International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 909-913 www.ijprajournal.com ISSN: 2456-4494

Anti-Oxidant and Anti-Cancer Study of AmorphousCurcuma longa

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Date of Submission: 25-03-2025 Date of Acceptance: 05-04-2025

ABSTRACT: Turmeric (Curcuma longa), a perennial herb from the Zingiberaceae family, is widely grown in tropical regions like India and China. It offers health benefits, including anti-inflammatory, antiprotozoal, and anti-tumor properties, making it valuable in cosmetics and pharmaceuticals. Its main bioactive compounds, curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin) are hydrophobic and dissolve in organic solvents, with ethanol yielding the highest extraction efficiency. In the present research we have studied the anti-oxidant and anti-cancer activity of Curcuma longa. Our results shows that Curcuma longacontains high amount of vitamin A, C and E. It also shows the high DPPH activity, indicating a prominent anti-oxidant properties. Our anti-cancer activity in MCF-7 cells shows that with increase in Curcuma longaconcentration there is increase in anti-cancer activity of Curcuma long.

I. INTRODUCTION:

Turmeric is a vibrant yellow-orange spice derived from the root of the Curcuma longa (CL) plant, commonly used in cooking, traditional medicine, and skincare (1). Native to South Asia, especially India, it is known for its earthy flavor and bioactive compound curcumin, which has powerful anti-inflammatory and antioxidant properties (2). Turmeric has been a key ingredient in Ayurvedic and Chinese medicine for centuries, aiding digestion, boosting immunity, and promoting overall health (3). Its versatility extends beyond food into herbal remedies, cosmetics, and even fabric dyeing, making it a highly valued natural resource (4).

Turmeric's anticancer activity is primarily attributed to curcumin, its key bioactive compound, which has been extensively studied for its potential to inhibit cancer cell growth and metastasis (5). Curcumin exhibits anti-inflammatory, antioxidant, and apoptosis-inducing properties that help suppress tumor development by targeting multiple cell signaling pathways (6). It has been found to reduce oxidative stress, inhibit angiogenesis (formation of new blood vessels in tumors), and modulate gene expression to prevent cancer progression (7). Studies suggest that curcumin may be effective against various cancers, inbreast, lung, colon, and prostate cancer, though its bioavailability remains a challenge, leading researchers to explore advanced delivery methods like nanoparticles and liposomes for enhanced therapeutic effects (8).

In the present research work we have studied the anti-oxidant and anti-cancer activity of Curcuma longafrom Marathwada Region. The results shows thatCurcuma longahashigh anti-oxidant activity and anti-cancer activity.

II. MATERIALS AND METHODS:

1. Preparation of Curcuma longabudextract:

Ground turmeric (50 g) was extracted with 500 mL of water, 50% ethanol, or 70% ethanol for 2 h at 100 °C. The extract was centrifuged at 6500 ×g and 4 °C for 10 min, and the supernatant was filtered through filter paper (No. 3, 110 mm, Whatman). The filtered solution was concentrated using a rotary evaporator under reduced pressure at 60 °C to remove ethanol (9).

2. Vitamin Analysis:

A. Vitamin A: The analysis was done as described previously (10).The test substance added with 1ml of chloroform & 5ml of antimony trichloride.Shaken well & observed for the color development. Vitamin A is present intest substance if it shows transient blue color.

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B. Tocopherol (Vitamin E): The analysis was done as described previously (11). The test substance was mixed with 2ml of ethanol, 0.2ml ofHNO3 followed by boiling for 5min. Tocopherol gives yellow to red color.

C. Vitamin C (Ascorbic Acid): The analysis was done as described previously (12). Test substance when added with 5ml distilled water, 5%w/v solution of sodium nitroprusside & 2ml dilute sodium hydroxide & mixed it with0.6ml Curcuma longa drop wise & stirred it well. Yellow color turns blue indicates presence of ascorbic acid.

3. DPPH Activity:

The analysis was done as described previously (13).Dilute ascorbic acid in methanol to obtain concentrations of 20, 40, 60, 80, and 100 µg/ml. Dissolve the plant extract in methanol at the desired concentration.Prepare a 0.1 mM solution of DPPH in methanol.Mix 2 ml of the prepared DPPH solution with 2 ml of the plant extract solution in a test tube.For the control, combine 2 ml of DPPH solution with 2 ml of methanol (without the plant extract).Shake the mixtures thoroughly and leave them at room temperature for 30 minutes, protected from light.Use a UV-Vis spectrophotometer to measure absorbance at 517 nm.Determine the antioxidant activity using the formula:

$$\text{Inhibition\%} = \frac{(\textit{Control Absorbance} - \textit{sample Absorbance})}{\textit{Control absorbance}} \; x\; 100$$

Where,

Abs control is the absorbance of DPPH radical + methanol

Abs sample is the absorbance of DPPH radical + extract/sample

4. Total Antioxidant Capacity:

The analysis was done as described previously (14).Dilute gallic acid in distilled water to obtain concentrations of 20, 40, 60, 80, and 100 µg/mL.Measure 0.1 mL of 100 µg/mL and 200 µg/mL Curcuma longa extract separately. Combine 1 mL of TAC reagent with each standard and sample solution. Prepare a blank by mixing 0.1 mL of distilled water with 1 mL of TAC reagent. Securely cap all tubes and incubate in a boiling water bath at 95°C for 90 minutes. Allow the tubes to cool to room temperature before taking measurements. Use a UV-Vis spectrophotometer to measure absorbance at 695 nm, using the blank as the reference. Construct a standard curve from gallic acid absorbance values. Express total antioxidant capacity as mg gallic acid equivalents (GAE) per gram of the sample

5. Cytotoxicity activity:

The analysis was done as described previously (15).In this assay, cells were seeded at a density of 5000 cells per well in 100 µL of medium in 96-well flat-bottom microtiter plates (Eppendorf Inc, USA). The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours to allow cell adherence.

CL bud extract was dissolved in dimethyl sulfoxide (DMSO) and further diluted with plain medium. The test samples were added in triplicates at final concentrations of 0.1, 1, 10, and 100 µM, followed by incubation for 48 hours at 37°C. Wells containing only cells served as the negative control. Each experiment was conducted in triplicate.After 48 hours, morphological changes in the cells were observed, and images of cells treated with the highest drug concentration were captured using a Phase Contrast Inverted Microscope (Model Ti-S, NIKON Co., Japan) equipped with a digital camera. The cellular proteins were then fixed using 10% trichloroacetic acid, stained with SRB dye, and subsequently eluted with 10 mM Trizma base. Absorbance was measured at 540 nm, with a reference wavelength of 690 nm, using a plate reader (Model Sunrise, Tecan Inc., USA). The percentage of cell growth was calculated based on

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the ratio of the average absorbance (O.D.) of the test wells to that of the control wells.

III. RESULTS AND DISCUSSION:

1. Determination of Vitamins:

Table 1. Vitamin content in Curcuma longabud extract

Vitamins	Results (mg/100g)
Vitamin A	421
Vitamin E	86.3
Vitamin C	512

The vitamin content is summarized in Table 1. Curcuma longabuds demonstrated significantly higher levels of vitamin A (421 mg/100 g) and vitamin C (512 mg/100 g) compared to vitamin E (86.3 mg/100 g). Among the vitamins analyzed, vitamin C was the most abundant, with higher concentrations in Curcuma longa buds (512 mg/100 g. Additionally, the results indicate that Curcuma longabuds has small amount of vitamin E.

2. Determination of DPPH Radical Scavenging Activity:

The findings of the DPPH radical scavenging activity are illustrated in Figure 1 comparing the results with the well-known antioxidant, Vitamin C. Based on the analysis of Figure 2, it is evident that CL budsexhibit strong DPPH radical scavenging effects. The antioxidant properties of CL buds were remarkable, with significantly lower IC50 values of 2.0280 µg/mL and 4.0697 µg/mL, respectively.The IC50 value represents the concentration required to reduce 50% of DPPH radicals and was calculated based on the percentage reduction of DPPH. Since IC50 values are inversely related to antioxidant activity, lower values indicate a higher antioxidant capacity. The results are presented as the mean of triplicate measurements at varying concentrations, with error bars indicating standard deviation. The chart also highlights significant variations in DPPH content among the samples from different locations. Additionally, Vitamin C, used as a standard, exhibited higher scavenging activity than all the tested samples.

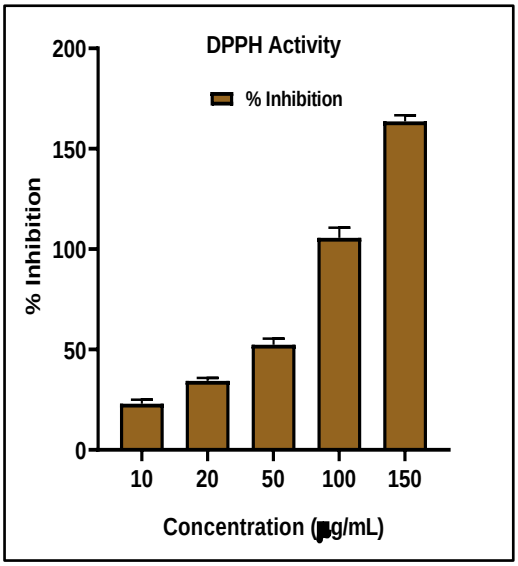

Fig.1 DPPH activity of Curcuma longa

3. Cytotoxic effect of Curcuma longain MCF-7 cells:

MCF-7 is breast cancer cells. We have tested anti-cancer activity of Curcuma longaextract in MCF-7 cells. Curcuma longaextract was dissolved in DMSO (0.01%) at 10, 20, 50, 100 and 150 µg/ml concentration. The extract then used to treat MCF-7 cancer cells. Our results shows that there is gradual increase in anti-cancer activity of Curcuma longawith increase in concentration of Curcuma longa. This anti-cancer activity of Curcuma longais because of free radical scavenging activity and nutritional activity of Curcuma longa.

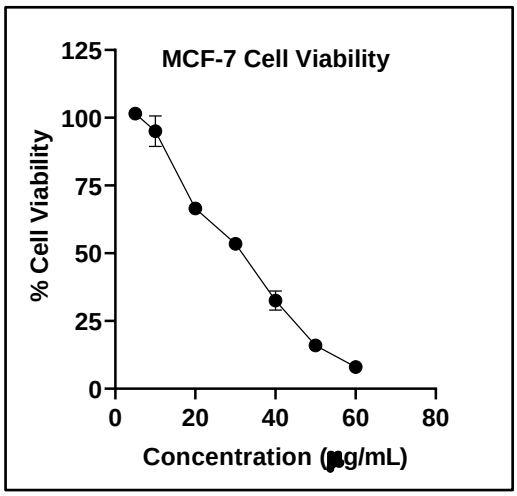

Fig.2 Cytotoxic effect of Curcuma longa

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IV. CONCLUSION:

In conclusion, presence of essential vitamins like vitamin A, ascorbic acid (vitamin C), and vitamin E in Curcuma longabuds highlights their potential in maintaining a healthy body and preventing various health conditions. These vitamins contribute to normal vision, protect against oxidative stress-related damage, regulate the immune response, facilitate wound healing, and provide relief from coughs and colds. Turmeric's rich vitamin content makes it a valuable resource in combating malnutrition.

Curcuma longa is a highly nutritious plant with significant antioxidantactivity. Itcontains essential nutrients, vitamins, and phytochemicalsthat can contribute to human health and nutrition. It also contributes to anti-cancer activity of Curcuma longa. Our results from anti-cancer activity of Curcuma longain MCF-7 shows that with increase in concentration of Curcuma longaresults into increase in cytotoxicity of Curcuma longa.

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