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Evaluating biochemical and pharmacological properties of Curcuma longa L. grown organically in two locations of Odisha, India: In vitro study

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Abstract: Organic farmers use nitrogen-fixing cover crops, herbicides, and biological fertilizers derived chiefly from animal and plant wastes. Curcumin levels are higher in this turmeric variety than in other types with differing pharmacological effects. Growing concerns about the safety of chemical fertilizers have heightened the need to identify locally adapted microbial strains that can be employed as a growth-promoting inoculum. Although research on the microbial diversity of the soil in Kandhamal and Koraput, India, was published, there is still a lack of information about the microbial community of the turmeric that is indigenous to these regions. This research compares and contrasts organically grown turmeric's growth-promoting and antibacterial activities with normally developed turmeric. This is a preliminary study that focuses on the microbiota communities of the study region. In vitro results showed that these properties have been shown to positively affect the development and nutritional content of studied turmeric plants; hence, they are regarded as key plant growth promoters. Using conventional and organic soil, both Kandhamal and Koraput rhizome sample was planted. The aqueous and methanolic rhizome extracts from both soil conditions were extracted. The Agar well diffusion method examined the rhizome extract's antibacterial activity. Bacteria were isolated and characterized from the rhizosphere and conducted various biochemical experiments. When tested against nine human pathogenic microorganisms, rhizomes harvested with vermicompost showed significantly increased antibacterial activity. The rhizospheric bacteria isolated from the organic soil region also help promote plant growth and development and provide adequate nutrients for growth and development. In every single experiment, the organically produced rhizome yielded superior results. The present study concludes that organic turmeric showed better and more effective results in increasing nutrient content, antibacterial activity and yield. Potential field applications necessitate more study into these rhizobacteria's molecular and functional characterization. Future improvements to biocontrol methods may come from studies examining the viability of deploying integrative, long-term bio-formulations in the field. The present research can potentially be used to investigate antibiotic synthesis by microbial communities in turmeric soil.

Introduction **Organic Farming**

Organic farming is multiplying as a viable substitute for traditional agriculture, utilizing eco-friendly tactics,

including organic fertilizers from plant and animal waste and insecticides from plant extracts and microbes (Jali et al., 2021). Organic agriculture is a technique of crop production management that prioritizes and enhances the



health of agricultural ecosystems (Singh, 2021). In addition, organic farming provides an environmentally acceptable alternative to the natural recycling of organic materials to prevent nutrient losses and waste accumulation. Farm manure, green manure, crop composting and other farm waste, vermicompost, oil cakes, and organic waste - animal dung are all examples of manure used in organic farming (Le campion et al., 2020).

Basics on Turmeric

The spice turmeric is widely used worldwide, primarily in tropical and subtropical areas. The plant is mostly produced in Asian countries, mainly India and China. It has long been used as a flavour and therapeutic herb in India. It is one of the most significant spices, especially among Easterners, and has a particular use for humans (Ravindran et al., 2007). With a short stem, the plant can reach a height of one metre. Curcuma longa (turmeric) is a rhizome-based spice related to ginger (Zingiberaceae). Rhizomes are underground stems that give rise to both roots and shoots. Turmeric gets its vibrant yellow colour from fat-soluble polyphenolic pigments called curcuminoids. The main curcuminoid present in turmeric is thought to be its most active Demethoxycurcumin component. bisdemethoxycurcumin are two more curcuminoids discovered in turmeric. It has had multiple therapeutic functions in India and has been used as a dye and condiment.

Curcumin's potential as a disease-preventative and therapeutic has been studied again in light of its discovery anti-inflammatory and anti-cancer capabilities. Originating in South Asia, the wild turmeric root remains in places like Indonesia and Sri Lanka. Most of the world's supply comes from the tropical regions of Asia and Africa. Turmeric grows naturally in the jungles of South Asia simultaneously in different countries, including India and Indonesia. India dominates global markets as a producer, consumer, and exporter of turmeric. In Bangladesh, China, Thailand and Cambodia, it is widely grown in Malaysia, Indonesia and the Philippines (Verma et al., 2018). The main curcuminoid present in turmeric is thought to be its most active component.

Therapeutic significance

The medicinal effects of *Curcuma longa* and its ingredients have been demonstrated in numerous highly effective ways. According to preliminary studies, "Curcumin" is the most significant phytochemical present in turmeric, and it may affect various diseases, including diabetes, cancer, arthritis, and other disorders. It was also

revealed that the *Curcuma longa* contains several other significant phytochemicals such as flavonoids, alkaloids, terpenoids, tannins, phenolic compounds, phytosterols, and saponins (Khatun et al., 2021; Sahoo et al., 2022a).

Curcumin has been shown to lower cholesterol and triglycerides, a type of fat circulating in the bloodstream, raising cardiovascular disease risk. Nutraceuticals such as curcumin can be used to treat prostate cancer because they help activate the D.N.A. damage response) (Horie, 2012). Curcumin's synergistic action is also well-known. Docosahexaenoic acid (D.H.A.) and curcumin inhibited breast cancer cell proliferation. The high efficacy of Curcuma oil in ischemic brain injury prevention has been demonstrated with a wide therapeutic window for Curcuma oil's neuroprotective properties. Curcumin can help prevent allergic reactions caused by nonspecific and specific mast cells (Yun-Ho et al., 2010; Sahoo et al., 2022a).

Carbs (69.4%), protein (6.3%), fat (5.1%), minerals (3.5%), and water (13.1 %) comprise the remaining curcuminoids, in addition to the curcumin (approximately 80%), desmethoxycurcumin (12%), and bisdemethoxycurcumin (12%) (Arawande et al., 2018; Sahoo et al., 2022). It is a powerful disinfectant for the digestive system. Measles can be treated with turmeric. It is known to be the most potent immunomodulator. Turmeric, like Silymarine, has antihepatotoxic properties that prevent liver damage and aid in removing scars and other types of skin marks. In addition, turmeric volatile oils can reduce muscular edoema by depositing extra watery fluid in tissue or capillaries (Akaberi et al., 2021).

Objectives

Evidence suggests that these rhizobacteria could be used to create inoculums or biofertilizers that boost crop yield and nutritional content in the field. It is impossible to grasp the distribution and variety of indigenous bacteria in a specific rhizosphere without first learning about the native bacterial community present, characterizing them, and recognizing them. Antibiotics are often produced from bacteria, and specific strains are chosen due to how simple they are to isolate, culture, maintain, and develop. Many chemicals isolated from rhizobacteria show significant promise as therapeutics and biocontrol agents.

The objective of my study is to analyze the antibacterial activity by taking two different rhizome extracts grown in conventional and organic soil conditions through the Agar well diffusion method against eight human pathogen bacteria and to identify the rhizospheric bacteria present in organic soil conditions that perform various biochemical activities such as

antibiotic sensitivity, catalase, starch hydrolysis etc. The other goal is to analyze the compounds in the rhizome, soil and leaves sample from different soil conditions through XRF analysis. The purpose was to characterize the rhizobacteria found in turmeric cultivated in other regions and to assess the effectiveness of their antagonistic activities, pharmacological activities. biocontrol capacity, and ability to stimulate plant growth. The benefits of this preliminary scientific inquiry can be fully realized by further exploitation for sustainable agriculture through detailed scientific investigation and exploitation of the capability of these strains as plant growth-boosting bacteria.

Materials and Methods

Methodological procedures were carried out according to the stages in Figure 1.

Study area

This experiment was conducted at Centurion University of Technology and Management, Bhubaneswar, a historical city in India's eastern state of Odisha. Khordha is between 20°15' N and 85°52' E in latitude and longitude. The climate of Bhubaneswar, which is 45 metres above sea level, is tropical. Temperatures average about 27.4 degrees Celsius (81.3 degrees Fahrenheit). The average yearly precipitation is 1505 millimetres (59.3 inches). The soil type is fertile red soil.

Collection of samples

The plant sample was collected from the Kandhamal and Koraput districts of Odisha. Summers in Kandhamal are hot and dry, while the winters are cold and dry. The district can be found in coordinates 19°34′-20°36′ N, 83°34′-84°34′ E. Temperatures as high as 45.5 degrees Celsius and as low as 2.0 degrees Celsius have been measured in the region. On average, 1522.95 millimetres of precipitation falls annually. A mainly mixed grey soil group makes up the soil. The district of Koraput is located in southern Odisha. It can be found in the Eastern Ghats. Koraput can be found between latitudes 19°10' north and 82°5' east and longitudes 83°23' and 84°00' east. The average high is 30.6 degrees Celsius, while the average low is 10.4 degrees Celsius, making the climate in this area hot and humid. Yearly precipitation averages 1521.8 mm throughout this region. The soil in the area is a mix of red and brown. The soil texture is sandy or clayey (Panda et al., 2019).

Authentication of plants

The selection of plants was based on a thorough analysis of the available literature. According to the available research, the selected plants have a background of traditional use in effectively treating diseases. Electronic sources such as PubMed and Scopus were searched for studies proving turmeric's efficacy. Various phytoconstituents are present in the selected parts of the plant with antibacterial properties. Historically, these plants have been essential in the Ayurvedic medical system. Detailed notes on the plants were compared to the herbarium and their taxonomic classification. We could ascertain the plants' authenticity based on these criteria.

Plantation of sample

The collected rhizomes were planted under conventional and organic soil conditions in the Centurion University of Technology and Management campus garden in Bhubaneswar, Odisha. Another sample was grown organically in the Kandhamal and Koraput districts to analyze chemical compounds. The rhizomes were nurtured to maturity for 3-4 months. The cultivated plants were delicately uprooted, and rhizomes were carefully separated from the plants.

Preparation of plant extract

The powder was prepared by collecting rhizomes from both organic and conventional soil. Using a Soxhlet extractor, the powder of the plant material was first steeped in methanol and aqueous solvents at a temperature below the solvent's boiling point. The extracts were then collected. The extracts were then kept in sterile vials in the refrigerator until needed. Higher extract yields were obtained using this method (Manikandan et al., 2019).

Antibacterial activity

Staphylococcus aureus (MTCC 96), Pseudomonas aeruginosa (MTCC 424), Escherichia coli (MTCC 443), Vibrio cholerae (MTTC 3906), Klebsiella pneumonia (MTTC 3906), Serratia marcescens (MTTC 4822), Staphylococcus coagulase-negative (MTCC 5856), Salmonella typhi (MTCC 735) bacterial strains were collected from S.C.B. Medical College and Hospital, Cuttack and were used to test antimicrobial activity. Using the Agar well diffusion technique, methanolic and aqueous rhizome extracts from both soil conditions were tested for their antibacterial efficacy against nine human pathogenic pathogens. The well was filled with plant extracts (100 mg/ml), and the sensitivity of the various microbial species was measured by observing the size of the inhibition zones on the agar plate around the discs. Microorganism inactivation levels were 8 mm or lower (Rojalin et al., 2020).

U.V. Spectroscopy analysis

The organic Kandhamal turmeric's methanolic extract was examined under visible and UV light for U.V.

spectroscopic analysis. The extracts were centrifuged at 10,000 rpm for UV-VIS analysis for 10 minutes. A wavelength scanner was used to scan the extracts. The wavelength range of Perkin Elmer is 200-600nm. Peak UV-VIS values were measured. To ensure accuracy, each analysis was repeated twice (Sharma et al., 2018).

Isolation and identification of Rhizospheric soil bacteria

Using a serial dilution process on 1g of fine rhizospheric soil, the rhizospheric soil bacteria were isolated. The resulting colonies were counted using a colony counter. The number of cells in a dilution was calculated by looking at the number of colonies on the plate. Gram staining was used to identify two colonies from a 48-hour-old culture plate (Jyotirmayee et al., 2021).

Study of biochemical activities of isolated bacteria Catalase test

2-3 drops of hydrogen peroxide solution were dropped onto the organism on the microscope slide. Bubble formation should be noticed quickly (Jyotirmayee et al., 2021).

pH tolerance test

The flat pH electrode's tip was put on the surface of the culture media sample to determine the pH. The values on the metre were taken when the pH and temperature had stabilized. Before measuring another sample, the pH electrode tip was cleaned with deionized or distilled water and blotted dry with soft tissue. Finally, the colony-forming unit (CFU) was determined (Mohapatra et al., 2015).

CFU/ml = (Number of colonies x Dilution factor) / Volume of the culture plate.

Temperature tolerance test

The isolated soil bacteria were inoculated into each test tube and incubated at temperatures of 23°, 30°, 37°, and 44° Celsius. CFUs were counted (Mohapatra et al., 2015).

CFU/ml = (Number of Colonies x Dilution factor) / Volume of the culture plate.

Starch hydrolysis test

Iodine solution was applied to the surface of the cultivated plate being incubated for the starch hydrolysis test. A clean zone surrounding the bacterial growth line indicated starch present (Sigmon, 2008).

Antibiotic sensitivity test

The surface of the plate was covered in streaks of the bacteria *V. cholerae*. The Agar well diffusion method assessed antibiotic sensitivity using Amikacin, methanol, aqueous, and petroleum ether extract. The inhibitory

Zone was determined and contrasted with other zones. Isolated soil microorganisms were streaked across the surface of another plate. The agar well diffusion method with Amikacin was used for the antibiotic sensitivity test (Mohapatra et al., 2015).

Phytochemical analysis

Extracts have been made using methanol, water, and ethanol for phytochemical testing (Swain and Padhy, 2015; Arawande et al., 2018).

Test for Carbohydrates

The plant extract was added to 5 ml of Benedict's solution and heated in a water bath. Carbohydrates can be identified by their characteristic precipitate colouration (red, yellow, or green).

Test for Tannins

Ferric chloride was added to separate test tubes containing plant extract. The colour changes show the presence of tannin.

Test for Saponin

A few droplets of the sodium bicarbonate solution (at a concentration of 5 percent) were added to a test tube containing plant extract. For three minutes, the mixture was vigorously agitated. The appearance of honeycomblike foam detected saponin.

Test for Flavonoids

Shinoda test: After combining the crude extract with pieces of magnesium ribbon and concentrated HCl, the mixture turned pink, showing the presence of flavonoid, after a few minutes.

Alkaline reagent test: The crude plant extract turned a bright yellow when treated with 2 ml of a 20% NaOH solution, but the colour faded after being treated with two drops of diluted acid. Positively, flavonoids were detected in this sample.

Test for Glycosides

Liebermann's test: The plant extract was combined with acetic acid and chloroform, each at a concentration of 2 millilitres. After the liquid had been cooled, concentrated H_2SO_4 was added to it. Glycosides' green representation of the steroidal aglycone.

Salkowski's test:

A plant crude extract was mixed with 2 ml of concentrated H_2SO_4 . Their reddish-brown colour denoted the presence of the steroidal aglycone constituent of the glycosides.

Test for Terpenoids

The plant extract (5 ml) was combined with the chloroform (2 ml) and evaporated over a water bath while the concentrated H₂SO₄ (3 ml) was heated. Greyish was the colour displayed by terpenoids.

Test for Steroids

Two ml of chloroform and one ml of concentrated hydrogen peroxide were added to five ml of crude plant extract. A red colour in the bottom chloroform layer

XRF analysis of rhizome and soil sample collected from different soil conditions

The Kandhamal and Koraput rhizome aqueous extract, the Koraput leaf aqueous extract, and 5 gm of fine soil

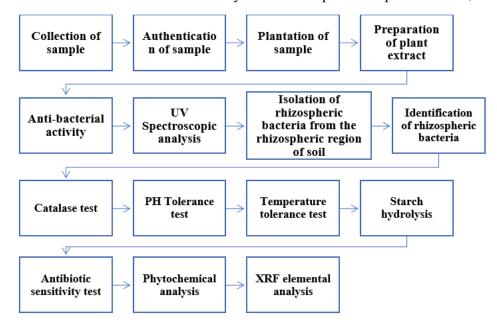


Figure 1. Flowchart showing the sequence in the current research approach

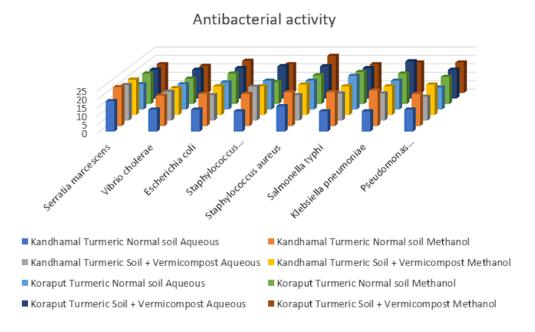


Figure 2. The graphical representation shows the antibacterial activity of both turmeric variety rhizome extracts under different soil conditions.

revealed the presence of steroids.

Test for Alkaloids

Plant extracts were taken in different test tubes. Dilute HCl was added to each test tube. Mayor's reagent was added to each test tube. As a result, light green colour formation indicates the presence of alkaloids.

from the plantation area were provided to the A.T.C. lab, CUTM, Bhubaneswar. (Mallick et al., 2021).

Results and Discussion Antibacterial activity

The antibacterial activity of both Kandhamal and Koraput turmeric extracts was tested against eight human pathogens and was measured using the bacterial growth was more effective against the pathogen *Salmonella typhi*. When tested on organically grown Koraput turmeric, the aqueous extract was shown to be more effective in inhibiting the growth of *Klebsiella pneumonia*. The methanolic extract of normally grown

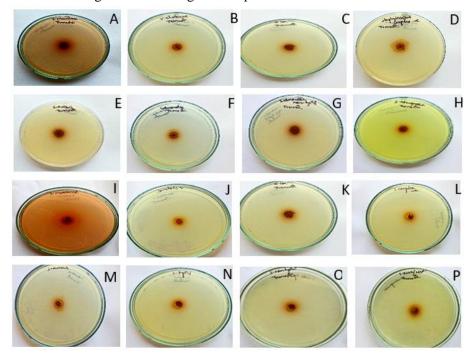


Figure 3. Zone of Inhibition of Kandhamal turmeric aqueous extract against different bacteria using normally grown rhizome (A) Serratia marcescens; (B) Vibrio cholerae; (C) Escherichia coli; (D) Staphylococcus coagulase -ve; (E) Staphylococcus aureus; (F) Salmonella typhi; (G) Klebsiella pneumoniae; (H) Pseudomonas aeruginosa; Zone of inhibition of aqueous extract against different bacteria using organically grown rhizome (I) Serratia marcescens; (J) Vibrio cholerae; (K) Escherichia coli; (L) Staphylococcus coagulase -ve; (M) Staphylococcus aureus; (N) Salmonella typhi; (O) Klebsiella pneumoniae; (P) Pseudomonas aeruginosa.

inhibition zones technique. In both normal and vermicompost-grown Kandhamal rhizome aqueous extracts, *Serratia marcescens* exhibited the most significant inhibitory zone. Kandhamal's methanolic turmeric extract revealed that *Serratia marcescens* inhibitory zone was the biggest among the two cultivars tested. Normally grown Koraput turmeric aqueous extract

Koraput turmeric had excellent antibacterial action against *Salmonella typhi*, having the highest ZOI. Methanolic extract of organic Koraput turmeric has a more significant effect against *Staphylococcus aureus*. The aqueous and methanolic turmeric extracts from both Kandhamal and Koraput have variations in antibacterial action against various human diseases (Fig. 2, 3, 4, 5, 6).

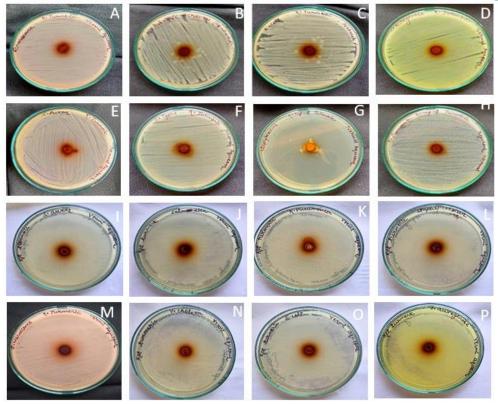


Figure 4. Zone of Inhibition of Kandhamal turmeric methanolic extract against different bacteria using normally grown rhizome (A) Serratia marcescens; (B) Vibrio cholerae; (C) Escherichia coli; (D) Staphylococcus coagulase-ve; (E) Staphylococcus aureus; (F) Salmonella typhi; (G) Klebsiella pneumoniae; (H) Pseudomonas aeruginosa; Zone of inhibition of methanolic extract against different bacteria using organically grown rhizome (I) Serratia marcescens; (J) Vibrio cholerae; (K) Escherichia coli; (L) Staphylococcus coagulase -ve; (M) Staphylococcus aureus; (N) Salmonella typhi; (O) Klebsiella pneumoniae; (P) Pseudomonas aeruginosa.

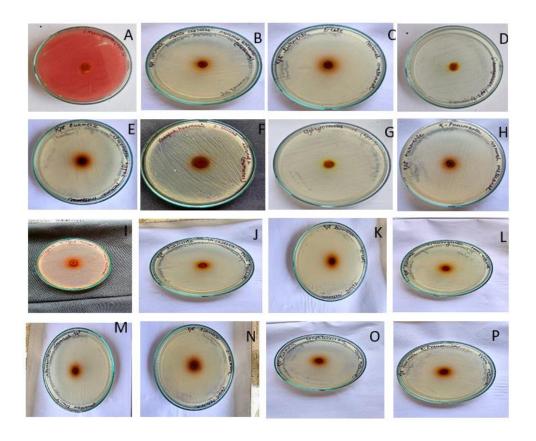


Figure 5. Zone of Inhibition of Koraput turmeric aqueous extract against different bacteria using normally grown rhizome (A) Serratia marcescens; (B) Vibrio cholerae; (C) Escherichia coli; (D) Pseudomonas aureginosa; (E) Staphylococcus aureus; (F) Salmonella typhi; (G) Staphylococcus coagulase -ve; (H) Klebsiella pneumoniae; Zone of inhibition of Koraput turmeric aqueous extract against different bacteria using organically grown rhizome (I) Serratia marcescens; (J) Vibrio cholerae; (K) Escherichia coli; (L) Pseudomonas aureginosa; (M) Staphylococcus aureus; (N) Salmonella typhi; (O) Staphylococcus coagulase -ve; (P) Klebsiella pneumoniae

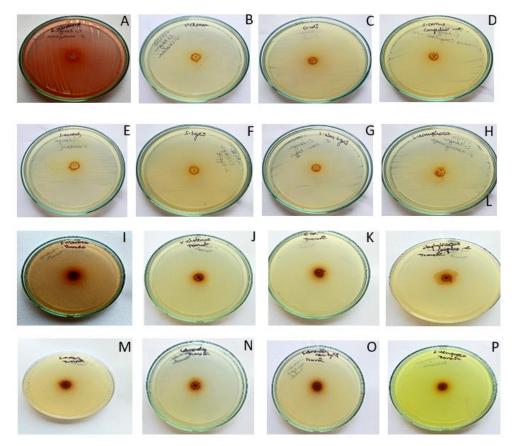


Figure 6. Zone of Inhibition of Koraput turmeric methanolic extract against different bacteria using normally grown rhizome (A) Serratia marcescens; (B) Vibrio cholerae; (C) Escherichia coli; (D) Pseudomonas aureginosa; (E) Salmonella typhi; (F) Staphylococcus aureus; (G) Staphylococcus coagulase -ve; (H) Klebsiella pneumoniae; Zone of inhibition of Koraput turmeric methanolic extract against different bacteria using organically grown rhizome (I) Serratia marcescens; (J) Vibrio cholerae; (K) Escherichia coli; (L) Pseudomonas aureginosa; (M) Staphylococcus aureus; (N) Salmonella typhi; (O) Staphylococcus coagulase -ve; (P) Klebsiella pneumoniae

U.V. Spectroscopy analysis

Due to the sharpness of the peaks and a suitable baseline, the UV-VIS profile of rhizome extract was collected at 200-600nm wavelength. Kandhamal turmeric methanolic extract UV VIS profile peaks at 420 nm, and

absorption of 0.411 was found in the extract (Fig. 7)

Isolation of rhizospheric bacteria through Serial dilution technique

A rhizospheric bacteria were isolated from the rhizosphere. The colonies had different physical

Table 1. Colonies with different morphological characters

Sl. No.	Name	Form	Colour
1.	C. longa (Kandhamal turmeric)	Irregular	White
2.	C. longa (Kandhamal turmeric)	Round	Yellow
3.	C. longa (Koraput turmeric)	Irregular	White

characteristics. From the mother culture plate, a single white colony of bacteria was taken for pure culture (Fig. 8; Table 1).

Gram-negative. After microscopy analysis, the strains were rod-shaped bacillus (Fig. 8; Table 2).

Catalase test

A catalase test was performed on two bacterial

Gram staining

Table 2. Gram staining of rhizobacterial strains of C. longa L.

Sl. No.	Name	Rhizobacterial strains	Gram Staining	Colour
1.	Kandhamal	White colony	Pink	Negative
	turmeric			
2.	Kandhamal	Yellow colony	Violet	Positive
	turmeric			
3.	Koraput	White colony	Pink	Negative
	turmeric			

Table 3. Catalase test of a white and yellow bacterial colony.

Sl. No.	Name	Rhizobacterial strains	Bubble formation	Catalase
1.	Kandhamal	White colony	Yes	Positive
	turmeric			
2.	Kandhamal	Yellow colony	Yes	Positive
	turmeric			
3.	Koraput	White colony	Yes	Positive
	turmeric			

Table 4. pH test of bacteria at different pH levels.

Sl. No.	Rhizobacterial strains	pH value	CFU/ml	Rate of growth
1.	Kandhamal turmeric	5	2.03×10 ⁵	Less
		7	8.7×10 ⁶	More
		9	6.2×10 ⁴	Less
2.	Koraput turmeric	5	2.5×10 ⁵	Less
		7	6.2×10 ⁵	More
		9	5.9×10 ⁵	Less

Gram staining was carried out on two bacterial colonies: yellow and white. According to the results, both colonies had different types of bacteria, with the yellow colony being Gram-positive and the white colony being

colonies of yellow and white. The findings revealed that the yellow colony produces bubbles, as does the white colony, indicating a positive response. After microscopy, the strains were rod-shaped (Fig. 8; Table 3).

pH tolerance test

Bacterial colonies were tested for pH tolerance at

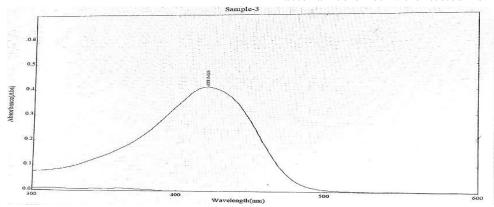


Figure 7. UV-Vis Spectrum of Kandhamal turmeric methanolic rhizome extract.

various pH levels. According to findings, bacterial colonies expanded faster at pH seven than other pH values. Some of them may not be able to develop at a certain pH. As a result, pH 7 is the ideal environment for bacterial colonies to thrive (Table 4).

Temperature tolerance test

The temperature tolerance of bacterial colonies was tested at various temperatures. When bacterial colonies were grown at 37°C, they clustered in clusters and produced more quickly than when grown at different temperatures. Some of them might not be able to grow at

a specific temperature. As a result, bacterial colonies grow best in temperatures between 37°C (Table 5).

Starch hydrolysis test

Based on the starch hydrolysis test results, iodine must be added to the Agar. The starch turns a deep brown colour after reacting with the iodine. Therefore, starch hydrolysis made conditions favourable for the proliferation of bacteria. These bacillus bacteria were found to hydrolyze starch. As a result, starch is detected in the bacillus bacterium (Fig. 8).

Antibiotic sensitivity test

Antibiotics showed a significant inhibition zone

Table 5. Temperature tolerance test of bacteria in different temperature conditions.

Sl. No.	Rhizobacteria l strains	Temperature value	CFU/ml	Rate of growth
1.	Kandhamal turmeric	23°C	4.2×10 ⁵	Less
		30°C	4.5×10 ⁵	Less
		37°C	8.5×10^6	More
		44°C	7.8×10 ⁵	Less
2.	Koraput turmeric	23°C	2.4×10 ⁵	Less
		30°C	3.2×10 ⁶	Less
		37°C	6.3×10^6	More
		44°C	5.1×10^5	Less

Table 6. Antibiotic sensitivity test by taking different extracts.

Sl. No.	Sample	Type of extract	Zone of inhibition
1.	Antibiotic	Amikacin	Sensitive
2.	Kandhamal turmeric	Methanol	Sensitive
		Aqueous	Moderately Sensitive
		Petroleum ether	Moderately Sensitive
3.	Koraput turmeric	Methanol	Sensitive
		Aguagua	Madagatalu Cancitius
		Aqueous	Moderately Sensitive
		Petroleum ether	Moderately Sensitive
4.	Soil rhizobacteria (Kandhamal turmeric)	Amikacin antibiotic	Sensitive
5.	Soil rhizobacteria (Koraput turmeric)	Amikacin antibiotic	Sensitive

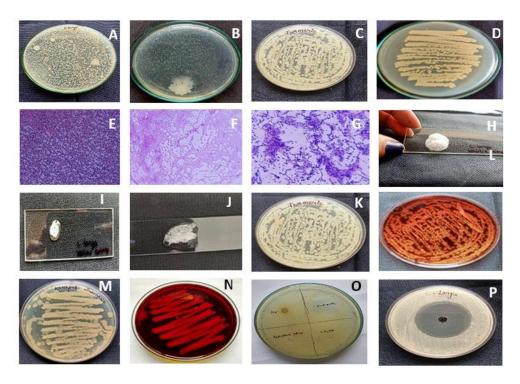


Figure 8. Colonies with different morphological characters. (A) Kandhamal Turmeric; (B) Koraput Turmeric; (C; D) Pure culture of the white colony; Rhizospheric bacteria after gram staining; (E) Yellow colony; (F) White colony; (G) White colony; bubble formation; (H) White colony; (I) Yellow colony; (J) White colony; Starch hydrolysis of soil bacteria. Kandhamal turmeric rhizobacterial strains (K) Before; (L) After; Koraput turmeric rhizobacterial strains (M) Before; (N) After. Growth Inhibition zone (O) Taking different rhizome extracts against V. cholerae; (P) Antibiotic against Kandhamal and Koraput turmeric rhizobacteria.



Figure 9. (A-F). Phytochemical analysis of leaf and rhizome extract.

besides the three extracts (methanol, petroleum ether, and aqueous) against *V. cholerae*. Methanol extract showed a higher inhibition zone than petroleum ether and water extract. The Amikacin antibiotic had the largest inhibition zone and was resistant, whereas the petroleum ether extract of *C. longa* had a minor inhibition zone (Fig. 7; Table 6). Antibiotic sensitivity test against soil bacteria

revealed that the antibiotic amikacin produced maximum Zone. Hence, the soil bacteria are susceptible to the antibiotic.

Phytochemical analysis of rhizome and leaf extract of organically grown turmeric

It was evaluated that different phytochemicals were present in the plant material collected from the

Table 7. Presence of phytoconstituent in leaf and rhizome extracts of organic turmeric.

Phytoconstituent		Leaf extract		Rhizome extract		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
Carbohydrates	++	++	++	++	++	++
Tannin	++	++	++	++	++	++
Saponin	++			++		1
Flavonoid	++	++	++	++	++	++
Glycosides	++	++	++	++	++	++
Terpenoids	++			++	++	++
Steroids		++	++		++	++
Alkaloid		++	++		++	++

organically grown Koraput turmeric in several solvents, including distilled water, ethanol, and methanol extracts (Fig. 9) (Table 7).

XRF Analysis Report

Conventionally grown Kandhamal turmeric rhizome

Rhizome analysis revealed the existence of numerous elements in varied estimating amounts, such as Si (0.183%), P (563.9ppm), S (304.8ppm), Cl (160.7ppm), K (325.3ppm), Ca (316.8ppm), Fe (23.7ppm), Gd (20.7ppm), H₂0 (99.646%) (Fig. 10).

Organically grown Kandhamal turmeric rhizome

The presence of various elements such as Si (743.1ppm), P (0.105%), S (272.4ppm), Cl (286.3ppm), K (0.107%), Ca (213.9ppm), Mn (0.0ppm), Fe (14.4ppm), Dy (20.9ppm), Eu (12.9ppm), Rb (2.5ppm), H₂O (99.632%) (Fig. 10).

Conventionally grown Koraput turmeric rhizome

In XRF analysis, the rhizome growing in regular soil displays its quantity of elements, i.e., Si (0.113%), P (0.173%), S (278.2ppm), Cl (257.9ppm), K (0.236%), Ca (223.0ppm), Mn (23.7ppm), Fe (20.1ppm), Co (0.0ppm), Zn (5.1ppm), Sn (56.1ppm), Eu (25.7ppm), Er (74.6ppm), Re (1.2ppm), H_2O (99.381%) (Fig. 10).

Organically grown Koraput turmeric rhizome

In XRF analysis, the rhizome growing in regular soil displays its quantity of elements, i.e., Si (0.106%), P (0.192%), S (269.1ppm), Cl (232.8ppm), K (0.206%), Ca (232.0ppm), Mn (0.2ppm), Fe (31.5ppm), Zn (7.8ppm), Sn (50.8ppm), Eu (37.9ppm), Re (0.8ppm), H₂O (99.410%) (Fig. 10).

Organically grown Koraput turmeric leaf

XRF analysis of Koraput turmeric leaf displaying its quantity of elements, i.e., Si (0.517%), P (646.0ppm), S (346.5ppm), Cl (526.8ppm), K (0.135%), Ca (930.1ppm), Ti (15.2ppm), Mn (3.6ppm), Fe (40.0ppm), Eu (20.8ppm), Er(39.6ppm), H₂O (99.092%) (Fig. 10).

Jajpur organic soil

The rhizome examination revealed the presence of several compounds in various estimating amounts, such as Al_2O_3 (9.800%), SiO_2 (76.553%), P_2O_5 (1.086%), SO_3 (0.128%), Cl (0.180%), K_2O (3.005%), CaO (1.697%), V_2O_5 (160.4%), TiO_2 (1.018%), Cr_2O_3 (396.6ppm), MnO (0.372%), Fe_2O_3 (5.830%), NiO (105.7ppm), CuO (105.7ppm), ZnO (193.9ppm), Ga_2O_3 (23.4ppm), As_2O_3 (3.7ppm), Br (18.3ppm), Rb_2O (176.9ppm), SrO

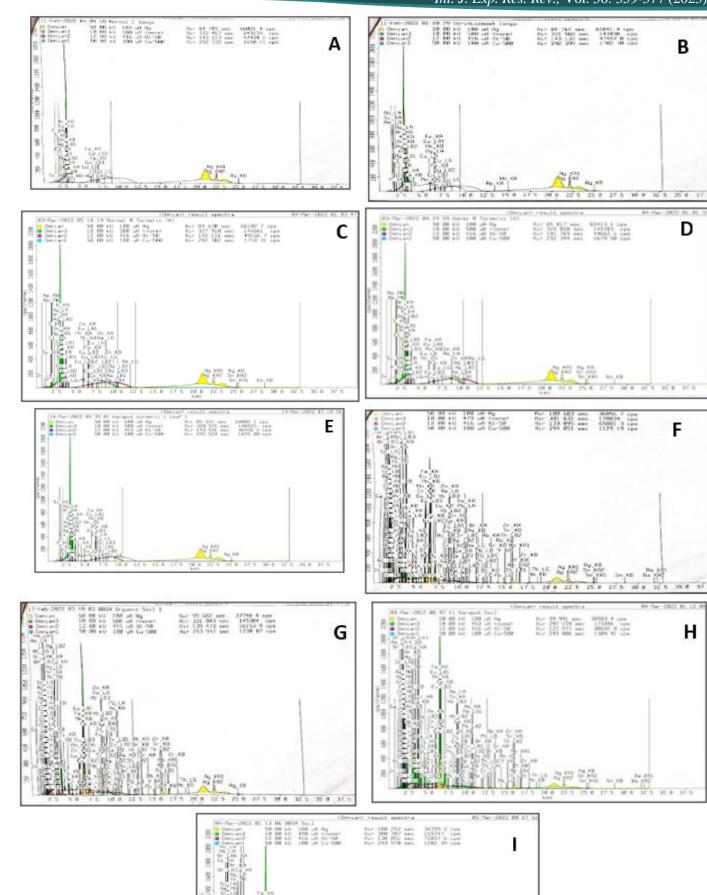


Figure 10. XRF analysis report.

Figure 10. XRF analysis report (A) Normal grown Kandhamal rhizome; (B) Organic grown Kandhamal rhizome; (C) Normal grown Koraput rhizome; (D) Organic grown Koraput rhizome; (E) Organic grown Koraput leaf; (F) Jajpur organic soil; (G) Bhubaneswar normal soil; (H) Bhubaneswar organic soil; (I) Koraput organic soil.

 $\begin{array}{lll} (201.2ppm), \ Y_2O_3 \ (45.0ppm), \ ZrO_2 \ (729.2ppm), \ Nb_2O_5 \\ (20.8ppm), \ Sn_2O_2 \ (90.7ppm), \ BaO \ (342.4ppm), \ Eu_2O_3 \\ (624.5ppm), \ Yb_2O_3 \ (7.2ppm), \ IrO_2 \ (4.9ppm), \ PbO \\ (36.9ppm), \ ThO_2 \ (23.9ppm), \ CO_2 \ (0.0ppm), \ Re \ (5.5ppm) \\ (Fig. 10). \end{array}$

Bhubaneswar organic soil

Rhizome examination revealed the presence of many compounds in various estimating amounts, such as Al_2O_3 (11.798%), SiO_2 (69.592%), P_2O_5 (1.565%), SO_3 (0.469%), Cl (0.252%), K_2O (2.186%), CaO (2.685%), TiO_2 (1.758%), V_2O_5 (475.9ppm), Cr_2O_3 (281.4ppm), Cr_2O_3 (281.4ppm), Cr_2O_3 (120.6ppm), Cr_2O_3 (120.6ppm),

Bhubaneswar conventional soil

In XRF analysis, organic soil displaying its quantity of compound, i.e., Al_2O_3 (20.930%), SiO_2 (59.615%), P_2O_5 (1.012%), SO_3 (0.495%), Cl (0.210%), K_2O (2.272%), Cl (2.101%), Cl (1.808%), Cl (0.210%), Cl (0.210%), Cl (0.210%), Cl (1.808%), Cl

Koraput organic soil

In XRF analysis, normal soil displays its quantity of compound, i.e., Al_2O_3 (16.154%), SiO_2 (61.541%), P_2O_5 (1.321%), SO_3 (0.405%), Cl (0.159%), Cl (0.159%), Cl (3.278%), Cl (2.41%), Cl (3.278%), Cl (2.41%), Cl (3.278%), Cl (3.278

According to research findings, bacterial colonies expanded faster at pH seven than other pH values and

developed in clusters at 37°C. Bacillus bacteria have been shown to hydrolyze starch. Bacillus bacteria help to stimulate plant growth and development and provide adequate nutrients for plant growth and development. Plants have antimicrobial properties due to terpenoids, steroids, saponins, tannins, and flavonoids in their bioactive compounds. Flavonoids, alkaloids, terpenoids, polyphenols, steroids, tannins, and saponins are only a few examples of phytochemicals with essential medical and nutritional applications. Polyphenols like flavonoids can boost the effectiveness of antibiotics against bacteria. Flavonoids are necessary and potent antibacterial substances because they bind to proteins and other extracellular components in bacterial cell walls. Terpenoids contribute to the breakdown of microbial membranes and weakening of cellular walls. When microorganisms come into contact with saponins, the enzyme proteins inside the cell begin to flow out. The steroid component of antimicrobials causes liposomes to rupture their lipid bilayer membranes (Sadhek and Abdullah, 2019).

Aqueous extracts of the rhizome and leaf and soil samples from both soil conditions were analyzed for trace elements in the Kandhamal and Koraput turmeric varieties using ED-X-ray fluorescence (XRF) technology. The sample tested positive for various elements, including Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Rb, Sr, Pb, etc. Various elemental concentrations have been provided (Table 8). The numerous trace elements and compounds found in plants and soil are thought to be vital in regulating diverse pharmacological activities (Table 9). Kandhamal conventional rhizome aqueous extract showed the highest Si concentration, followed by P, while organic Kandhamal rhizome aqueous extract displays the highest P concentration, followed by Si. Koraput turmeric rhizome aqueous extracts showed K as the most effective content, followed by P, in both soil conditions. However, the aqueous extract of Koraput leaves contained the highest percentage of Si, followed by K. Several substances discovered in soil samples from various places affected the plant sample's trace element composition. Differences in structure, soil cultivated area, plant age, environmental conditions, and irrigation methods are the primary causes of these variations in element concentrations (Yelmate et al., 2022).

Si activates transcription factors in a complicated process connected with the plant defence system, increasing plant tolerance to biotic stress. Si can act as a delivery mechanism for herbicides and fertilizers in plants, and it has the potential to be utilized as a fertilizer on its own for some crops. Since Si use in farming has the potential to aid in the creation of high-yielding, hightech crop types, it could also contribute to global food safety and security. Silicon has the potential to offer pollution-free, environmentally beneficial substitutes for many synthetic fertilizers (Song et al., 2021). One of the most important elements for plant growth is phosphorus. It plays a crucial role in cellular division and the formation of the growing tip of plants and is, therefore, a component of plant cells. Phosphorus is an essential mineral for plant growth. It follows nitrogen as the most critical yet limited macronutrient (N). From germination to grain formation to maturity, biological reactions (e.g., photosynthesis) are propelled by phosphorus-containing macromolecules like adenosine triphosphate (ATP). Potassium (K) is essential for healthy plant development and is the most common inorganic cation. It's crucial to the development of yield and quality enhancement. K is also necessary for plant development because it helps cells divide and multiply. It is evident that K has a major effect on plants' ability to absorb and use other nutrients and that the optimal K level varies greatly among different crops (Xu et al., 2020).

The research reveals that some rhizobacterial strains can reduce the negative impacts of water stress on highwater-use crops, allowing them to maintain their normal productivity levels under these conditions (Pereira et al., 2020). White turmeric plants, in particular, could benefit from rhizosphere bacteria because of the bacteria's capacity as a bio-stimulant, biofertilizer, biodegradation agent, and biocontrol product. The study's findings can be used in cultivating plants, particularly white turmeric, by promoting the use of superior isolates of rhizosphere bacteria as a secure and multi-functional biostimulant. Co-inoculation of arbuscular mycorrhizal fungi and Zincsolubilizing bacteria had a positive effect on plant growth, soil dehydrogenase activity, soil respiration, and the overall composition of bacteria in the rhizosphere of turmeric (Curcuma longa), compared to treatments that relied solely on ZSB2 (Bacillus megaterium) or AM (Rhizophagus sp.), respectively (Sarathambal et al., 2022).

According to a study finding, most of the PGPR looked promising for commercialization, and they might

be employed for plant growth stimulation and control of turmeric rhizome rot disease. Rhizome treatments, including Pseudomonas sp. and B. subtilis isolates, showed the highest biocontrol efficiency and the lowest percentage of symptom severity. To combat rhizome rot in turmeric, these isolates with strong antagonistic potential can be utilized in biocontrol programmes (Kharshandi and Kayang, 2023). Dual inoculation with nematodiphila RGK and Serratia Pseudomonas plecoglossicida RGK had greater efficacy than either inoculation alone. These results also indicate that the bioinoculants applied to the turmeric rhizosphere are successful since they show that phenolic compounds and flavonoids have a positive relationship with anti-radical activities. These phytochemicals, alone or in cooperation with others, hold great promise as a future treatment for various medical conditions and drug formulations (Jagtap et al., 2023).

S. sioyaensis TM32 is a novel strain discovered in the rhizospheric region of the turmeric plant (Curcuma longa L.). It showed potent antibacterial properties against a wide range of human and plant diseases, including the antibiotic-resistant pathogen Staphylococcus haemolyticus MR-CoNS, and represents a promising new lead for the identification of useful and novel bioactive chemicals. Antibacterial chemicals are produced by a novel bacterium found in the endophytic and rhizospheric regions of the medicinal plant Acalypha indica Linn. Rhizosphere bacterial strain RU112B and RU315B were the most effective at preventing K. pneumoniae from growing (Rahmawati, 2021).

Native Fluorescent Pseudomonads (FLPs) were isolated from the rhizospheres of many crops such as rice, turmeric, chickpea, mustard, pea, barley, brinjal, and lady finger, and their unique characteristics and prospective uses were recently revealed. The found isolates have useful characteristics such as antibiotic resistance, phosphate solubilization, generation of siderophores, and disease control, providing useful possibilities for long-term agricultural sustainability and disease management. IISR-TB4 (NCBI-MT192800) strain of *B. safensis* has growth-promoting and antifungal properties that could be further utilized to lessen the need for fungicides in turmeric cultivation, making it more environmentally friendly (Roshani et al., 2023).

Increased synthesis or semi-synthesis of secondary metabolites like curcuminoid, ascorbic acid, thymol, gallic acid, etc., has been using for PGPRs as biofertilizers, weed control along with therapeutic applications (Sahoo et al., 2022b). An attractive strategy

Table 8. The detected elemental concentration of aqueous extracts of Kandhamal and Koraput

samples

Elements	Kandhamal Turi	Candhamal Turmeric Rhizome		Koraput Turmeric Rhizome	
	Covnventional	Organic	Covnventional	Organic	Organic
Si	0.183 %	0.07431 %	0.113 %	0.106 %	0.517 %
P	0.05639 %	0.105 %	0.173 %	0.192 %	0.0646 %
S	0.03048 %	0.02724 %	0.02782 %	0.02691 %	0.03465 %
Cl	0.01607 %	0.02863 %	0.02579 %	0.02328 %	0.05268 %
K	0.03253 %	0.107 %	0.236 %	0.206 %	0.135 %
Ca	0.03168 %	0.02139 %	0.0223 %	0.0232 %	0.09301 %
Ti	-	-	-	-	0.00152 %
Mn	-	0%	0.00237 %	0.00002 %	0.00036 %
Fe	0.00237 %	0.00144 %	0.00201 %	0.00315 %	0.004 %
Co	-	-	0 %	-	-
Zn	-	-	0.00051 %	0.00078 %	-
Rb	-	0.00025 %	-	-	-
Sn	-	-	0.00561 %	0.00508 %	-
Re	-	-	0.00012 %	0.00008 %	-
Eu	-	0.00129 %	0.00257 %	0.00379 %	0.00208 %
Gd	0.00207 %	-	-	_	_
Dy	-	0.00209 %	-	-	-
Er	-	-	0.00746 %	-	0.00396 %

Table 9. The detected compound concentration of soil samples was collected from different regions

Compounds	Jajpur Organic soil	Bhubaneswar Conventional soil	Bhubaneswar Organic soil	Koraput Organic soil
Al_2O_3	9.800 %	20.930 %	11.798 %	16.154 %
SiO_2	76.553 %	59.615 %	69.592 %	61.541 %
P_2O_5	1.086 %	1.012 %	1.565 %	1.321 %
SO ₃	0.128 %	0.495 %	0.469 %	0.405 %
Cl	0.180 %	0.210 %	0.252 %	0.159 %
K ₂ O	3.005 %	2.272 %	2.186 %	3.278 %
CaO	1.697 %	2.101 %	2.685 %	4.441 %
V_2O_5	160.4 %	0.05023 %	0.04759 %	0.03163 %
TiO ₂	1.018 %	1.808 %	1.758 %	1.420 %
Cr ₂ O ₃	0.03966 %	0.02329 %	0.02814 %	0.02416 %
MnO	0.372 %	0.192 %	0.304 %	0.299 %
Fe ₂ O ₃	5.830 %	10.899 %	0.0008957 %	10.533 %
NiO	0.01057 %	0.01622 %	0.01206 %	0.0125 %
CuO	0.01057 %	0.01524 %	0.01591 %	0.01795 %
ZnO	0.01939 %	0.02129 %	0.04256 %	0.02926 %
Ga_2O_3	0.00234 %	0.00508 %	0.0047 %	0.004 %
As_2O_3	0.00037 %	0.00157 %	0.00174 %	0.00045 %
Br	0.00183 %	0.00103 %	0.00134 %	0.00349 %
Rb ₂ O	0.01769 %	0.01641 %	0.01767 %	0.0212 %
SrO	0.02012 %	0.00952 %	0.01454 %	0.01924 %
Y_2O_3	0.0045 %	0.00785 %	0.005 %	0.0055 %
ZrO ₂	0.07292 %	0.173 %	0.145 %	0.08603 %
Nb ₂ O ₅	0.00208 %	0.00521 %	0.0052 %	0.00383 %

SnO_2	0.00907 %	0.01332 %	-	0.01311 %
Nd_2O_3	-	0.02519 %	-	-
BaO	0.03424 %	-	-	0.08623 %
Eu_2O_3	0.06245 %	0.0588 %	0.06962 %	0.07346 %
Yb_2O_3	0.00072 %	0.00756 %	0.00708 %	0.00611 %
IrO_2	0.00049 %	0.00099 %	0.00072 %	0.00083 %
PbO	0.00369 %	0.00887 %	0.00581 %	0.00692 %
ThO_2	0.00239 %	0.00569 %	0.00749 %	0.0032 %
CO_2	0 %	0 %	0 %	0 %
Re	0.00055 %	0 %	0 %	0 %

for achieving sustainable agriculture, reviving soil health and production, and promoting the production of secondary metabolites is to employ microbial treatments

for boosting agricultural output via rhizospheric engineering. When added to the spent grain, Azospirillum is highly suggested for ameliorating saline-sodic soil. It is more successful than compost when used to improve the fertility of saline-sodic soils (Ahkami et al., 2017). Organic agriculture approaches have been discovered to give improved growth, nutrient content, and production. As a result, better quality Kandhamal and Koraput turmeric can be grown organically. Organic farming necessitates the utilization of natural wastes components in its production, which is an advantage for tribal peoples. Turmeric grown organically is both safe and valuable for human health. The rhizospheric bacteria isolated from the organic soil region also aid in plant growth and development, providing adequate nutrients for growth and development. It showed better and more effective results on the increase, expansion, nutrient content, antibacterial activity and yield. It is concluded that using an organic farming technique is better for better growth, nutrient content, and outcome. Organic rhizome contributes better activity towards medicinal purposes. Therefore, it is suggested to use pure organic rhizomes for better health and development.

Conclusion

Full traditional knowledge of turmeric may be validated by modern pharmacological investigations focusing on its chemical nature, its influence on various factors, in-depth analyses of the mechanisms behind the observed biological actions, and the findings of molecular investigations. Although this information is insufficient to prove a natural product's safety and usefulness, it warrants additional research. Studies on molecular, phytochemical, and pharmacological effects better understand the aspects contributing to the medicine's safe usage, such as interactions with other medications and

nutritional considerations. If these findings support and explain the traditional uses, a clinical trial in volunteers or patients may be justified for the desired outcome. Koraput turmeric is second only to Kandhamal turmeric regarding phytoconstituents and curcumin concentration. When taken correctly, it has a powerful therapeutic impact. According to the results, organically farmed turmeric is more effective than conventionally grown turmeric. Organic turmeric has more potent antibacterial activity against various human diseases than regular turmeric rhizomes.

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Conflict of Interest

Authors declare no conflict of interest.

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