

ANTIMICROBIAL, ANTIBACTERIAL AND GC-MS ANALYSIS OF CURCUMA AMADA

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology and Engineering

By

SHRISTI DHARA (37230083)



DEPARTMENT OF BIOTECHNOLOGY

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY (DEEMED
TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC | 12B Status by UGC | Approved by AICTE

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March - 2021



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Ms **SHRISTI DHARA (37230083)** who carried out the project entitled "**ANTIMICROBIAL, ANTIBACTERIAL AND GC-MS ANALYSIS OF CURCUMA AMADA**" under my supervision from November 2020 to March 2021.

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DECLARATION

I **SHRISTI DHARA (37230083)** hereby declare that the Project Report entitled **ANTIMICROBIAL, ANTIBACTERIAL AND GC-MS ANALYSIS OF CURCUMA AMADA** done by me under the guidance of Dr. Prabhavathy (Internal) and C. Sivraj (External) at ARMATS BIOTEK Training and Research Institute Department of CSE at Sathyabama Institute Of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Curcuma amada is a rhizomatous aromatic herb that belongs to the species of *Curcuma* genus and the family of Zingiberaceae. It is commonly known as mango ginger. *C. amada* is of East Indian origin. It has been extensively employed in the food industry and alternative medicines. It has a popular spice and vegetable due to its rich flavor, which is described as sweet with subtle earthy floral and pepper overtones and similar to that of raw mango. Like other members of the *Curcuma* genus, *C. amada* also has a kind of therapeutic properties. The root contains an essential oil and pungent principles. It strengthens the stomach and digestive system, is carminative and expectorant. It gives relief in digestive complaints such as abdominal gas problems, constipation, colic, indigestion, bad breath, stomach pain, loss of appetite, wind, indigestion, and hiccups. It effectively reduces and cures bronchitis, asthma, and cough internally. The external application of mashed and grated root of *C. amada* is useful in the treatment of sprains, wounds, bruises, and ulcers. The objectives of the study were to evaluate the antioxidant and antibacterial activities of ethanol rhizome extract of *C. amada*. Antioxidant activity methods such as radical DPPH radical scavenging assays and reduction assays such as Fe^{3+} reducing power assay were to be carried out for ethanol rhizome extract of *C. amada*. The antibacterial activity is to be carried out by the well diffusion method. *Curcuma amada* tuber extract as a natural antioxidant due to presence of ellagic acid, 4H-1-Benzopyran - 4-one, 7-hydroxyle-2-phenyl, isoflavone, flavones identified by GC-MS analysis.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Medicinal plants

Throughout history, mankind has always been interested in naturally occurring compounds from natural sources. Various extracts of flowers, plants and insects have been used for isolating compounds whose taste, color and odour could be used for various purposes (Ikan, 2007). Recently, there has been a literary expression of Thornton Wilder in his book, *The Eighth Day*, "Nature never sleeps. The process of life never stands still (Calvin, 1969).

The use of natural products as medicinal agents presumably predates the earliest recorded history as the earliest humans used various, but specific plants to treat illness (Ballick and Cox, 1996). In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. This has led to a system of health care known as Ayurvedic medicine. One useful plant from this body of knowledge is snakeroot, *Rauwolfia serpentina*, used for centuries for its sedative effects. Today, the active components in snakeroot are widely used in Western medicine to treat high blood pressure (Kong *et al.*, 2003). Theophrastus (370-285 BC) began the scientific classification of plants, and Dioscorides *De Materia Medica* ("Regarding Medical Matters") (77 AD) reported the uses, medicinal and otherwise, of over 600 plants. Western medicine can be traced back to the Greek physician Hippocrates (460-377 BC), known as the Father of Medicine. Hippocrates believed that a disease had a natural cause and used various herbal remedies in his treatments. Ibn al-Baitar (1197-1248) listed over 1400 drugs and medicinal plants in his *Corpus of Simples*. In Europe, after the tenth century, much of the medicinal lore was based in the church, particularly the monastic orders, but by the 1500's, after the invention of the printing press, herbals available to the general public were popular, particularly in England (Ballick and Cox, 1996). By the late 1700's, studies like William Withering's *An Account of the Foxglove and its Medicinal Uses* (1785) began to appear. These were based on case histories, described specific doses and gave administration instructions for herbal remedies. The people of India use more than 10,000

plants as medicines, out of which approximately 7500 are used in folk and tribal system, 1900 in Ayurveda, 500 in Siddha, 400 in Unani and 300 in Amchi (Tiwari et al., 2004).

Until the late 1800's, organic chemistry was used almost exclusively for the study of natural products. The natural products that were studied and used tended to be the compounds that occurred in the largest amounts, mostly in plants, and were most easily isolated in a pure or sometimes not very pure form by techniques such as simple distillation, steam distillation, or extraction with acid or base. Originally, teas or decoctions (aqueous extracts) or tinctures or elixirs (alcoholic extracts) were used to prepare, administer herbal remedies and these were usually the starting points for isolation work. Now different solvents are employed, for e.g., ethanol to extract, hexane to concentrate non-polar constituents, methanol to concentrate polar constituents, and modern isolation techniques include all types of chromatography, often guided by bioassays, to isolate the active compounds. Stereochemistry was not often determined. Now, structures are elucidated primarily by spectroscopic techniques, and the stereochemistry is an important feature of the structure.

1.2. The chemistry of natural products

Among the recent outstanding contributions to the chemistry of natural products, the most important one is the conformational analysis designed by Derek Barton. He used it for the structural determinations of many complex molecules such as β -amyrin and cycloartenol. In 1945, Robert B. Woodward involved in the structural determinations of penicillin, strychnine, patalin, terramycin, aureomycin and the synthesis of Vitamin B₁₂. Extracts of toxic plants have been used for hunting and murder throughout the world for thousands of years. Thus, Strychnos and Chondrodendion (both containing strychnine) were used in arrow poisons. The Colombian arrow poison consists of toxins from the legs of frogs. When rye is infected by the fungus, Claviceps purpurea, the toxin ergotamine and a number of ergot alkaloids are produced. These compounds cause serious illnesses (Geissman, 1969).

The rapid and impressive development of organic chemistry in the 19th century had a tremendous effect on the discoveries of natural products. Towards the end of the 19th century, microbiology has developed enormously. In 1877, Alexander Fleming discovered penicillin which was active against Tuberculosis and was first isolated by him in 1929. In 1944, Waxman isolated streptomycin which is used for the treatment of tuberculosis. In 1952, Bloch and Woodward suggested a mechanism for the cyclization of squalene to cholesterol. In 1962, Francis Crick and James Watson described the double helix structure of proteins (Croat, 1969).

The mankind discovered the idea that certain plants has healing potential, they contain antioxidant and antimicrobial principles, was all well accepted. Plants provide the predominant ingredients of medicines in most medical traditions. Medicinal plants play a vital role in the health care system all around the world. India has a rich flora that is widely distributed throughout the country. Since ancient times, man has used the plants to treat the common infectious disease. Herbal medicines are the fundamental method adopted in traditional methods to cure various diseases and physiological conditions such as Ayurveda, Unani and Siddha. Some of the traditional medicines are still used by man for their habitual treatment for various diseases. For example, tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich *et al.*, 2004). The essential oil of this plant is use to treat acne and other infectious problems of the skin.

Among all five kingdom, plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. Plants are now occupying the important position in allopathic medicine, herbal medicine, homeopathy and aromatherapy. In this modern world the medicinal plants are the sources of many important drugs. Many of the indigenous medicinal plants are used as spices and also sometimes added to food which is meant for pregnant mothers for medical purpose. For example black pepper is known as the "King of Spices". It is the oldest and highly valuable medicinal spice in India (Madhuri and Pandey, 2009).

Plants are one of the most important sources of medicine. Medicinal plants are used in two different areas of health management *i.e.*, traditional system of medicine and modern system of medicine. The traditional system of medicine mainly consist of two types (1) local or folk or tribal stream (2) codified and organized Indian system of medicines like Ayurveda, Siddha and Unanni etc.(Prakash *et al.*, 2010).

In India, approximately around 3000 plants species are known to have medicinal properties. Our traditional systems of medicines, viz., Ayurveda, yunani and Siddha etc. use herbs for treatment. According to world health organization (WHO), about 80% of the world's population relies on traditional forms of medicine for health care. The use of medicinal plants are not only cost effective, they are safe for health care and almost free from serious side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use (Sofowa, 1993).

The leaves of plant contain the medicinal attributes of many plants. They are used for chronic ulcers, chronic nervous diseases, chronic piles and fistula. The dried leave is given with milk in mental disability and fresh juice of leaves is for jaundice and fevers.

It is estimated that over 9,000 plants are known to have medicinal applications in various culture and countries (Farnsworth and Soejarto, 1991). Medicinal plants are used by household women to take care of their families by applying classical traditional systems of medicine at the village level. According to world health organization (WHO), over 80% of world's population depends directly on medicinal plants for their primary health care. Many plant materials are not only used in homeopathic medicine but also play a vital role in allopathic medicine (Srivastava *et al.*, 1995).

The concept of Ayurvedic was developed between 2500 and 500BC in India. The literal meaning of Ayurveda is "science of life". According to Ayurveda, a good health means metabolically well-balanced human beings and the diseases develop from the body is due to external factors. The practice of Ayurveda therapeutics consisted of 8 sections which is divided in to 180 chapters and listed 314 plants, which are used as medicine in India (Subhose *et al.*, 2005).In India, around 20,000, medicinal plants have been

recorded (Dev,1997) but traditional communities are using only 7000-7500 plants to cure various diseases(Perumal and Ignacimuthu, 1998 and 2000). Today, majority of medicines are prepared using plants, animal products and minerals etc. This Ayurvedic system of medicine is widely accepted and practiced in many countries like India, United States and Japan (John, 1984). For the preparation of Ayurvedic medicine, major pharmaceutical industries rely on plant products (Veale *et al.*, 1992).

1.3. Phytochemicals

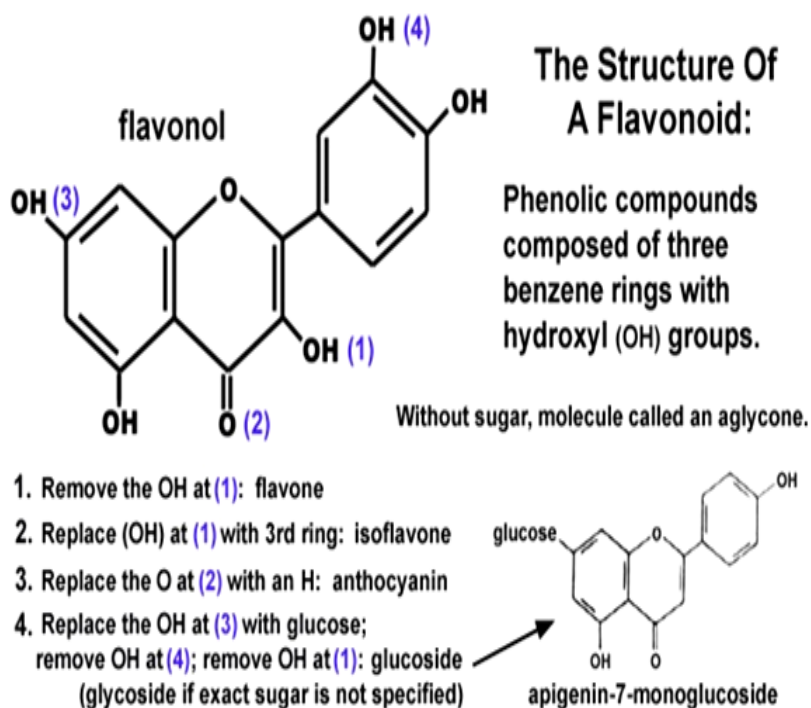
Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, which mean they are not required by the human body to sustain life. Plant produces these chemicals to protect them but recent research showed that they can also protect human against diseases. Phytochemicals are found in plant-based foods such as fruits, vegetables, beans, and grains. There are many phytochemicals and each work differently. Some of the well known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, phenolic compounds, tannins and glycosides. The anti-inflammatory, cytotoxic, antiplatelet, scavenger and antioxidant properties of prenylflavonoids isolated from the *Artocarpus* plants were reported (Liou *et al.*, 1993; Lin *et al.*, 1993).

Some phytochemicals have either antioxidant or hormone-like actions. There is some evidence that a diet rich in fruits, vegetables, and whole grains reduces the risk of certain types of cancer and other diseases. Current research is now directed towards finding the specific compounds in these foods that may account for these healthful effects in humans. Phytochemicals are used for the prevention and treatment of many health conditions, including cancer, heart disease, diabetes, and high blood pressure. There is some evidence that certain phytochemicals might help to prevent the formation of potential carcinogens (substances that causes cancer), block the action of carcinogens on their target organs or tissue, or act on cells to suppress cancer development.

There are several major groups of phytochemicals

1.2.1. Flavanoids

A Flavanoid function in the plant world seems to attract pollinators to flowers or animals that eat the fruits with the intention that they can disperse the seeds. Flavanoids are found only in plants, so to obtain these components we must eat a diet rich in vegetables. Flavanoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-[allergic](#), [anti-inflammatory](#), [antioxidant](#), [anti-microbial](#) ([antibacterial](#), [antifungal](#), and [antiviral](#)), anti-[cancer](#) and anti-[diarrheal](#) activities.



1.2.2. Carotenoids

Among the 600 or more carotenoids in foods, beta-carotene, lycopene and lutein are well-known leaders to reduce the damage from free radicals. Foods high in carotenoids may be effective allies against prostate cancer (beta-carotene); cancers of

the mouth, pharynx, esophagus, stomach, colon and rectum (lycopene); and may help decrease your risk of macular degeneration (lutein).

Foods high in carotenoids include red, orange, deep-yellow and some dark-green leafy vegetables, like tomatoes, carrots, spinach, Brussels sprouts, sweet potatoes, winter squash and broccoli.

1.2.3. Terpenoids

Terpenoids (also called “isoprenoids”) are secondary metabolites occurring in most organisms, particularly in plants. More than 40,000 individual terpenoids are known to exist in nature with new compounds being discovered every year. A large number of terpenoids shows cytotoxicity against a variety of tumor cells and cancer cells. This review critically examines the potential role of naturally occurring terpenoids, from diverse origins, in the chemoprevention and treatment of liver tumors.

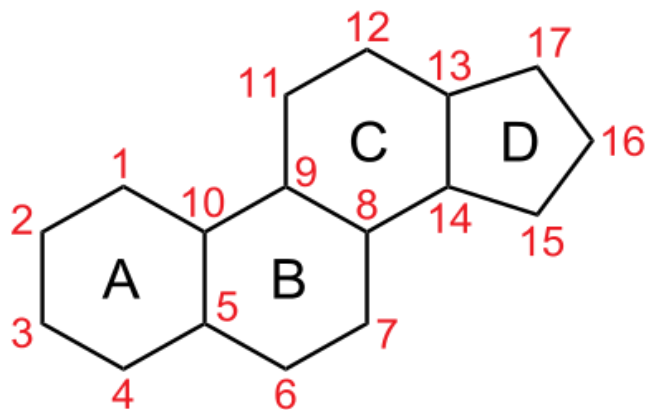
1.2.4. Alkaloids

Alkaloids are one of the largest groups of chemical arsenals which are produced by plants. Many of these metabolic by-products are derived from amino acids and include an enormous number of bitter, nitrogenous compounds. According to R.F. Raffauf (1996), more than 10,000 different alkaloids have been discovered in species from over 300 plant families. Alkaloids often contain one or more carbon rings, usually with a nitrogen atom in the ring. The position of the nitrogen atom in the carbon ring varies with different alkaloids and with different plant families. In some alkaloids, such as mescaline, the nitrogen atom is not within a carbon ring. Actually, it is the position of the nitrogen atom that affects the properties of these alkaloids. Although they undoubtedly existed long before humans, some alkaloids have remarkable structural similarities with neurotransmitters in the central nervous system of humans, including dopamine, serotonin and acetylcholine. The amazing effect of these alkaloids on humans has led to

the development of powerful pain-killer medications, spiritual drugs, and serious addictions by people who are ignorant of the properties of these powerful chemicals.

1.2.5. Steroids

A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary lipid cholesterol, bile acids, the sex hormones estradiol and testosterone and the anti-inflammatory drug dexamethasone. The core of steroids is composed of seventeen carbon atoms bonded together that take the form of four fused rings: three cyclohexane rings (designated as rings A, B and C in the figure to the right) and one cyclopentane ring (the D ring). The steroids vary by the functional groups attached to this four-ring core and by the oxidation state of the rings. Sterols are special forms of steroids, with a hydroxyl group at position-3 and a skeleton derived from cholestane (Moss, 1989).



These steroids are similar to hormones that your adrenal glands make to fight stress associated with illnesses and injuries. They reduce inflammation and affect the immune system.

Corticosteroids also treat:

- ❖ Arthritis
- ❖ Asthma

- ❖ Skin conditions such as eczema and rashes
- ❖ Some kinds of cancer
- ❖ Steroids are strong medicines, and they can have side effects, including weakened bones and cataracts. Because of this, Steroids are usually taken for as short a time as possible.

1.2.6. Antioxidant properties:

Normal body functions such as breathing or physical activity and other lifestyle habits such as smoking produce substances called free radicals that attack healthy cells. When these healthy cells are weakened, they are more susceptible to cardiovascular disease and certain types of cancers. Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements (Subhashini *et al.*, 2011). Antioxidants, such as vitamins C and E and carotenoids, which include beta-carotene, lycopene and lutein help to protect healthy cells from damage caused by free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources (cheeseman, 2003). Free radical oxidative stress caused a wide variety of clinical disorders. A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress.

1.2.7. Anticancerous properties:

Many phytochemicals have been tremendously effective in the treatment of cancer. It is known that many inhibit the growth of cancer cells. Ancient Indian medicinal science was based on the natural products of plant origin. Recently, numerous bioactive compounds have been isolated from plant sources and several of them are currently in clinical trials. Plant-derived compounds have been a vital source of varied clinically useful anti-cancer agents: Camptothecin derivatives [1], Topotecan and Irinotecan, Etoposide, derived from Epipodophyllotoxin [2], and Paclitaxel (taxol) [3]. Furthermore, other potent molecules

include Vinca alkaloids (Vinblastine, Vincristine) [4], Flavopiridol, a semi-synthetic analogue of the chromone alkaloid and Rohitkine, a pyridoindole alkaloid derived from leaves of Ochrosia species [5]. Presently, research on anticancer drug development is largely dependent on exploring potential phytochemicals. Indian sub-continent is rich in its diversity of flora, being a tropical country with a large spread of rain forests and river basins. It is floristically rich with about 33 percent of its botanical wealth (over 15,000 species of higher plants) being endemic [6]. Hence, analyzing this potential, InPACdb initiative is taken to expose the potent anticancer phytochemicals of Indian Origin in international arena.

1.2.8. Anti-inflammatory:

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. Anti-inflammatory are the drugs or medicines which are used to relieve swelling, heat, pain and other symptoms of inflammation. These used to reduce the inflammatory response to infections agents, Trauma, Surgical procedures or in musculoskeletal muscle (Mitchell and Cotran, 2000).

1.2.9. Antimicrobial:

An **antimicrobial** or **antibiotic** is an agent that kills microorganisms or inhibits their growth.^[1] Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungals are used against fungi. They can also be grouped according to their function. Antimicrobials that kill microbes are called microbicidal and those that merely inhibit their growth are called microbiostatic. Antiseptics will help to reduce infection during surgery. Disinfectants such as bleach are nonselective antimicrobials. They kill a wide range of microbes and are valuable for cleaning inanimate surfaces to prevent the spread of illness, but they are generally not medicinal.

CHAPTER 2

LITERATURE SURVEY

Mango ginger (*Curcuma amada*) is a rhizomatous aromatic herb of the family Zingiberaceae and is cultivated throughout India, Sri Lanka, Bangladesh and in many South-East Asian countries for its rhizomes that are used as flavouring for pickles and other dishes and also valued for their medicinal properties. The fresh as well as dried rhizomes are used for flavouring curries. The fresh cut rhizomes have the flavour and the colour of mango, hence the name mango ginger.

The herb attains 60–90 cm height, leaves are long, petiolate, oblong-lanceolate, tapering at both ends, glabrous, green on both sides; flowers are white or pale yellow in spikes that occur in the centre of the leaves, lip is semi-elliptic, yellow, three-lobed, the middle lobe emarginated.

Taxonomic classification:

Domain – *Eukaryota*

Kingdom – *Plantae*

Subkingdom – *Viridiplantae*

Superdivision – *Embryophyta*

Division – *Tracheophyta*

Class – *Magnoliopsida*

Subclass - *Liliana*

Order – *Zingiberales*

Family – Zingiberaceae

Genus - *Curcuma* L



Figure 1: Habitat of *Curcuma amada*

CHAPTER 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

Aim

The aim of the present study was to evaluate antioxidant, antibacterial activities and GC-MS analysis of ethanol rhizome extract of *Curcuma amada*

Objectives

The present study was undertaken with the following objectives:

- To determine the qualitative phytochemical analysis of rhizome extract of *Curcuma amada*.
- To determine the quantitative phytochemical analysis of rhizome extract of *Curcuma amada*
- To determine the antioxidant activity of rhizome extract of *Curcuma amada*
- To determine the antibacterial activity of rhizome extract of *Curcuma amada*
- To perform GC-MS analysis of rhizome extract of *Curcuma amada*

CHAPTER 4

EXPERIMENTAL OR MATERIALS AND METHODS

Preparation of Extract

Rhizomes of *Curcuma amada* were collected at Koyambedu, Chennai, Tamilnadu, India. The rhizomes were crushed and about 20 g of the rhizomes was soaked in ethanol for 72 h at room temperature. The supernatant was filtered and condensed at room temperature which yields pale yellow gummy extract.

Qualitative phytochemical analysis

The rhizomes extract of *Curcuma amada* was subjected to preliminary phytochemical screening using specific reagents as followed in standard methods. The different qualitative chemical tests were performed for establishing the profile of given extract for its chemical composition. The tests were done individually for crude extract. The following tests were performed to detect various phytoconstituents present in it.

Detection of alkaloids (Evans, 1997)

Solvent free extract (50 mg) was stirred with few mL of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows:

Mayer's test (Evans, 1997)

To a few mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Hager's test (Wagner *et al.*, 1996)

To a few mL of filtrate, 1 or 2 mL of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicates the test positive.

Dragendorff's test (Waldi, 1965)

To a few mL of filtrate 1 or 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicates the test positive.

Stock solution

Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few minutes with 50 mL Glacial acetic acid. After 12 hours the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. Clear, red brown filtrate, 40 mL was mixed with 160 ml of ethyl acetate and 1 ml of water and stored in amber - colored bottle.

Detection of carbohydrates and glycosides (Ramakrishnan *et al.*, 1994)

3. The extract (100 mg) was dissolved in 5 mL of water and filtered. The filtrate was subjected to the following tests.

Fehling's test

One mL of filtrate was boiled on water bath with 1 mL each of Fehling solutions I and II. A red precipitate indicated the presence of sugar.

Fehling's solution

Fehling's solution I: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 mL with distilled water.

Fehling's solution II: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made up to 500 mL.

Benedict's test

To 0.5 mL of filtrate, 0.5 mL of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicated the presence of sugar.

Benedict's reagent

Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 mL of distilled water and boiled to make it clear. Copper sulphate (17.3 g) dissolved in 100 mL distilled water was added to it.

Detection of glycosides

50 mg of extract was hydrolysed with concentrated HCl for 2 h on a water bath, filtered and the hydrolysate was subjected to the following tests.

Borntrager's test (Evans, 1997)

To 2 mL of filtrate hydrolysate, 3 mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Detection of saponins

Foam test (Kokate, 1999)

The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

Detection of phenolic compounds

Ferric chloride test (Mace, 1963)

The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Detection of Flavonoids

To the methanolic extract, NH_3 solution was added. Bright yellow color appears and addition of excess amount of conc. H_2SO_4 , the yellow color disappears.

Test for Terpenoids

To 5 mL of methanolic extract, 2 mL of chloroform was added and mixed well. Add a little of conc. H_2SO_4 was carefully added to form a reddish brown layer.

Detection of phytosterols (Finar, 1986)

Libermann-Burchard's test

The extract (50 mg) was dissolved in 2 mL of acetic anhydride. To this, one or two drops of concentrated H_2SO_4 was added slowly along the sides of test tube. An array of colour changes showed the presence of phytosterols.

C. Lead acetate test

The extract (50 mg) was dissolved in distilled water and to this, 3 mL of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Determination of total phenols

Folin-Ciocalteu reagent method was used to determine the total phenolic compounds⁶ with slight modifications. One hundred μL of rhizome extract of *Curcuma amada*

(1mg/mL) was mixed with 900 μL of methanol and 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% (w/v) of Na_2CO_3 solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent ($\mu\text{g}/\text{mg}$ of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of curcuma amada was determined using aluminium chloride method with slight modification as described by Liu et al⁷. Five hundred μL of extract (1mg/mL) was mixed with 0.5 mL of methanol and 0.5 mL of 5% (w/v) sodium nitrite solution. Then, 0.5 mL of 10% (w/v) aluminium chloride solution was added, shaken well, followed by 100 μL of 1 M NaOH solution was added. The absorbance was measured at 510 nm and the result was expressed as ($\mu\text{g}/\text{mg}$ of extract) quercetin equivalent, which is a common reference compound.

***In vitro* antioxidant assays**

DPPH[•] radical scavenging activity

The antioxidant activity of curcuma amada extract was measured by DPPH (1, 1- diphenyl 2-picrylhydrazyl) free radical scavenging activity⁸. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (100-600 $\mu\text{g}/\text{mL}$) of aqueous curcuma amada extract. The mixture was then allowed to stand for 30 min incubation in dark. One mL of DPPH solution mixed with 1 mL of methanol was used as the control. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as positive control. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

Phosphomolybdenum reduction assay

The antioxidant capacity of aqueous dried curcuma amada extract was assessed as described by Prieto *et al*¹⁰. Extract with different concentrations (50 - 300 µg/mL) was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of Phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

Ferric (Fe³⁺) reducing power assay

The reducing power of aqueous dried Rose petals extract was determined by the slightly modified method of Yen and Chen¹¹. One mL of extract with different concentrations (50 – 300 µg/mL) was mixed with 1 mL of 1% (w/v) potassium ferricyanide [K₃Fe (CN)₆] solution and 1 mL of phosphate buffer (0.2 M, pH 6.6). The mixtures were then incubated at 50°C in a water bath for 20 min. Five hundred µL of 10% (w/v) trichloroacetic acid solution was added to each mixture followed by 1 mL of 0.1% (w/v) freshly prepared FeCl₃ solution was added and shaken well. The absorbance was measured at 700 nm and ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

Antibacterial activity

The microorganisms of Gram-positive bacteria such as *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus subtilis* were used for the evaluation of the antibacterial activity.

Agar well diffusion method

Nutrient broth agar medium was prepared according to the standard methods (peptone- 5 g, yeast extract-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g). The required amount of ingredients was weighed and suspended in the required volume of 100 mL of distilled water and then autoclaved at 15 lbs and 121°C for 15 min. The hot medium was poured in sterile petri plates which were kept in the aseptic Laminar chamber. The medium was allowed to solidify for 15 min.

Antibacterial activity of aqueous curcuma amada extract was carried out using agar well diffusion method ¹². The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which are previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The root tuber extract was then poured into each well containing 250, 375, and 500 µg/mL concentrations. All the plates with extract loaded wells were incubated at 37°C for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (30 µg) was used as the positive control.

Gas chromatography–Mass Spectrometry (GC–MS)

The aqueous dried Curcuma amada extract were injected into an HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as the carrier gas, the flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; the mass range of 50-600 mass units¹³. The database of the National Institute of Standard and Technology (NIST) was used for the interpretation of the mass spectrum of GC-MS.

CHAPTER 5

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical analysis of rhizome extract of *Curcuma amada* showed the presence of alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, , tannins and glycosides.

Table 1: Qualitative analysis of *Curcuma amada* extract

Phytochemicals	Test	Inference	Result
Alkaloids	Hager's test: To the extract, saturated aqueous solution of picric acid was added and shaken well.	Yellow precipitate	+
Terpenoids	Salkowski test: To the extract, chloroform was added and mixed well. Then, few drops of Conc.H ₂ SO ₄ were added along the sides of the test tube.	Red ring appears.	+
Steroids	Libermann-Burchard's test: To the extract, 1 ml of acetic anhydride was added and shaken well. To this, few drops of Conc.H ₂ SO ₄ were added along the sides of the test tube.	Dark violet colour appears	+
Phenolic compounds	Neutral Ferric chloride Test: To the extract, few drops of neutral ferric chloride solution was added and shaken well.	Purple colour appears	+
Flavonoids	Alkaline Reagent test: To the extract, few drops of 2% NaOH solution was added and shaken well.	Yellow colour appears	+
Tannins	Lead acetate test: To the extract, few drops of 5% Pb(CH ₃ COO) ₂ solution was added and shaken well.	White colour appears	+
Glycosides	Legal's test: To the extract, few drops of pyridine and few drops of alkaline sodium nitroprusside solution was added and shaken well.	Blood red color appears.	+
Saponins	Foam test: To the extract, 3 mL of distilled water was added and shaken vigorously.	Foam appears	-

Total phenols and flavonoids

Flavonoids and phenolic acids make up one of the most pervasive groups of plant phenolics. Due to the tremendous biological activities of phenolic acids and flavonoids, they are used as therapeutic agents. Plants and herbs consumed by humans may contain thousands of different phenolic acid and flavonoid components¹⁴. The dietary phenolics are important due to their antioxidative and possible anticarcinogenic activities. Phenolic acids and flavonoids also function as reducing agents, free radical scavengers, and quenchers of singlet oxygen formation¹⁵. Besides, flavonoids and phenolic acids components play important roles in the control of cancer and other human diseases. Oxidative stress is considered to be substantial, if not crucial, in the initiation and development of many diseases, including inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, arteriosclerosis, and aging¹⁶. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer, and the aging process. Phenolic compounds are physiologically active against herbivores or pathogens and degenerative diseases. Flavonoids can prevent injury caused by free radicals and are oxidized by radicals, resulting in a more stable, less-reactive radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made to be inactive. Antioxidants are specific compounds that protect human, animal, and plant cells against the damaging effects of free radicals. The phenols and flavonoids are good antioxidants, quantified in the aqueous dried curcuma amada extract. The total phenolic content was 495.47 $\mu\text{g}/\text{mg}$ of GAE and the total flavonoid was 40.48 $\mu\text{g}/\text{mg}$ of QE (Table 1).

Table 2: Quantitative estimations of *Curcuma amada* extract

S. No	Phytochemicals	Amount (µg/mg)
1.	Phenols	495.47 GAE
2.	Flavonoids	40.48 QE

DPPH[•] radical scavenging activity

Scavenging of DPPH free radicals is one of the popular antioxidant methods. DPPH radical scavenging assay is a decolorization assay that will measure the capacity of antioxidants to directly scavenge DPPH[•] radicals¹⁷. The ability of curcuma amada tuber extract to scavenge free radicals was assessed by the DPPH method. The maximum DPPH[•] radical scavenging activity of curcuma amada tuber extract was 51.36% at 600 µg/mL concentration (Table 2, Fig 2) and the IC₅₀ was 584.11 µg/mL concentration. It was compared with the standard of ascorbic acid (IC₅₀ = 11.98 µg/mL concentration). In this method, the purple colour 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was reduced to yellow colour 1,1-diphenyl-2-picrylhydrazine, and the reducing capacity increases with increasing concentration of the extract.

Table 3: DPPH[•] radical scavenging activity of *Curcuma amada* extract

S. No.	Concentration (µg/mL)	<i>Curcuma amada</i> extract
		% of inhibition
1	100	26.36
2	200	32.87
3	300	37.67
4	400	41.78
5	500	46.57
6	600	51.36

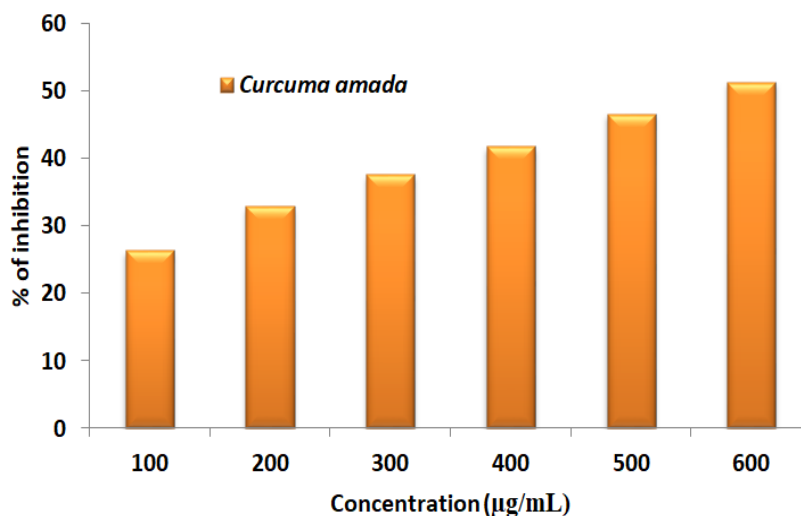


Figure 2: DPPH[•] radical activity of *Curcuma amada* extract

Phosphomolybdenum reduction activity

The total antioxidant activity of curcuma amada extract was measured by phosphomolybdenum reduction assay method. In this method, Mo (VI) ion reduced to Mo(V) by the extract and the formation of green phosphate/Mo (V) complex at acidic pH¹⁹. The maximum phosphomolybdenum reduction was 89.16 % at 300 µg/mL concentration

(Table 3, Fig 3) and the RC_{50} was 57.62 $\mu\text{g/mL}$ concentration. It was compared with the standard ascorbic acid ($RC_{50} = 6.34 \mu\text{g/mL}$ concentration). PM assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant, and molybdenum ligand. It involves thermally generating auto-oxidation during the prolonged incubation period at a higher temperature.

Table 4: Phosphomolybdenum reduction activity of *Curcuma amada* extract

S. No.	Concentration ($\mu\text{g/mL}$)	<i>Curcuma amada</i> extract
		% of reduction
1	50	43.39
2	100	71.69
3	150	82.49
4	200	86.99
5	250	88.98
6	300	89.96

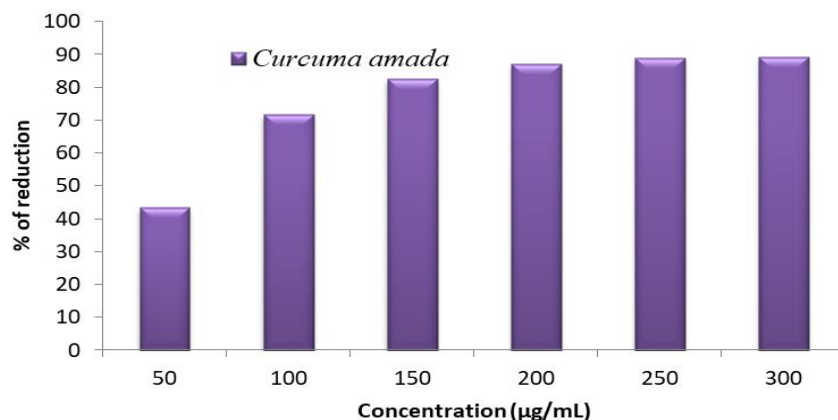


Figure 3: Phosphomolybdenum reduction activity of *Curcuma amada* extract
Ferric (Fe^{3+}) reducing power activity

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the aqueous dried Rose petals extract and the subsequent formation of Ferro-ferric complex. The reduction ability increases with an increase in the concentration of the extract²⁰. The maximum Fe^{3+} reduction was 60.68% at 300 µg/mL concentration (Table 3, Fig 3) and the RC_{50} was 155.28 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} = 7.72 µg/mL concentration). Also in this assay, higher absorbance of the reaction mixture indicates higher reduction potential and a significant indicator of its potential antioxidant activity. The educing capacity of the extract was performed using Fe^{3+} to Fe^{2+} reduction assay as the yellow colour changes to green or blue colour depending on the concentration of antioxidants. The antioxidants such as phenolic acids and flavonoids were present, a considerable amount in the curcuma amada extract, and showed the reducing capacity in a concentration dependant manner.

Table 5: Ferric (Fe^{3+}) reducing power activity of *Curcuma amada*

S. No.	Concentration ($\mu\text{g/mL}$)	<i>Curcuma amada</i> extract
		% of reduction
1	50	21.09
2	100	38.70
3	150	48.30
4	200	56.07
5	250	60.40
6	300	60.68

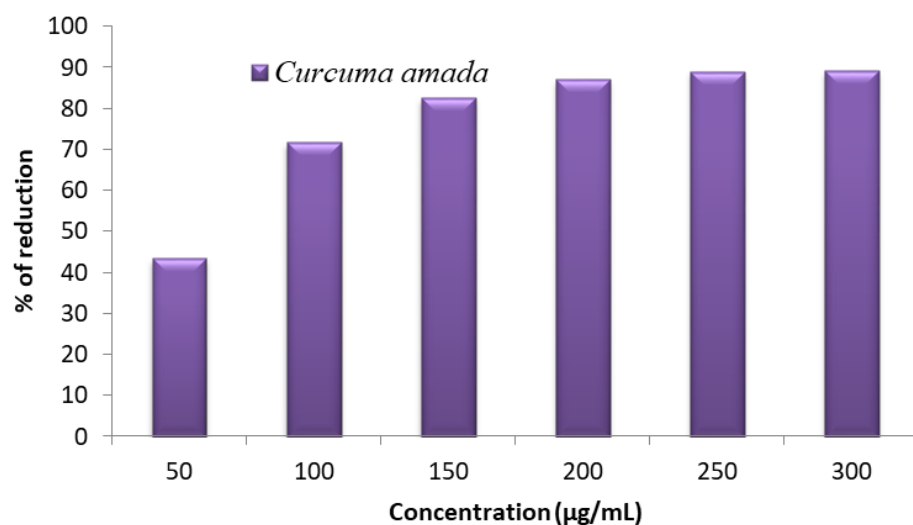


Figure 4: Ferric (Fe^{3+}) reducing power activity of *Curcuma amada*

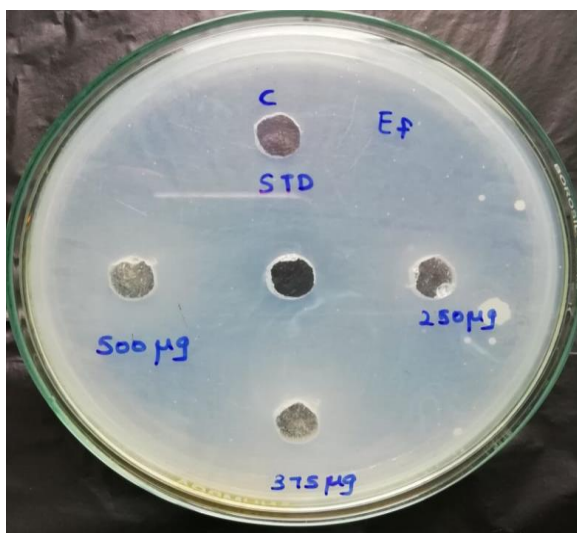
Antibacterial activity by agar well diffusion method

The *Curcuma amada* extract was tested for its antibacterial activity against bacteria such as *Enterococcus faecalis*, *Staphylococcus aureu* *Escherichia coli*, and *pseudomonas*

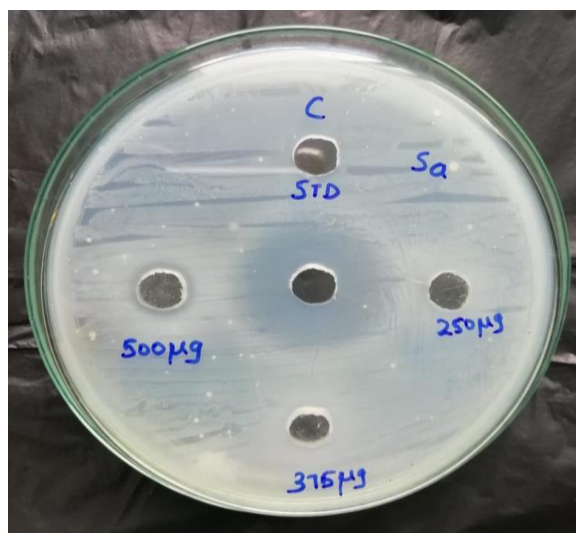
aeruginosa. The antibacterial activity showed a maximum zone of inhibition of 20 mm against *Escherichia coli* at 500 µg/mL concentration of the extract. The antibacterial activity may be due to the presence of phenolic compounds, terpenoids, which inhibit bacterial cell wall biosynthesis by binding to a highly conserved motif of lipid II and lipid III, two key precursors of bacterial cell-wall polymers as peptidoglycan and teichoic acid that adversely affect the growth of microbes²¹.

Table 4: Antibacterial activity of *Curcuma amada* extract

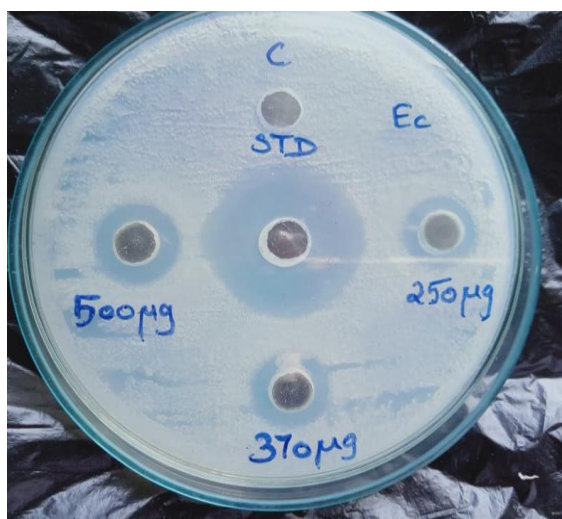
Organisms	250 µg	375 µg	500 µg	Standard (Tetracycline-30 µg)
<i>Enterococcus faecalis</i>	-	10	11	22
<i>Staphylococcus aureus</i>	-	10	12	18
<i>Escherichia coli</i>	12	14	20	20
<i>pseudomonas aeruginosa</i>	10	11	14	18



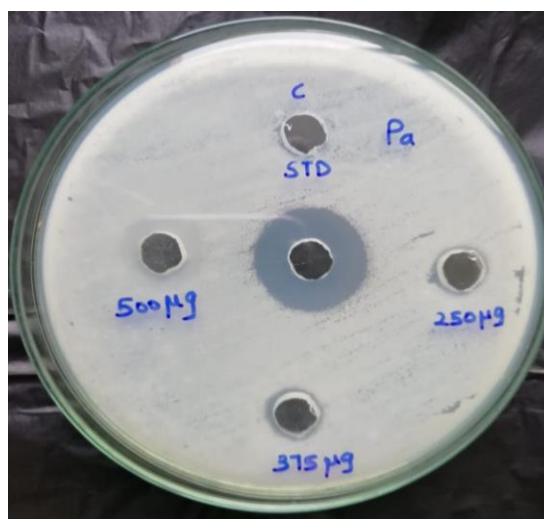
Enterococcus faecalis



Staphylococcus aureus



Escherichia coli



Pseudomonas aeruginosa

Figure 5: Antibacterial activity of *Curcuma amada* extract

GC-MS analysis

GC-MS analysis of aqueous dried Rose petals extract was shown in Table 5 and the chromatogram was represented in Figure 6. Antioxidant compounds such as flavone (5,7-

dihydroxy-3-phenylchromen-4-one), oleic acid, and antibacterial compound Pyrimidine,5-ethyl-2-[4-[4-ethylcyclohexyl]phenyl]²² were eluted and recorded.

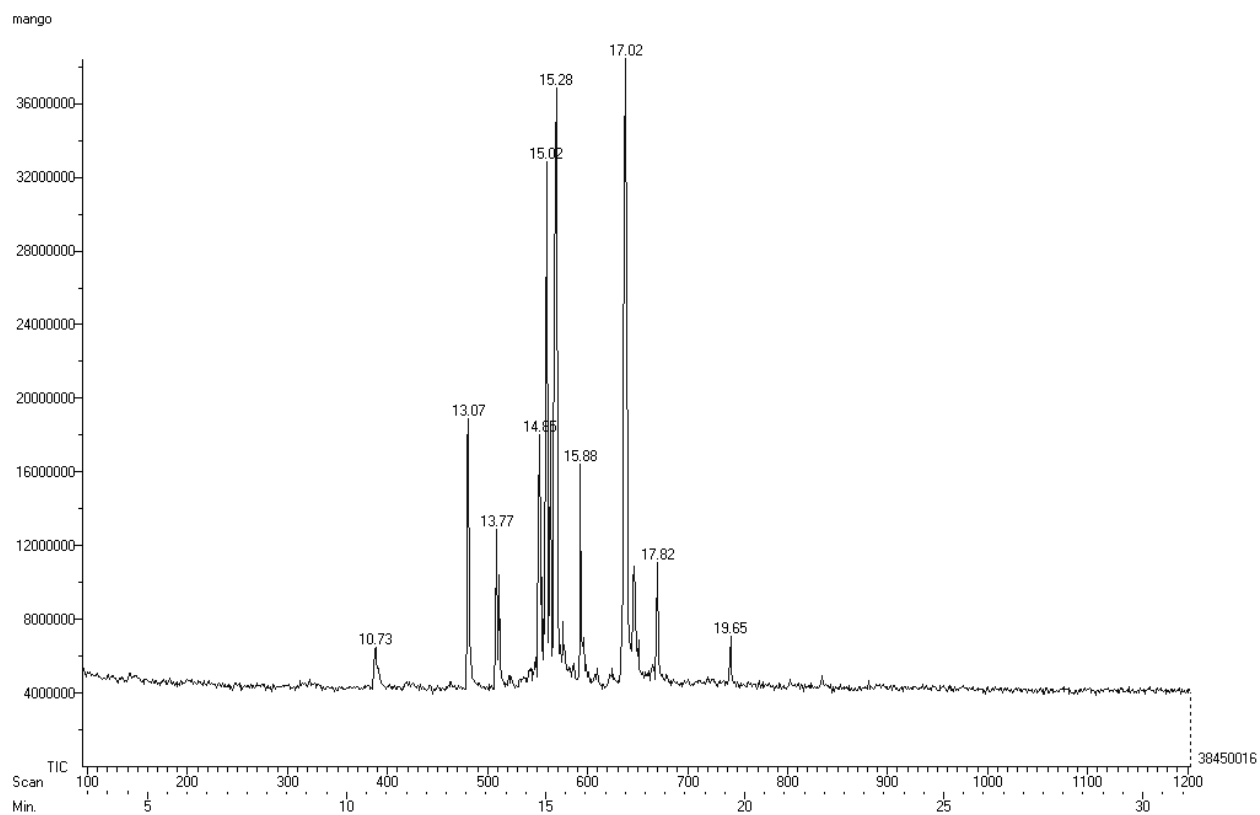
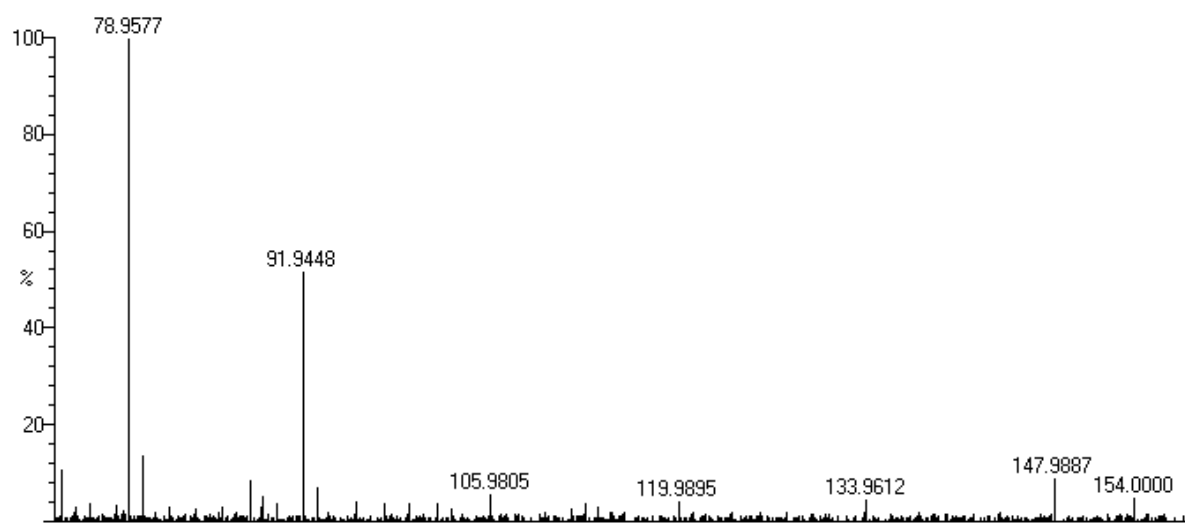


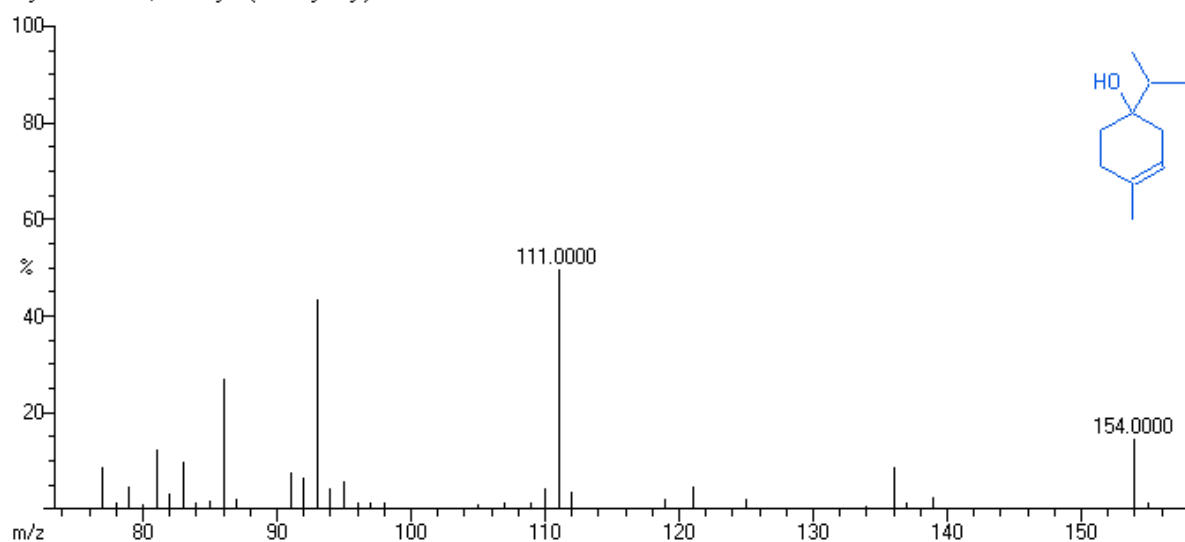
Figure 6: Gas chromatogram of curcuma amada extract

mango

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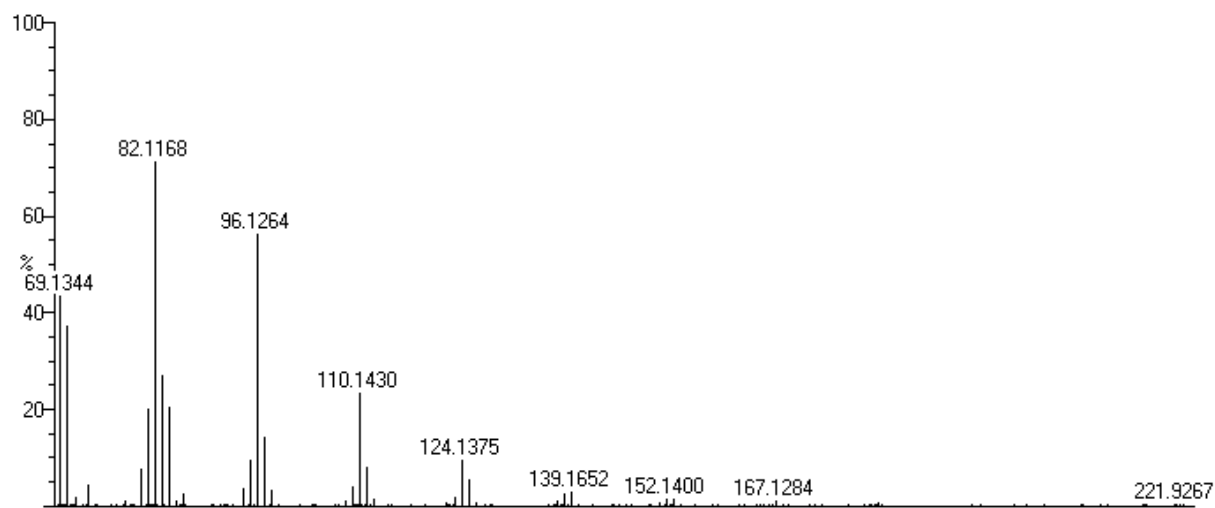


NIST CAS# 562-74-3 #ions=112
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-



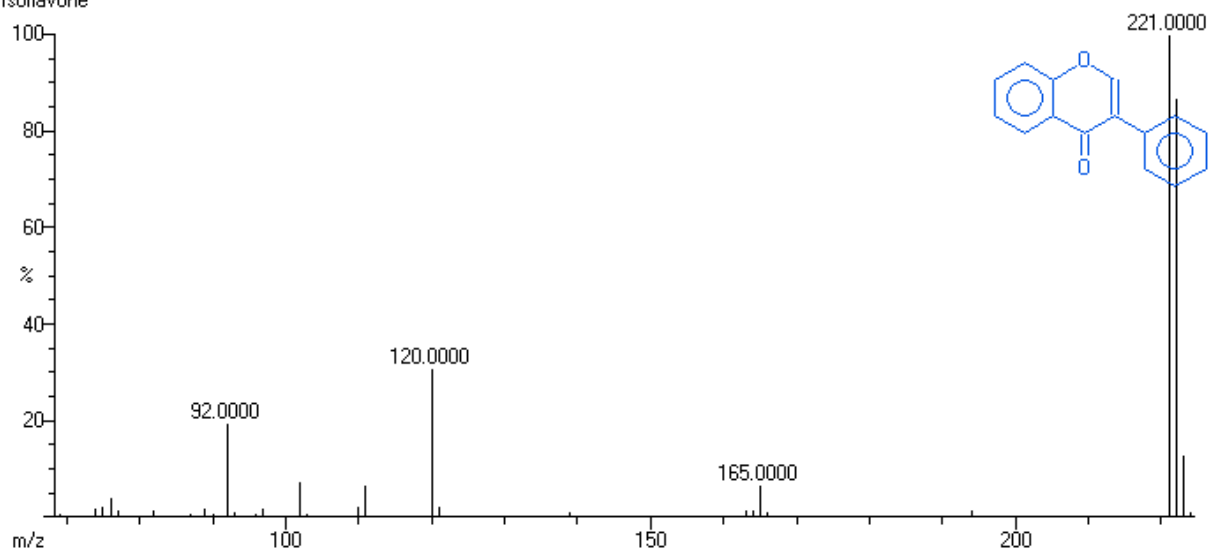
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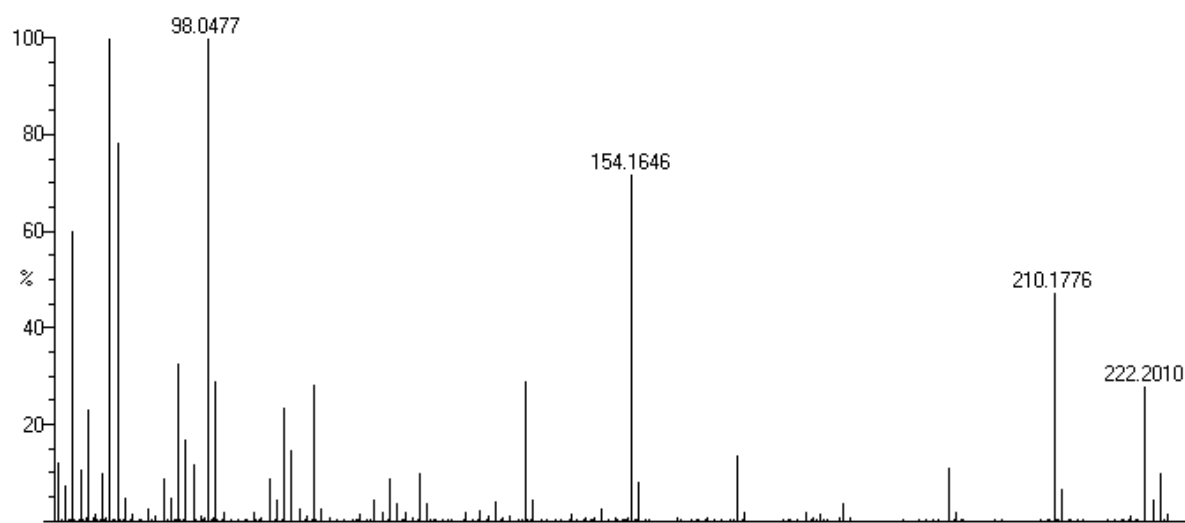
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Isoflavone



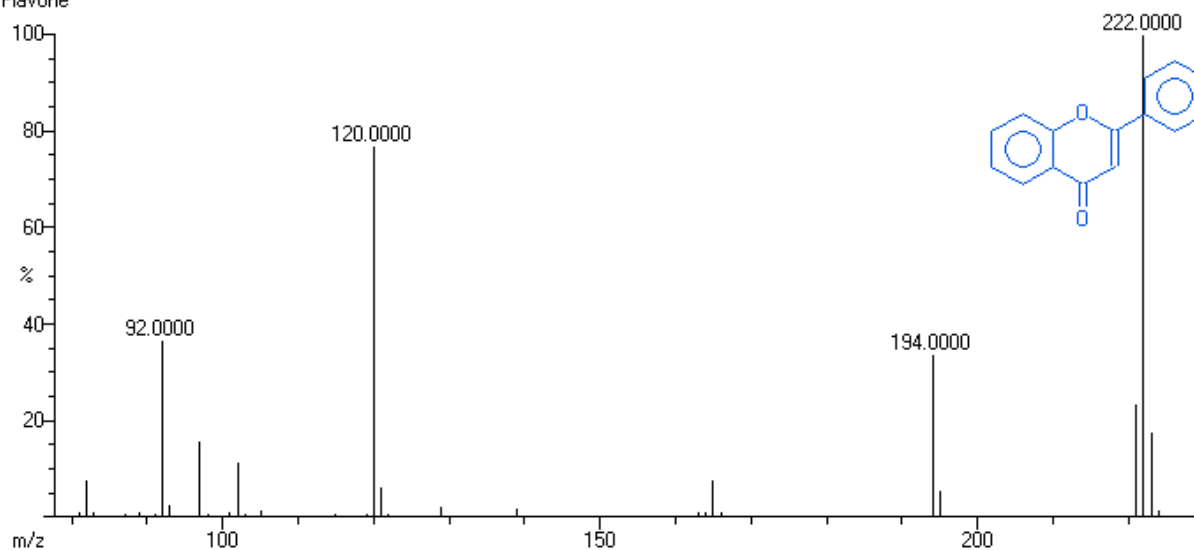
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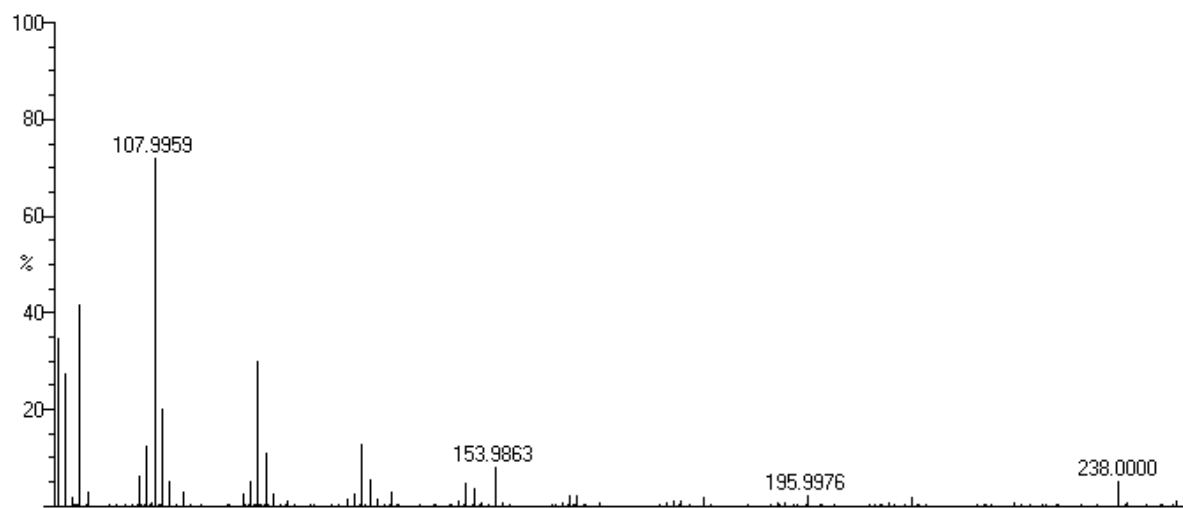
NIST CAS# 525-82-6 #ions=78

Flavone

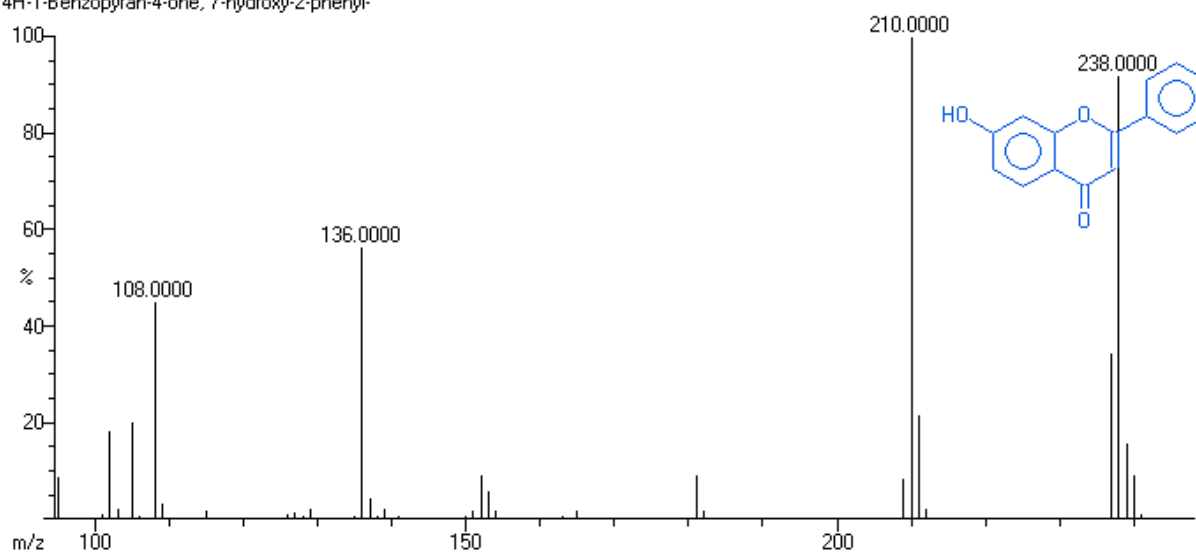


mango

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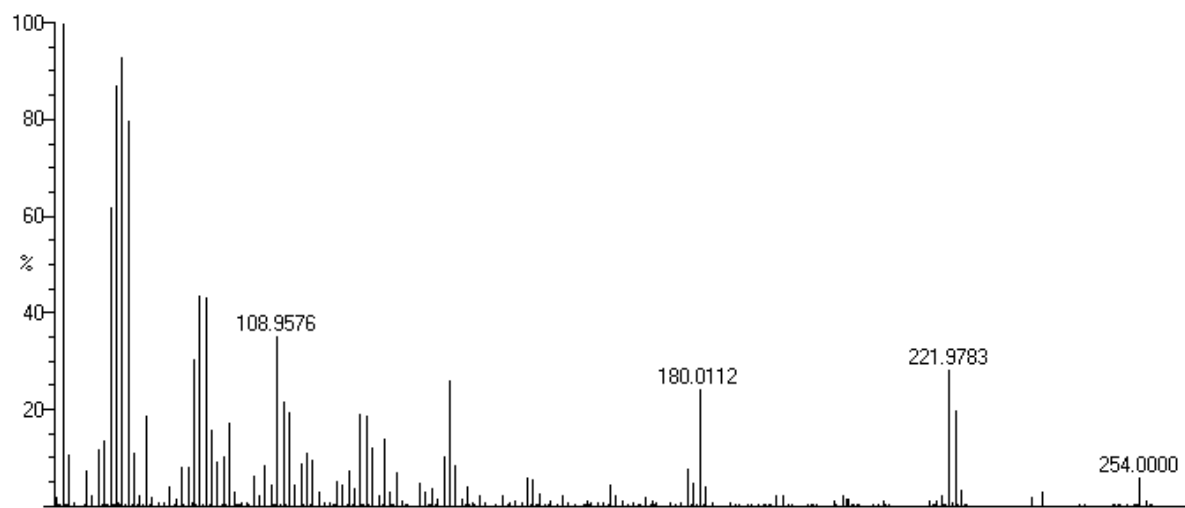


NIST CAS# 6665-86-7 #ions=211
4H-1-Benzopyran-4-one, 7-hydroxy-2-phenyl-

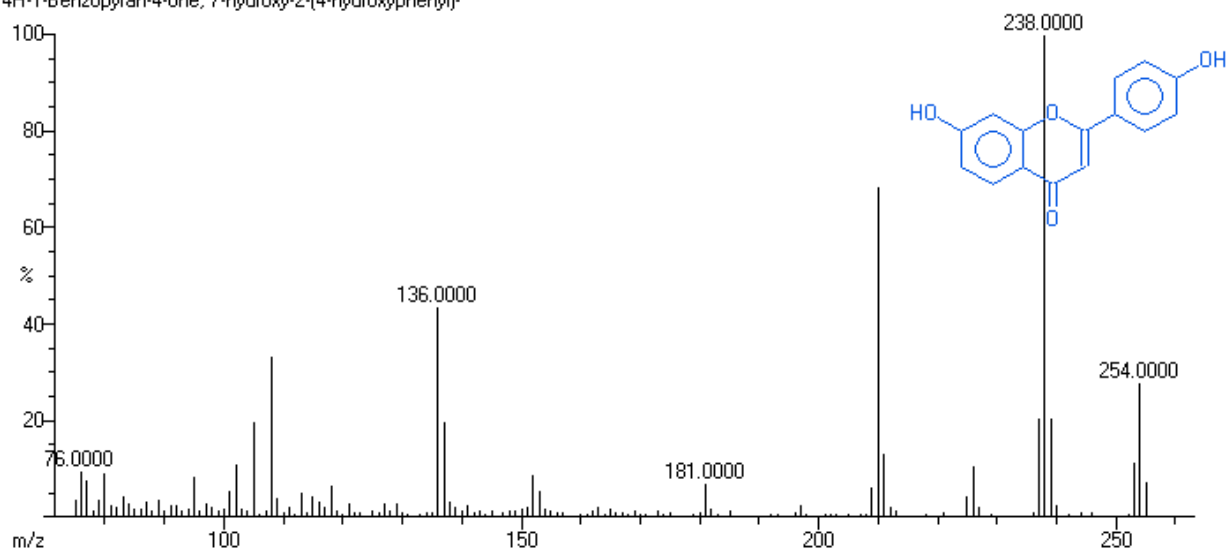


mango

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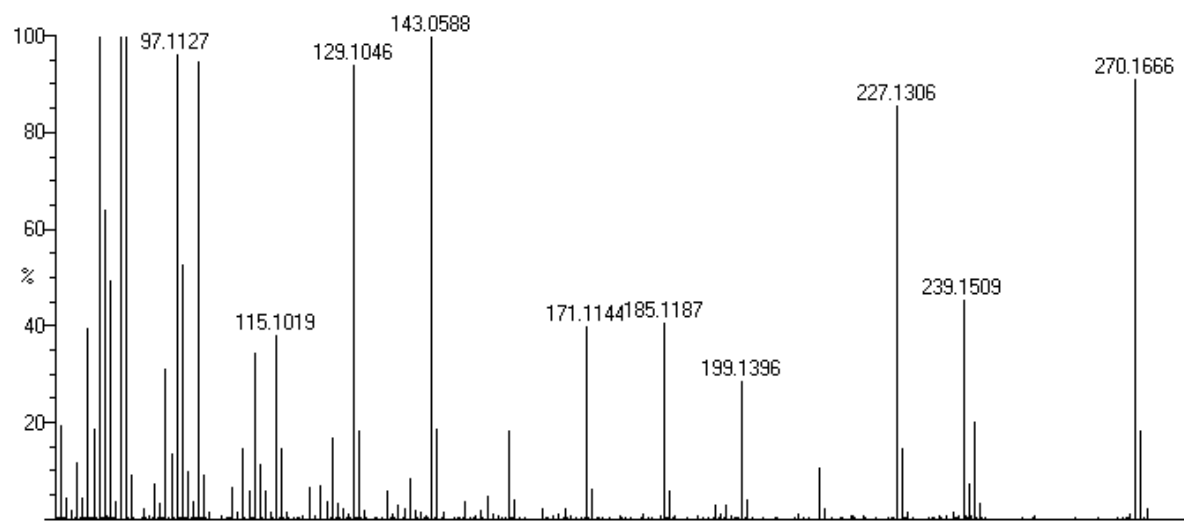


NIST CAS# 2196-14-7 #ions=181
4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-hydroxyphenyl)-

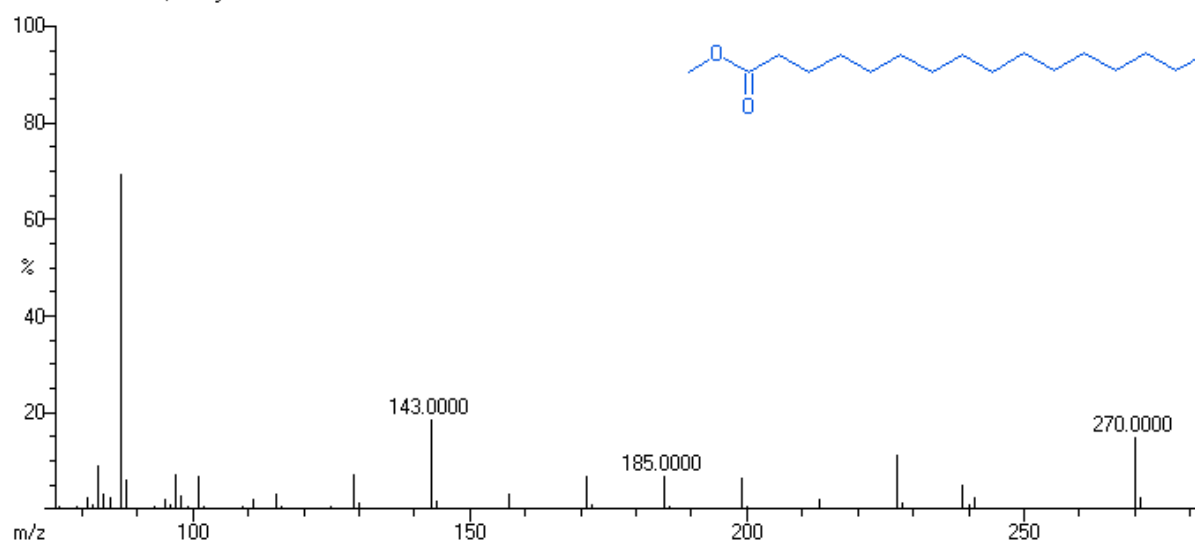


mango

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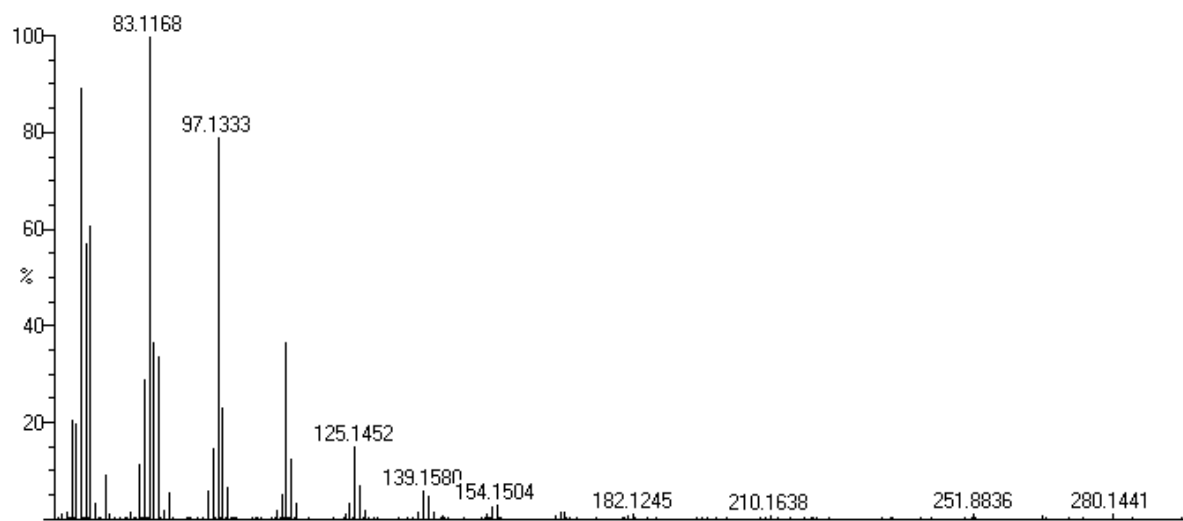


NIST MS 1 of 40 (112-39-0) #ions=152
Hexadecanoic acid, methyl ester

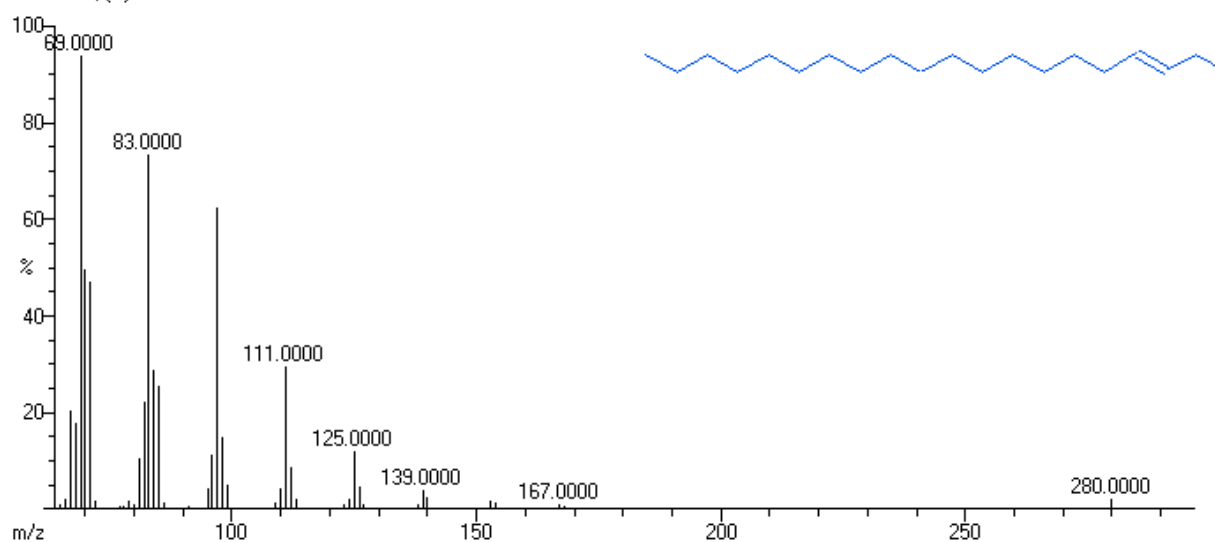


mango

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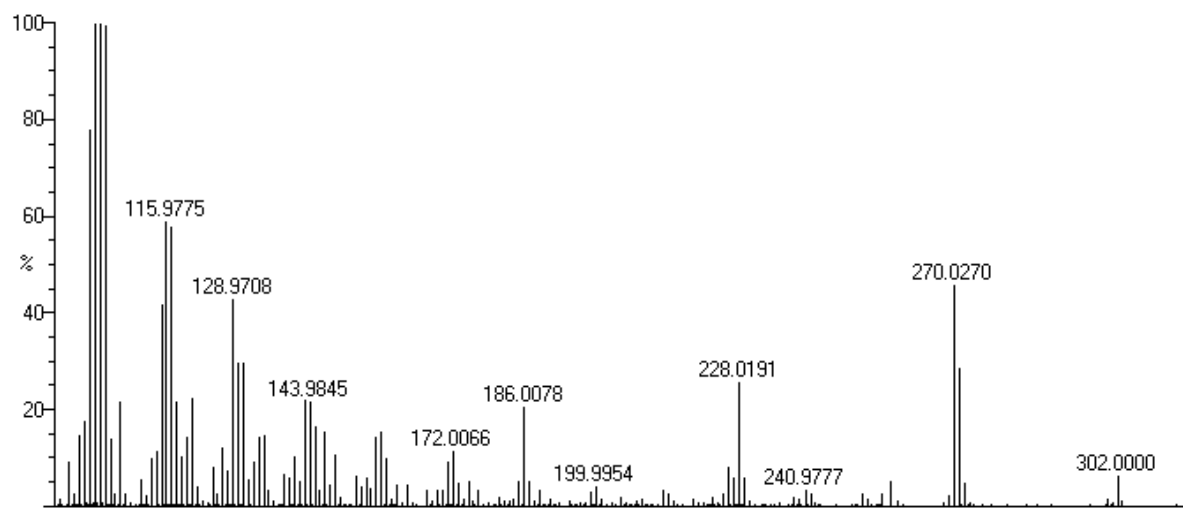


NIST MS 3 of 40 (74685-33-9 #ions=121
3-Eicosene, (E)-

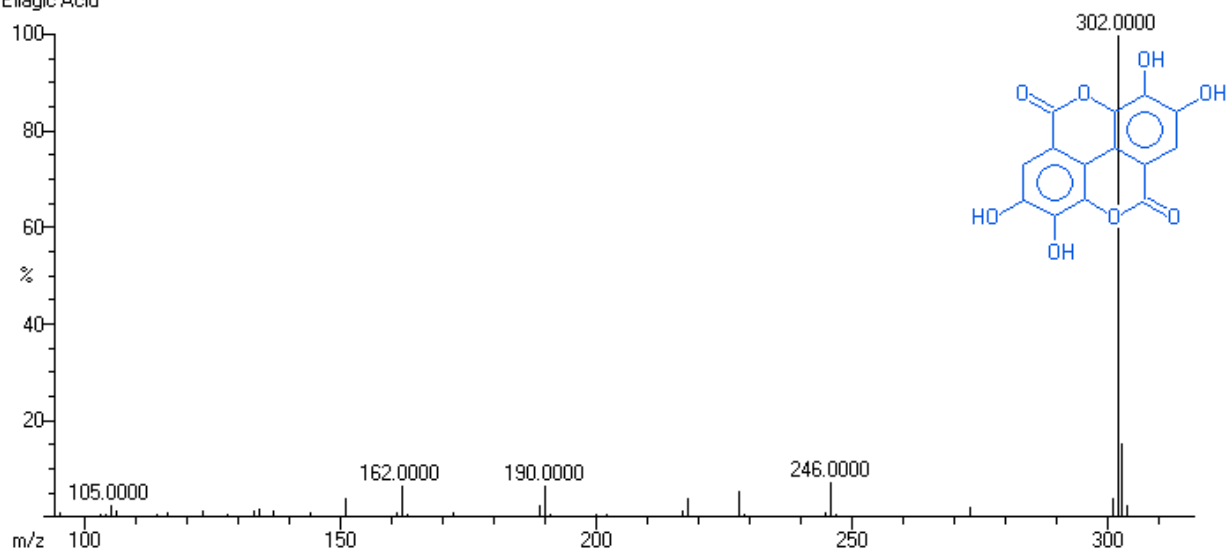


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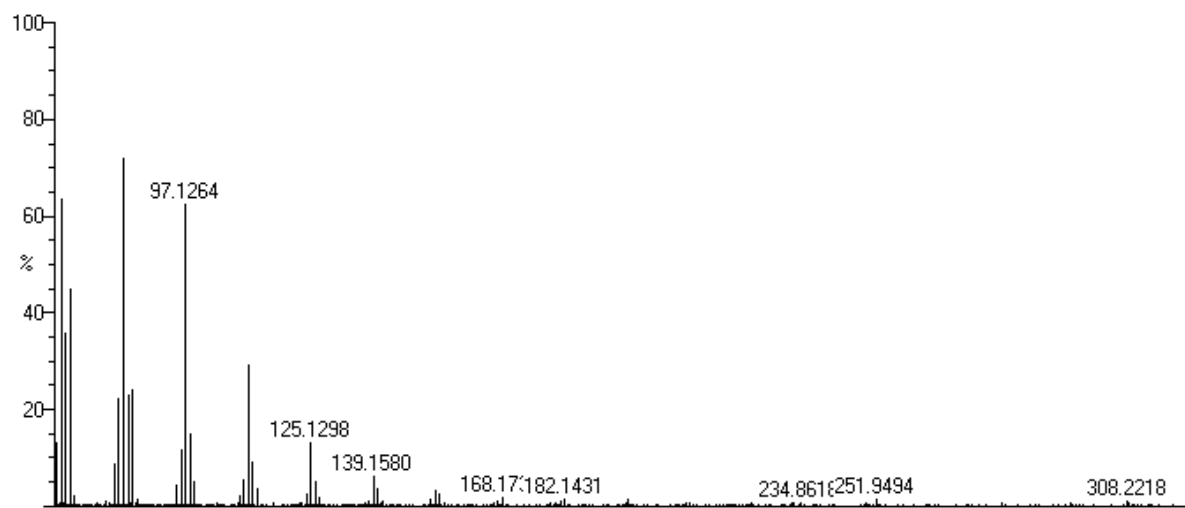


NIST CAS# 476-66-4 #ions=163
Ellagic Acid

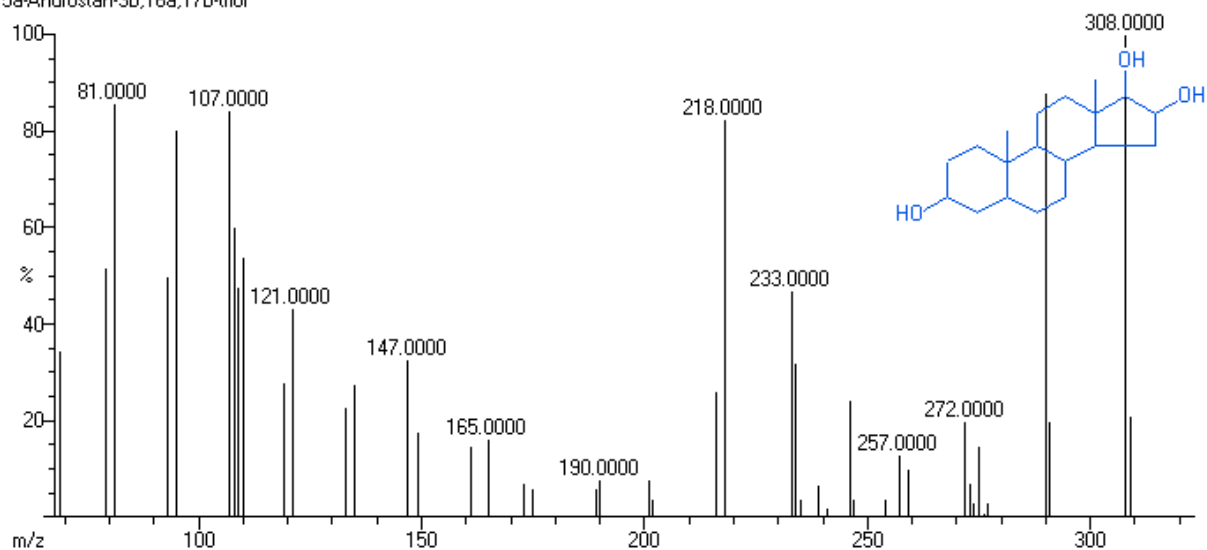


mango

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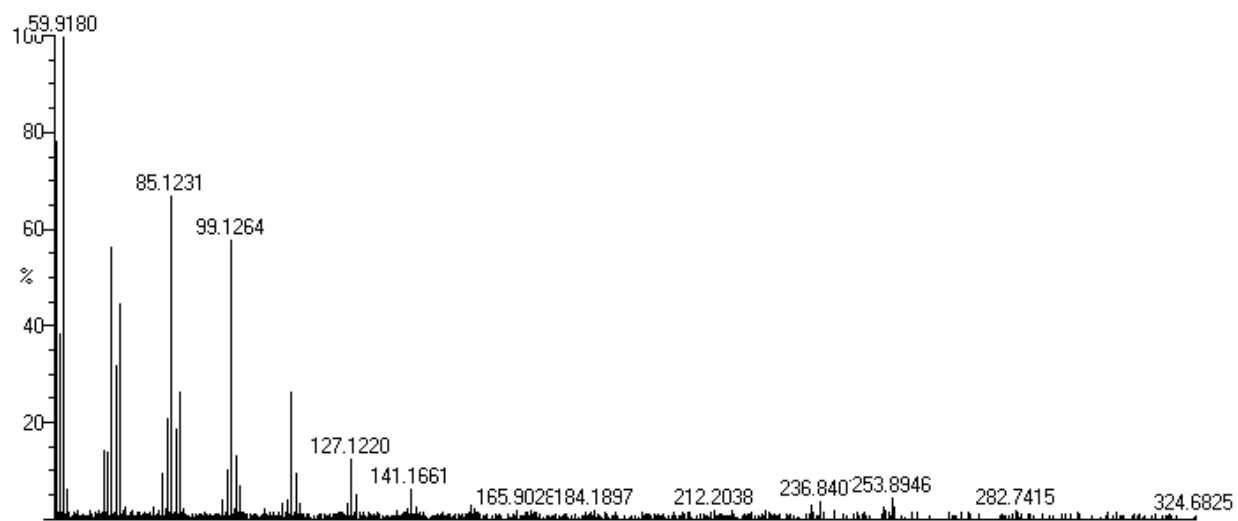


NIST MS 43 of 620 (DB# 1372 #ions=50
5a-Androstan-3b,16a,17b-triol



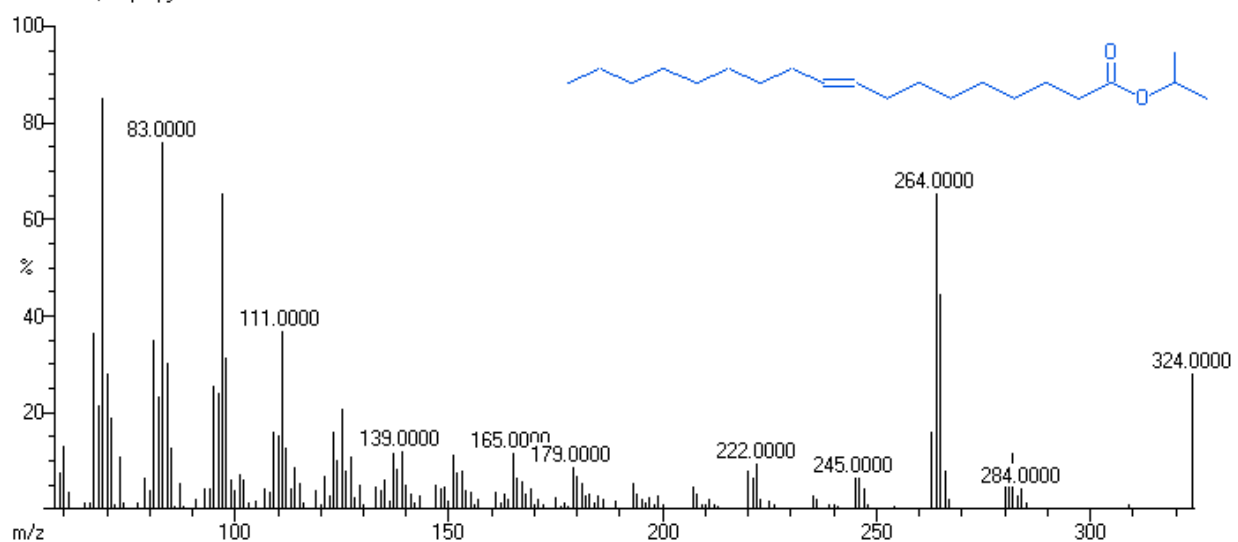
mango

Scan: 743 TIC=7103600 Base=28.9%FS #ions=1597 RT=19.65



NIST MS 17 of 555 (22147-34 #ions=182

Elaidic acid, isopropyl ester



CHAPTER 6

SUMMARY AND CONCLUSION

Antioxidants are substances that significantly delay or prevent the oxidation of an oxidizable substrate when present in low concentrations. Antioxidants help protect human body cells from the formation of radicals. Radicals, also known as free radicals are aggressive and toxic and are produced typically during cell metabolism. They are common transient intermediaries in chemical reactions with cell components, causing permanent damage and they are believed to be the source of aging and the cause of several degenerative diseases. Plants are potential sources of natural invaluable food antioxidants. Antioxidants react with free radicals and form a complex that prevents cell destruction and is easily cleaned out. The results of the present study indicate that *Curcuma amada* tuber extract has significant antioxidant activities to reduce the harmful effect of radicals. The results of the present study provide promising hope to use *Curcuma amada* tuber extract as a natural antioxidant due to presence of ellagic acid, 4H-1-Benzopyran -4-one, 7-hydroxyle-2-phenyl, isoflavone, flavone.

REFERENCES

1. Moein M, Karami F, Tavallali H, Ghasemi Y. Composition of the essential oil of *curcuma amada* Mill. From south of Iran. Iran J Pharmaceut Sci. 2010; 6:59–62.
2. Kaul VK, Singh V, Singh B. Damask rose and marigold: prospective industrial crops. J Med Aromat Plant Sci. 2000; 22:313–318.
3. Yassa N, Masoomi F, Hadjiakhoondi A. Correspondence chemical composition and antioxidant activity of the extract and essential oil of Rosa damascena from Iran, Population of Guilan. Daru. 2009; 17:175–180.
4. Leenen R, Roodenburg AJC, Tijburg LBM, Wiseman SA. A single dose of tea with or without milk increases plasma antioxidant activity in humans. Eur J Clin Nutr. 2000; 54:87–92.
5. Heim KE, Tagliaferro AR, Bibilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem. 2002; 13:572–84.
6. Spanos, G.A., and Wroslad, R.E. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of Agricultural & Food Chemistry*, 1990; .38:1565-1571.
7. Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. and Yan, G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. From *Ginkgo biloba*. *Food Chemistry*. 2007; 105: 548-554.
8. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*, , 1958; 29: 1199-1200.
9. Ravishankara MN, Shrivastava N, Padh H, Rajani M. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus*. *Phytomedicine*. 2002; 9:153–60.
10. Prieto, P., Pineda, M and Anguilar, M. Spectrophotometric quantisation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. *Anal. Biochem*, 1999; 269: 337-341.
11. Yen, G C, and Chen, H Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agri. Food Chem*, , 1995; 43: 27-32.
12. Kubo, I., Muroi, H. and Himejima, M. Antimicrobial activity of green tea flavor components and their combination effects. *Journal of Agric Food Chem*. 2002; 40: 245-248.

13. Harini V, Vijayalakshmi M, Sivaraj C, Arumugam P. Antioxidant and Anticancer Activities of Methanol Extract of *Melochia corchorifolia* L. Int. J. of Sci. and Res., 2017; 6(1): 1310-1316.
14. Lukyanova LD, Storozheva ZI, Proshin AT Corrective effect of flavonoid containing preparation extra life on the development of Parkinson's syndrome. Bull. Exp. Biol. Med., 2007; 144: 42-45.
15. Duan, X., Wu, G., Jiang, Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules. 2007; 12: 759–771.
16. Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 1998; 46: 1887–1892.
17. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 2011; 48:412–22.
18. Gülçin, İ.; Oktay, M.; Küfrevioğlu, Ö.İ.; Aslan, A. Determination of antioxidant activity of lichen *Cetraria islandica* (L.) Ach. Journal of Ethnopharmacology, 2002; 79 (3): 325-329.
19. Loo, A.Y., Jain, K., Darah, I. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem. ., 2008; 107: 1151–1160.
20. Siddhuraju, P., Becker, K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem. 2007; 101: 10–19.
21. Maher Obeidat, Mohamad Shatnawi, Mohammad Al-alawi, Enas Al-Zu`bi, Haneer Al-Dmoor, Maisa Al-Qudah, Jafar El-Qudah and Ismael Otri. Antimicrobial Activity of Crude Extracts of Some Plant Leaves. Research Journal of Microbiology, 2012; 7: 59-67.
22. Sharma, V., Chitranshi, N., & Agarwal, A. K. Significance and biological importance of pyrimidine in the microbial world. International journal of medicinal chemistry, 2014;. 2014: 202784. <https://doi.org/10.1155/2014/202784>.

Aggarwal, B.B., Sundaram, C., Malani, N., Ichikawa, H. 2007. "Curcumin: the Indian Solid Gold". *Advances in Experimental Medicine and Biology*. 595,1-75.

Akowuah, G.A., Z. Ismail, I. Norhayati and A. Sadikun. 2005. The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chemistry*, **93**: 311-317.

Cockbill, S., and Frpharms, F.C.P.P., 2000. Wound the healing process. *Hospital Pharmacist*, 9: 255-260.

Czygan, F.C., 1993. Kulturgeschichte and Mystic des Johanniskrautes, *Zeitschrift fur Phytotherapie*, **5**: 276 - 282.

Decker e.A., Welch B., 1990. role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and food chemistry* 38, 674-677.

Dinis TCP, Madeira VMC, Almeida MLM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch. Biochem. Biophys.* 315: 161-169.

Gorby GL, Peacock JE, Jr. *Erysipelothrix rhusio-pathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev Infect Dis* 1988; 10: 317-25.

Hiremarh IG, Ahn YJ, Kim Soon-II. Insecticidal Activity Of Indian Plant Extracts against *Nilaparvatalungens*(HomopteraDelphacidae). *App EnomolZool*, 1996.

Kala, C.P and B.S. Sajwan. 2007. Revitalizing Indian systems of herbal medicine by the National Medicinal Board through institutional networking and capacity ,*Current science*,.93,(6) 797-806.

Kala, C.P. 2005. Indigenous uses, population density, and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. *Conserv. Biol*,19, 368–378.

Krafczyk, N; Woyand, F; Glomb, MA (2009). "Structure-antioxidant relationship of flavonoids from fermented rooibos.". *Molecular nutrition & food research* **53** (5): 635–42.

Kunin, W.E., and Lawton, J.H., 1996. Does biodiversity matter? Evaluating the case for conserving species. In: Biodiversity, (Editor Gaston, K.J.), Blackwell Science LTD, UK. pp. 283-308.

Lai PK, Roy J (June 2004). "Antimicrobial and chemopreventive properties of herbs and spices". *Curr. Med. Chem.* **11** (11): 1451–60.

Maenthaisong R, Chaiyakunapruk N, Niruntraporn S, Kongkaew C (2007). "The efficacy of aloe vera for burn wound healing: a systematic review". *Burns* **33** (6): 713–718.

Mahdi, JG; Mahdi, AJ; Mahdi, AJ; Bowen, ID (April 2006). "The historical analysis of aspirin discovery, its relation to the willow tree and antiproliferative and anticancer potential.". *Cell proliferation* **39** (2): 147–55.

Maity, Hansda, Bandyopadhyay, Mishra, 2009, Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos*(L.) Corr, Indian Journal of Experimental Biology, Vol 47, pp 849-861.

Manjeshwar S B, Harshith P Bhat ,Nandhini Josepha , FarhanFazala. Phytochemistry and medicinal uses of the bael fruit (*Aegle marmelos* Correa). Exotic Fruits: their composition, Nutraceutical and Agroindustrial Potential, V44, I 7, (2011).

Mavi A, Terzi Z, Ozgen U et al. 2004. Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. *verum* (Rubiaceae), *Urtica dioica* (Urticaceae). *Biol Pharm Bull* **27**: 702-705.

Mazumder, R., S. Bhattacharya, A. Mazumdrer, A.K. Pattnaik, P.M. Tiwary and S. Chaudhary, 2006. Antidiarrhoeal evaluation of Aeglemarmelos (Correa) Linn. root extract. *Phytother Res.*, 20: 82-84.

Mitra, R. 1980. Silent pharmacognostical characters of Varuna, important drugs of Ayurveda. *Bull.Med. Etheno. Bot. Res.*, 1: 80-98.

Ody, P. 1993. The Complete Medicinal Herbal. New York: DK Publishing.192pp p.

Ordonez, A. A. L.; Gomez, J. D.; Vattuone, M. A.; Isla, M. I. **2006**. *Food Chemistry*, **97**: 452–458.

Ozgoli, G; Goli, M; Simbar, M (March 2009). "Effects of ginger capsules on pregnancy, nausea, and vomiting.". *Journal of alternative and complementary medicine* 15 (3): 243–6.

Parmar, C., M.K. Kaushal, Aeglemarmelos In:Wild fruits of the sub-Himalayan region, Kalyani Publishers, New Delhi, India (1982).

Patel, Axay R.; Garach, Dipak; Chakraborty, Manodeep; Kamath, Jagdish V. Aeglemarmelos (Linn.): A Therapeutic Boon for Human Health. *International Journal of Research in Ayurveda & Pharmacy*; Mar2012, Vol. 3 Issue 2, p159.

Pieroni, A., Quare, C.L., Villanelli, M.L., Mangino, P., Sabbatini, G., Santini, L., Boccetti, T., Profili, M., Ciccio, T., Rampa, L.G., Antonini, G., Girolamini, C., Cecchi, M, and Tomasi, M., 2004. Ethnopharmacognostic survey on the natural ingredients used in folk cosmetics, cosmeceuticals and remedies for healing sting diseases in the inland Marches, Central-Eastern Italy. *Journal of Ethnopharmacology*, **9**: 331- 344.

Policegoudra R S, Abiraj K, Channe Gowda D and Aradhya S M 2007b Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome; *J. Chromatogr. B* 854 40–48.