Research Article

Exploring the Therapeutic Potential: A Comprehensive Study on *Curcuma longa* Linn Antioxidant, Cytotoxic and Anti-inflammatory Properties

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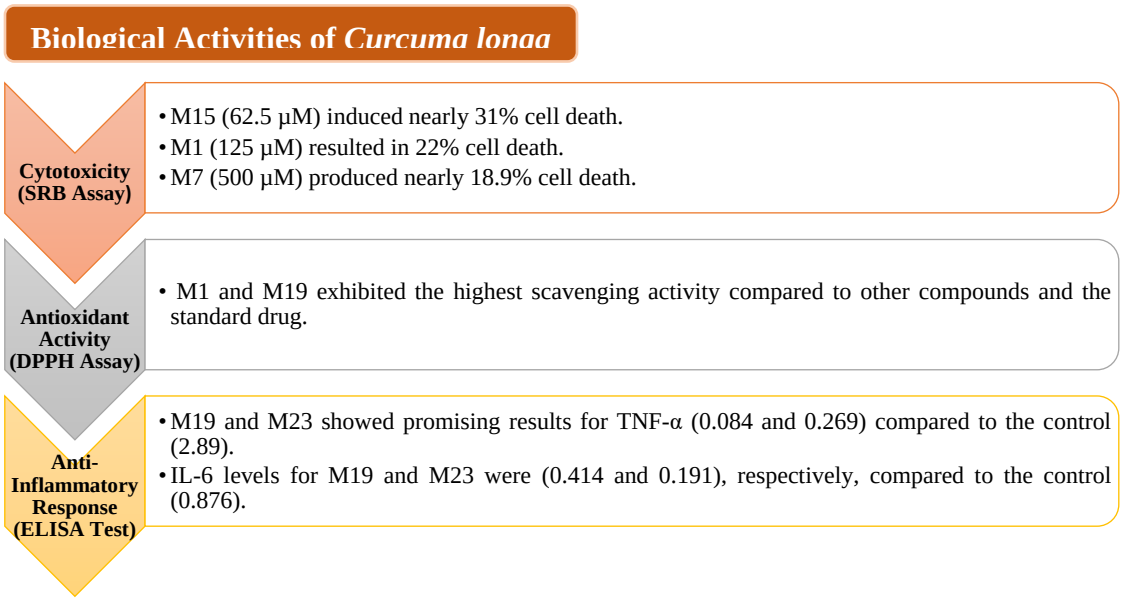
Abstract: *Curcuma longa* Linn, a member of the Zingiberaceae family, has been known for its numerous health benefits since ancient times. This study aims to explore the cytotoxic, antioxidant, and anti-inflammatory properties of extracts obtained from *Curcuma longa* at different time zones, specifically samples labelled as 'M1, M3, M5, M7, M9, M11, M13, M15, M17, M19, M21, and M23.

- Methods: Curcuma longa extracts (M1, M3, M5, M7, M9, M11, M13, M15, M17, M19, M21, and M23) were analysed for cytotoxicity using the SRB assay, measuring % cell viability and % Growth inhibition. DPPH radical scavenging activity revealed M1 and M19 with the highest scavenging activity compared to other compounds and a standard drug. ELISA tests for TNF-α and IL-6 concentrated on the results of M19 and M23.
- Results: In the SRB assay, M15 at 62.5 µM demonstrated approximately 31% cell death, M1 at 125 µM showed 22% cell death, and M7 at 500 µM produced nearly 18.9% cell death. DPPH radical scavenging activity indicated that M1 and M19 exhibited the highest scavenging activity compared to other compounds and the standard drug. In the ELISA test, compounds M19 and M23 showed promising results for TNF-α, with values of (0.084) and (0.269) respectively, compared to the control value of (2.89). For IL-6, M19 and M23 demonstrated values of (0.414) and (0.191) respectively, in contrast to the control value of (0.876).
- Discussion and Conclusion: This study underscores the notable cytotoxic, antioxidant, and anti-inflammatory properties of Curcuma longa extracts, particularly highlighting the promising outcomes of compounds M19 and M23. These findings indicate potential therapeutic benefits in terms of cell viability, radical scavenging, and cytokine regulation. Further research is essential to unravel the mechanisms behind these effects, paving the way for potential applications in health and medicine.

Graphical Abstract:



Key words: *Curcuma longa*, DPPH assay, SRB assay, ELISA, TNF-α, IL-6

1. INTRODUCTION

Throughout the course of history, a wide range of mineral dyes obtained from turmeric (*Curcuma longa* L.), paprika (*Capsicum annuum*), sassafras (*Sassafras albidum*), and other sources have been employed to impart colour to food products and to manufacture pharmaceutical preparations and related goods. During the mid-nineteenth

century, there was a significant development in the field of organic dyes, resulting in the creation of a wide range of coloring additives that held commercial significance. However, it was the advent of natural dyes or pigments that brought about a revolutionary change in the market [1]. While several turmeric species have been documented for their antioxidant activity, there exists a dearth of comparative data regarding the various species and variants of turmeric. Moreover, the process of isolating putative antioxidant components from *Curcuma longa* has not been conducted as of yet [2]. Curcumin has been observed to possess anti-mutagenic and anti-carcinogenic characteristics due to its antioxidant activity [3]. *Curcuma longa* Linn, a member of the Zingiberaceae family, is a perennial herb usually referred to as turmeric. The rhizomes and oils of this substance hold significant relevance. It is commonly utilized as a culinary spice in household food preparation. In conjunction with other natural dyes, it is additionally employed as a pigment for textiles, medicines, confectionery, and cosmetics [4]. Multiple research studies have been conducted on spices subjected to dosages ranging from 10±15 kGy, revealing that there are no significant alterations observed in terms of volatile oils, spicing power (IAEA, 1992), and antioxidant characteristics [5]. Topical administration of this substance has been employed for the treatment of several skin conditions, including wounds, blistering disorders such as pemphigus and herpes zoster, parasitic skin infections, and acne. Oral administration has been employed for the treatment of several conditions such as the common cold, liver disorders, urinary tract diseases, and as a means of blood purification [6]. Ayurveda and traditional Chinese medicine have been extensively documented for their efficacy in treating a diverse range of maladies, encompassing stomach issues, inflammatory conditions, hepatic disorders, gynecological complications, infectious diseases, sprains, and boils, respiratory ailments such as cough and cold, asthma, as well as dental problems. Over the course of the last twenty years, there has been a significant amount of preclinical and clinical investigation that has established a scientific foundation for the utilization of this particular spice in the treatment of many human ailments. Moreover, these studies have also demonstrated that it is safe for human consumption at gram-level quantities. The present study investigated the anti-inflammatory properties of turmeric in rats using three different models of inflammation: cotton pellet granuloma, formalin-induced arthritis, and granuloma pouch. The efficacy of turmeric was shown to be similar to that of hydrocortisone, a corticosteroid medication commonly used for the management of many inflammatory and allergic disorders [7]. The anti-inflammatory characteristics of C. longa can be ascribed to its capacity to hinder the production of inflammatory prostaglandins from arachidonic acid, as well as its ability to modulate neutrophil function in the context of inflammatory conditions. The topical application of curcumin has been found to have potential in mitigating inflammation and irritation commonly observed in inflammatory skin disorders and allergies. However, caution should be exercised to avoid the yellow color of curcumin from staining clothing.

Turmeric, a perennial herb with antioxidant properties, is used in various industries for its culinary spice, pigment in textiles, medicines, confectionery, and cosmetics. It has no significant alterations in volatile oils, spicing power, or antioxidant characteristics. It is safe for human consumption at gram-level quantities and has anti-inflammatory properties due to its ability to inhibit inflammatory prostaglandin production.

2. MATERIALS AND METHODS

a. General Experimental:

The positive controls and compounds utilized in the cytotoxicity, antioxidant, and anti-inflammatory experiments were procured from Sigma Chemical Company, unless explicitly specified differently. All solvents used in this study were of ACS reagent quality and were procured from Aldrich Chemical Co., Inc. Merck provided the methanol and chloroform. The remaining components were all of an analytical grade. For every experiment, double-distilled water (DDW) was used. RAW264.7 cells in SRB assay were procured from the ATCC

b. Plant material

The plant materials, *C. longa* rhizome portions, were collected in the village Cherukumpakala near Lambasingi, Alluri Sitaramaraju District, Andhra Pradesh. It was collected and processed and extracts prepared under personal supervision of M. Bapuji. The material collected is not a hybrid variety. It is a native variety, organically cultivated. Traditionally harvested after 2 years where as the hybrid varieties are harvested after 9 months. It is reported by modern farmers that native variety cultivated by tribals has higher curcumin content than hybrid variety.

c. Soxhlet Extraction Process

The C. longa rhizome should be dissected into its components and then given a thorough washing with water to remove any contaminants. Before being processed into a fine powder using a 60 mesh size, the samples were freshly dug outward cut immediately into small pieces and preserved in pure methanol. In order to get rid of the fatty impurities, powdered materials were defatted with petroleum ether that was 98% strong. With the use of the hot continuous extraction (soxhlation) procedure, the components that had previously been rendered defatted were removed. The soxhlation procedure took 9 hours and required 250 mL of chloroform and 100 mL of methanol to complete. The methanolic fraction thus obtained was filtered. After heating the filtrate to 40 °C in a rotating evaporator under a vacuum, the filtrate was concentrated and stored at 40 °C for later use.

d. In Vitro Bioassays for Cytotoxicity (SRB Assay)

The RAW264.7 cell line was acquired from the American Type Culture Collection (ATCC) and was kept alive in DMEM with 10% fetal bovine serum (FBS) as an addition. A 96-well plate containing the cells was seeded with 3 x 103 cells per well. They were then put into a CO2 incubator that was preheated to 37°C. The cells were treated with the test chemicals at four different concentrations (62.5, 125, 250, and 500 M) after a 16-hour incubation period. The cells were subjected to the SRB assay after a 24-hour incubation period in order to gauge their viability. The SRB assay involves fixing cells in an ice-cold, 10% solution of trichloroacetic acid (TCA) for an hour at a temperature of 4°C. The fixing stage keeps cellular proteins intact and stops them from being lost during subsequent washing processes.

The excess trichloroacetic acid (TCA) was then removed from the fixed cells by gently running tap water over them.

The cells were then given a 30-minute incubation period with a 0.057% (w/v) solution of sulforhodamine B (SRB). A chromophoric substance called sulphorhodamine B has an affinity for biological proteins, making it easier to test them. The cells underwent a washing phase using a 1% acetic acid solution after being incubated with the Sulforhodamine B (SRB) solution to remove any dye that was not bound to the cells. The accurate assessment of cell viability may be hampered by the presence of an excessive amount of unbound SRB dye.

A 10 mM Tris base solution was added to the cellular milieu in order to interfere with the SRB-protein interaction. Then, the spectrophotometer was used to gauge the dissolved SRB-protein complex's absorbance at a wavelength of 562 nm. The measurement of absorbance provides information about the RAW264.7 cells' growth and viability after being exposed to various concentrations of the test chemicals.

e. Antioxidant assay

i. DPPH solution preparation: A solution of 0.5 mM concentration was prepared by dissolving 4.9 mg of DPPH in 25 mL of methanol.

ii. DPPH assay procedure: Triplicate samples of test compounds at various concentrations (0.31, 0.62, 1.25, 2.5, and 5 mg/mL) were added to 96-well plates. Following the addition of samples, a solution containing 100nM Tris-HCl and 0.5mM DPPH was introduced. Subsequently, the plate was placed on a shaker for duration of 30 seconds and incubated in darkness at room temperature for a period of 20 minutes. The subsequent kinetics of DPPH was measured spectrophotometrically at a wavelength of 517 nm for duration of 5 minutes, and the IC50 value was determined.

f. Anti-inflammatory Assay

The concentration of TNF-α was determined using a sandwich ELISA technique. The capture antibody was employed in conjunction with a sodium carbonate buffer of 0.1 M concentration, maintained at a pH of 9.5. In order to create a point of reference, internal standards were utilized in the form of serial dilutions of TNF-α standard. These dilutions ranged from 0 to 1000 pg/mL and were prepared using a diluent consisting of 10% BSA in PBS. Samples were procured from a variety of sources, namely ‘M1, M3, M5, M7, M9, M11, M13, M15, M17, M19, M21, and M23’. The detection of TNF-α was accomplished by employing a biotinylated secondary antibody and the detection reagent consisted of an avidin peroxidase conjugate in combination with TMB. After a 30-minute period of incubation, the reaction was brought to an end by the addition of 2 M H_2SO_4. The resulting absorbance was then determined at a wavelength of 450 nm.

Table 1: Name of Cytokine, Catalogue number and sample dilution

SC. No	Name of cytokine	Catalogue no.	Sample dilution
1.	TNF-α	DY410-05 (R&D Systems)	1:25
2.	IL-6	DY406-05 (R&D Systems)	1:20

To assess the anti-inflammatory characteristics, a research was done to examine the release of the pro-inflammatory cytokine IL-6. For the in vitro tests, a concentration of 1 x 10^6 peripheral blood mononuclear cells (PBMCs) per milliliter (mL) were introduced onto a 24-well plate. The cells were either maintained in an inactive state using growth medium alone or stimulated for a duration of 30 minutes with a concentration of 5 g/mL of the mitogen concanavalin A (ConA) prior to their exposure to plant extracts. The peripheral blood mononuclear cells (PBMCs) were subsequently distributed into duplicate wells in 96-well microtiter plates, with a concentration of 2 x 10^5 cells/mL. The two groups consisted of cells that had undergone pretreatment with ConA and cells that had not received any treatment. The experimental groups were administered distinct amounts of diverse plant extracts (100 g/mL, 50 g/mL, and 10 g/mL), either individually inside a cultivating substrate or left untreated as a control. The cultures were subsequently placed in an incubator for a duration of one night, maintaining a temperature of 37°C and a carbon dioxide concentration of 5%. To facilitate the implementation of an enzyme-linked immunosorbent test (ELISA), the supernatants derived from both the experimental and control cell cultures were procured. Negative controls were used in this study to include cells that had not undergone any treatment, regardless of whether they had received ConA therapy prior to the experiment or not. The quantities of IL-6 cytokines in a sample of peripheral blood mononuclear cells (PBMCs) supernatant were assessed using an enzyme-linked immunosorbent assay (ELISA), as per the manufacturer's instructions provided by eBioscience, located in San Diego, USA. The measurement was conducted on a volume of 100 L. All incubation procedures were performed at the standard room temperature. The measurement of optical density at a wavelength of 450 nm was conducted using a microplate reader manufactured by Biotek, a company headquartered in Winooski, Vermont, inside the United States. The measurement was corrected by referencing a wavelength of 570 nm. Cytokine assays were standardized using globally recognized criteria set by the World Health Organization. A detection threshold of 4 pg/mL was chosen for each assay measuring human cytokines and IL-6. The statistical analysis incorporated the calculation of means derived from three distinct experiments. The researchers employed a one-way analysis of variance (ANOVA) to ascertain any significant differences among the groups for each plant extract. Subsequently, statistical analysis was conducted using GraphPad Prism 5 software (GraphPad Software, La Jolla, USA) by performing paired t-tests to ascertain the level of statistical significance between the groups. The data's statistical significance was determined to be significant at a p-value of less than 0.01. The provided data were presented

in the form of means accompanied by standard error of the mean.

3. RESULTS AND DISCUSSION

a. Cytotoxic activity

The outcomes of the SRB assay provide an assessment of cellular viability or growth inhibition in relation to the substances being evaluated. A greater absorbance value signifies a bigger number of viable cells, whilst a lower value implies the presence of growth inhibition or cytotoxic effects of the tested substances on the cells. Typically, the outcomes obtained from the SRB assay exhibit a high degree of reproducibility. The optical density (OD) observed at the conclusion of the experiment is a matter of utmost importance. Firstly, it should be noted that the linearity of measurement is compromised when optical density (OD) values surpass a threshold of 2. Furthermore, in the event of cultural wells becoming overgrown, it is plausible that the growth medium could be depleted, resulting in a partial cessation of cellular growth and subsequent cell death. This phenomenon is most likely to occur in control wells and wells containing lower concentrations of the drug. When considering these factors collectively, it is likely that there will be a notable alteration in the IC50 value, primarily resulting in an underestimating. The data presented in Table 2 allows for the calculation of cell viability and growth inhibition percentages. It is shown that M15 at a concentration of 62.5 µM resulted in approximately 31% cell death. Similarly, M1 at 125 µM and M7 at 500 µM exhibited cell death percentages of 22% and 18.9% respectively, as depicted in Figure 1.

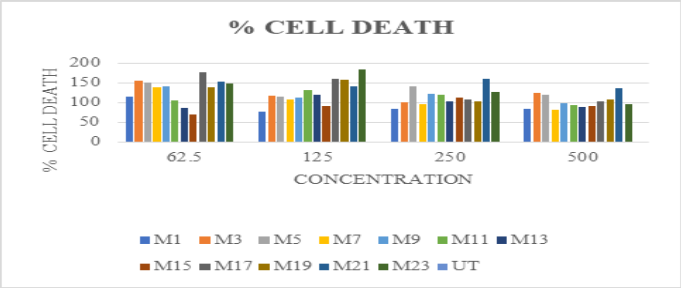


Figure 1: Percentage cell death vs. concentration of M1-M23

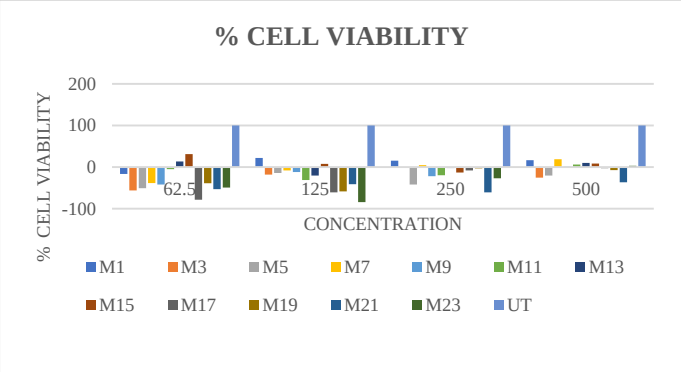


Figure 2: Percentage cell viability vs. concentration of M1-M23

b. Antioxidant activity

i. DPPH radical scavenging activity: The results of each test sample are presented in Table 3. In this experiment, each test sample was obtained at concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 mg/ml. The assay was conducted for all extracts, namely 'M1, M3, M5, M7, M9, M11, M13, M15, M17, M19, M21, and M23' which were extracted from *curcuma longa*. It was observed that M1 and M19 exhibited the highest scavenging activity compared to the other compounds and the standard drug (Figure 1&2).

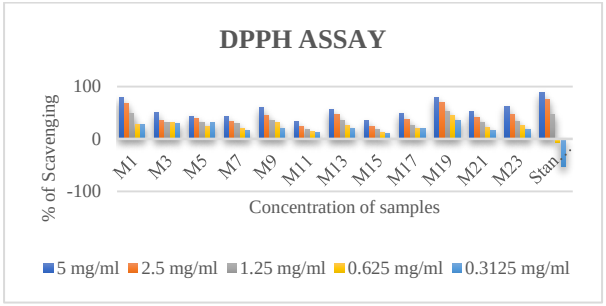


Figure 3: DPPH radical scavenging activity of extracts collected at different time zones at different concentrations with standard.

c. Anti-inflammatory Activity

It is possible to learn a lot about a drug's potential anti-inflammatory qualities by comparing the levels of tumour necrosis factor (TNF) and interleukin-6 (IL-6) in the experimental conditions with and without the substance of interest. If the substance significantly lowers the levels of TNF and IL-6 when compared to the control, this suggests that it may have anti-inflammatory properties by preventing the release or generation of these pro-inflammatory cytokines. Less inflammation is frequently associated with lower levels of the inflammatory proteins interleukin-6 (IL-6) and tumour necrosis factor (TNF). Figures 4 and 5 show that the M19 and M23 extract had an inhibitory effect on TNF- production. In comparison to the standard, the examination of extract M23 demonstrates a more pronounced manifestation of IL-6's influence. As shown in Figures 4 and 5, a number of television programs had a moderate impact on M19 and M13.

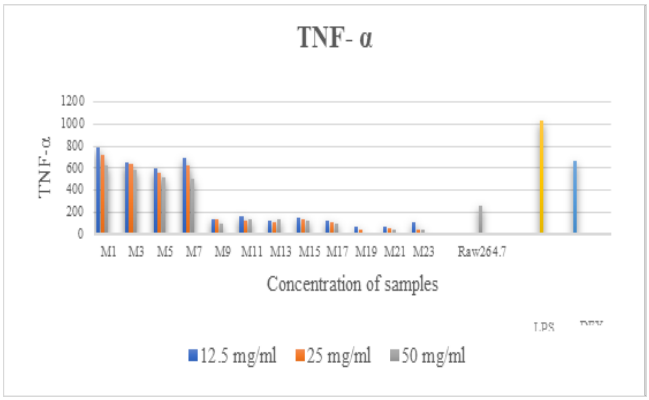


Figure 4: ELISA TNF-α Results for M samples

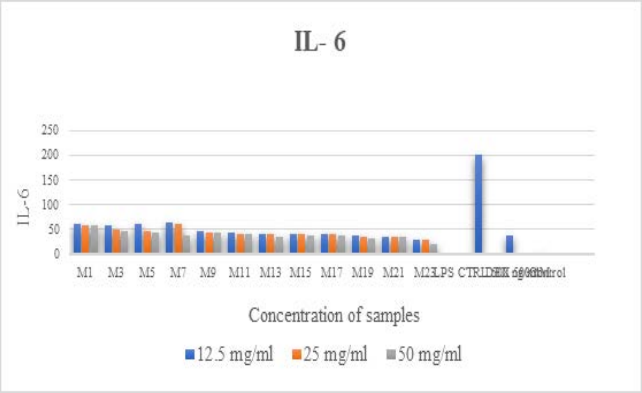


Figure 5: ELISA IL-6 results for M samples

4. CONCLUSION

In conclusion, the evaluation of the biological activities of *Curcuma longa* has demonstrated the tremendous potential of curcumin, a naturally occurring chemical abundantly present in a variety of fruits, vegetables, and herbs, particularly turmeric. Curcumin has been found to have strong anti-inflammatory, antioxidant, and cytotoxic properties after thorough research on a variety of biological activities. The main attribute of this substance's outstanding properties is curcumin, which is also known as the active ingredient.

The DPPH assay findings show that there is significant antioxidant activity present, which is supported by the assay results. Additionally, when compared to established benchmarks, the SRB assay yields favourable results. The capacity of the curcumin extract to lower inhibitory levels of TNF- and IL-6 further contributes to its positive anti-inflammatory effects. This suggests that it may have therapeutic use in the management of inflammatory illnesses.

However, it is crucial to recognize that even though these results are encouraging, more pharmacological research is required to confirm and improve these first results. Curcumin exhibits a variety of biological actions, making it an interesting subject for on-going research and development in the field of natural compounds and their potential medicinal benefits.

ACKNOWLEDGEMENT

We are thankful to Bapuji Maringanti KL College of Pharmacy, Guntur, for helping us to finalize the project.

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