cardiac glycosides (Keller-Killani Test): A small amount of ferric chloride and two milliliters of glacial acetic acid were added to the plant extract. A successful test result is indicated by a brown ring.

Quinones: Red color formation when 1 milliliter of extract and 1 milliliter of concentrated H_2SO_4 mixed together indicates the presence of quinones

Antibacterial activity

Test organism: Microorganisms were acquired from Annamalai University, Department of Microbiology. A combination of three gram-negative strains of *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* and two gram-positive strains of *Bacillus subtilis* and *Staphylococcus aureus* were employed. The bacterial cultures were utilized as stock cultures and were stored in the proper agar slants at 4°C during the investigation.

Agar well diffusion method: To measure the antibacterial activity, agar-well diffusion method was employed. Following the preparation of the Muller-Hinton agar medium, the medium was poured on sterile petri plates and allowed to settle for 10 to 15 minutes at room temperature, or until the agar attained an uniform thickness. When the organism is grown rapidly overnight at 35–37°C, this approach works effectively. On agar plates, sterilized cotton swabs containing the bacterial culture were swabbed. After inoculation, sterilized 6-mm-diameter corkborers were used to drill wells into the agar. As the positive control, azithromycin (10 µg/ml) was used to treat both gram-positive and gram-negative bacteria, while DMSO was used as the negative control. Three distinct concentrations of crude extracts (100, 50, and 25 µg/ml) were pipetted into the wells. The plates were incubated at 37°C for 24 h. The antibacterial activity was measured using its inhibitory length. Every experiment was run in triplicate.

Antioxidant activity

DPPH radical scavenging activity: The assay for free radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was employed (Brand *et al.*, 1995) to quantify antioxidant activity. It was determined by using spectrophotometer (Hitachi-U-20). Spectrophotometric monitoring of the purple hue decaying at 517 nm indicates the existence of an antioxidant that can donate an electron to DPPH, causing the characteristic purple color to disappear. The crude extracts were dissolved in methanol and 5% ethanol, respectively, and different concentrations of each extract (100, 200, 300, 400 and 500 µg/mL) were utilized. A 0.2 mM DPPH solution in methanol was made, and 100µl of this solution was added to methanolic leaf and root extracts. The absorbance of the solution was measured at 517 nm after 30 minutes of incubation at 25°C. As a positive control, ascorbic acid was used.

Following equation was used to compute the radical scavenging activity.

% scavenging activity

$$x = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} X\ 100$$

Where, $Abs_{control}$ is the absorbance of the control reaction and Abs_{sample} is the absorbance of the test extract samples.

Statistical analysis

The antibacterial study results are presented as the mean standard deviation of three repeats for each chemical analysis. The statistical analysis was conducted using one-way analysis of variance (ANOVA) and Duncans comparison. The relationship between the concentration of phytochemicals and their capacity to scavenge free radicals was ascertained using Pearson analysis.

Results and discussion

Extract yield

The yield was highest in methanolic extract (18.2%) of *Curcuma angustifolia* followed by hexane extract (9.1) and lowest in petroleum ether (5.7%), suggesting that methanol is effective for phytochemical extraction compared to other extracts.

Phytochemical analysis

A qualitative analysis based on precipitation and staining reactions by particular reagents was used to identify the major components of the secondary metabolites in the crude rhizome extract. As indicated in Table 1, phytochemical screening assays for several extracts of *Curcuma angustifolia* rhizome demonstrated the presence of active phytoconstituents such as saponin, flavonoid, alkaloid, quinone, cardiac glycosides, steroid, tannin, and coumarin. Phytochemicals were present or absent depending on the solvent medium used for extraction; the methanol extract contained the highest concentration of phytochemical components.

Flavonoids are naturally occurring phenolic compounds that are helpful to human health because they act as antioxidants and neutralize free radicals in the diet (Del *et al*., 1997). Localized tumors are caused by a class of polymeric phenolic compounds known as tannins. *Alpinia nigra* was found to contain terpenoids and steroids, both of which have been shown to have antibacterial action (Okwu, 2004). Red blood cell precipitation and coagulation, as well as anti-inflammatory effects, are characteristics of saponins (Shi *et al*., 2004). Alkaloids are found in medications that lower fever and headache pain. These are credited for having analgesic and antibacterial qualities. *Curcuma angustifolia* was found to be devoid of carbohydrates, as shown in Table 2, and its hexane extract contained fewer phytochemical compounds than other solvent extracts.

The presence of saponin, flavonoid, alkaloid, quinone, cardiac glycosides, steroid, tannin, and coumarin suggests the potential of *Curcuma angustifolia* to exhibit relevant biological activities.

Table 1. Phytochemical constituents in different extracts of *C. angustifolia*

Sl. No	Name of phytochemical compound	Methanol	Hexane	Petroleum ether
1.	Alkaloids	+	+	+
2.	Flavonoids	+	+	-
3.	Steroids	+	-	+
4.	Proteins	+	-	+
5.	Saponins	+	+	+
6.	Phenols	+	-	-
7.	Tannins	+	-	+
8.	Coumarins	+	-	-
9.	Cardiac glycosides	-	+	-
10.	Quinones	+	-	+

(+) = Detected, (-) = Not detected, *C. angustifolia* = *Curcuma angustifolia*

Antibacterial assay

Analysis was done in the crude extracts against three gram-negative bacteria, *Pseudomonas aeruginosa, Escherichia coli,* and two gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*. Table 2 shows the inhibition of bacterial growth. The inhibitory zone was measured to ascertain the antibacterial activity. Gram-positive and gram-negative bacteria were tested against extracts at various doses of 25, 50, and 100 µg/ml. Rhizome extracts exhibited a zone of inhibition ranging from 12.65±1.05 mm to 19.31±1.03 mm for gram-positive bacteria and from 10.25±0.98 to 16.26±0.60 mm for gram-negative bacteria.

In general, the dose and kind of bacterial strains used determine the antibacterial activity of plant crude extracts. Further evidence for a connection between the chemical components in the crude extracts and their antibacterial effect can be found in studies by Akharaiyi (2011) and Sekar *et al*., (2010). The crude extracts contained flavonoids and tannins, which are beneficial substances. Nonetheless, these bioactive substances were stimulating the antibacterial and antioxidant responses. Because the active ingredients in the crude extract have the power to inactivate enzymes, cell membrane transport proteins, microbial activity, and other substances (Sekar *et al*., 2012; Alabri *et al*., 2014), it is possible to dilute or raise the concentrations of these ingredients by fractionation (Anyasor *et al*., 2010). The results of this study demonstrate that the crude extract of *Curcuma angustifolia* possesses significant antibacterial activity against a range of pathogenic bacteria, suggesting its potential as a natural antimicrobial agent.

Table 2. Antibacterial activity of *Curcuma angustifolia* against the test microorganisms

Sl. No.	Pathogenic bacteria	Experimental units	Inhibition zone (mm) ± standard deviation
Gram-positive bacteria			
1.	*Bacillus subtilis*	C_1	12.65±1.05
		C_2	14.43±0.85
		C_3	15.99±0.48
		PC	19.11±0.78
		NC	0
2.	*Staphylococcus aureus*	C_1	14.45±0.77
		C_2	16.59±0.71
		C_3	19.31±1.03
		PC	20.82±0.68
		NC	0
Gram-negative bacteria			
3.	*Klebsiella pneumoniae*	C_1	10.25±0.98
		C_2	11.46±0.70
		C_3	12.83±0.77
		PC	15.32±0.52
		NC	0
4.	*Escherichia coli*	C_1	11.99±0.70
		C_2	13.06±0.57
		C_3	13.75±0.66
		PC	15.32±0.72
		NC	0
5.	*Pseudomonas aeruginosa*	C_1	14.42±0.83
		C_2	15.02±0.37
		C_3	16.26±0.60
		PC	17.17±0.86
		NC	0

C_1= 25%, C_2 = 50%, C_3 = 100%, PC = Positive control, NC = Negative control

Antioxidant activity

Fig. 1 presents the findings of the plants DPPH scavenging activity. *Curcuma angustifolia* rhizome methanolic extracts were tested at various concentrations of 100, 200, 300, 400, and 500 µg/ml for their antioxidant activities. Spectrophotometric measurement of quenched DPPH at 517 nm was used to calculate the proportion of free radical scavenging of DPPH following reaction with materials. The extracts exhibited free radical scavenging efficiency ranging from 19.88% to 38.51%. Several investigations have demonstrated that the presence of hydroxycinnamic acids, caffeic acid phenyl ester, flavonoids, and the phenolic content significantly affect the samples ability to scavenge free radicals (Al-Abd *et al*., 2015). The rate of change in color gradually decreases, indicating the scavenging capacity of the antioxidant. Flavonoids, tannins, saponins, and aromatic compounds are present in crude extracts of *Curcuma angustifolia*. All these bioactive materials must have caused DPPH solution to become discolored because of their ability to donate hydrogen as reported in earlier studies (Sekar *et al.*, 2012; Ayoola *et al*., 2008).

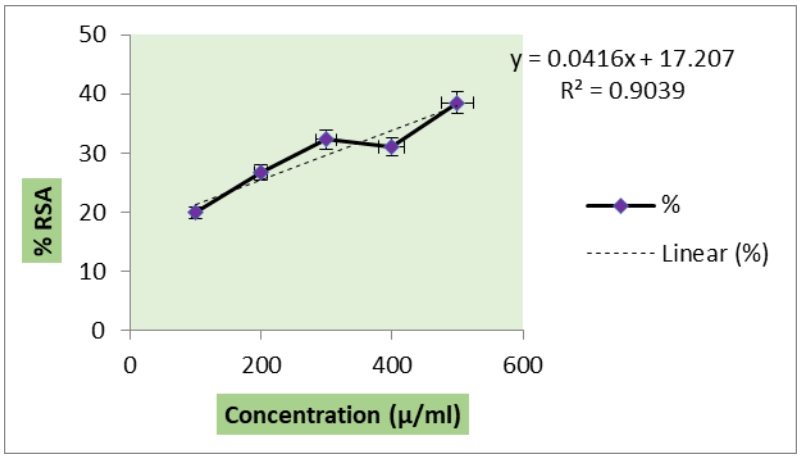

Fig. 1. Graph showing DPPH radical scavenging activity (% of control) of *Curcuma angustifolia*

The results of the *in-vitro* antioxidant activity of *Curcuma angustifolia* rhizome crude extract suggest that the phytochemicals present in the plant crude extract are effective in scavenging free radicals, thereby contributing to its antioxidant properties. In general, the overall findings obtained from the present study pave way for the upcoming extensive research works which can be conducted on the pharmacological assay and phyto-compound investigations of *Curcuma angustifolia* rhizome in the near future.

Conclusion

The antibacterial, antioxidant, and phytochemical components of *Curcuma angustifolia* rhizomes were investigated in this study. The presence of phytochemicals such as alkaloids, steroids, carbohydrates, phenols, cardiac glycosides, flavonoids, and tannins must have contributed for the

observed antioxidant and antibacterial properties of the crude extracts of *Curcuma angustifolia*. The plant also showed strong antibacterial action against specific gram-positive and gram-negative bacteria. Therefore, it is clear that *Curcuma angustifolia* rhizomes have a significant therapeutic value. Additional investigation is required to isolate and identify the active principles found in the extracts that may have therapeutic applications.

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