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Phytochemical Screening And Antimicrobial Potential Of Turmeric Varieties Grown In Uttarakhand Region

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Abstract
Curcuma longa is a -well-known medicinal herb that has been used to cure a variety of illnesses in traditional medicine. In the present study, four different turmeric varieties procured from Almora and Pithoragarh district of Uttarakhand were evaluated for antimicrobial activity and curcumin content. The antibacterial activity of turmeric species were tested against Bacillus species, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa at different concentrations of the extract by using a disc diffusion method. Vancomycin and Lenezoid was the control. The ethanolic extract with compared to aqueous plant rhizome extract, Curcuma longa shown greater potential to suppress test pathogenic bacteria and fungi, with zones of inhibition ranging from 3 mm to 10 mm and 4 mm to 9 mm, respectively. The in vitro inhibition effect was determined by the agar well diffusion method. The findings demonstrated that the turmeric plant had antibacterial properties against specific species and might be very beneficial to the pharmaceutical business in developing medications to treat illnesses and managing the serum lipid profile of the sector. Therefore, it is advised that more research be done on it in order to determine whether it may be used as a plant medicinal treatment, particularly at this time when herbal medications are becoming more and more significant as antibacterial agents.

Key Words: *Turmeric, Curcumin, Zingiberaceae, Curcuma longa, Curcuma zedoaria etc.*

INTRODUCTION

Turmeric also referred to as Harida in Sanskrit, Yellow ginger Jianghuang in Chinese, Kyo in Japanese. Turmeric is a rhizomatous perennial plant of the *Zingiberaceae* family extensively cultivated in tropical and subtropical regions of the world. India is the largest global producer of turmeric. *Curcuma longa* is the most commonly recognized species of the genus *Curcuma*. Nevertheless, *Curcuma zedoaria, Curcuma aromatica,* and *Curcuma rakatakanta* are further species in this genus. (Ciuca *et al.*, 2023). It is widely grown throughout India and is commonly known as "haldi" there. Turmeric is well-known for its therapeutic qualities and is frequently used as a spice and coloring ingredient (Luthra *et al.*, 2001). Curcumin, sometimes referred to as diferuloylmethane, is the primary active component of *Curcuma longa L.* (Turmeric), a yellow Indian spice that is native to Southeast Asia and is derived from the ginger family (Zingiberaceae). It is utilized in Worldwide as spice and coloring ingredient in textiles, medicines, confections and cosmetics in its dried and powdered form (Manasa et al.,2023). Many botanical supplements available on the market contain turmeric powder or extract, and turmeric in the form of different crude extracts has demonstrated remarkable antibacterial efficacy against a range of gram-positive and gram-negative strains, such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* (R.K. et al., 2010).

Many *Curcuma longa* species have long been utilized for their medicinal qualities. *Curcuma longa, Curcuma zedoaria, Curcuma aromatic,* and *Curcuma amada* are among the species whose antifungal, antibacterial, and anti-inflammatory properties have been documented in numerous literatures (Apisariyakulet al., 1995; Yoshioka et al., 1998). The aim of this work was measurement of curcumin content and preliminary phytochemical screening of turmeric extracts and in vitro evalution of antibacterial activity of different vareties of turmeric extracts.

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MATERIAL AND METHODS

The current investigation was conducted at SGRR University's School of Basic & Applied Sciences laboratory. Following methodology was adopted for the work.

1 Collection of plant material.

In the present study four different turmeric verities procured from Almora and Pithoragarh district of Uttarakhand was evaluated for antimicrobial activity and curcumin content (Fig.4.1). The rhizomes of Pragti, white, black and Lakadong turmeric were used in the study.

Fig.1 (a-d): Rhizomes of different varieties of Turmeric

Sample Preparation.

The 250g of each variety of turmeric rhizomes were sliced and air dried for one week (Fig.4.2).

The dried rhizomes were further used for extract preparation and assessment of the curcumin content.

Fig.2: The sliced turmeric rhizomes.

Preparation of ethanolic extract

The process of extracting turmeric powder was completed as per methodology described by Subashini and co-workers (2020), with slight modifications. The samples were powdered using a pestle into a coarse powder and used further for exaction using 70% ethanol. Ethanolic extracts were prepared by adding 10gm powder into the 100ml ethanol (70%) in 250mL conical flasks. Each flask was kept for 72 hrs at 160 rpm in a rotary shaker. The mixtures were filtered significantly using Whatman No. 1 filter paper and the filtrates obtained were evaporated througha water bath at 40 °C (Subashini *et al.*, 2012).

In –vitro antimicrobial evaluation of turmeric extracts

The antibacterial activity of the various turmeric extracts was assessed using the agar well diffusion method. DMSO served as the negative control and the antibiotics Vancomycin (VA 30 mcg) and Linezolid (LZ 30 mcg) as the positive controls.

Microbial strains used for the antimicrobial analysis

Two bacterial strains selected for the antimicrobial analysis were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonasaeruginos.*

Inoculums preparation

Inoculum was prepared by taking 4-5 colonies of all four test organisms from 24 hours incubated plates, were added in 5 ml sterile normal saline and mixed well. The turbidity of bacterial suspension was visually adjusted with 0.5 McFarland standards.

Antimicrobial assay

The antibacterial activity of various extracts was detected using the Agar well diffusion method (Harit et al., 2013). The whole surface of Muller Hinton Agar plates was covered with a sterile swab that had been submerged in the bacterial suspension. To make sure that the inoculums were distributed evenly, the plates were rotated by around 60 degrees. A sterile borer was used to create wells (6 mm in diameter) in the MHA plates, 2 mm from the plate's edge. Different types of turmeric ethanol extract were added to the wells. Turmeric extracts 50mg/ml and 100mg/ml concentrations were inoculated in wells. Standard antibiotic discs (Vancomycin and Linezolid) 30mcg were also placed on the MHA plates as a positive control and DMSO was used as a negative control. The Plates were incubated at 37° for 18-24 hours and noted for the appearance of zones of inhibition. The zones of inhibition were measured with the scale and noted in mm. Isolates were classified as sensitive and resistant as per zone diameter interpretative criteria of CLSI.

Phytochemical Screening

For the Screening of certain chemical ingredients, the individual extract was put through a qualitative phytochemical screening process. According to conventional protocol, phytochemical tests were conducted (Sawant et al., 2013).

1. **Test for Alkaloids: Mayer's Test-** 3ml of concentrated extract and 1ml of HCl were placed in a test tube. The mixture was then heated gradually for 20 minutes, allowed to cool, and filtered for the subsequent test. A few drops of Mayer's reagent are added to 1 ml of filtrate at the test tube's side. The presence of alkaloids was indicated by a white or creamy precipitate.
2. **Test for Flavonoids: Alkaline Reagent Test-** After the extract was treated with a 10% sodium hydroxide (NaOH) solution, the presence of flavonoids was shown by the production of an intense yellow or red color.
3. **Test for Phenols: Ferric Chloride test-** The test extract was treated with four drops of a solution of alcoholic $FeCl_3$. The development of a bluish-black hue signifies the existence of phenol.
4. **Test for Carbohydrates: Iodine Test-** 5 drops of iodine solution were added to 2ml of extract; the blue color indicates a positive test.

Test for Tannins: When 4 ml of extract were treated with 4 ml of $FeCl_3$, a green color formed, signifying the presence of tannins.

Curcumin Estimation

All the varieties of turmeric used in the study were tested for curcumin content. The samples were sent to Indian Spice Board, Narella Delhi. The curcumin level was evavaluated as per ASTA method.

RESULTS AND DISCUSSION

Extract Preparation Turmeric samples were procured from Almora and Pithoragarh district of Uttarakhand and evaluated for their antimicrobial activity and presence of phytochemicals. The rhizomes of Pragati, Black Turmeric, White Turmeric, and Lakadong turmeric were used in the study (Table5.1). In order to test for antimicrobial activity, fresh rhizomes of *Curcuma longa* (turmeric), *Curcuma caesia* (black turmeric), *Curcuma amada* (mango ginger), and *Curcuma aromatic* (van turmeric) were collected from the Almora and Pithoragarh districts of Uttarakhand, India.

Table 5.1: Details of turmeric varieties used in study

S.No.	Varieties	Binomial	Weight before drying	Weight after drying

1.	Lakadong	*Curcuma longa*	250gm	67.79gm
2.	IISR Pragati	*Curcuma longa*	250gm	60.70gm
3.	White Turmeric	*Curcuma zedoaria*	250gm	54.33gm
4.	Black Turmeric	*Curcuma caesia*	250gm	63.79gm

The samples were powdered using a pestle into a coarse powder and used further for exaction using 70% ethanol (Fig 5.1.1). Ethanolic extracts were prepared by adding 10gm powder into the 100 ml ethanol (70%) in 250 mL conical flasks (Fig5.1.2). Each flask was kept for 72 hrs at 160 rpm in a rotary shaker. Following full evaporation, the crude extracts were placed in a dark glass bottle and preserved at 4°C until they were needed for additional examination. For antimicrobial analysis, the ethanolic extracts were further diluted in 100% DMSO (Fig5.1.3).

Using a Soxhlet device, a powder sample was extracted successively using ethanol and water. A vacuum evaporator was used to evaporate these extracts until they were completely dry. Before being used, the vacuum-dried extract was kept in airtight vials. 20 mg of dry powder were dissolved in one of the appropriate solvent (ethanol and water) to create stock solutions for each extract (Harit et al., 2013).

The antibacterial qualities of turmeric *Curcuma longa* against certain microorganisms were assessed in a study by Odo and colleagues. Using two solvents—ethanol and water—different quantities of turmeric extract (100, 50, 25, and 12.5% mg/mL) were made. Using the disc diffusion method, the extract's antibacterial activity was evaluated against *Pseudomonas aeruginosa*, *Bacillus species*, *Staphylococcus aureus* and *Escherichia coli* at varying extract concentrations. Ciprofloxacin served as the control.

Ethanol and aqueous extracts showed zones of inhibition against test organisms ranging from 1 to 10 mm. With zones of inhibition that ranged from 3 to 10 mm against Bacillus species, 4 to 9 mm against S aureus, and 1 to 7 mm against E coli, the ethanolic extracts outperformed the aqueous extracts. P aeruginosa was not inhibited in any way. The water extract and ethanol extracts differed significantly ($P < .05$). According to this study, the turmeric plant possesses antibacterial properties against specific organisms and could be very helpful to the pharmaceutical industry in developing medications to treat illnesses and regulate aberrant blood lipid profiles (Odo et al., 2013).

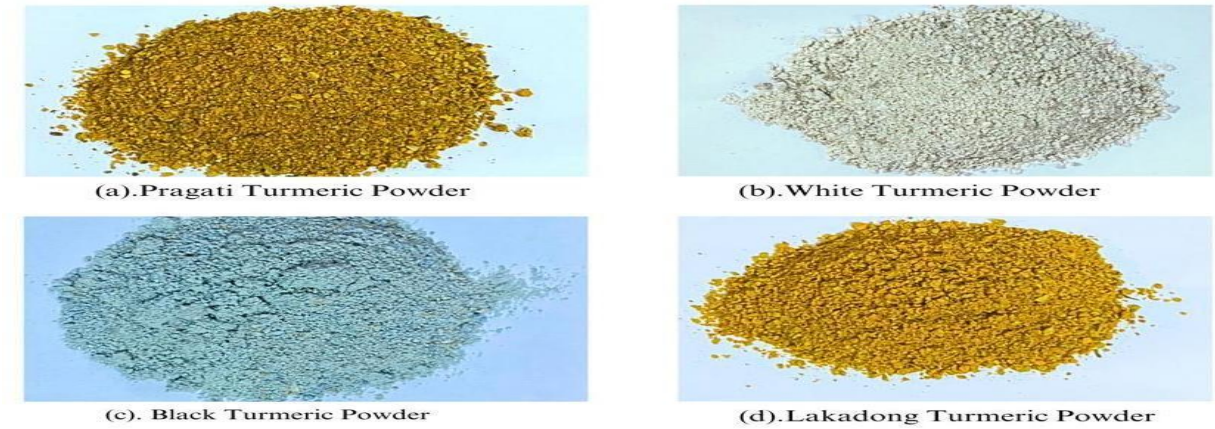

Fig.5.1.1 Turmeric powder used for preparation of ethanolic extracts.

Fig.5.1.2 Conical flasks (250ml) having 100ml Ethanolic extracts.

Fig.5.1.3 (a-d) Overview of extraction process.

Antibacterial activity of different varieties of turmeric:

This study examined the effects of ethanolic extracts from four distinct plants belonging to the same species on three gram-negative and one gram-positive bacteria (S. aureus, E. coli, K. pneumoniae, and P. aeruginosa). The zone of inhibition of bacterial growth at a specific concentration was identified using the Agar well diffusion method. Significant inhibitory actions were exhibited by all types. All investigated bacterial strains showed inhibition (Table 5.2.1).

The study conducted by Ilham and colleagues (2018) sought to ascertain the antibacterial activity of turmeric leaf extract. The extraction process with maceration utilizing water, methanol, and ethyl acetate as the solvent, and concentrations of turmeric leaf extract of 20%, 40%, 60%, and 80% were the two parameters in the fully randomized design. *Shigellady senteriae, Staphylococcus aureus*, and *Escherichia coli* were all inhibited in their growth by turmeric leaf extract, while Lactobacillus acidophilus was not (Ilham et al., 2018).

Different fractions extracted from the rhizome of *Curcuma longa* were tested for their *in vitro* antibacterial activity against clinical isolates and standard strains of Staphylococcus aureus. Compared to the reference strain of *Staphylococcus aureus* the clinical isolates were found to be more sensitive for various fractions. According to observations made using a scanning electron microscope, the test pathogen treated with *Curcuma longa* extract displayed morphological abnormalities, including a partial absence of the cytoplasmic membrane, which causes cell disintegration. The broad spectrum antibacterial capability of *Curcuma longa* extracts, which may be used to treat microbial illnesses, is demonstrated by their capacity to stop the growth of test pathogens.

Additionally, Harit and his colleagues employed the agar well diffusion method to detect the antibacterial activity of various extracts. Tetracycline and fluconazole were used as positive controls in the antifungal and antibacterial tests. Bacterial cultures of *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC96) and fungal cultures of *Candida albicans* (MTCC 3017) and *Aspergillus flavus* (MTCC 277) were used as a test organism for antimicrobial activities. All microbial cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. The agar well diffusion method was used to measure the inhibitory effect, and the results indicated that ethanolic plant extracts were more effective than aqueous plant rhizome extracts at inhibiting test pathogenic bacteria and fungi. The aqueous extraction method was ineffective for isolating the active antibacterial component from curcuma since the water fractions of the four chosen plants exhibited no inhibition against any of the bacteria and fungus examined in this investigation (Harit et al., 2013).

The results of inhibitory activity of extracts of turmeric rhizomes were quite impressive. The zones of inhibition were observed with ethanolic extracts of turmeric rhizomes against *Staphylococcus* strains, *E.coli, K. pnuemoniae*, and *Pseudomonas*. The diameters of the zones were smaller than the positive control when compared to positive controls (Table 4.2).In our study, the white turmeric ethanolic extract of the turmeric had effective inhibitory activity (inhibition zone 24mm at 100mg/ml) against *Klebshiella*, although big zones of inhibition were observed. Similar,

effect shown in lakadong ethanolic extracts. On *E.coli*, all ethanolic extracts had shown moderate inhibitory effect. And on *S. aureus*, and *Pseudomonas*, quite strong results seen by all turmeric ethanolic extracts as shown in (Fig.5.2.2 to 5.4.5).Based on Table 5.2.1, it can be seen that the antimicrobial effect of white turmeric rhizome ethanol extract is more effective in inhibiting the growth of the *Klebsiella pneumoniae*. This can be seen from the diameter of inhibitory zones in the *K. pneumoniae* at 100mg/ml. For *Escherichia coli* bacteria, It works better at concentrations of 50mg/mL and 100mg/mL, while *Staphylococcus aureus* is more efficient at concentrations of 100 mg/mL. According to Davis and Stout, explain that the criteria for antibacterial inhibition consistency of ≥20 mm is very strong, 10-20 mm is strong, 5-10 mm is moderate and ≤ 5 mm is weak.However, Harit and his colleagues' studies demonstrated that the ethanol extract of C. longa (inhibition zone 13 mm against B. subtilis at concentration 20 mg ml–1) exhibited the most dramatic action, with inhibition zones exceeding 11.0 mm. Furthermore, at a dosage of 20 mg ml–1, the ethanol extract of C. longa had notable effectiveness against S. aureus, A. flavus, and C. albicans, exhibiting inhibition zones of 12.0, 11.0, and 10.0 mm, respectively. At a concentration of 20 mg ml–1, the ethanol extracts of Candida aromatica showed a moderate suppression of B. subtilis, S. aureus, and Candida albicans at 11 mm each. Ethanolic extract of the C. aromatic was the only extract which shows inhibition activity against *P. aeruginosa* at 10 mm. While none of the other three plants' ethanolic or aqueous extracts demonstrated any efficacy against P. aeruginosa.According to the study, the turmeric plant possesses antibacterial properties against specific species and might be very helpful to the pharmaceutical industry in creating medications to treat illnesses. Although large zones of inhibition were seen, the white turmeric ethanolic extract demonstrated strong inhibitory efficacy against Klebshiella in our investigation (inhibition zone 24 mm at 100 mg/ml).

Table 5.2.1 : Results of antimicrobial tests of the investigated Turmeric varieties in Agar diffusion assay.

Sr. No.	Turmeric Sample	Test organisms	Zone of inhibition (mm) for ethanolic extract (50mg/ml) With mean ± SD	Zone of inhibition (mm) for ethanolic extract (100mg/ml) with mean ± SD	Positive Control		Negative control used as (DMSO)
					Antibiotic (30mcg)	Zone of Inhibition (mm) with mean ± SD	
1.	P_1	*E.coli*	17.66 ± 0.577	19.33 ± 0.57	LZD	33.6 ± 1.52	0
		Pseudomonas aeruginosa	17.66 ± 0.577	17.66 ± 1.52	LZD	15 ± 1	0
		Klebsiella pneumonia	17.33 ± 0.577	23.66 ±1.52	LZD	38 ± 2	0
		S. aureus	16.66 ± 0.577	1.966 ± 0.57	VAN	19 ± 1	0
2.	L_1	*E.coli*	14.33 ± 5.13	20.66 ± 0.57	LZD	35.33 ± 1.15	0
		Pseudomonas	19 ± 1.73	19.66 ± 1.52	LZD	12.66 ± 1.154	0
		K. pneumoniae	21 ± 2.64	23.22 ± 1.54	LZD	35.33 ± 1.154	0
		S. aureus	16 ± 1	18.66 ± 0.57	VAN	17.3 ± 1.54	0
3.	B_1	*E.coli*	16.33 ± 0.577	17.33 ± 1.54	LZD	33.3 ± 1.54	0
		Pseudomonas	19 ± 1	17.33 ± 0.57	LZD	19 ± 1	0
		K. pneumoniae	19.6 ± 0.577	18.33 ± 0.57	LZD	34.66 ± 2.30	0
		S.aureus	18 ± 1	16.6 ± 1.154	VAN	16 ± 2	0
4.	W_1	*E.coli*	17.66 ± 0.577	17.33 ± 1.54	LZD	31.3 ± 1.154	0
		Pseudomonas	19.6 ± 0.577	1.66 ± 1.54	LZD	14 ± 2	0
		K. pneumoniae	18.66 ± 1.15	24.33 ± 0.57	LZD	34 ± 1	0
		S. aureus	19.66 ± 0.577	21.3 ± 0.577	VAN	18.3 ± 0.57	0

SD : Standard deviation ; **LZD** : Linezolid ; **VAN** : Vancomycin ; **DMSO** : Dimethyl sulfoxide ;
mcg : microgram.

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https://www.theaspd.com/ijes.php

(a) (b)

Fig. 4.2.2 (a-b) Inhibitory activity of ethanolic extract of Pragati (P_1) against *Staphylococcus aureus* and *Escherichia coli* which is demonstrated by the zones of inhibition around the discs and antibiotic have been included as positive control, DMSO as a negative control.

(c) (d)

Fig. 5.2.3 (c-d) Inhibitory activity of ethanolic extract of Lakadong (L_1) against *Staphylococcus aureus* and *Escherichia coli* which is demonstrated by the zones of inhibition around the discs and antibiotic have been included as positive control, DMSO as a negative control.

(e) (f)

Fig.5.2.4 (e-f) Inhibitory activity of ethanolic extract of White turmeric (W_1) against *Staphylococcus aureus* and *Escherichia coli* which is demonstrated by the zones of inhibition around the discs and antibiotic have been included as positive control, DMSO as a negative control.

(g) (h)

Fig 5.2.5 (g-h) Inhibitory activity of ethanolic extract of Black turmeric (B_1) against *Staphylococcus aureus* and *Escherichia coli* which is demonstrated by the zones of inhibition around the discs and antibiotic have been included as positive control, DMSO as a negative control.

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Phytochemical analysis of turmeric extracts

The chemicals listed in Table 5.3.1 are present in the crude ethanolic extract of turmeric, according to the results of the initial phytochemical screening. According to Table 5.3.1, phytochemical screening of the ethanolic extract of turmeric indicates the presence of a respectable quantity of phenols, alkaloids, tannins, flavonoids, and reducing sugars. However, it is also depicted in Figure 5.3.2 (a-e).

Table 5.3.1: Analysis of phytochemical in turmeric ethanolic extracts.

Phyto-constituents	**Name of the Test**	**Observations**
Flavonoids	**Alkaline Test**	+
Tannins	**Ferric chloride Test**	-
Alkaloids	**Mayer's Test**	+
Carbohydrates	**Iodine Test**	-
Phenols	**Ferric chloride Test**	+
Representations: + = Present, - = Absent or not detectable.		

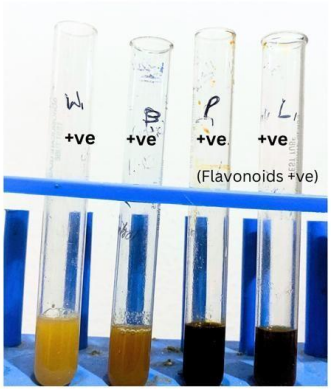

Fig. (a.) Test for flavonoids

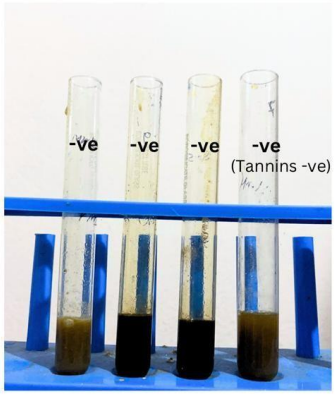

Fig. (b) Test for tannins

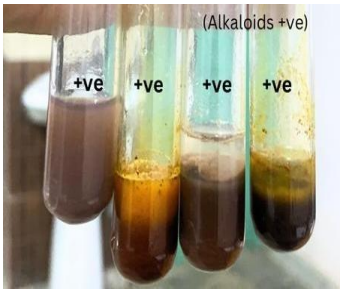

Fig. (c.) Test for alkaloids

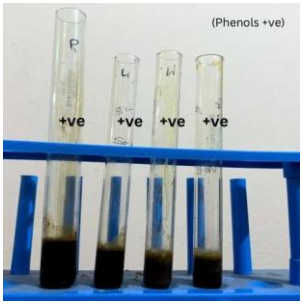

Fig. (d.) Test for phenols

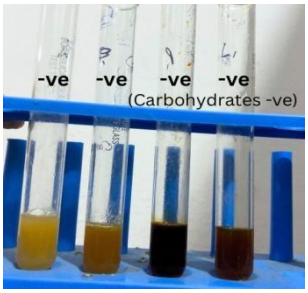

Fig. (e.) Test for carbohydrates

Fig 5.3.2 (a-e) Phytochemical analysis of turmeric extracts.

The phytochemical examination of C. longa extract demonstrated antibacterial activity in a study by Gupta and his colleagues (2015), revealing the presence of various active components in various extracts. Alkaloids, tannin, flavonoids, glycosides, and carbohydrates were all present in the extract of Curcuma longa. According to some sources, the chemicals that give higher plants their antibacterial properties are flavonoids and alkaloids. (Gupta and others, 2015).The presence of a respectable quantity of alkaloids, saponin, tannins, coumarin, flavonoids, diterpenes, phlobatannins, cardiac glycosides, phenols, steroids, anthraquinones, reducing sugars, anthocyanins,

and terpenoids is revealed by phytochemical screening done on the ethanolic extract of turmeric by Oghenejobo and his colleagues (2017).

In a study, Saxena and his colleagues (Saxena *et al.,* 2012) displayed the findings of phytochemical screening for three distinct species of curcuma. The outcome offers an empirical foundation for the plant's possible application in the production of novel medications. The extract from Curcuma caecia contained components that are known to have therapeutic effects against pathogens that cause disease, including carbohydrates, flavonoids, steroids, phenol alkaloid tannins, amino acids, and glycosides. Therefore it could be used pharmacologically to develop new compounds for the heath benefit.

The phytochemical screening results are similar to those achieved by Saxena et al., Oghenejobo et al., and Gupta et al. Turmeric's chemo preventive and physiological effects in numerous tumour bioassays, as well as the decreased enhanced development of tumour cells, have been attributed to the presence of flavonoids and curcumin. The non-nutritive parts of plants, known as phytochemical, shield the plant from pests and illnesses while also shielding people from a range of illnesses.

Results of curcumin estimation of turmeric samples.

Curcumin estimation was done by ASTA Method and carried out by Indian Spice Board, Narella, Delhi and the following results were seen (Table no. 5.4).

Table no. 5.4: Curcumin estimation %

S. No.	Samples	Curcumin percentage%
1.	L_1	1.48%
2.	B_1	0.01%
3.	P_1	3.66%
4.	W_1	0.14%

CONCLUSION

Based on the current research, it is determined that the white turmeric ethanolic extract had effective inhibitory activity(inhibition zone 24mm at 100mg/ml) against *Klebshiella*, although big zone of inhibition were observed. Similar, effect shown in lakadong ethanolic extracts. On *E.coli,* all ethanolic extracts had shown moderate inhibitory effect. And on *S.aureus*, and *Pseudomonas*, quite strong results seen by all turmeric ethanolic extracts as shown in (Fig.5.2.2 to 5.2.5). This study reveal that Turmeric plant has antibacterial power against certain organisms and may be of significant value of pharmaceutical industry for the creation of drugs to cure diseases. Turmeric's chemopreventive and physiological effects in numerous tumor bioassays, as well as the decreased enhanced development of tumor cells, have been attributed to the presence of flavonoinds and curcumin. The non-nutritive parts of plants, known as phytochemicals, shield the plant from pests and illnesses while also shielding people from a range of illnesses.

There have also been claims that turmeric offers anti-diabetic properties. By lowering the amount of glucose-6-phosphate, it is said to lower the risk of developing diabetes. The major classes of secondary metabolites found in plants are known to be alkaloids. They may be used as pain relievers and are said to have strong effects on people. The turmeric extract contains a little amount of tannin (Table 5.3.1). However, its use in the treatment of intestinal problems such as dysentery and diarrhea may be due to the presence of tannin. Turmeric's strong antibacterial and antioxidant qualities, as well as its ability to lower cholesterol and stop the spread of cancer, may be due to its curcumin content.

From the results obtained in this study it is found that Turmeric is a plant that could be cultivated because of its usefulness as an antibacterial agent. It is recommended therefore that further research should be done on it for its use as a plant medicinal especially in this period that herbal drugs are becoming significant as antimicrobials.

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