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Research Article

Phytochemical and Biological Studies on *Curcuma longa* L. in Pattoki (Kasur), Pakistan

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Abstract: *Curcuma longa* (Turmeric, Haldi) is an important medicinal and traditional plant belonging to the family *Zinger beraceae.* Current studies were performed to explore the phytochemical composition, nutritional value, antioxidant and antimicrobial potential of *Curcuma longa* rhizome collected from Pattoki (Kasur), Pakistan. Phytochemical investigations have shown the presence of secondary metabolites such as saponins, terpenoids, glycosides, tannins, and alkaloids. The extract displayed fantastic antioxidant potential with 70.34% total phenolic content (TPC). The highest antioxidant potential 87.92% was observed with a 50µg/mL concentration of methanolic extract. The plant extract has shown an excellent nutritional potential due to the presence of significant quantities of carbohydrates, protein, fibers, fat, and energy content of 101.12 Kcal/100g. However, it displayed insignificant antimicrobial potential against tested bacterial *(Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, and Escherichia coli)* and fungal *(Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, and Alteraria alternata)* strains.

Keywords: *Curcuma longa*, Chemical ingredients, Antioxidant, Antibacterial, Antifungal

1. INTRODUCTION

Plants are widely investigated throughout the world owing to their nutritional, antioxidant, antimicrobial, and pharmaceutical potential [1-4]. *Curcuma longa* L. (Turmeric) is also a medicinal plant of the Zingiberaceae family [5]. It is a well-known herb that is used in medicine in the Ayurvedic and Unani systems of medicine [6]. Turmeric is a perennial plant having medium height and an underground stem. Rhizomes are short-branched, ovate, oblong, and pyriform [7]. The plant has unique pharmaceutical characteristics and is widely grown in tropical areas including Pakistan, India, China, and Peru. In South Asia, India, and China, this plant is frequently used as a preservative, coloring, and flavoring agent [8]. It is also considered a food additive throughout the world [9]. It is commonly known as Haridraor Haldi (in the subcontinent), Manjal (in the Tamil language). Turmeric is also considered as the Indian saffron because it is generally used as a substitute for costly saffron spices [10].

Turmeric species have been used in traditional medicines for the treatment of diabetes, ulcer, cough, enlarged spleen and liver, hepatic disorder, chest pain, skin diseases blood purifier, boils, and rheumatism [9]. *Curcuma longa* consists of a variety of compounds that are important for spice, cosmetics, and are medicinally important [11]. It is rich in bioactive compounds including flavonoids, polyphenols, and antioxidants, and can be used as a substitute for antibiotics in food items. Turmeric contains nonvolatile constituents (e.g., curcuminoids) and volatile components (e.g., curlone, ar-turmerone, zingiberene, and turmerone) [12]. Curcuminoids are predominant phenolic compounds and are responsible for the distinguishing color of turmeric. They are majorly

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comprised of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin [13]; other essential macromolecules are sugars, protein, and resins. The main and useful active ingredient is curcumin that consists of 0.3-5.4% of raw turmeric. Curcumin is a crystalline and orange-yellow colored component that is water-insoluble and is considered to be the main ingredient responsible for the biological functions of turmeric due to its high powerful bioactivity (Fig. 1) [13, 14]. Curcumin is a potent bioactive antioxidant of turmeric acting as an antioxidant, anti-inflammatory, anti-platelet, cholesterol decreasing, antibacterial, and antifungal properties [15]. It also prevents the growth of *Helicobacter pylori*, which causes gastric ulcers [16] and may act as an anti-inflammatory in conditions like arthritis, bursitis, and back pain. Curcumin can attach to heavy metals like cadmium and lead, and hence lowering the toxicity of these heavy metals [14].

Turmeric has multipurpose uses in food flavoring, medicines, cosmetics (due to its skin caring effects), and textile industries (for dying purposes) [17-19]. Its most commonly used commercial products include oleoresins, extracts, and turmeric powder [17]. Various parts of turmeric plants are used widely in many Asian countries either in cooked (as vegetables) or in raw form. Turmeric is also reported to be a nutritional valued species because the plants contain different minerals, vitamins, fats, proteins, carbohydrates, and starch [15]. Keeping in view a very large number of widespread uses and tremendous applications of *Curcuma longa*, current studies were focused to investigate the phytochemical composition, antioxidant and antimicrobial studies of this plant locally collected from Pattoki region of Kasur (Pakistan).

2. MATERIALS AND METHODS

Fresh rhizomes of *Curcuma longa* L. were obtained from the village near Pattoki. The species were identified from the Department of Biology, Lahore Garrison University, Lahore, Pakistan. The rhizomes were washed, cut into small pieces, and dried under shade for one week. The extract of fine powder was prepared in methanol through maceration and stored for further studies.

The pyrex origin glassware was used in

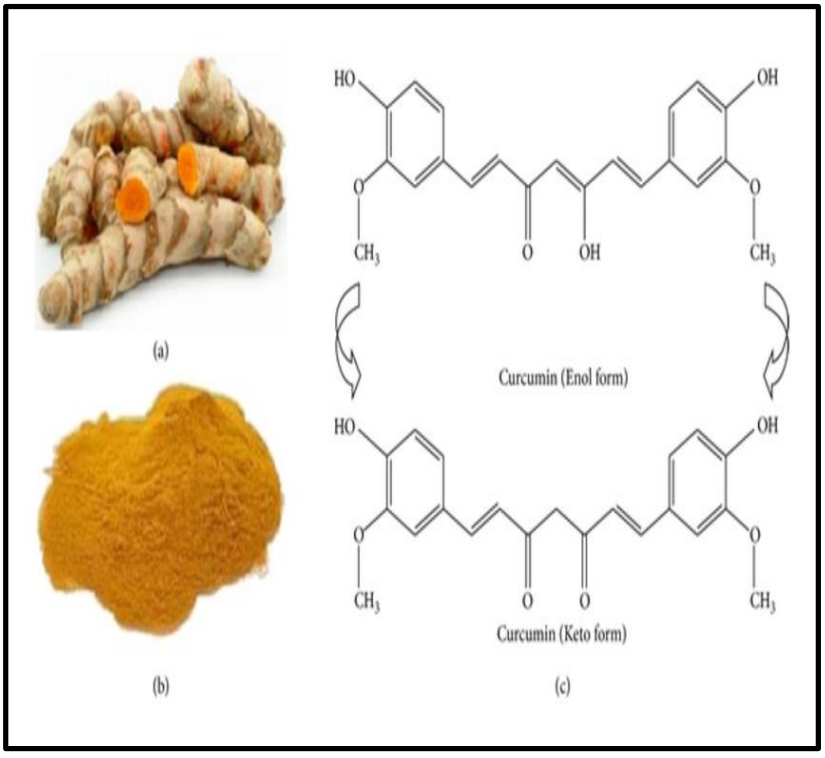

Fig 1. The root of turmeric. (b) Crystallized powder of curcumin. Curcumin is thought to be the main active ingredient derived from the root of turmeric. (c) The enol and keto forms of curcumin are common structures of the drug

experimental work. All the chemicals and the solvents were of analytical grade. TPC and DPPH methods were used to investigate the antioxidant potential [20]. Folin-Ciocalteu reagent and distilled water were used for the determination of total phenolic content. Then different dilutions were prepared from a stock solution to measure its calibration curve. 5 % sodium carbonate and Ciocalteu reagent were also prepared by distilled water as a solvent. The antioxidant activity of leaves and pulp extracts was determined by a UV-Visible spectrophotometer using the DPPH radical-scavenging activity assay.

The extracts were subject to antibacterial and antifungal activity evaluations by disc diffusion method [21, 22].

2.1 Extract Preparation

Fresh *Curcuma longa* L. rhizomes (250 g) were taken and cut into small pieces. Then methanol was added up to the mark in a 1000 mL round bottom flask. After this Soxhlet extraction apparatus used to separate its extract by funnel and Whatsman's filter paper, No. 41 was used for filtration. Then distillation occurred and its extract was prepared for further analysis and tests.

2.2 Qualitative Analysis of Phytochemicals

Phytochemical analysis was used by reported procedures [23-26] to determine the presence of saponins (foam test), terpenoids (Salkowski test) [23], glycosides (Borntrager's test) [24, 25], tannins (ferric chloride test) [26] and alkaloids (Dragendorff's test) [24] in methanolic extract of *Curcuma longa* L. rhizomes. These results were used to determine the presence and absence of phytochemicals.

2.3 Determination of Total Phenolic Content (TPC)

Curcuma longa L. extract with Folin-Ciocalteu reagent was used to determine total phenolic content. This reagent was used by Singleton and Rossi in 1965 for the first time. Firstly 2 mL sample of turmeric extract was inserted into different test tubes and mixed with a 5ml Folin-Ciocalteu reagent. After this, 4 mL of 5% sodium carbonate Na_2CO_3 solution was added and remained for half an hour. Gallic acid was involved as the standard. The absorbance was measured at 765 nm by using a UV-Visible spectrophotometer [20].

2.4 Determination of Antioxidant Activity by DPPH Method

2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution with methanol is monitored on a UV-Visible spectrophotometer is a much effective, simple, and accurate method. The absorbance of DPPH occurred at 517 nm at the maximum level. The color change occurred from purple to yellow due to absorption from an oxidant [20].

Antioxidant potential of methanolic extract of fresh rhizome of *Curcuma longa* L. was determined by DPPH radical scavenging activity with UV-Visible spectrophotometer (1700, Shimadzu, Japan). The DPPH solution was made with methanol as solvent and DPPH as a solute. The sample was used to prepare different concentrations of 50, 40, 30, 20, and 10 µm respectively with methanol. Then various concentrations of the sample were added to 3ml methanolic solution of DPPH solution in different test tubes. After this, the absorbance of the blank and resultant solution was determined at 517 nm by a UV-Visible Spectrophotometer at normal temperature under light protected area. Then percentage inhibition was determined by using the following equation.

$$\% \, Inhibition = \frac{\text{Abs. of Control} - \text{Abs. of Sample}}{\text{Abs. of control}}$$

2.5 Antibacterial and Antifungal Activities of *Curcuma longa* L. rhizomes

Antibacterial activity was performed against Gram-positive bacteria *Salmonella typhi, Bacillus subtilis,* and *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli*. The tested strains (ATCC) of fungus and bacteria were obtained from Applied Chemistry Research Centre (ACRC), PCSIR Labs Complex, Ferozpur Road Lahore-54000- Pakistan PCSIR, Lahore.

The antifungal studies were performed against fungal strains such as *Aspergillus flavus, Fusarium oxysporum, Aspergillus niger,* and *Alternaria alternata* were used. Streptomycin (0.1 %) was used as a standard against bacterial strains in petri plates with turmeric methanolic extract samples. Sodium azide was used against fungal strains in petri plates with turmeric extract samples. These bacterial and fungal strains were collected from a laboratory in Lahore.

2.5.1 Preparation of Nutrient Agar and Potato Dextrose Agar

Nutrient agar (3.4 g) was dissolved in 100 mL of distilled water. Then the temperature was maintained at 100^{O}C in an autoclave for 15 minutes and decreased to 60 ^{O}C temperature. 2.8 gram of potato dextrose agar was dissolved in 100 mL of distilled water. Then the heat was also applied in an autoclave for 15 minutes to make both solutions.

2.5.2 Preparation of Slant

5ml of nutrient agar (NA) and potato dextrose agar (PDA) was taken in test tubes. Both were heated on auto-clave before addition in test tubes and remained for at least 24 hours. The media was solidified in the form of a slant. The tilted position of test tubes was used to increase the surface area to observe more bacterial growth [21].

2.5.3 Antibacterial and Antifungal Activity using Disc Diffusion Method

The disc diffusion method was used for determining antibacterial activity. A nutrient agar (for antibacterial) and potato dextrose agar (for antifungal) thoroughly mixed in water was poured into petri plates. Then it was solidified by cooling it. Turmeric extract was poured into 6mm thick petri plates. Then bacterial/fungal strains were added into Petri plates from the suspension of test tubes of slant. The plates were incubated at a temperature of 37 ^{O}C for 24 hours for bacterial strains and 25 ^{O}C for 24 hours for fungal strains. The zone of inhibition was measured from the diameter of a disc in mm from the disc plate against *Curcuma longa* L. extract of fresh rhizome [22, 27].

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical Analysis (Qualitative)

Phytochemical screening of the methanolic extract of *Curcuma longa* L. fresh rhizomes have shown the presence of secondary metabolites including saponins, glycosides, terpenoids, alkaloids, and tannins in the rhizome of turmeric.

The presence of these phytochemical compounds showed that the *Curcuma longa* L. plant has many medicinal properties and is used in many pharmacological applications such as antibacterial, antifungal, antioxidant activities, etc. That is the reason it has been used as a traditional medicine in different countries for many centuries.

Similar investigations have shown the presence of curcuminoids (polyphenolic pigments) mainly deferulolyl methane (curcumin) which is responsible for the yellow color of the rhizome. Other curcuminoids include bisdemethoxycurcumin and dimethoxy curcumin [28]. Turmeric is a natural antiseptic and is sometimes known as Indian saffron. The presence of non-nutritive chemical compounds renders it disease preventive properties [28]. The phytochemistry of *Curcuma longa* enables it to find a wide range of biological applications due to its digestive, hepatoprotective, cardioprotective, antiseptic, antioxidant, antiviral, antibacterial, anticancer, and anti-inflammatory potential [29].

3.2 Nutritional Analysis (Quantitative)

Turmeric is rich in minerals, antioxidants, and energy so it has become very important to be entered into the system of human nutrition. It shows the excellent nutritive value and many other beneficial properties. Its root powder finds flavoring properties as a spice and as a food medicine [28]. Turmeric finds applications as a coloring agent in butter, cheese, and other foods [30]. It has been used to given a golden color to boiled white rice. It also finds uses in various food products such as gelatins, sauces, cereals, cake icings, sweets, popcorn, biscuits, orange juice, yogurt, yellow cakes, ice cream, baked products, dairy products, and canned beverages. It also finds uses in sweet dishes, savory, and as fresh turmeric pickle [31].

Nutritional analysis of the rhizome of *Curcuma longa* L. has shown the presence of varying amounts of important nutritional contents. Moisture was present in excess amount. The plant rhizome was found to contain carbohydrates, fats, proteins, fibers, and ash content in different percentages. It has a large amount of energy. Nutritional analysis data of *Curcuma longa* are given in Table 1.

3.3 Determination of Total Phenolic Content

In the quantitative phytochemical analysis, total phenolic content was determined. It was performed by using a UV-Visible spectrophotometer at 765 nm. Methanolic extract of fresh rhizomes from Curcuma *longa* L. were obtained. Different concentrations of gallic acid were made and then their absorbance was determined (Table 2). Gallic acid was used as a standard. Total phenolic content was determined by using the calibration curve against concentrations and absorbance obtained from a UV-Visible spectrophotometer. The absorbance of turmeric extract was used to calculate its concentration by using a calibration curve of Gallic acid. The linear graph with different concentrations of turmeric at 765 nm is displayed in Fig. 2.

3.4 Determination of antioxidant activity by DPPH method

Curcuma longa acts as a natural antioxidant for the treatment of oxidative species in our body that decreases the production of oxidative species. DPPH was used as a stable free radical for the determination of antioxidant. Natural and artificial antioxidants convert DPPH free radicals into

Table 1. Nutritional analysis of *Curcuma longa* L.

Constituents	Amounts
Moisture	71.43%
Ash	1.95%
Protein	2.76%
Fat	4.40%
Fiber	6.85%
Carbohydrate	12.62%
Energy	101.12 Kcal/100g

Table 2. Absorbance at different concentrations of Gallic acid

S. No.	Concentration of Gallic acid (ppm)	Absorption
1	10	0.003
2	20	0.005
3	30	0.008
4	40	0.01
5	50	0.012

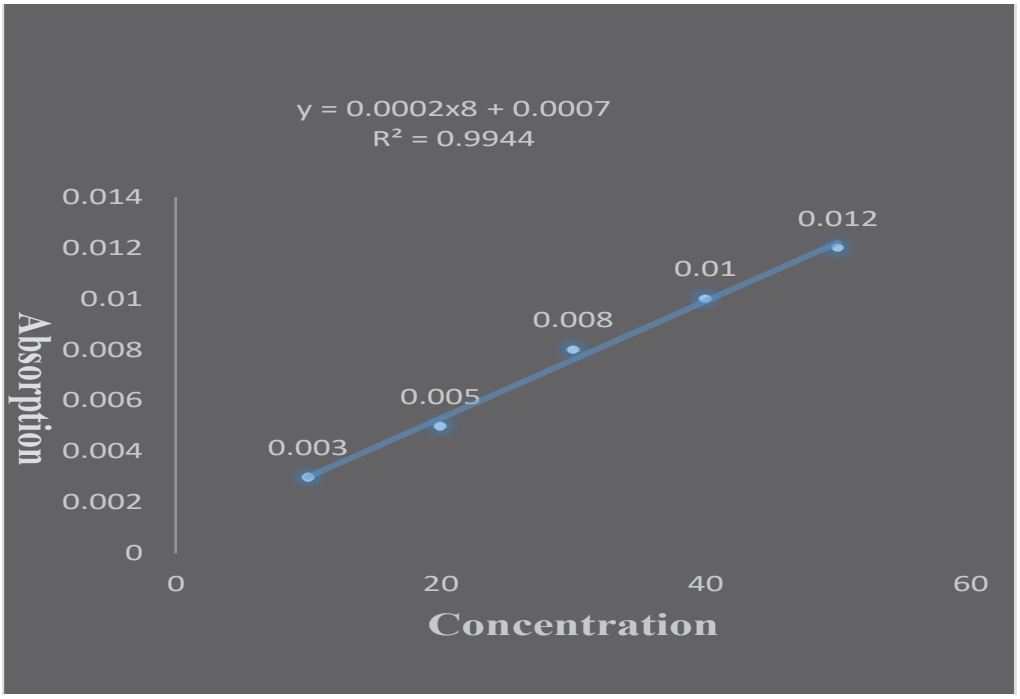

Fig 2. Calibration curve for different concentrations of Gallic acid; % Yield of TPC = 70.34%

diphenyl picryl hydrazine which was yellow.

Different studies have investigated that turmeric species are important sources of natural antioxidants that provide significant protection against free radical damage [32].

The antioxidant activity was determined for the methanolic extract of fresh rhizomes of *Curcuma longa* L. by the DPPH method. The obtained data are summarized in Table 3. The investigated extract has shown the strongest antioxidant potential (87.92%) at 50 µg/mL. When the concentration was increased up to 50 µg/mL then % inhibition was also increased.

Thus *Curcuma longa* L. fresh rhizomes can be used in the treatment of different diseases due to their strong antioxidant activity.

Table 3. Antioxidant activity of Methanolic extract of fresh rhizomes of *Curcuma longa* L.

S. No.	Conc. µg/mL	Absorbance	% Inhibition
1	10	0.5450	49.19
2	20	0.3635	66.11
3	30	0.1820	83
4	40	0.1557	85.48
5	50	0.1295	87.92

3.5 Antibacterial activity and Antifungal activity of *Curcuma longa* L.

Curcuma longa L. fresh rhizome extract was tested by disc diffusion method against four bacterial strains such as Gram-positive bacteria (*S. typhi, S. aureus*, and *B. subtilis*) and Gram-negative bacteria (*E. coli*). The antifungal activity was performed against four fungal strains (*A. niger, A. alternata, F. oxysporum*, and *A. flavus*). Bacteria were at a temperature of 37 °C and 25 °C, respectively. The zones of inhibition were measured in millimeters (mm) by a zone reader [27] and are given in Table 4.

The methanolic extract of *Curcuma longa* L. has shown insignificant antibacterial and antifungal potential. The zone of inhibition of methanolic extract of turmeric was comparatively small (3mm) against Escherichia coli as compared to that (5 mm) displayed against *Bacillus subtilis, Salmonella typhi*, and *Staphylococcus aureus* (Table 4). The lower activity of the methanolic extract was rendered to the solvent effect. The OH group in the phenolic content of *Curcuma longa* L reacts with the OH group of methanol and this caused the decreased behavior of antibacterial activity which dissolves in sugars present in bacterial disc plate. The maximum zone of antifungal inhibition (22 mm) was displayed against *Aspergillus flavus* while the minimum zone of inhibition (15 mm) was shown against *Fusarium oxysporum* (Table 4).

4. CONCLUSIONS

The methanolic extract of fresh rhizome of *Curcuma longa* (turmeric) collected from Pattoki (Kasur), Pakistan had shown excellent antioxidant activities. The 10, 20, 30, 40 and 50 µg/mL solutions of the extract have shown % inhibition of 49.19%, 66.11%, 83%, 85.48% and 87.92%, respectively. The % yield of TPC was found to be 70.34%. The presence of secondary metabolites such as alkaloids, glycosides, saponins, terpenoids, and tannins was also verified in the extract. The extract has shown

Table 4. Antimicrobial data of extract of *Curcuma longa* L.

Antibacterial Activity Data				Antifungal Activity Data		
Pathogen Bacteria	Gram +/-	Zone of inhibition (mm)	Standard (Streptomycin 0.01%)	Pathogen Fungi	Zone of Inhibition (mm)	Standard (Sodium Azide) mm
E. coli	-	3	20	*F. oxysporum*	5	15
B. subtilis	+	5	12	*A. niger*	5	20
S. aureus	+	5	21	*A. flavus*	10	22
S. typhi	+	5	15	*A. alternata*	10	21

an excellent nutritional value due to the presence of significant amounts of carbohydrates, protein, fibers, fat, and energy content of 101.12 Kcal/100g. However, the extract has shown insignificant antimicrobial potential against the tested bacterial and fungal strains. This lowest activity is rendered to the reaction of phenolic/sugar -OH group of turmeric with the -OH moiety of methanol.

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