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Phytochemical Analysis and Biological Activities of Curcumin

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Abstract

Curcumin is a bright yellow phytochemical derived from the rhizome of Curcuma longa. Curcumin possesses a wide range of biological effects such as antimicrobial, anti-proliferative, antioxidant, anti-inflammatory, anti-diabetic and neuroprotective activities. Such versatility makes curcumin a promising lead compound for the development of new derivatives that may have a role in the management of numerous illnesses like cancer, diabetes and Alzheimer's disease. These biological effects of turmeric have been attributed to its constituent curcumin that has been widely studied for its anti-diabetic, anti-obesity, anti-oxidant, wound healing and anti-cancer effects and phytochemical analysis of methanolic extract was done. As a result of extensive epidemiological, clinical, and animal studies several molecular mechanisms are emerging that elucidate multiple biological effects of curcumin. The purpose of the study was concentrated on in vitro and in vivo studies on the biological effects of Curcumin, preliminary phytochemical screening of the methanolic extract of the turmeric rhizome and determined the antioxidant activity of curcumin by employing in vitro antioxidant assays such as 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH)scavenging. The solvent extracts tested positive for the presence of bioactive constituents alkaloids, flavonoids, phenols, saponins, Terpenoids, cardiac glycosides and fixed oils and fatty acid. DPPH demonstrated total antioxidant activity.

Keywords: Curcumin, Curcuma longa, Phytochemicals, Antioxidant, Anti-cancerous, Anti-diabetic, DPPH, spectrophotometer, Free Radical Scavenging.

1. Introduction

Curcuma longa (turmeric) belonging the ginger family Zingiberaceae which is native to the tropical South Asia.

Turmeric is known as the 'golden spice' as well as the 'spice of life' . It has been used in India as a medicinal plant and held sacred from time immemorial. India is the largest producer, consumer and exporter of turmeric with 82% of world production and 45% of export. It requires temperatures between 20-30˚c and good rainfall to grow. Turmeric plants and rhizomes are gathered annually.

The plant extracts are known to have anti inflammatory, anti-diabetic, imunomodulatory, anti-oxidative, anti-mutagenic activities, protective effect against induced toxicity, anti-bacterial activities and anti human immunodeficiency virus activity .

Turmeric is an important food preparations preservative that preserves freshness and gives a characteristic flavours. Curcuma rhizomes have a characteristic dark yellow color, and it has been found to be a rich source of phenolic compounds, i.e. curcuminoids. Curcuminoids contain three different curcuminoids Curcumin, demethoxy curcumin, and bisdemethoxycurcumin. In this study we performed the extraction and preliminary phytochemical analysis of the methanolic extract of Curcuma longa Rhizome.

Recently powder of turmeric used as traditional medicine against gastrointestinal diseases, especially for biliary and hepatic disorder, Diabetic wounds, Alzheimer, inflammation, sinusitis, anorexia and cough. Turmeric which act as anticancer, anti-diabetic, antioxidant, anti-obesity, anti-inflammatory, antimicrobial, anti-fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant and possess anti HIV activity to cope up.

Oxidative stress is an imbalance between free radicals and antioxidant systems to cause chronic diseases such as cancer, Alzheimer's and Parkinson's1. Reactive oxygen species (ROS) and free radicals, such as hydroxyl radicals and hydrogen peroxide are produced in the human body during normal metabolic pathways and exposure to exogenous stress such as ionizing radiation and air pollutions can induce adverse effects on the normal physiological activity of cells. The body's system is equipped with antioxidant defense and enzymes which neutralize the ROS. Unfortunately, enhancement of ROS levels or less ability of detoxification of the antioxidant defense system, can lead to increased oxidative stress and turn cell damage. Antioxidants act as protective effects on the cells so that they can protect it from damages caused by uncontrollably produced ROS. Although a number of synthetic and natural antioxidant compounds have already been identified, the search for effective antioxidant and lesser side effects and toxicity is being continued. Turmeric is one of the plants that contain natural active ingredients and safe to use. Curcumin is a hydrophobic polyphenol derived from the rhizome of the Curcuma longa. The present study was designed to investigate free radical scavenging, reducing power potency of curcumin with in vitro models and phytochemical analysis of curcumin from the turmeric rhizome.

Medicinal uses

Rhizome: Purifies blood, used as tonic to brain and heart, used to treat leucoderma, piles, bronchitis, asthma, tumours, tuberculous glands on the neck, enlargement of spleen, diabetes, obesity.

Preliminary phytochemical screening

The chemical evaluation includes qualitative chemical tests which have been used for identification of various phytoconstituents present in the powdered crude drug. The Preliminary phytochemical investigations of aqueous extract, acetone extract, ethanolic extract, chloroform extract and methanolic

extract of Curcuma longa rhizome using commonly employed precipitation and coloration reactions were performed which reveals the presence such as carbohydrates, proteins, alkaloids, glycosides, terpenes, steroids, flavonoids, tannins and saponins. The corresponding test performed which are described below.

2. Materials and Methods

2.1 Materials

DPPH (1,1-diphenyl-2-picryl-hydrazyl),Spectrophotometer, Turmeric powder, hydrochloric acid, Wagner's reagent, Fehling's solution A and B, Sodium hydroxide, Ferric chloride solution, Distilled water, Chloroform and conc. Sulphuric acid and Glacial acetic acid.

2.2 Methods

Preparation of the Extract

The rhizomes of Curcuma longa were collected and sun dried, cut into small pieces. The small piece of dried rhizome was then grinded to get a fine powder, which is ready for use.

Test for Alkaloid

The extract was mixed with 3ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with the Wagner test.

Wagner Test: 1ml or 2ml of the filtrate extract was treated with Wagner's reagent; formation of brown reddish precipitate shows positive result of alkaloids.

Test for Tannins

Lead test: 20mg turmeric was dissolved in 1ml of distilled water in a test tube and 1-3 drops of Ferric chloride were added to the solution. Then the mixture was observed for blue or green color.

Test for Cardiac Glycosides

20mg of turmeric was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. 0.5ml of conc. Sulphuric acid was slowly added along the sides of the tube. A brown ring at the interface indicates the presence of glycosides.

Test for Saponins

Foam Test: The presence of Saponins was found when 40mg turmeric was dissolved with 5ml of distilled water and shaken vigorously and then heated to boil. Frothing shows the presence of saponins.

Test for Flavonoids

Ferric Chloride Test: 20mg of turmeric was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution was added to it. Conc. Sulphuric acid was added later. A yellow color indicates the presence of flavonoids. The yellow color disappeared on allowing the solution to stand.

Test for Terpenoids

Salkowaski's test: 20mg turmeric was dissolved in 1ml of chloroform and 1ml conc. Sulphuric acid was added to it. A reddish brown discoloration at the interface showed the presence of Terpenoids.

Test for Phenol

Ferric Chloride Test: 4 drops of Alcoholic FeCl3 solution were added in the test extract. Appearance of bluish black color indicates the presence of phenol.

Test for carbohydrates

Fehling's test few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

Test for protiens

Biuret's test: Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet color indicates presence of proteins.

Fixed oils and fatty acid

Spot test: Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil & fats.

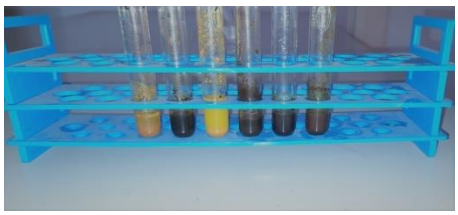


Fig 2.2: Phytochemical Screening of Curcuma longa

2.3 DPPH Free Radical Scavenging Assay

The scavenging activity for DPPH free radicals was measured according to the procedure. Curcumin of concentrations (1 to 3 ml) was added separately to each 3ml of 0.1 mm methanolic solution of DPPH and allowed to stand for 30 min at room temperature. The absorbance of each solution was determined at 517 nm using spectrophotometer.

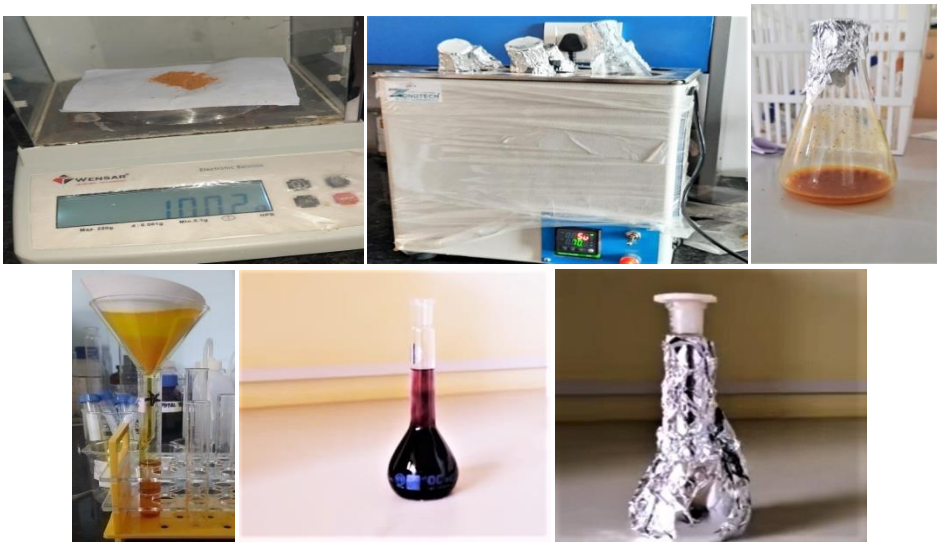
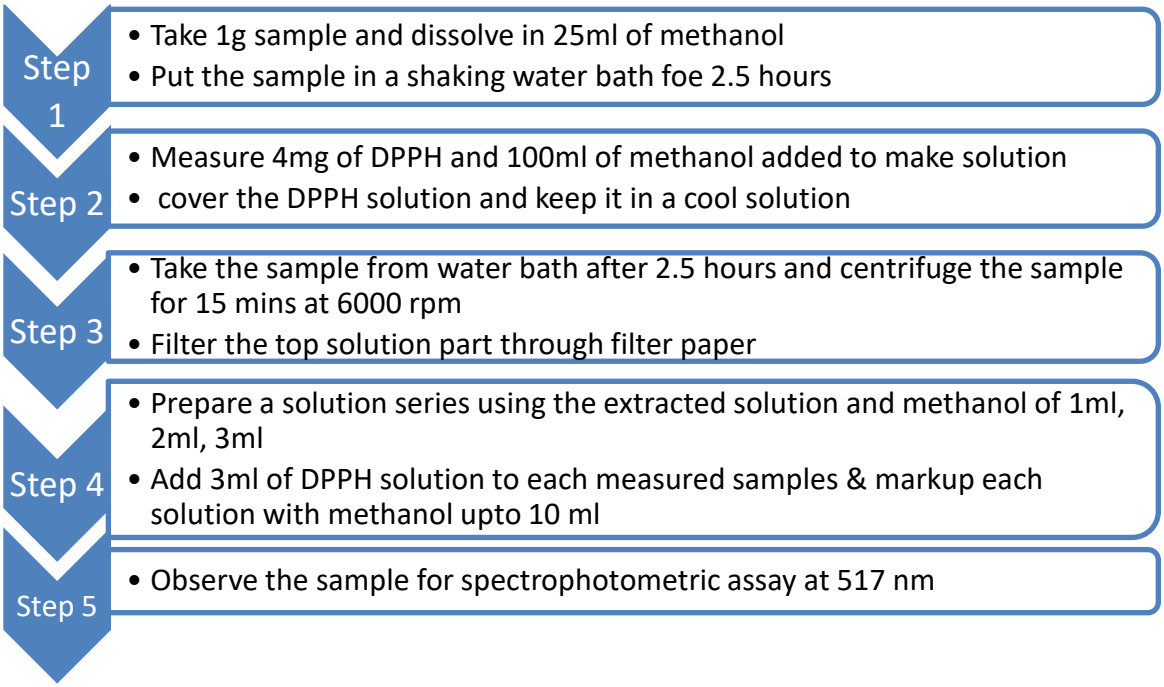


Fig 2.3 a): Showing Sample preparation for DPPH Free Radical Scavenging Assay

Fig 2.3 b): Method for DPPH Free Radical Scavenging Assay



2.3 Biological Activities of Curcumin: A systematic Review

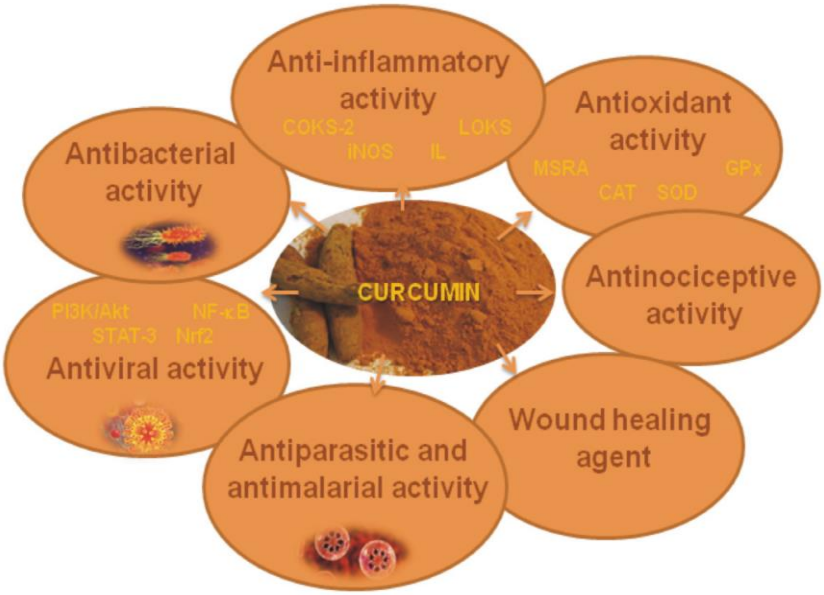


Fig 2.4 a): Showing Biological Activities of Curcumin

Antioxidant activity

ROS and reactive nitrogen species (RNS) are generated in the human body in various endogenous systems, in pathophysiological conditions or exposure to various physical and chemical factors. Free radicals can change lipids (lipid peroxidation), proteins (loss of enzyme activity), and DNA (mutagenesis and carcinogenesis); they contribute to ageing and many human diseases. Curcumin shows strong antioxidant activity. The antioxidant property is attributed to the presence of various functional groups, including methoxy, phenoxy, and carbon–carbon double bonds in its structure. Curcumin is a classic phenolic antioxidant that donates H atoms from phenolic groups. It was found that curcumin can

inhibit oxidative damage caused by stress in the brain, liver, and kidneys of rats. Lipid peroxidation is significantly reduced in rats treated with curcumin before applying γ-radiation. Curcumin increases enzymatic antioxidant activity by increasing the expression of methionine sulfoxide reductase (MSRA) and increasing the levels of the enzymes MSRA, SOD(superoxide dismutase), CAT(catalase), GPx(glutathione peroxidase). Curcumin may act as an antioxidant against oxidative stress in rats with diabetes mellitus by increased SOD expression in cochlear fibroblasts. The antioxidant activity of curcumin was assessed using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical assay. The percentage of free radical removal using curcumin was 69 at a concentration of 0.1 mM. Curcumin has shown a large capacity to remove smaller oxidative molecules such as H_2O_2, HO•, ROO•. Curcumin can be used as an effective antioxidant to protect against ROS in the cytoplasm of cells. Curcumin formulations with different carriers that are stable and protected from various influences are used as antioxidants.

Anti-diabetic Activity

A Pilot Study performed in Mexico showed evidence that dietary supplementation with curcumin can reduce oxidative stress in patients with non-diabetic or diabetic proteinuric CKD(Chronic Kidney Disease). Curcumin significantly improved the elimination of free radicals activity in individuals with non-diabetic proteinuric CKD and reduced the plasma levels of malondialdehyde(MDA). Another study, which investigated the effect of nano-curcumin in T2DM(Type 2 Diabetes Mellitus) individuals, was adequately randomized, with no significant loss of participants, and even with a dose that the authors considered low, the effects of curcumin were positive. The fasting blood glucose (FBG), Hemoglobin A1c (HbA1C), body mass index (BMI), Estimated Average Glucose (eAG), total cholesterol (TC), LDL-c(Low Density Lipoprotien), HDL-c(High Density Lipoprotien), and triglyceride (TG) were compared between the two groups after the intervention. Curcumin improved FBG, HbA1c, BMI, and eAG, but did not affect LDL-c, HDL-c, TG, and TC.

Anti-cancerous Activity

Curcumin has long been used as a dietary ingredient with known health benefits. Extensive research over the last decade has shown that curcumin possesses anticancer activities and could be used as a preventive or treatment agent against cancers, either as a single or combination therapy with chemotherapeutic agents. Curcumin exhibits biological activities in various stages of carcinogenesis including inhibition of oncogene activation, prevention of cancer-related inflammation, inhibition of cancer cell proliferation, induction of apoptosis and prevention of metastasis, and sensitization of cancer cells to chemotherapy. Curcumin has been established as a chemopreventive agent that has the ability to suppress or retard the carcinogenic process induced by various chemical carcinogens. In animal models of gastric and colon cancer, curcumin inhibits the development of cancerous and precancerous lesions induced by N-methyl-N'-nitro-N-nitrosoguanosine (MNNG), a known mutagenic agent causing DNA methylation. The study, MNNG was given in drinking water at the concentration of 100 ppm for 8 weeks, and then 0.2% or 0.5% of curcumin was fed to the rats for 55 weeks. The results showed that the number of atypical hyperplasia in curcumin-treated rats was significantly less than that in the control group.

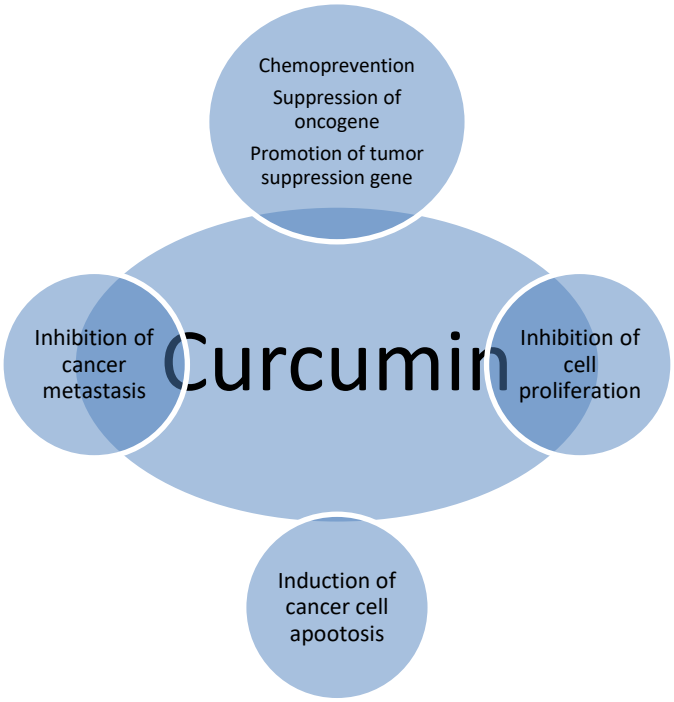


Fig 2.4 b): Anticancer Properties of Curcumin

Anti-obesity Activity

Obesity has been classified as a growing epidemic and its associated metabolic disorders are considered a major risk to the health system. Curcumin interacts with specific proteins in adipocytes, pancreatic cells, hepatic cells, macrophages, and muscle cells, where it suppresses several cellular proteins such as transcription factor NF-kB, STAT-3(NF-κB transcription factors and the signaling pathways are central coordinators in innate and adaptive immune responses. STAT3 regulates the expression of a variety of genes in response to cellular stimuli, and thus plays a key role in cell growth and apoptosis), Wnt/β-catenin(The **Wnt/β-catenin** pathway comprises a family of proteins that play critical roles in embryonic development and adult tissue homeostasis. The deregulation of Wnt/β-catenin signalling often leads to various serious diseases, including cancer and non-cancer diseases) and activates PPAR-γ, Nrf2 (Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated receptor in the nuclear hormone receptor family that synergistically interacts with the nuclear factor erythrocyte 2-related factor 2 (Nrf2) pathway to promote the expression of related genes and inhibit ferroptosis) cell signaling pathway. In addition, curcumin downregulates the inflammatory cytokines, resistin and leptin, and upregulates adiponectin as well as other associated proteins. The interactions of curcumin with several signal transduction pathways reverse insulin resistance, hyperglycemia, hyperlipidemia, and other inflammatory symptoms associated with obesity and metabolic diseases.

3. Result and Discussion

India has about 20,000 known traditional medicinal plants in the world. Rural populations depend on the medicinal plants for treatment of the ailments. The results of the preliminary phytochemical analysis provide an verifiable basis for the use of medicinal plants in therapeutic therapy. The phytochemical constituents are responsible for the biological and pharmacological actions of curcumin. Alkaloids have antibacterial activities. Curcumin, demethoxy curcumin and bis-demetho-hydroxycurcumin, are three pharmacologically important curcuminoids that have been isolated from Curcuma longa. They have

been shown to possess several biological activities such as anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-fungal, anti-viral and anti-cancer properties. Curcumin prevents radiation induced damage and help repair cells by anti oxidative, free radical scavenging activity.

TABLE 3.1: Results of the preliminary phytochemical analysis of Methanolic extract of Curcuma longa Rhizome:

Phytochemicals	Test	Observation	Interference
Alkaloids	Wagner's Test	Red precipitate	Present
Tannins & Phenolic Compounds	Lead Test	Green Color	Present
Terpenoids	Salkowaski's Test	Reddish brown color	Present
Saponins	Foam Test	Presence of emulsion	Present
Flavonoids	Ferric Chloride Test	White Precipitate	Present
Cardiac Glycosides		Brown ring	Present
Fixed oils and Fatty Acids	Spot Test	Presence of spot	Present

TABLE 3.2: Results of the Reactive Scavenging Activity (RSA) of Curcumin

Concentration(ml)	Control	Sample	%RSA
1ml	0.52	O.278	46.53
2ml	0.52	0.197	62.11
3ml	0.52	0.943	81.34

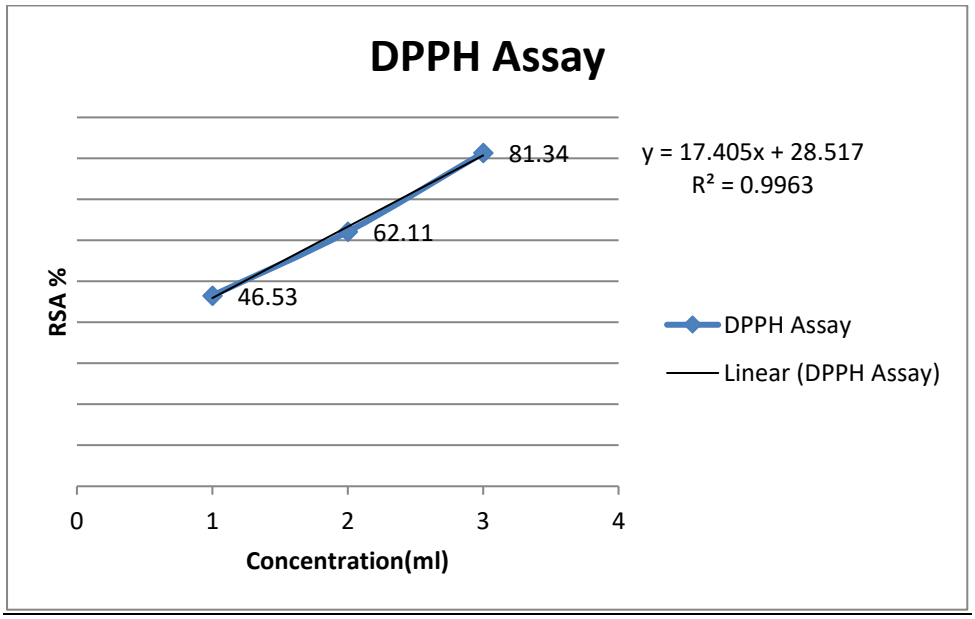


Fig 3.1: DPPH Assay of Curcumin

Conclusion

The preliminary phytochemical analysis of methanolic extract of Curcuma longa showed presence of some important Phytochemicals like alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids (Table No. 3.1). These phytoconstituents have important pharmacological

activities like anti-carcinogenic, anti-inflammatory, antimicobial, and antioxidant properties. Curcumin was found to be an effective antioxidant in different in vitro assays including: DPPH and radical scavenging. Fig. 3.1 shows the total antioxidant activity of curcumin. Curcumin is a widely studied natural compound that has exhibited enormous in vitro and in vivo therapeutic potential in prevention of diseases. Curcumin is a natural anti-inflammatory, anti-diabetic, anti-obesity and anti-cancerous agent representing a safe and low-cost alternative for this condition's therapeutic approach. The modulation of several cellular transduction pathways by curcumin has recently been extended to elucidate the molecular basis for obesity and obesity-related metabolic diseases. The relatively low cost of curcumin, safety and proven efficacy make it advisable to include curcumin as part of healthy diet. Studied have proven that 500-2000 mg of doses of curcumin daily helps in managing and preventing life style diseases.

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