"Phytochemical Screening and Antimicrobial Investigation of Ethanolic Extract of Curcuma Caesia Roxb"

Sunil S V[1]; Arpitha H P[1]; Pruthvi K V[1]; Varuna kumara J B[1*]; Nethravathi A M[2]
[1] Department of Biochemistry, Sahyadri Science College, Shivamogga
[2] Department of Biotechnology, Sahyadri Science College, Shivamogga

Corresponding Author: **Varuna kumara J B**

Abstract: The investigation into black turmeric (Curcuma caesia) has yielded promising results across several analytical dimensions, highlighting its potential as a valuable natural resource. Phytochemical analysis revealed that black turmeric is rich in a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids. These compounds are known for their therapeutic properties, suggesting that black turmeric could be a significant source of natural bioactive with potential health benefits. In antimicrobial testing, black turmeric extract demonstrated considerable activity against a range of pathogenic microorganisms, indicating its potential as a natural antimicrobial agent. This efficacy supports its application in developing new treatments or preventive measures for infections. Additionally, the antioxidant activity assays confirmed black turmeric's strong ability to neutralize free radicals which is crucial in combating oxidative stress and related diseases. The observed high radical scavenging activity and favourable lethal values underscore its potential as a potent antioxidant. Collectively, these results underscore black turmeric's significant potential in various applications including pharmaceuticals, nutraceuticals, and functional foods. The comprehensive data supports further research and development to fully explore and harness the therapeutic and preventive capabilities of black turmeric which could lead to valuable contributions to health and wellness.

Key Words: Phytochemical; Curcuma caesia Roxb; Antioxidants; Antimicrobial activity

1. Introduction

Herbal medicine or phytomedicine is the major element of the Ayurveda (Muthukaviya et al., 2024), the ancient Indian medical practice that primarily relies on herbs and plants for maintaining good health (Rapoliene & Matuleviciute 2024). Despite being used in India since the Vedic era, the use of herbal remedies has become increasingly popular in the contemporary world in recent years because of its many advantages and little-known drawbacks. The demand for more participation and funding in research and development to find novel medications is growing along with the use of synthetic medications in daily life.(Singh et al., 2024).

Curcuma caesia Roxb, sometimes known as black turmeric, is a rare perennial herb that has therapeutic uses(Kaur et al., 2024).These are majorly present parts of north east and

central India. In India these are found in West Bengal, Madhya Pradesh, Orissa, Chhattisgarh and Uttar Pradesh states. Additionally, it can be found in small amounts in the Himalayan root hills, the North Hill Forest of Sikkim, and the Papi Hills of East Godavari, West Godavari, and Khammam district of Arunachal Pradesh. Compared to yellow turmeric (Curcuma longa), which has been extensively researched and its many benefits have been established, this plant has received less attention. It is a member of the Zingiberaceae family which consists of more than 70+ species of resembling rhizome herbs.The rhizome of black turmeric has tremendous medicinal properties(Ibrahim et al., 2023; Kanglom et al., 2023).

It has historically been used to treat a variety of conditions, including gonorrhoeal discharge, inflammation, anthelmintic, aphrodisiac, leprosy, cancer, epilepsy, fever, wounds, vomiting, menstrual disorders, smooth muscle relaxation, hemorrhoids, and inflammation (Chadalavada et al., 2017). According to Madhya Pradesh, the plant is considered extremely fortunate and a person who owns it will never run out of food or cereals(Behar et al., 2014).

The plant rhizome paste wasusefulfor bruises, rheumatic pains, anti-diarrhea and to relieve stomach-aches.The group of some tribes in Lohit district uses a rhizome paste to treat scorpion or snake bites. In Assam the cattle'sare treated with rhizome juice combined with mustard oil while havingdysentery.The fresh rhizome scents strong and camphoraceous. It tastes gritty and bitter, and these pastes used forcommon colds, coughs and pneumonia. The herb's rhizomes are used as a rubeficient to massagethe body. The rhizome of C. caesia is also used to treat asthma and fever. In north eastern India, rhizome powder is applied as a face mask. For migraine relief, fresh rhizomes are mashed and applied as a paste to the forehead; for injures and bruises, it is administered to the body. Rhizomes have anti-cancer, anti-epilepsy, anti-leukoderma, and anti-HIV/AIDS properties. Menstrual issues are said to be cured and stomach gasses released by consuming a modest amount of rhizome paste. There are several uses for the plant's aromatic, essential oil-containing rhizome(Sumi et al., 2024; Borah et al., 2020; Haida et al., 2023; Sharma et al., 2019).

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Fig 1. Black turmeric plant& tuber

2. Materials and Methods

2.1 Collection of plant material

Fresh rhizomes of Black turmeric were collected from the farm regions of Kyasanooru of Shivamogga district, Karnataka, India in the month of May.

2.2 Extraction process of sample 1

Collected rhizomes were washed thoroughly and chopped into small pieces and shade dried and coarsely powdered, the powdered rhizome was extracted with Soxhlet extraction method by using 99% ethanol until the solvent become discolour in tumble. The obtained extract was concentrated by evaporating ethanol.

2.3 Extraction process of sample 2

Freshly collected rhizomes were washed thoroughly and grinded them in a motor and pestle with sufficient water.Collect the turmeric homogenate into a beaker and added enough water. Filter the homogenate by using a muslin cloth and allow filter to settle.The white settled starch like substances at the bottom were separated by decanting the supernatant carefully. Wash the settled particles 3-4 times by using filtered water and decant the supernatant again, Collect the compact white material mass wasdried and used as for analysis.

2.4 Solubility test for sample

In this test we use different types of solution like phosphate buffer (pH 7), saline (pH 7), 5% NaOH, 10 % NaOH, 5% HCl, 10% HCl, and distilled water to check the solubility of the extracted Curcuma caesia Roxb (Black Turmeric) samples.

2.5 Estimation of total carbohydrates

The total carbohydrate were estimated by using DNS reagent assay. About 0.5gm/ml of extract was weighed accurately and homogenised with distilled water. From the homogenised mixture (200µg, 400µg, 600µg, 800µg and 1000µg) concentrations of aliquots were taken and 0.5 ml of DNS reagent was added, the mixture was incubated for 10 minutes in a boiling water bath. After incubation the tubes were cooled to room temperature and diluted with 6ml of distilled water agitated and the absorbance was measured at 540 nm. The volume of extract was replaced with solvent to create the blank and the results are compared with the standard maltose curve (Sarkar et al., 2024).

2.6 Estimation of total protein

The total protein was estimated by using the Folin-Coicalteu method (Lawag et al., 2023), 0.5gm/ml of extract was weighed accurately and homogenised with distilled water. From the homogenised mixture (20µg, 40µg, 60µg, 80µg and 100µg) concentrations of aliquots were taken, for this aliquots 5ml of alkaline solution and 0.5ml of 1:1 diluted Folin-Ciocalteu reagent was added and agitated, the mix was further incubated at room temperature for 30minutes and absorbance was measured at 720nm by using UV- visible

spectrophotometer. The extracts were substituted with the equal volume of distilled water to make the blank. Similar procedures are carried for both the samples and the results are compared with the standard BSA.

2.7 Phytochemical analysis

Phytochemicals analysis of extracts of Curcuma caesia Roxb (Black Turmeric) samples were carried qualitatively to detect the existence of different phytoconstituents(Gonfa et al.,2020). The test sample extracts for both the sample 1 and 2 were prepared by addition 1:1 w/v of extract plus distilled water. The solution was agitated to homogenise and further taken for the qualitative tests.

2.8 Estimation of total phenols

The Folin–Ciocalteu reagent test was used to estimate the total phenol concentration(Perez et al.,2023).The extracts of different concentrations (20µg, 40µg, 60µg, 80µg and 100µg) were prepared with methanol. Distilled water about6 mL and 500 µL of Folin–Ciocalteu was added to this and assorted thoroughly. After proper mixing 1.5 mL of 20% Na_2CO_3 and 1.9 mL of distilled water was added and incubated in dark area for two hours. After incubation the absorbance of light was measured at 760 nm.The same process was conceded for standard Gallic acid. Volume of extract was replaced with methanol to create the blank. The experiments are carried in duplicates for both samples.

2.9 DPPH Radical Scavenging Assay

In accordance with Asfaw et al., 2024, DPPH radical scavenging activity will be quantified. The 20µL of extract is incubated with 140µL of DPPH solution (6.2 mg in 100 mL of 100% ethanol) for 30 minutes at room temperature in the dark. After 30 minutes, the absorbance was measured at 536 nm and the optical density values are compared with ascorbic acid standards.The findings will be presented as a percentage of the activity that scavenges free radicals.

2.10Detection of Curcumin by TLC

About 6-10gm of TLC grade Silica gel was weighed accurately, gently mixed with 10-15 ml of distilled water till the consistency of the solution into slurry. The mixture was poured on clean glass plate to form a thin layer of silica gel, activationof TLC plate was carried by incubating at 100°C for overnight further it was utilised for the chromatographic identification of curcumin.0.2gm/ml of ethanol and water homogenate, sample 1 and sample 2 respectivelyofblack turmeric was prepared.Spot 1-2µL of extracts onto the TLC plate using capillary tube, about 1cm above the solvent (5ml of chloroform in 95ml of methanol). Place the TLC plate in the closed chamber with the mobile phase. Allow the mobile phaseto go $3/4^{th}$ upwards(depending on the solvent and plate).After taking the plate out of the chamber, allow it to dry and expose the plate to UV light (254 nm) in UV light chamber to detect curcuminoid spots. Compare the sample spots with standards.

Identify the presence of curcuminoid based on matching by observing the spots(Janssen &Gole 1984).

2.11Estimation of total tannins

The Folin-Ciocalteu method was used to determine the total crude tannins (Zhang et al., 2023).A clean test tube with 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, and 1 mL, of 35% sodium carbonate solution was taken and 0.1 mL of rhizome extract was added, (sample 1 & 2 were added separately) total volume was made to 10 mL with distilled water, agitate and kept in room temperature for 30min and recorded the absorbance. Tannic acid standard solutions (20, 40, 60, 80, and 100 µg/mL) were made in concurrence with the procedure. By using UV/visible spectrophotometer the absorbance of the test samples and standard solutions was measured at 725 nm in relative to the blank. Estimations are carried in duplicates and further the total tannin content (TTC) was compared with the standard graph.

2.12 Antimicrobial Assay

To conduct antibacterial activity nutrient broth was prepared by adding 0.6gm of beef extract, 1gm of peptone, 0.1gm NaCland made up to 200ml with distilled water with pH 7 and these mixes are gently agitated and poured into clean test tube and were closed with cotton plug, packed and sterilized. Sabourauds dextrose broth was prepared for antifungal activityand the composition consist of 0.5gm of peptone, 2gm of dextrose dissolved and made up the final volume to 50ml with distilled water, pH 5.6 and these mix are gently agitated and poured into clean test tube and were closed with cotton plug, packed and sterilized.Bacterial cultures such asEscherechia coli, Staphylococcus aureus, Enterococcus fecalis and fungal cultures such as Pencillium, SclerotiumandAspergillus flavus are inoculated into specific medium and added with different concentration (25%, 50%, 75% and 100%) of both sample 1 and 2seperately. Antibacterial activity was carried by incubatingrespective tubes at 37°C for 16 hrs and for antifungal activity respective tubes were incubated at 32°C for 32-48 hrs. After the incubation, the percentage of turbidity was determined by comparing with the blank tube and the results interprets that turbidity is directly proportion to growth of microbes. These results are compared with the positive standard antibiotics (Sunil et al., 2022).

3 Results

3.1Sample preparation

Black turmeric rhizome powder | Polar Solvent (Ethanol) | Soxhlet extraction and filtration | Extracted sample

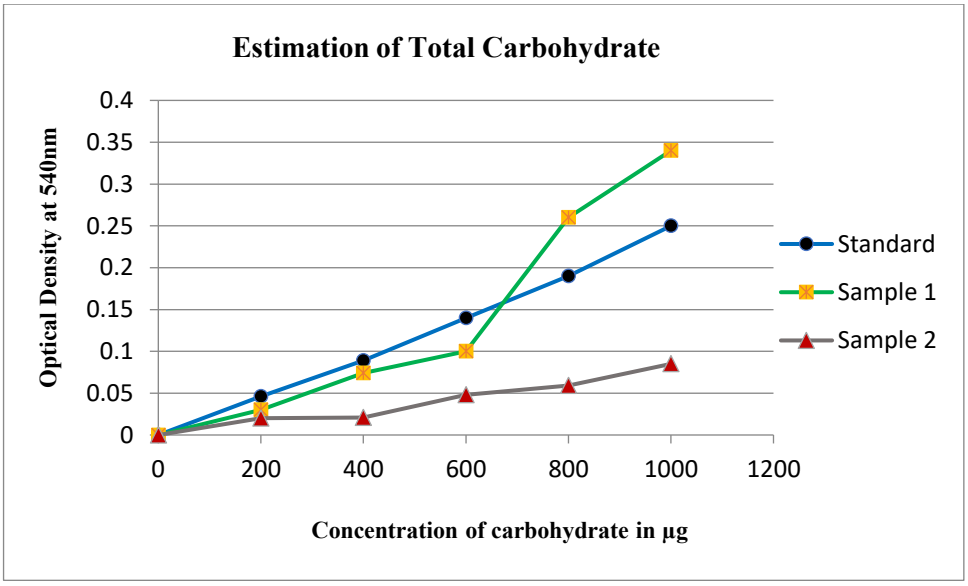
Black turmeric rhizome powder was weighed accurately and extracted using soxhlet extraction method with polar (Ethanol)solvent. For 50 gram of black turmeric rhizome powder 8 gram of extract was obtained.

3.2 Solubility test

Solutions	ample 1	ample 2
Phosphate buffer	Positive	Negative
Saline	Positive	Negative
5% NaOH	Positive	Negative
10%NaOH	Positive	Negative
5% HCl	Positive	Positive
10% HCl	Positive	Positive
Distilled water	Negative	Positive

Table 1: The given table showing the black turmeric extract sample 1 & sample 2 solubility in different solutions.

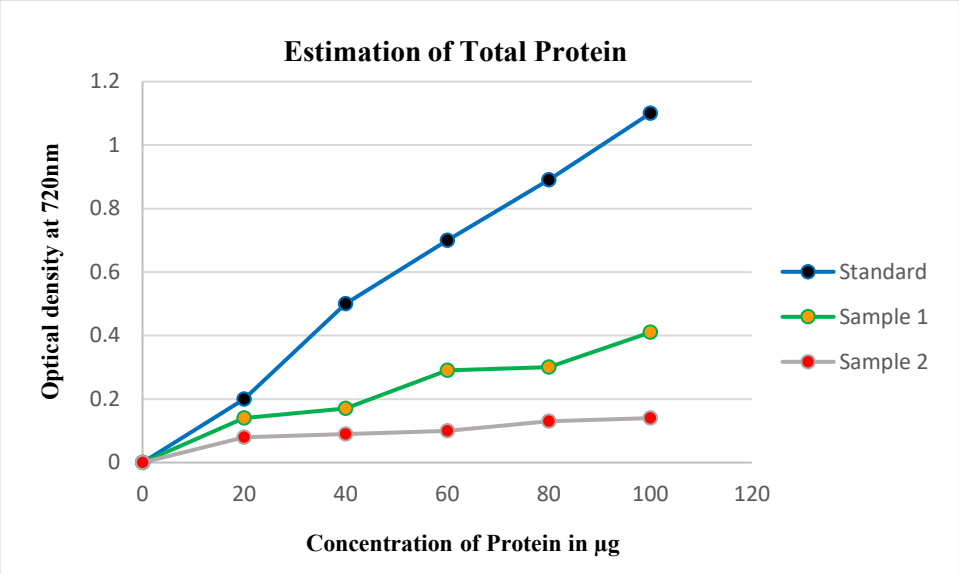
3.2 Estimation of total carbohydrates



Graph 1: The graph represents the total carbohydrates estimated in black turmeric extracts. In x axis, the concentration of maltose was taken and in y axis the absorbance

was measured at 540nm. Sample 1 shows higher concentration and Sample 2 shows lower concentration of total crude carbohydrate compare to Standard maltosecalibration curve.

3.2 Estimation of total Proteins



Graph 2: The graph represents the total proteins estimated in black turmeric extracts. In x axis, the concentration of standard total proteins was taken and in y axis the absorbance was measured at 720nm and the sample 1 and sample 2 shows very less amount of crude protein compared to Standard BSA.

3.3 Phytochemical analysis

Test		Procedure	Observations for presence	Inference	
				Sample 1	Sample 2
Detection of alkaloids					
	Picric acid test	0.1mL sample+ 3-4 drops of 2% picric acid solution	An orange coloured complex	Negative	Negative
	Iodine Test	To 3mL of test sampleadd2-3 drops of iodine solution	A deep blue colour formation, which disappears after boiling and reappears after cooling	Negative	Negative
Detection of Carbohydrates					
	Barfoed's test	1mL sample + 1mL Barfoed's reagent+ heat for 2 min.	A red precipitate at the bottom of test tubeinfers {monosaccharides}	Negative	Negative
2)	Molisch's test	2mL sample+ 2 drops of α–Naphthol + 1mL conc. H_2SO_4 along the sides of test tube	A violet ring between the junction of two liquid	Positive	Positive

	Seliwanoff's Test	1mL sample + 3mL seliwanoff's reagent+ heat on water bath for 5 min.	cherry red colour rmation {ketoses}	Negative	Negative
	Resorcinol test	2mLsample + 1-2 crystals of resorcinol + equal volume of conc. HCland heat	A cherry red colour formation {ketones}	Positive	Negative
	Test for starch	3ml sample + 5mL 5% KOH solution	A cinary colouration	Negative	Negative

Detection of Reducing sugars					
	Benedict's test	0.5mLsample + 0.5mLBenedict's agent+ Boil for 2min on water bath.	Brick red colour/ light green colour /yellow colour formation	Positive	Negative
	Fehling's test	mL of Fehling's solution & &B+ 1mL sample+ boil in water bath for 5min	A red coloured precipitate	Negative	Negative
Detection of Glycosides					
1)	0% NaOH test	mL dil. H_2SO_4 + 0.2mL ample + boil for 15min, left for cooling and neutralize with 10% aOHthen add 0.2mL of ehling's solution A & B	A brick red coloured precipitate	Negative	Negative
2)	queous NaOH test	ample dissolved in 1mL of water and add few rops of aqueous NaOH solution	A yellow colour formation	Positive	Negative
3)	Concentrate H_2SO_4 test	5mL sample + 2mL glacial acetic acid + a drop of 5% $FeCl_3$and conc. H_2SO_4along the sides of the test tube	brown ring formation etween the junction of two liquid	Positive	Positive
Detection of Cardiac Glycosides					
	eller-Killani test	1mL sample + 1.5mL glacial acetic acid + 1 rop of 5% ferric chloride olution + conc. H_2SO_4 (along the side of test tube)	blue coloured solution (in acetic acid layer)	Negative	Negative
	Kedee's test	mL of testsample add1-2mL methanol + 1-2mL alcoholic potassium ydroxide solution + 3-4 rops of 1% alcoholic 3,5-initrobenzene and heat the mixture	A disappearing violet colour {Cardenolides}	Negative	Negative
	Bromine water test	sample + 0.1mL of bromine water	A yellow precipitate formation	Negative	Negative

Detection of Proteins and Amino acids					
	Biuret test	To 2mL sample + 1 drop of 2% copper sulphate sol. Add 1mL of 95% ethanol + KOH pellets	Pink coloured solution (in Ethanolic layer)	Negative	Negative
	Ninhydrin test	mL sample + 2 drops of Ninhydrin solution	A purple coloured solution {Amino acids}	Negative	Negative
	Xanthoproteic test	Sample + Concentrated Nitric acid	A yellow coloured solution	Negative	Negative
Detection of Flavonoids					
	Alkaline reagent test	mL sample + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour becomes colourless on addition of dilute acid	Positive	Negative
		Sample + 10% ammonium hydroxide solution.	A yellow fluorescenceappearance	Negative	Negative
	ead acetate test	mL sample + few drops of 10% lead acetate solution	A yellow precipitate	Negative	Negative
	Ammonia test	Sample + 5mL dil. Ammonia solution + conc. H_2SO_4	A yellow colour	Negative	Negative
	onc. H_2SO_4 test	Sample + conc. H_2SO_4	An orange colour	Negative	Negative
Detection of Phenolic compounds					
1)	Iodine test	mL Sample + few drops of dil. Iodine solution	A transient red colour	Positive	Positive
2)	Ferric chloride test	Sample + few drops of 5% ferric chloride solution	Dark green colour or bluish black colour	Negative	Negative
3)	Gelatin test	Sample + 1% gelatin solution + 10% NaCl	A white precipitate	Negative	Positive
4)	ead acetate test	Sample + 3mL of 10% lead acetate solution	A white precipitate	Negative	Negative
5)	llagic Acid Test	Sample + 5% glacial cetic acid + 5% sodium nitrite solution	Solution turns muddy Niger brown precipitate	Positive	Negative
6)	Potassium	Sample + few drops of	A dark colour	Positive	Positive

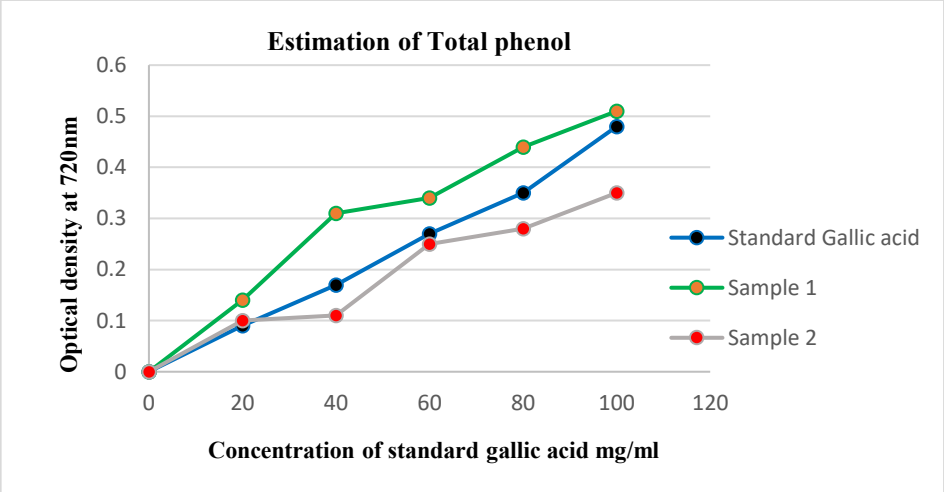
	lichromate test	potassium dichromate solution			
7)	Test for Cartenoids	(1gm extract + 10mLchloroform, vigorously shaken and ltered) obtained filtrate dded with conc. H_2SO_4	A blue colour at the interface	Negative	Negative
Detection of Tannins					
1)	Gelatin test	Sample + 1% gelatin solution + 10% NaCl	A white precipitate	Negative	Negative
2)	Braymer's test	1mL Sample + 3 drops 10% Ferric chloride solution	Blue-green colour	Negative	Negative
3)	0% NaOH test	4mL Sample + 4mL 10% NaOH + shaken well	Formation of emulsion {Hydrolysable tannins}	Negative	Negative
4)	Bromine water test	ml of bromine water + 0.5gm extract	Decolouration of bromine	Negative	Positive
5)	ead sub acetate test	mL Sample + 3 drops of lead acetate solution	A creamy gelatinous precipitate	Positive	Positive
Detection of Phlobatannins					
1)	HCl test	2mL sample + 2mL 1% Cland boil the contents	A red precipitate	Negative	Negative
Detection of Saponins					
1)	Foam test	0.5gm extract + 2mL water (vigorously shaken)	Persistent foam for 10 min.	Negative	Negative
		2mL water + 0.5gm extract (vigorously shaken for 15 min.)	ormation of thick layer of foam	Negative	Negative
		0.2gm extract + 5mL distilled water; shaken well; heated to boiling	Appearance of creamy miss of small bubbles	Negative	Negative
2)	$NaHCO_3$ test	ample + 0.1mL sodium bicarbonate solution + distilled water (vigorously shaken)	Stable honeycomb like froth	Negative	Negative
Detection of Phytosterols					
1)	alkowski's test	Sampl	Red colour (in lower	Positive	Positive

		e + few drops of conc. H_2SO_4 (Shaken well and allowed to stand)	layer)		
	esse's response	5mL sample + 2mL hloroform + 2mL conc. H_2SO_4	ink ring / Red colour (in ower chloroform layer)	Positive	Positive
	ılphur test	Sample + pinch of sulphurpowder	Sulphur sinks to the bottom	Positive	Positive
Detection of Triterpenoids					
	ılkowski's test	Sample + few drops of onc. H_2SO_4 (Shaken well and allowed to stand)	Golden yellow layer (at the bottom)	Negative	Negative
Detection of Diterpenes					
	opper acetate st	Sample + 3-4 drops of copper acetate solution	Emerald green colour	Positive	Negative
Detection of Quinones					
	lcoholic KOH st	1mL Sample + 0.1mL alcoholic KOH	Red to blue colour	Negative	Negative
	onc. HCl test	Sample + conc. HCl	A green colour	Negative	Negative
Detection of Anthraquinones					
	ornträger's test	10mL 10% ammonia solution + 0.5ml Sample (shaken vigorously for 30 sec.)	A pink, violet, or red coloured solution	Negative	Negative
Detection of Anthocyanins					
	Cl test	mL Sample + 2mL 2N HCl (+ 0.1mL ammonia)	ink-red sol. which turns lue-violet after addition of ammonia	Negative	Negative
Detection of Carboxylic acid					
	ffervescence st	mL Sample + 1mL sodium bicarbonate solution	Appearance of Effervescence	Negative	Negative
Detection of Gums and Mucilage's					
	lcohol test	Dissolve 100mg extract in 10mL distilled water + 25mL absolute alcohol (constant stirring)	White or cloudy precipitate	Negative	Positive

Detection of Resins					
1)	Turbidity test	1mL Sample + 2 drops cetone, poured into10mL istilled water + 20mL 4% HCl	Turbidity	Negative	Positive
Detection of Fixed Oils and Fat					
1)	Spot test/ Stain test	.2gm of extract is pressed n between to filter papers	Oil stain on the paper	Negative	Negative
2)	Saponification test	2oml sample + 2oml of 0.5N alcoholic KOH + .2ml of phenolphthalein boiledgently with reflux)	oap formation or partial neutralization of alkali	Negative	Negative

Table 2: The table showing the phytochemical constituents present in the extract of black turmeric sample 1 and sample 2.

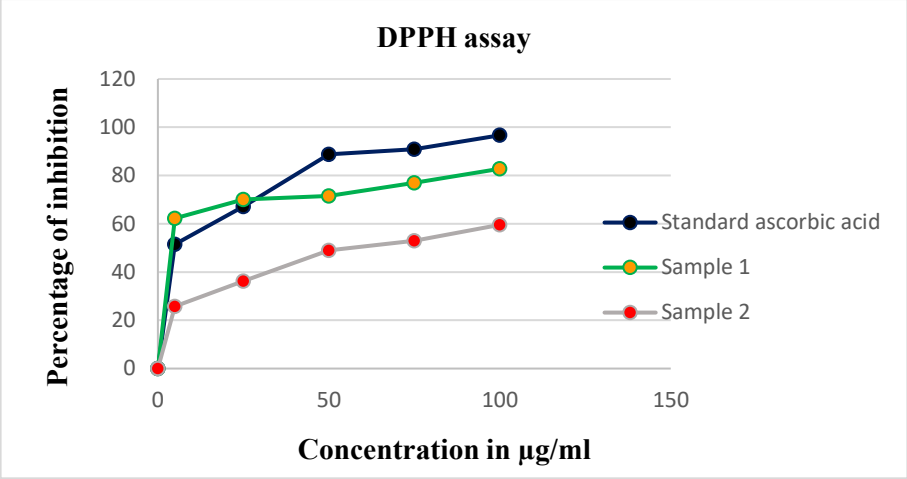
3.6 Estimation of total Phenols



Graph 3: The graph represents the total phenol content estimated in black turmeric extracts. In x axis, the concentration of standard Gallic acid mg/ml was taken and in y axis the absorbance was measured at 720nm and the sample 1 shows higher phenol content than standard and sample 2 shows lesser phenol content compared to standard.

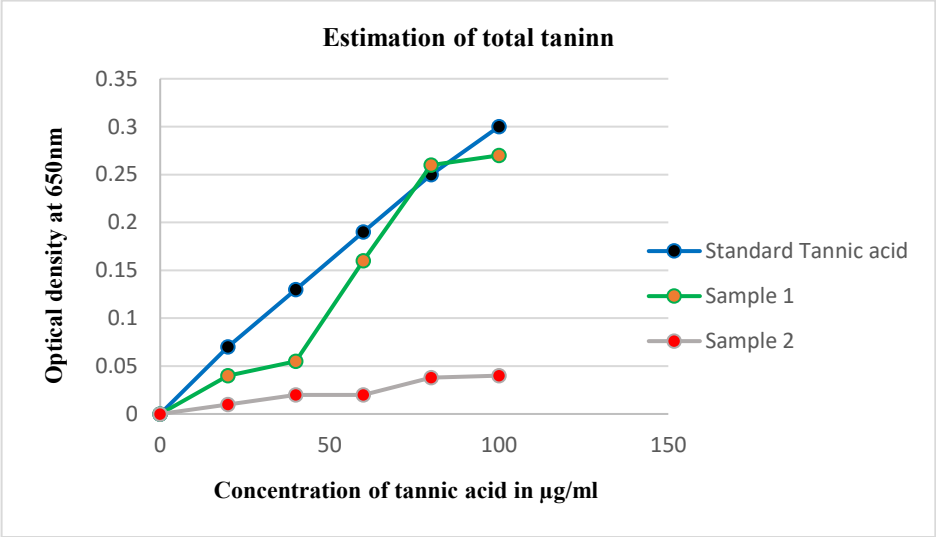
3.7DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was done to evaluate the antioxidant activity of black turmeric samples. The percentage of DPPH radical inhibition was calculated for each concentration of the samples and the results were shown in the graph.



Graph 4: The graph represents the DPPH assay or radical scavenging activity of black turmeric extracts. In x axis, the concentration of standard ascorbic acid was taken and in y axis percentage of inhibition was taken. The sample 1 and sample 2 both shows radical scavenging activity and the percentage of inhibition is directly proportional to concentration of extract.

3.8 Estimation of total Tannins



Graph 5: The graph represents the total tannins estimated in black turmeric extracts. In x axis, the concentration of standard total tannic was taken and in y axis the absorbance was measured at 650nm. The sample 1 and sample 2 shows presence of tannins in crude extracts.

3.9 Detection of Curcumin by TLC

After developing the TLC plate in a combination of methanol: chloroform solvents, yellow spots observed under the UV light.On the developed TLC plate, yellow coloured stains are spotted for theboth sample 1 andsample 2. This results shows the presence of curcumin in black turmeric.

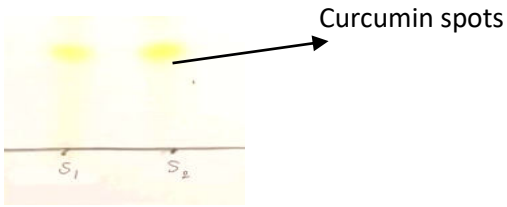


Fig 2. TLC plate with curcumin spots

3.10 Antimicrobial Assay

3.10.1 Anti-bacterial activity

The most pronounced activity was shown by sample1- ethanol extract maximum activity against Escherichia coli at all different concentration. Compared to sample 2 maximum activity was shown by sample 1 against Staphylococcus aureus with 75% concentration and maximum activity of sample 2 against Staphylococcus aureus was seen in 100% concentration, sample 1 against Enterococcus fecaliswith 25%, 75% and 100% concentration shown maximum activity, sample 2 shows the minimum activity against Enterococcus fecalisat all concentration. This concludes that the maximum activity was shown by the ethanol extract sample 1 than that of sample 2 showed less activity.

3. 10.2 Anti-fungal activity

The maximum activity was shown by the sample 1 with 25%,50%, and 100% concentration than the sample 2.Sample 1 shown maximum activity against Pencillium with 75%, 100% concentration extract and with 50%, 100% concentration showed activity against Sclerotium while sample 2 shown the maximum activity against Sclerotium with 25%, 75%, 100% concentration extract. Sample 1 shown the maximum activity against Aspergillus flavus with 25%, 50%, 75%, 100% concentrations, while sample 2 shown the maximum activity at 75% concentration only. This shows that sample 1 shown the maximum activity against Aspergillus flavus and Pencillium compared to sample 2. Sample 2 shown the maximum activity against Sclerotium than sample 1.

4 Discussion

Herbal medicines may be used as alternative to the allopathic medicine in future days, as because to overcome side effects that are causing by the allopathic medicines. Hence from the ancient Indian traditional medicine practice is stick to the herbal medicine to treat disease and infections and also to enhance the health and wellbeing. The Curcuma caesia Roxb (Black Turmeric), plant and their products (active, natural principles and crude extracts) that have been mentioned or used in the Indian traditional system of medicine and have shown experimental, clinical and pharmacological activities. Although many phytochemicals have good medicinal qualities, they are not stable in aqueous solutions or adequately soluble in pure water. Because of their low water solubility, which poses serious hazards of poor bioavailability, high dose requirements and unfavourable side effects. Phytochemicals expansion in the pharmaceutical industry

is constrained. This, in turn, hinders the full utilization of these plant resources. Enhancing the solubility and bioavailability of phytochemicals through the creation of biocompatible solvents with high dissolving capacities is an easy and effective technique that demonstrates extremely important practical significance to meet widespread application requirements. In this contrast we checked for solubility (Table 1) of black turmeric extracts and we obtain that the sample1 is soluble in phosphate buffer, saline, 5% NaOH, 10% NaOH, 5% HCl and 10% HCl. Sample 2 is soluble in 5% HCl, 10% HCl and Distilled water.The carbohydrates are usually seen very much in the leaves of plants than the other parts of plants. These carbohydrates are very much important molecules which are directly involved in transfer & storage of energy, structural support, plant growth & support, involved in photosynthesis, acts as elicitors and signalling molecule like phytohormones and carbohydrate binding lectin molecules or proteins plays a role in plant immunity. In our studies the total carbohydrate was estimated and reported that the black turmeric extracts have very good amount of carbohydrate in higher concentration of extract than in lower. (Graph 1). Proteins are the another molecules which are seen commonly with the carbohydrates plants these are also very much important molecules involved as enzymes in biosynthesis & photosynthesis, plant proteins such as thionins, lectins and plant defensin are majorly takes part as plant defensive molecules against various plant pathogens. Mainly plant proteins are involved in plant cell growth, structure and ionic regulations. In our studies the concentration of protein are observed that it is directly proportional to amount of extracts taken (Graph 2). The table (2) represents the different antioxidants/phytochemicals that are present in black turmeric crude extract samples. Which interprets that it has many phytochemicals/ antioxidants that definitely results in the free radical scavenging activities and summarises that the work consisting of extract, is a promising and may be a potent antioxidant agent. Antioxidants that are present in the plant play a vital role in controlling the cell damage by inhibiting the free radical activities. Phenolic compounds present in plants induce a redox properties that imparts the antioxidant activity in the plants. The release of phenolic compounds or polyphenols such as defensive molecules are due to the aggression of parasites, predators, harmful pathogens and ultraviolet radiations. These phenols are also called as the secondary metabolites responsible for plant colouration. In our study the presence of phenols in both the samples are compared with the standard Gallic acid (Graph 3).The DPPH assay was used to determine the scavenging capacity of antioxidants that are present in the samples. In our studies both the samples are having antioxidants hence they shown a very good results for scavenging capacity and percentage of inhibition at the higher concentration than the lower one compared to standard ascorbic acid graph (Graph 4). Tannins are another protective molecules that are present in plants. These molecules act as toxic substances against the pathogens. Tannins are generally present in the leaves, roots, geminating buds-seeds, majorly protects plants from biotic stress and helps in wound healing. Tannins are also acts as pigment structural components with antioxidant properties. In our studies the black turmeric extract sample 1 have good amount of tannin at higher concentration than

the sample 2 (Graph 5) this results shows that both the samples are having tannin contents that involved in plant protection.Curcumin are potent molecule that are abundantly present in the turmeric species. In our studies the presence of curcumin was identified (Fig 2) and thus it confirms that the black turmeric extracts are having another supporting polyphenol or antioxidant molecule that known as a plant derived immunomodulatory molecules that involved in scavenging free radicals such as reactive oxygen species and nitrogen species also inhibit the enzymes responsible for ROS generation and thus upregulate plant health management and plant defence system. By comparing with all the different percentage extract samples 1 and 2, the results shows that the black turmeric is having a potent antimicrobial activity in both the samples and results in inhibiting the growth of the pathogenic microbial mass.From the study it was known that black turmeric rhizome is one of the potent herbal medicinal tuber that is having very good free radical scavenging activity and antimicrobial activity present in it and in future it may be used as traditional herbal medicinal product.

5 Conclusion

The investigation into black turmeric (Curcuma caesia) has yielded promising results across several analytical dimensions, highlighting its potential as a valuable natural resource. Phytochemical analysis revealed that black turmeric is rich in a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids. These compounds are known for their therapeutic properties, suggesting that black turmeric could be a significant source of natural bioactives with potential health benefits. In antimicrobial testing, black turmeric extract demonstrated considerable activity against a range of pathogenic microorganisms, indicating its potential as a natural antimicrobial agent. This efficacy supports its application in developing new treatments or preventive measures for infections. Additionally, the antioxidant activity assays confirmed black turmeric's strong ability to neutralize free radicals which is crucial in combating oxidative stress and related diseases. The observed high radical scavenging activity and favorable lethal values underscore its potential as a potent antioxidant. Collectively, these results underscore black turmeric's significant potential in various applications including pharmaceuticals, nutraceuticals, and functional foods. The comprehensive data supports further research and development to fully explore and harness the therapeutic and preventive capabilities of black turmeric which could lead to valuable contributions to health and wellness.

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