Volatile chemical profiling and antioxidant activity of Curcuma longa (Linn)

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I. PHYTOCHEMISTRY

Phytochemistry is in the study of phytochemicals, the chemicals which are derived from plants. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. They are secondary metabolites produced by plants in which some have medicinal uses (Hotta *et al.*, 2020). The plant chemicals that protect plant cells from environmental hazards such as pollution stress, drought UV exposure and pathogenic attack are called as phytochemicals. More than 4,000 phytochemicals have been identified and are classified by protective function, physical characteristics and chemical characteristics and about 150 phytochemicals have been studied in detail (Saxena *et al.*,2013).

1.1. Essential oils

Essential oil is a concentrated hydrophobic liquid containing volatile (easily evaporated at normal temperatures) chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea or simply as the oil of the plant from which they were extracted, such as oil of clove. An essential oil is "essential" in the sense that it contains the "essence of" the plant's fragrance the characteristic fragrance of the plant from which it is derived. Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Essential oils are often used for aromatherapy, a form of alternative medicine in which healing effects are ascribed to aromatic compounds. Aroma of essential oil is the basis for their use in perfumery, cosmetics and the food industry (Butnariu *et al.*, 2018). Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavour and odour (Firn, 2010). Aromatherapy may be useful to induce relaxation, but there is not sufficient evidence that essential oils can effectively treat any condition. Common extraction methods are hydrodistillation, steam distillation, solvent extraction, CO2 extraction, maceration, enfleurage, cold press extraction and water distillation.

1.2. Primary and Secondary metabolites

1.2.1 Primary Metabolites

Primary metabolites are microbial products produced continuously during the exponential phase of growth and are involved in primary metabolic processes such as respiration and photosynthesis. Essential molecules for normal physiologica growth and energy

requirements of plants is called primary metabolism and the products are called primary metabolites. They are widely distributed in nature and are also utilized as food by man.

1.2.2 Major secondary metabolites

Secondary metabolites are organic compounds produced by bacteria, fungi, or plants which are not directly involved in the normal growth, development, or reproduction of the organism.Plant secondary metabolites are classified by their chemical structure.

1.2.2.1. Phenolics

Phenolics are largest group of plant secondary metabolites.They share the presence of one or more phenol groups as a common characteristic and range from simple structures with one aromatic ring to highly complex polymeric substances.They are widespread in plants where they contribute significantly to the color, taste and flavor of many herbs, foods and drinks. Some phenolic are valued pharmacologically for their anti-inflammatory activities such as quercetin or anti-hepatotoxic properties such as silybin (Hussein *et al.*, 2018). Plant phenolics are biosynthesized by two basic pathways, Shikimic acid path way and malonic acid path way (Kumar *et al*., 2018). Curcumin contain phenolic

compounds are curcuminoids (Sarangthem and Haokip, 2010). Many of the phenolic molecules are also effective antioxidants and free radical scavengers.

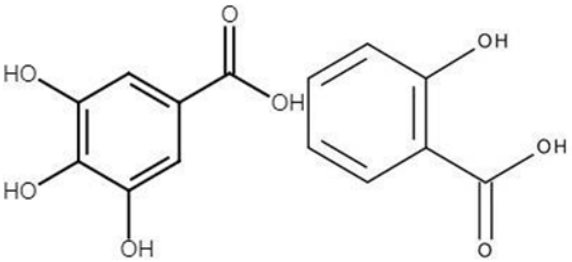

Figure 1.1. Gallic acid and Salicylic acid

1.2.2.2. Flavonoids

Flavonoids (or bioflavonoids) (from the Latin word flavus, meaning yellow, their colour in nature) are a class of polyphenolic molecules. Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings and a heterocyclic ring. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche *et al*., 2016). Flavonoids are synthesized through the phenylpropanoid pathway, transforming phenylalanine into 4-coumaroyl-CoA, which finally enters the flavonoid biosynthesis pathway. The first enzyme which is specific for the flavonoid pathway, chalcone synthase, produces chalcone scaffolds from which all flavonoids derive (Ferreyra *et al.,* 2012).

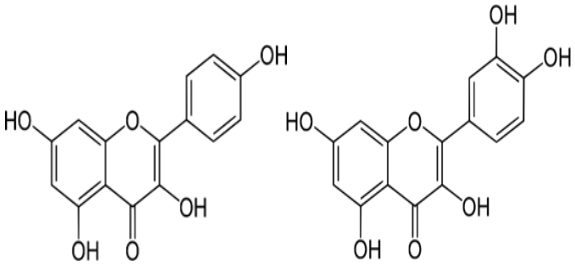

Figure 1.2. Kaempferol and Quercetin

1.2.2.3. Terpenes

Terpenes are the largest and most diverse group of plant secondary compounds. The name "terpene" is derived from the word "turpentine," which in turn comes from the old French ter(e)bintb, meaning "resin". They are all derived chemically from 5-carbon isoprene units assembled in different ways (Hussein *et al.,* 2018). Simple terpenes are volatile, evaporating quickly, and are considered the essential oils that imbue plants unique odour's these odours may attract or repel other organism as needed for survival. More complex terpenes consisting of several isoprene units may be precursors to bioactive molecule like cholesterol, steroid hormones, or waxy substances that act as protective coverings. Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8 (Monika *et al.*,2008). Among terpenoids, monoterpenoids (C_{10}), sesquiterpenoids (C_{15}) and diterpenoids (C_{20}) were detected in *Curcuma longa* (Lee *et al*., 2014).

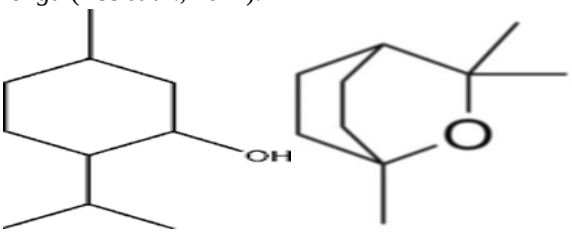

Figure 1.3. Menthol and 1,8- Cineole

1.2.2.4. Saponins

Saponins are compounds that possess a polycyclic aglycone moiety with either a steroid (steroidal saponins) or triterpenoid (triterpenoidal saponins) attached to a carbohydrate unit (a monosaccharide or oligosaccharide chain). These sugar units are composed variously of pentose's, hexoses or uronic acids. This hydrophobic-hydrophilic asymmetry means that these compounds have the ability to lower surface tension and are soap-like. The foaming ability of Saponins is caused by the combination of a hydrophobic (water insoluble) sapogenin and hydrophilic (water soluble) sugar parts. Saponins have a bitter taste and some are toxic and are known as sapotoxin (Biren, 2009). The aglycone portion of the saponin molecule is called the genin or sapogenin. Saponins are widespread among plants, having been reported from more than 500 plants from at least 90 different families; these substances have been isolated from all parts of plant (Hussein *et al*., 2018).

1.2.2.5. Coumarin

Coumarins are a family of benzopyrones. The four

most widely distributed coumarins are coumarin, umbelliferone, esculetin (aesculetin) and scopoletin. Coumarins used as perfumes, cosmetics and industrial additives (Matos *et al*.,2015). Simple coumarins are biogenetically derived from shikimic acid, via cinnamic acid.The specificity of the process is the C-2 hydroxylation,producing a break (β-oxidation) of the side chain (i.e. Salix sp.) or chain isomerization and subsequent lactonization, generating the umbelliferone (Hussein *et al* .,2018).

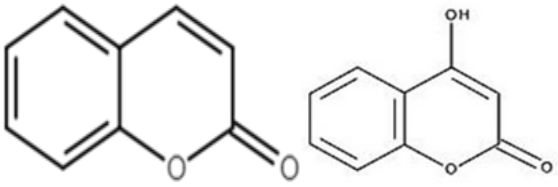

Figure 1.4. Coumarin and 4-hydroxycoumarin

1.2.2.6. Alkaloids

Alkaloids are organic compounds with at least one nitrogen atom in a heterocyclic ring. Their definition is problematic, as they do not represent a homogeneous group of compounds from any standpoint, whether chemical, biochemical, or physiological. Except for the fact that they are all nitrogen-containing compounds, no general definition fits all alkaloids. Alkaloids can be divided according to their basic chemical structure into different types. The following are basic types of alkaloids: acridones, aromatics, carbolines, ephedras, ergots, imidazoles, indoles,bisindoles,indolizidines,manzamines,oxindoles ,quinolines,quinozolines,phenylisoqui nolines, phenylethylamines, piperidines, purines, pyrrolidines, pyrrolizidines, pyrroloindoles, pyridines and simple tetrahydroisoquinolines (Hussein *et al* .,2018)

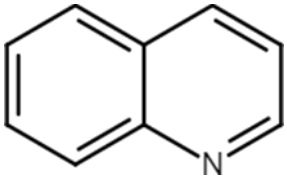

Figure 1.5. Quinoline

1.3. Extraction, isolation and separation of secondary metabolites

1.3.1 Extraction

(a) Solvent extraction

Various solvents have been used to extract different phytoconstituents. The plant parts are dried in a range of temperature (50-60°C) or dried preferably in shade so as to bring down the initial large moisture content to enable its prolonged storage life. The plant material is then extracted in a Soxhlet apparatus or by soaking in water or alcohol (95% v/v). This can then be concentrated and used to determine the presence of phytoconstituents.

(b) **S**upercritical fluid extraction (SFE)

This is the most technologically advanced extraction system (Patil and Shettigar, 2010). Super Critical Fluid Extraction (SFE) involves use of gases, usually CO_2 and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. Solvent properties of CO_2 can be manipulated and adjusted by varying the pressure and temperature that one works at. The advantages of SFE are, the versatility it offers in pinpointing the constituents, you want to extract from a given material and the fact that your end product has virtually no solvent residues left in it (CO_2 evaporates completely). There are many other gases and liquids like liquid SO_2 that are highly efficient as extraction solvents when put under pressure (Patil and Shettigar, 2010).

c) Microwave-Assisted extraction

An innovative, microwave-assisted solvent-extraction technology known as Microwave- Assisted Processing (MAP) has been reported recently (Patil and Shettigar, 2010). MAP applications include the extraction of high-value compounds from natural sources including phytonutrients, nutraceutical and functional food ingredients and pharmaceutical actives from biomass. By using closed vessels, the extraction can be performed at elevated temperatures accelerating the mass transfer of target compounds from the sample matrix. A typical extraction procedure takes 15–30 min and uses small solvent volumes in the range of 10–30 ml. These volumes are about 10 times smaller than volumes used by conventional extraction techniques. In addition, sample throughput is increased as several samples can be extracted simultaneously. In most cases recoveries of analytes and reproducibility are improved compared to conventional techniques, as

shown in several applications (Eskilsson *et al.,* 2000).

(d) Solid phase extraction

This involves sorption of solutes from a liquid medium onto a solid adsorbent by the same mechanisms by which molecules are retained on chromatographic stationary phases. These adsorbents, like chromatographic media, come in the form of beads or resins that can be used in column or in batch form. They are often used in the commercially available form of syringes packed with medium (typically a few hundred milligrams to a few grams) through which the sample can be gently forced with the plunger or by vacuum. Solid phase extraction media include reverse phase, normal phase, and ion-exchange media. This is method for sample purification that separates and concentrates the analyte from solution of crude extracts by adsorption onto a disposable solid-phase cartridge (Patil and Shettigar, 2010). Advanced device formats facilitate processing of problem samples combined with a high level of automation. Approaches to computer-aided method development promise to replace tedious trial-and-error procedures with fast simulations based on suitable kinetic and retention models (Poole, 2003).

1.4.2. Chromatography

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid) and affinity or differences among their molecular weights (Coskun 2016). There are different type of chromatographic techniques such as column chromatography, thin layer chromatography, HPLC, HPTLC, GC-MS etc.

a) Column chromatography

Column chromatography is a common chromatographic technique. In traditional column chromatography a sample to be purified is placed on the top of a column containing some solid support, often silica gel(Roge *et al.,* 2011).A chromatographic separation ,which is achieved by distributing substances in a mixture between two phases: a moving phase and a stationary phase(Scott 1983).Adsorption chromatography that is widely used for the separation of individual components of interest present in mixture.When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slow are eluted out last. The adsorption of solute molecules to the column occurs in a reversible manner.

b) Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them.MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them (Hussain *et al.,*2014). GC/MS begins with the gas chromatograph, where the sample is volatized. This effectively vaporizes the sample (the gas phase) and separates its various components using a capillary column packed with a stationary (solid) phase. The compounds are propelled by an inert carrier gas such as argon, helium or nitrogen. As the components become separated, they elute from the column at different times, which is generally referred to as their retention times. Once the components leave the GC column, they are ionized by the mass spectrometer using electron or chemical ionization sources. Ionized molecules are then accelerated through the instrument's mass analyser, which quite often is a quadrupole or ion trap. It is here that ions are separated based on their different mass-to-charge (m/z) ratios. The final steps of the process involve ion detection and analysis, with compound peaks

appearing as a function of their m/z ratios. Peak heights, meanwhile, are proportional to the quantity of the corresponding compound. A complex sample will produce several different peaks, and the final readout will be a mass spectrum. Using computer libraries of mass spectra for different compounds, researchers can identify and quantitate unknown compounds and analytes. GC-MS is widely used in pharmaceutical industries for analytical research and development,

quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients, bulk drugs and formulations (Al-Rubaye *et al.*,2017).

c) Thin-layer chromatography

Thin-layer chromatography is a "solid-liquid adsorption" chromatography. Thin layer chromatography (TLC) is used to separate non-volatile mixtures, is performed on a sheet of glass, plastic or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina) or cellulose**.** This layer of adsorbent is known as the stationary phase (Narayana *et al*., 2018).The solvent travels up the thin plate soaked with the solvent by means of capillary action. During this procedure, it also drives the mixture priory on the lower parts of the plate with a pipette upwards with different flow rates. Thus the separation of analysts is achieved. This upward travelling rate depends on the polarity of the material, solid phase, and of the solvent. Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given substance, determine the purity of a substance (Bele *et al.*,2011). The rate of the movement of the components is expressed as;

Rf = the distance travelled by solute/ the distance travelled by solvent Rf is the retardation factor

d) High performance liquid chromatography

High performance liquid chromatography (HPLC) is a form of liquid chromatography which is used to separate the individual components of interest present in mixture or dissolved in sample solution. It is based on pumping of mobile phase through the packed column under high pressure (Akash and Sajid, 2020). The basic principle involved in HPLC is based on the phenomenon of column chromatography in which the mobile phase is pumped through a packed column by applying high pressure.

1.4. The Genus *Curcuma*

Curcuma genus is in the family Zingiberaceae. *Curcuma longa, Curcuma aeruginosa, Curcuma pseudomontana, Curcuma aromatica, Curcuma xanthorrhiza, Curcuma zedoaria, Curcuma angustifolia* are the pharmacologically active species (Kanse *et al.*, 2018). They occur in wild and cultivated forms and widely distributed throughout the Tropics of Asia, Africa, Australia (Saxena *et al.*, 2013). India is popularly known as the "Spice Bowl of the World" as a wide variety of spices with premium quality is grown in the country since ancient times. In India Kerala, Tamil Nadu, Andhra Pradesh is the largest producer of *Curcuma longa.* It is commonly known as Turmeric, Haridra in Sanskrit, Haldi in Hindi, and Manjal in Malayalam. The project targets to study the phytochemical screening, GC-MS analysis, and antioxidant potentiality of rhizome extracts of *Curcuma longa*.

1.5. Plant Profile

Figure 1.6. *Curcuma longa* (Linn)

Taxonomical Classification

Kingdom	: Plantae
Phylum	: Spermatophyta
Subphylum	: Angiospermae
Class	: Monocotyledonae
Order	: Zingiberales
Family	: Zingiberacea
Genus	: Curcuma
Species	: Curcuma longa
Scientific name	: *Curcuma longa* (Linn)

1.6. Aim and relevance of the work

A perfect example of medicinal plant credited with innumerable medicinal qualities validated by modern science and used since ancient times is *Curcuma longa*. The present work involved collection and identification, isolation of essential oil, identification of volatile compounds from the essential oil of rhizome of *Curcuma longa*. The present study is also an attempt that examines the antioxidant activity of *Curcuma longa* extracts.

The present study was carried out by the following objectives;

- Identification and quantification of volatile compounds from the rhizome essential oil of *Curcuma longa* (Linn)
- Phytochemical screening of *Curcuma longa* (Linn)
- Antioxidant studies of *Curcuma longa* (Linn) extracts

II. GENUS CURCUMA

Genus *Curcuma* is best known for being an essential source of coloring and flavoring agents in the, traditional medicines, spices, dyes, perfumes, cosmetics and ornamental plants. The genus originates in the Indo-Malayan region and it is broadly spread all over the world across tropical and subtropical areas, approximately 100 *Curcuma* species reported. The rhizome is the most commonly used part of the plant.The rhizomes of *C. aeruginosa, C. amada, C. aromatica, C. longa, C. pierreana, C. pseudomontana, C. purpurascens, C. xanthorrhiza*, and *C. zedoaria* are also employed as dye, spice, and food flavoring and coloring agent in food preparations mainly due to their exotic aroma (Rajkumari *et al.,* 2017). *C. longa* is one of the most studied species. India is also the largest producer, consumer, and exporter of turmeric in the world.

Figure 2.1. *Curcuma longa* (Linn)

2.1. Commercial uses

2.1.1. Food stuff and colorant

In most of the Asiatic countries the largest quantity of it is utilized as a food adjunct in vegetable, meat and fish preparations. It is necessary culinary ingredient gives musky flavour and yellow colour to curries. There are hardly few recipes in which Turmeric Powder is not used in Indian Dishes. It can be used as a substitute for saffron hence it is called as Indian Saffron. It is used to flavour and colouring cheeses and butter, margarine, pickles, mustard, liquor, fruit drinks, sauces, cakes, biscuits, popcorn, cereals, jellies, fruit dishes and other foodstuffs. Turmeric powder is one of the most significant colouring materials of India in all kinds of cuisine to give light to golden yellow colour. Previously, in India, turmeric was largely used for dyeing wool, silk and cotton, to impart a yellow shade, in an acid bath. It is still used to dyeing cotton. The dye is also employed as colouring material in pharmacy, confectionery, rice milling and food industries. For thousands of years it has been used in the Hindu religious ceremonies. Considerable quantities of turmeric are converted as kumkum used for tilak which stands for wealth, fortune, prosperity and opulence (Sabale *et al.*,2013).

2.1.2. Indicator

Turmeric paper, also called curcuma paper or in German literature, Curcumapapier, is paper steeped in a tincture of turmeric and allowed to dry. It is used in chemical analysis as an indicator for acidity and alkalinity. The paper is yellow in acidic and neutral solutions and turns brown to reddish-brown in alkaline solutions with transition between pH of 7.4 and 9.2. In case of strong acid and strong base, volume consumed with phenolphthalein and curcuma longa are almost similar, differing only 0.5 ml and pH at end point was same both cases (Patil *et al.* 2018).

2.2. Phytochemical Studies

Turmeric rhizome typically contains carbohydrates, protein, fat and minerals. The phytochemical analysis of methanolic extract of *Curcuma longa* showed presence of some important phytochemicals like

alkaloids, tannins, phenolic compounds, phytosterols, terpenoids, saponins and flavonoids (Rajesh *et al*.,2013).

Essential oil analysis of *C. longa* reported with major components: ar-turmerone, β- turmerone and curlone (Mehra *et al.,* 2019). α-phelladrene , terpinolene ,undecanal , p- cymene which were present as major components in the leaves essential oil were observed as trace constituents in the rhizome oil (Awasthi *et al.,*2009). ar-Turmerone, the major volatile component in the rhizomes of *C. longa* showed potent α-glucosidase inhibitory activity (Lekshmi *et al.,* 2012). Curcumin is an important pharmacological agent that is isolated from turmeric rhizome and the compound belongs to the group of curcuminoids i.e. diphenylheptanoids. Curcumin is the most active and the well-studied component in *C. longa.* Other characteristic compounds identified from *C. longa* are demethoxy curcumin and bisdemethoxy curcumin **(Figure 2.2)** (Stanojevic *et al.,*2015). Climatic conditions and topography could have significant impact on the content of curcumin in *C. longa*. Previous reports show that the curcumin content in *C. longa* in Kerala varied between 0.12% to 2.35%, which is very low compared to other states in India (Geethanjali *et al.,*2016).

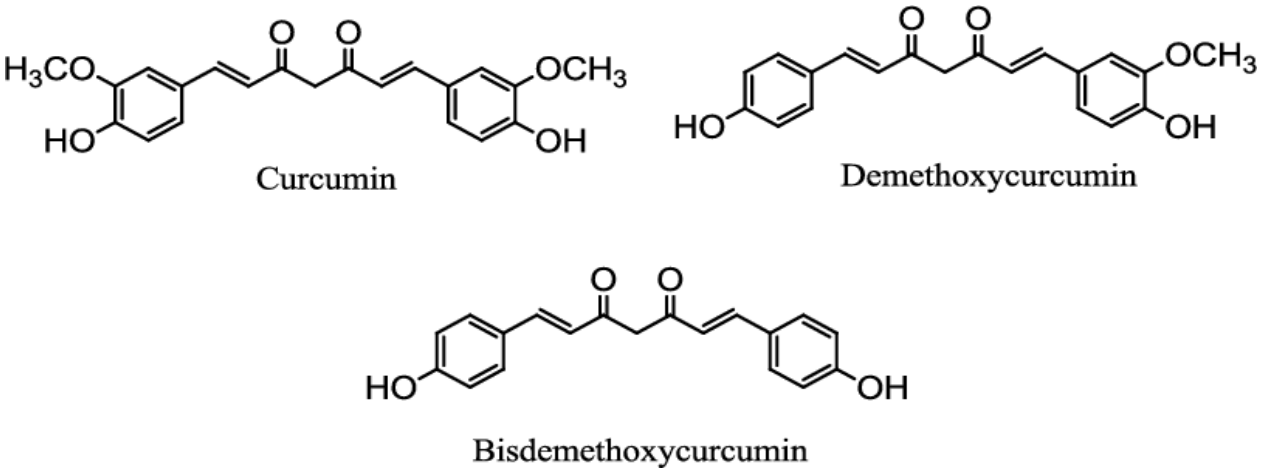

Figure 2.2: Characteristic compounds identified from *C. longa*

2.3. Biological and pharmacological activities of *C. longa*

Pharmacological action of *Curcuma longa* is mainly due to its main active constituent curcumin present in *C. longa*. Different extract of turmeric such as ethanolic, aqueous, ether, and petroleum ether shows different types of activities. For hundreds of years, members of *Curcuma* have been used in traditional medicine for treating respiratory complaints, pain, digestive disorders, inflammatory conditions, wounds, hypercholesterolemia, hypertension, hematologic and circulation abnormalities, infectious diseases and cancer prevention (Dosoky *et al.,*2018). Curcumin was said to have a cholesterol-lowering, antidiabetic and antioxidant properties and to have an anticancer activity in both in vitro and in vivo models. Then, with the clinical studies conducted with humans, it was determined that curcumin was safe and effective (Kocaadam and Sanlier 2017).

2.3.1. Antioxidant activity

Curcumin exhibits strong antioxidant effect through free radical scavenging activity. It acts as a scavenger of oxygen free radicals. It can protect haemoglobin from oxidation. *In vitro*, curcumin can significantly reduce speed of the production of reactive oxygen species such as superoxide anions, hydrogen peroxide and nitrite radical production. This is done by activated macrophages, which take part in a vital job in inflammation. Different extracts of turmeric such as water and fat soluble and its curcumin content exhibit strong antioxidant activity (Ashraf *et al.,* 2018). The powerful antioxidant property of curcumin can prevent

rancidity of foods and provide foodstuffs containing less oxidized fat or free radicals (Kulkarni *et al*., 2017).

2.3.2. Anti-inflammatory activity

Curcuma longa plays very vital role in reducing inflammatory swelling. Oral administration of curcumin was found to be as effective as cortisone or phenylbutazone in acute inflammation. Anti-inflammatory properties of *C. longa* may be endorsed to its skill to restrain both biosynthesis of inflammatory prostaglandins from arachidonic acid and neutrophil function during inflammatory states (Ashraf *et al*., 2018). Turmeric oil displayed significant anti-inflammatory activities in the acute and chronic models of inflammation. NO, superoxide and their reaction product peroxynitrite are generated in excess during the host response against infections and inflammatory conditions (Liju *et al*., 2011).

2.3.3. Anticoagulant activity

Curcumin has found to possess anticoagulant activity. Its mechanism of action is to inhibit collagen and adrenaline induced platelet aggregation in vitro as well as in vivo in rat thoracic aorta (Ashraf *et al*., 2018). ar-turmerone in *C. longa* has been shown to display antiplatelet activity (Naz *et al.,* 2010).

2.4.4 Anti-venom activity

A fraction consisting of ar-turmerone, isolated from *C. longa* was tested for its anti-venom activity and the results revealed that the extract neutralized both the hemorrhagic activity and lethal effect of venom in mice. In this study ar-turmerone was capable of abolishing the haemorrhagic activity of *Bothrops* venom and about 70% of the lethal effect of *Crotalus* venom. ar-Turmerone can act as an enzymatic inhibitor in the case of venom enzymes, with proteolytic and hemorrhagic activities (Araujo *et al.*, 2001).

2.4.5 Anti-diabetic activity

The limited human studies indicate that curcumin administration can improve glucose homeostasis and reduce the diabetic phenotype with reduced blood glucose levels and reduced insulin resistance (Hartogh, *et al.*, 2020). Curcumin also shows an ability to increase the level of insulin in plasma and the sensitisation of lipoprotein lipase (Bodalska *et al.*,2017). Curcumin prevents galactose-induced cataract formation at very low doses. Both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes in rat.

Curcumin also decreases advanced glycation end products induced complications in diabetes mellitus (Chattopadhyay *et al.*, 2004).

2.4.6. Anti -cancer activity

Curcumin has been found to possess anticancer activities via its effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis (Krup *et al*., 2013). However, leukaemia, breast, colon, hepatocellular and ovarian carcinoma cells go away through apoptosis in the occurrence of curcumin, lung, prostate, kidney, cervix and central nervous system malignancies and melanoma cells illustrate fight to cytotoxic effect of curcumin. Production of rat thymocytes also decreases by Curcumin. These powerfully involve that cell growth and cell death contribute to a common lane (Ashraf *et al.,* 2018). Due to the low potency of curcumin and its derivatives, higher doses are required to see a therapeutic response, which increases the adverse effects and reduces the patient compliance (Tomeh *et al*.,2019).

2.4.7 Anti-microbial activity

Curcuma longa has good promise as an antifungal agent that could be used as a therapeutic remedy against human pathogenic fungi on account of its various *in vitro* and *in vivo* antifungal properties and strong fungicidal action, long shelf-life, its tolerability of heavy inoculum density, thermo stability, broad range of anti dermatophytic activity and absence of any adverse effects (Krup *et al*.,2013). Ether and chloroform extracts and oil of *C. longa* have antifungal effects. Crude ethanol extract also possesses antifungal activity. Turmeric oil is also active against *Aspergillus flavus*, *A. parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum* (Chattopadhyay *et al.*, 2004).

Curcumin and the oil fraction suppress growth of several bacteria like *Streptococcus*, *Staphylococcus*, *Lactobacillus*, etc. The aqueous extract of turmeric rhizomes has antibacterial effects. Curcumin also prevents growth of Helicobacter pylori CagA+ strains *in vitro* (Chattopadhyay *et al.,* 2004). The ethanol extract of the rhizomes has anti- Entamoebahistolytica activity. Curcumin has anti-Leishmania activity in vitro. Several synthetic derivatives of curcuma have anti- *Leishmania amazonensis* effect. (Chattopadhyay *et al.,* 2004). The anti-protozoal activity was reported in the ethanolic extract of turmeric against

Plasmodium falciparum and *Leishmania* (Nirajan *et al* .,2008).

2.4.8. Pharmacodynamics

Animal study shows that the absorption of curcumin after oral administration varies from 25- 60%, Based on a clinical trial, report that a daily dose of 3.6 g curcumin may achieve pharmacologically efficacious levels. The standard quantity curcumin is 400-600 g for 3times per day (Dei *et al.*,2019).

2.4. Turmeric adverse reaction

Clinical trials report few adverse reactions (e.g. Ulcer). Rare cases of contact dermatitis and anaphylaxis have been reported. An increased risk of kidney stone Formation is theoretically possible in susceptible individual. There are some negative side effects have been reported. Seven subjects receiving 500–12,000 mg in a dose response study and followed for 72 h experienced diarrhea, headache, rash, and yellow stool (Hewlings *et al.,* 2017). In another study, some subjects receiving 0.45 to 3.6 g/day curcumin for one to four months reported nausea and diarrhea and an increase in serum alkaline phosphatase and lactate dehydrogenase

III. PLANT MATERIAL

Curcuma longa rhizomes were collected from Regional Ayurveda Research Institute for Lifestyle Related Disorders, Poojapura, Thiruvananthapuram.

3.1. Isolation of essential oil

200 g of fresh rhizomes were hydrodistilled using a Clevenger-type apparatus for 3h. The essential oil obtained was dried over anhydrous sodium sulphate and stored at 4^0C till further analysis. Essential oil yield was also noted.

3.2. Essential oil analysis

(a) GC-FID analysis: GC-FID analysis was carried out on a Shimadzu GC-2010 Plus Gas Chromatograph with AOC-20i auto injector and FID (Shimadzu, Japan). The column used was Rxi-5 Sil MS capillary column (Cross bond 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness, Restek USA) and the GC operation conditions were; injection mode, split; split ratio, 1:50; injector temperature, 270˚C; oven temperature programme, 60-250˚C (3˚C/min); hold time 2 min. at 250˚C; carrier gas, N_2 at 3 mL/min; detector temperature 270˚C. Relative percentages of individual components were obtained from the peak area percent report of volatiles from GC/FID data.

(b) GC-MS analysis**:** The essential oils from fresh and oven dried samples were obtained by hydrodistillation using a Clevenger type apparatus. The oils were analyzed by GC/MS on a Shimadzu TQ triple quadrupole gas chromatograph fitted with a Cross bond 1,4- bis(dimethylsiloxy)phenylene dimethyl polysiloxane Rxi-5 Sil MS capillary column (30 m x 0.32 mm, film thickness 0.25 m) coupled with a Nexis 2030 series mass selective detector. The constituents were identified by MS library search (Wiley 275), relative retention indices (RRI) and by literature reference (Adams, 2007).

3.3. Determination of RRI and Identification of cōmpounds

Relative retention indices (RRI) of essential oil constituents were determined on the Rxi-5 Sil MS column using C5-C30 straight chain alkanes as standards. Individual constituents in the leaf oil were identified by WILEY and NIST database matching, comparison of mass spectra with literature and comparison of their RRI's calculated with respect to homologous of n- alkanes (Adams.2007). Retention Indices were calculated using the below equation

$$RRI = 100[(Ex – Hn)]/ (HN+1 – Hn)] + 100 n$$

Where Ex is the retention time of the compound (oil constituents), HN and HN+1 are retention times of reference hydrocarbons with n and n+1 carbon atoms respectively.

3.4. Preparation of extracts

C. longa rhizomes were washed dried and powdered in a mill. 2g of the rhizome powder was extracted with hexane followed by methanol using a Soxhlet apparatus.

3.5. Phytochemical screening of extracts

Hexane and methanol extracts of *C. longa* rhizomes were screened for various secondary metabolites by the following tests.

a) Steroids and Terpenoids (Liebermann-Burchard test)

10 mg of extract was dissolved in dry chloroform. Few drops of acetic anhydride were added followed by 1mL conc. sulphuric acid. Appearance of pink colour confirms the presence of terpenoids whereas, green colour in the chloroform layer shows the presence of steroids.

b) Alkaloids

- Dragendroff's test: 10 mg of extract was dissolved in methanol and few drops of Dragendroff's reagent were added. Orange red precipitate shows the presence of alkaloids.
- Mayer'test:10 mg of extract was dissolved in con. HCl and filtered. A few drops of solution were poured into the centre of a watch glass. Mayer's reagent was added in drops to the sides of the watch glass with the help of a glass rod. Formation of a gelatinous white precipitate at the junction of two liquids shows the presence of alkaloids.
- Wagner's test: 100 mg of extract was dissolved in methanol and few drops of wager's reagent were added. The brown flocculent precipitate confirms the presence of alkaloids.

c) Flavonoids

- Shinoda's test: 10 mg of extract dissolved in methanol. Mg turnings were added into this followed by few drops of con.HCl. A magenta colour shows the presence of flavonoids.
- One drops of above extract was placed on a filter paper and then exposed to ammonia vapour. Yellow colour shows the presence of flavonoids.

d) Coumarins

10 mg extract was dissolved in methanol and alcoholic KOH was added. Appearance of yellow color which decolorizes while adding conc. HCl shows the presence of coumarins.

e) Saponins

Extract was dissolved in water and shaken well. Froth formation which lasts for a long time shows the presence of saponins.

f) Carbohydrates (Molisch's test)

About 10 mg of extract was dissolved in 1 ml water. 2 drops of 1% alcoholic solution of α- naphthol is added. Then carefully add 1 ml con. Sulphuric acid along the sides of the tube so that it forms a heavy layer at the bottom. Deep violet color at the junction of 2 liquids indicates the presence of carbohydrates.

3.6. Antioxidant studies

3.6.1. Total phenolic content estimation

Principle: Folin-ciocalteu's reagent is used for phenolic estimation. This reagent is formed from a mixture of phosphor tungstic acid and phosphor molybdic acid, which after oxidation of phenols, is reduced to a mixture of blue oxides of tungsten and molybdenum. The blue colouration produces has a max. absorption range of 750nm, and this is proportional to the total quantity of phenolic compounds originally present. As the concentration increases colour increases.

Method: C. longa rhizome methanol extracts (1 mg/mL) at different concentrations (25µl and50µl) is made up to 1ml with distilled water. To this 5ml F.C. Reagent was added. After 5 minutes add 4ml 20% Na_2CO_3 solution. After 30 minutes OD of the solution is measured at 750 nm. Gallic acid at the concentrations between 20-100 µg. Result is expressed as mg/g GAE of the extract. All the experiment was done in triplicate and result were expressed as mean±SD

3.6.2. DPPH radical scavenging activity assay

To prepare 100 ml 0.2mM DPPH solution, 7.83mg DPPH was accurately weighed and dissolved in 100 ml methanol

Principle: The pink colour of DPPH solution diminishes to light pink or changes to yellowish brown or decolourised depending on activity. The radical scavenging capacity of different
extracts can be estimated in comparison with ascorbic acid. The antioxidant capacity was expressed as % radical scavenging activity.

Method: C. longa rhizome methanol extracts at different concentrations (10-50µl) are made up to 1ml using methanol. To this 1ml DPPH solution was added. A control was also prepared with 1ml methanol and 1ml DPPH solution. After an incubation of 30 mins, absorbance was measured at 517nm in a UV-Visible spectrometer. The activity of the extracts was expressed as percentage scavenging using the formula;
% scavenging = [(Acontrol-Asample)/Acontrol] x 100.

The activity was reported as IC50 value; the

concentration of sample required to scavenge 50% of DPPH radical, relative to the control (DPPH without antioxidant agents added). Ascorbic acid was used as the standard. All the experiment were done in triplicate and result were expressed as mean±SD

IV. VOLATILE CHEMICAL PROFILING OF CURCUMA LONGA RHIZOME

4.1.1. Essential oil yield

A yellow coloured essential oil was isolated from rhizome of *Curcuma longa* by hydrodistillation. Percentage yield of oil was calculated from the volume of oil obtained and the weight of the substance taken for hydrodistillation. Essential oil yield of the leave was 0.75ml % (v/w)

4.1.2. Analysis of essential oils

GC-MS analysis of essential oil, subsequent data base matches and interpretation of data resulted in identification of 27 compounds comprising 97.8% of the total oil composition. Major compounds in essential oil have been identified as *ar*-turmerone (38.2%), Neryl isobutanoate(10.2%) andα-santalene (6.9%) based on its mass spectra (Table 4.1, Figure 4.1). Previous studies reported that *Curcuma longa* volatiles were dominated by *ar*-turmerone, curlone, α-turmerone, β-sesquiphellandrene, α-zingiberene, germacrone, terpinolene, *ar*- curcumene and α-phellandrene and showed four distinct chemical clusters (Dosoky *et al.,* 2019). In dry rhizome oil ar-turmerone, α-santalene and ar-curcumene were the major compounds, which results that α-turmerone, a major component in fresh rhizomes is only minor one in dry rhizomes (Singh *et al.,*2010). The rhizome oil of *C. longa* from northern plains of India was reported to contain 59.7 % of ar-turmerone (Naz *et al.,* 2010). α- Phellandrene, p-cymene, α-terpinolene and p-cymen-8-ol were characterized as the major compounds in rhizome oil of *C. longa* (Sharma *et al*; 2019). Mass spectra and structure of major compounds in the *C. longa* essential oil are shown below (Figure 4.2 and4.3).

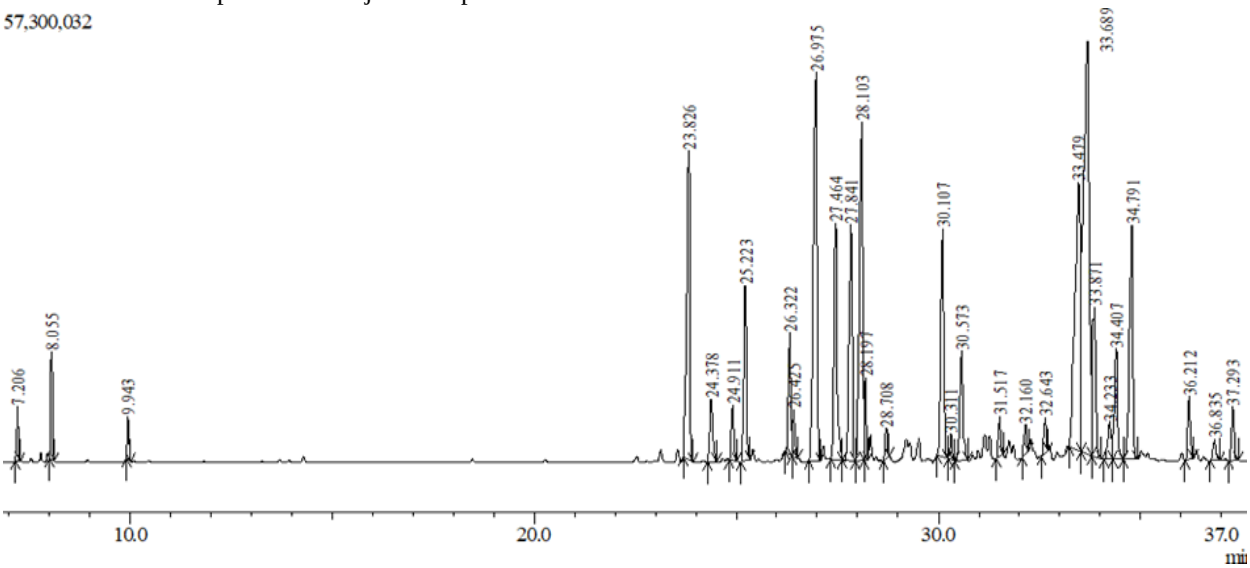

Figure 4.1. Gas chromatogram of *Curcuma longa* rhizome essential oil

Table 4.1. Essential oil composition of *Curcuma longa* rhizomes

Sl No.	RT	RRI $_{lit}$	RRI $_{comp}$	Compound	Percentage
1	7.207	1002	1006	α-Phellandrene	0.6
2	8.057	1026	1031	1,8-cineole	1.3
3	9.943	1086	1084	Terpinolene	0.5
4	23.833	1416	1417	α-santalene	6.9
5	24.377	1411	1430	α-cis-Bergamotene	1.3

6	24.910	1445	1443	epi-β-Santalene	3.2
7	25.220	1458	1451	E-β-Farnesene	2.0
8	26.323	1479	1478	ar-Curcumene	0.7
9	26.423	1490	1480	Neryl isobutanoate	10.2
10	26.983	1505	1494	α-Zingiberene	5.2
11	27.470	1505	1506	β-Bisabolene	4.9
12	28.110	1521	1522	β-Sesquiphellandrene	1.2
13	28.197	1529	1524	E-γ-Bisabolene	0.5
14	28.707	1532	1533	γ-Cuprenene	4.9
15	30.107	1576	1573	Santalenone	0.4
16	30.310	1582	1578	Neryl isovalerate	2.6
17	30.573	1585	1585	cis –Carvyl angelate	0.7
18	31.517	1606	1610	Geranyl isovalerate	0.6
19	32.163	1627	1627	1-epi-Cubenol	0.6
21	33.487	1668	1662	ar-Turmerone	38.2
22	33.873	1672	1672	5-isocedranol	0.9
23	34.240	1683	1682	epi-α-Bisabolol	2.4
24	34.407	1693	1687	Germacrone	5.9
25	34.797	1706	1697	E-γ-Alantone	1.0
26	36.207	1740	1736	6R,7R-Bisabolone	0.4
27	37.290	1777	1767	E-α-Atlantone	0.7
				Total	97.8
				Monoterpene hydrocarbons	1.1
				Oxygenated monoterpenes	1.3
				Sesquiterpene hydrocarbons	23.9
				Oxygenated sesquiterpenes	71.5

Major compounds present in *Curcuma longa* rhizome essential oil

a) ar-Turmerone

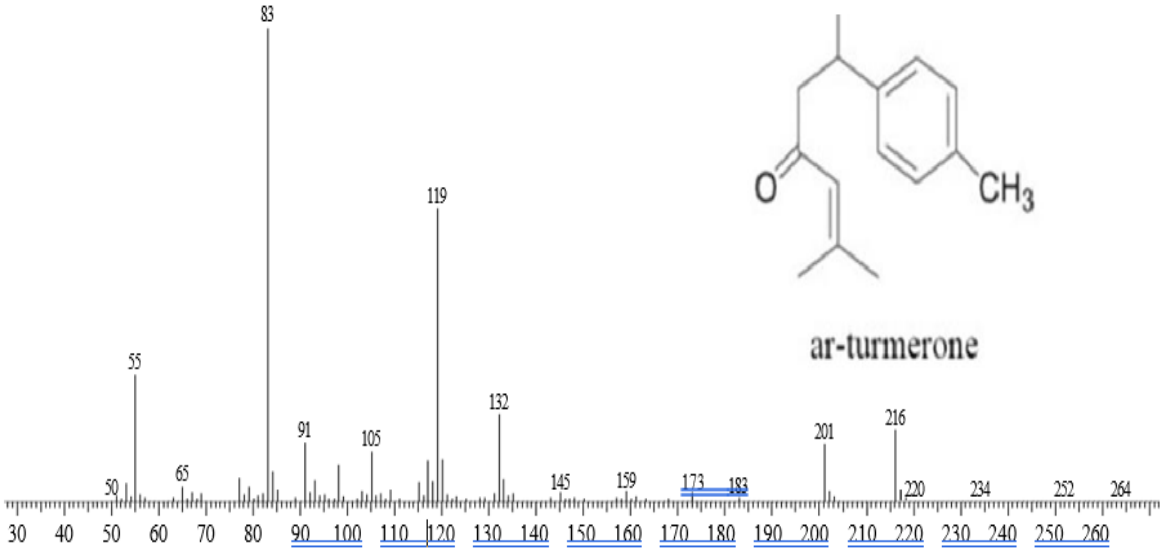

Figure 4.2. Mass spectrum and structure of ar-Turmerone

b) Neryl isobutanoate

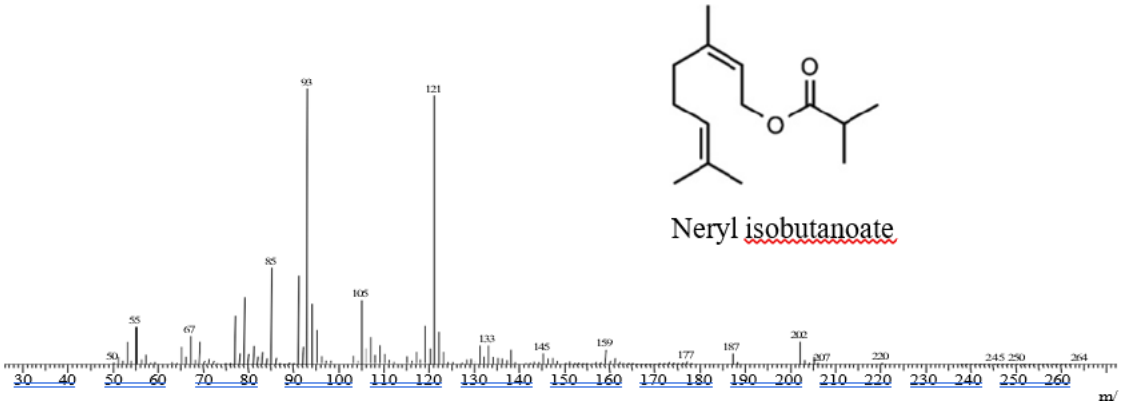

Figure 4.3. Mass spectrum and structure of Neryl isobutanoate

4.2. Phytochemical screening of *Curcuma longa* rhizome extracts

Rhizomes of *C. longa* were screened for various phytochemical compounds. The hexane extract showed positive test result only for flavonoids, terpenoids and coumarins whereas, methanol extract gave positive result for carbohydrates, coumarins, terpenoids and flavonoids.

Table.4.2: Phytochemical screening of *Curcuma longa* rhizome extracts

Sl, No	Phytochemical Constituents	Hexane extract	Methanol extract
1	Alkaloids	-	-
2	Flavonoids	+	+
3	Steroids	-	-
4	Terpinoids	+	+
5	Coumarins	+	+
6	Saponins	-	-
7	Carbohydrates	-	+

+ Presence - Absence

4.3. Antioxidant activity studies

4.3.1. Total phenolic content

Total phenolic content of methanol extract of *C. longa* rhizome was estimated using Folins- Ciocalteu's reagent colorimetric method and the results were expressed as mg/g GAE. The study revealed that, *C. longa* rhizome contains 366.4±1.7 mg/g GAE phenolics. Previous studies have reported that total phenolics in extracts of 60% aqueous methanol and 80% aqueous methanol as 523.87 mg GAE/ 100 g and 682.43 mg GAE/ 100 g respectively (Nisar *et al*., 2015). More than 500 mg GAE/100g phenolics were reported from 96 % methanolic extract followed by 50 % methanolic extract (Ramkumar *et al.*, 2016).

4.3.2. *In vitro* antioxidant assay

The *in vitro* antioxidant activity of methanolic extract of *C. longa* rhizomes were estimated by DPPH assay, where ascorbic acid was used as standard. The antioxidant activity is expressed as % radical scavenging activity. The IC_{50} value of ascorbic acid was 12.33±1.68 µg/µl while, that for *C. longa* rhizomes were 41.25± 0.99 µg/µl. The results show that the antioxidant activity of *C. longa* rhizomes is moderate compared to standard ascorbic acid (Table 4.3, Figure 4.4). Previous reports showed that 96 % methanolic extract had the highest DPPH radical scavenging activity followed by 50 % methanolic extract and aqueous extract with mean value of 61.80 µg/µl and 37.35 µg/µl respectively (Ramkumar *et al.*, 2016).

Table.4.3. DPPH assay of ascorbic acid and methanolic extract of *C.longa* rhizomes

Sl No	Concentration of ascorbic acid (µg/mL)	% Radical scavenging	Concentration of *C. longa* rhizome methol extract (µg/mL)	% Radical scavenging
1	20	51.17	10	14.01
2	40	63.14	20	25.88
3	60	65.49	30	41.56
4	80	74.6	40	46.47
5	100	81.47	50	59.41

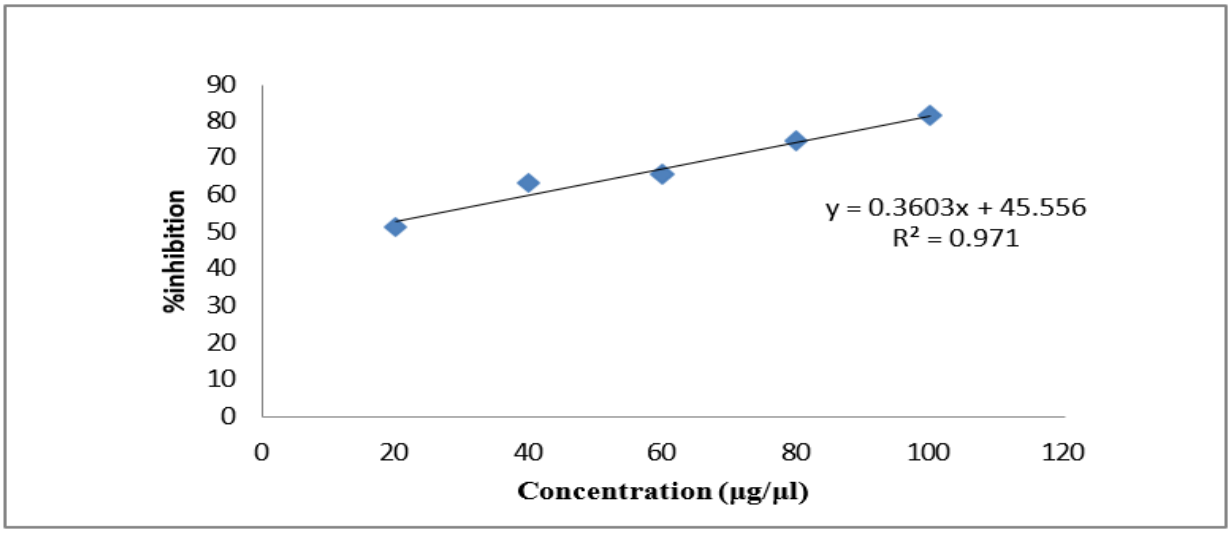

Figure 4.4. IC_{50} value of std Ascorbic acid for DPPH radical scavenging

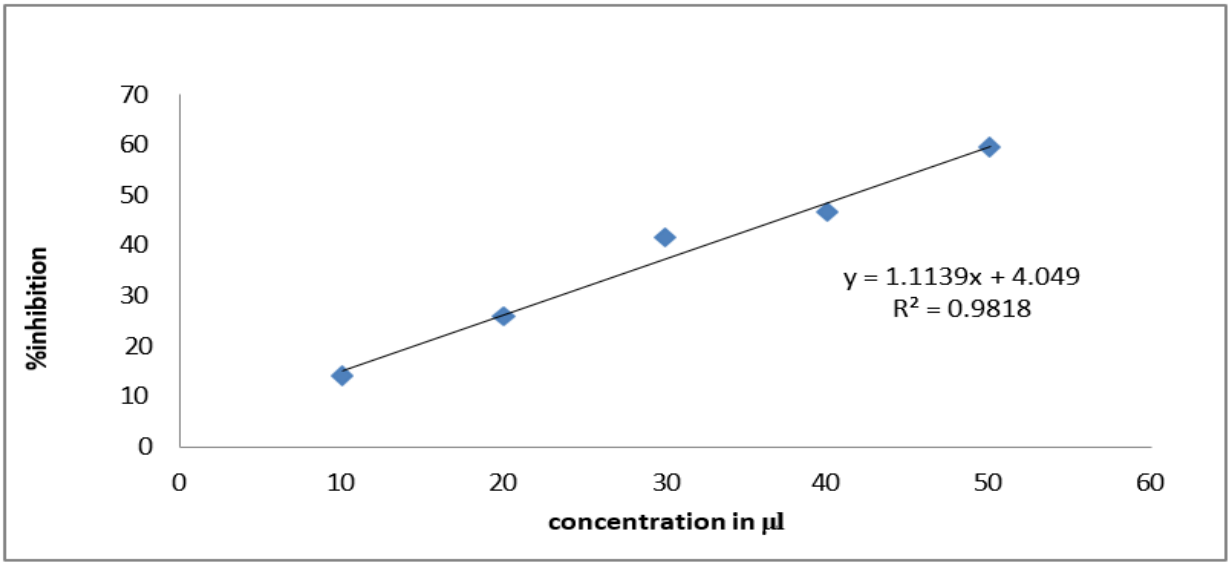

Figure 4.5. IC_{50} value of *C. longa* rhizome methanol extract for DPPH radical scavenging activity

V. CONCLUSION

The plant kingdom represents on extraordinary reservoir of molecules synthesized from plants that can be useful in various forms like bioactive compounds, flavouring agents, aroma compounds etc. The genus *Curcuma* is one among most explored group of plants. The present study reports the essential oil composition and antioxidant activity of *Curcuma longa* rhizomes collected from Thiruvananthapuram,

Kerala. The study revealed the plants as rich source of volatile compounds, ar-turmerone, β-sesquiphellandrene, α-phellandrene, ar- curcumene. Content of ar-turmerone in *C. longa* rhizome oil was moderately high up to 38.3% and the oil yield was 0.75% (v/w). The antioxidant evaluation by DPPH radical scavenging assay revealed moderate activity for the rhizome extracts. Unlike previous studies Neryl isobutanoate andα-santalene were of the major compounds in the present study and the results substantiate the importance of more rigorous studies on well-known medicinal plants.

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