

**PHYTOCHEMICAL CHARACTERAZATION OF
AMARANTHUS SPINOSUS AND *ACALYPHA INDICA***

**ABIRAMI T
(20PBT003)**

**A Thesis submitted to
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore – 641 043**

**In Partial Fulfilment of the Requirement for the Degree of
Master of Science in Biotechnology**

May 2022

Certificate

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Introduction

1. INTRODUCTION

From prehistoric period a big a part of the population recognizes the remedial belongings of several plant life and the identical has been proved with the aid of using the scientific science. India a land of vast alters in soil alongside with climate circumstance is an idyllic role supposed for the gardening of fantastic range of plant life with medicinal residences and which may be utilized in cosmetics, agrochemical and perfumery additionally in pharmaceutical industries. In the start of the human society and at gift too natural remedy may be used for the remedy of various disease. Rig Veda which became written among 4500-1600 BC being the oldest depository of the man or women consciousness describes the scientific use of plants in Hindu ethnicity. In traditional and in western drug treatments plant life performs an essential part. Derivative rug from the plant is assisting the human from the prehistoric length. (Nag1a *et al.*, 2018).

In present day's human civilization, the most widely used, accepted and recognised form of medicine is plant derived medicinal products throughout the world. Plants produce several types of secondary metabolites that are biosynthetically formed from primary metabolites, and these phyto compounds are the main sources of herbal, pharmaceutical and nutritional supplements. The medicinal and nutritional requirements of these plants are lies in various Phyto molecules with distinct biological actions on the organ system of humans. Human beings utilise to produce phytomedicine from diverse parts of the plants and these are working as panacea for modern cultures, civilizations and societies (Poddar *et al.*, (2020)). About 80% of humans in growing international locations rely upon conventional medicinal drug for their number one fitness care needs, due to their low costs, effectiveness, often insufficient provision of contemporary-day medicinal drug cultural and spiritual preferences. Studies on conventional medicinal flora have turn out to be increasingly treasured with inside the improvement of healthcare and conservation applications in exclusive components of the world (Kumkhale *et al.*, 2018).

Plants with therapeutic potential are widely employed as folk remedies in different indigenous system of medicine like Siddha, Ayurveda and Unani and also as processed product of pharmaceutical industry. There are around 4.5 million plant species in India, among them only 250,000-500,000 plant species have been investigated phytochemically for biological or pharmacological activity. Plants extracts can be used to treat a variety of

ailments and these would be utilised as a new formulation for the novel drug discovery in pharmaceutical industry (Bamola N *et al.*, 2018).

Traditional medicinal practices primarily based totally on herbal plant life had been diagnosed by the World Health Organization (WHO, 2002) as dependable medicinal reassess for healing activities. The medicinal plant life are available round backyards, settlements, spreading along roadsides, and residence compounds Medicinal plant life are diagnosed as potential supply of bioactive compounds. More than 80% of current capsules are derived at once from reassess of plant life and microbes. Natural products derived from medicinal plant life have extensive variety of pharmacological significance. Bioactive compounds as they comprise healing, and their complicated nature will capable of engage with mammalian cell targets (Chekuri S *et al.*, 2020).

Current years, Secondary plant metabolites (Phytochemicals), formerly with unknown pharmacological activities, has been substantially investigated as a supply of medicinal retailer. Nature has been a supply of medicinal retailers because instances immemorial. The significance of herbs with inside the control of human illnesses can't be over emphasized. Some essential phytochemicals consist of alkaloids, flavonoids, phenolic, tannins, saponins, steroids, glucosides, terpenes, etc. which can be allotted in diverse components of the Plants. Nature is a precise supply of systems of excessive phytochemical variety representing phenolic (45%), terpenoids and steroids (27%) and alkaloids (18%) as principal agencies of phytochemicals. Although, those compounds appear to be non-important to the plant generating them, they play a critical position in survival via way of means of mediation of ecological interactions with competitors, shield them from diseases, pollution, stress, UV rays and additionally make contributions for color, aroma and flavor with recognize to the plant. The metabolites produced via way of means of the plant to shield themselves against biotic and abiotic stresses have become drugs that human beings can use to deal with diverse diseases (Shaikh *et al.*, 2020).

Acalypha indica is one in every of weed plant life that comprise vital medicinal values for human fitness applications. It may be located normally in India, Sri Lanka, Thailand and Pakistan. The extracts of numerous components of the plant, leaves, roots and stem components are used for medicinal functions to deal with numerous sicknesses along with the attention infections, breathing issues, rheumatism, and pores and skin issues and to lower blood sugar level (Chekuri *et al.*, 2020).

The *Amaranthus spinosus* Line. Plant ('Spiny amaranth', 'Prickly amaranth', or 'Spiny pig weed') is a medicinal plant that's discovered in abundance in India and round the sector. This plant is typically discovered as a weed in rice agriculture. Historically, this plant has been used considerably in Ayurveda, the conventional people's medicinal drug of India, to deal with diabetes, jaundice, and diverse different ailments. Previous research has proven this plant to own numerous medicinal houses, consisting of anti-inflammatory, anti-diabetic, anti-diarrheal, antibacterial, anti-microbial and anti-malarial houses. Hence, the medicinal houses of this plant are of eager hobby to the clinical community. While previous phytochemical research was achieved on person elements of this plant, inclusive of the leaves, the houses of the complete plant are exceedingly unknown to the sector of science, and there has been little try to put together pharmaceutical pills from this plant (Sharma *et al.*, 2021).

Clitoria ternatea, commonly referred to as butterfly pea, may be a perennial herbaceous plant from the Fabaceae family. It's recently attracted a lot of interest as it has potential applications both in Modern medicine and agriculture, and as a source of natural food colorants and antioxidants. *C. ternatea* has long been cultivated as a forage and fodder crop, and early studies assessed the Plant for these purposes. Numerous Field trials in Queensland, Australia, eventually led to the registry of *C. ternatea*, the sole cultivar in Australia that was released for grazing purposes (Conway and Doughton,2005). Additionally, *C. ternatea* has been widely utilized in traditional medicine, particularly as a Supplement to reinforce cognitive functions and alleviate symptoms of numerous ailments including Fever, inflammation, pain, and diabetes (Oguis, *et al.*,2019).

The genus Basil belongs to the Lamiaceae family. There are about 150 species in *Ocimum*.The Genus *Ocimum* has many species used to treat it various kinds of diseases, especially from ancient times, especially the Species *Ocimum basilicum* . If not, it is known as a sweet basil. It's a universally cultivated herb, Perennial plant. It is a popular herb used in Italy. It has Numerous powerful activities due to existing metabolites present in it. This is due to its toxic metabolites It is also used as a traditional medicine an ornamental plant. *Ocimum basilicum* essential oil extract used as a food flavor. Used as Cooking herbs, ornamental herbs. It also has been used as a commercial fragrances and flavor to improve the shelf life of food (Purushothaman *et al.*,2018).

Amaranthus viridis L. (Family Amaranthaceae) is Distributed in the warmer parts of the world. In addition, the Whole plant possesses analgesic and anti-pyretic properties and is

used for the treatment of pain and fever respectively in Traditional systems of medicine. *A. viridis* is possibly of Asian origin but now a cosmopolitan weed in the tropical and Subtropical regions of the world, also ubiquitous faraway to Temperate regions (e.g. in Europe, North America, Asia and Australia). In equatorial Africa it is also a profound and common weed. It is occasionally cultivated. *A. viridis* Linn. Is Erect or Ascending annual or short-lived perennial herb up to 1 m tall; Glabrous, angular, bear branches, stem slender to sparsely Pubescent in upper part with multicellular hairs (Reyad *et al.*,2015).

Diabetes Mellitus is a metabolic ailment characterized by hyperglycemia as a result of defects in both insulin secretion or insulin resistance or both. There are two fundamental styles of diabetes- Type-1 (insulin-established DM) and Type-2 (noninsulin-established DM). Type-I DM happens whilst the human immune system destroys pancreatic β -cells, which can be responsible for secreting insulin. Insulin awareness can correctly be controlled via non-stop injection in well timed dosages. Elevated post-prandial blood glucose degrees are broadly identified as one of the earliest ailment markers withinside the prediction of next microvascular and macrovascular headaches that could development to complete symptomatic Type-2 Diabetes (T2DM). Type-2 DM debts for approximately 90% of the diabetic instances and commonly begins as insulin resistance till the pancreas slowly loses its cap potential to supply insulin. Globally, an anticipated of 422 million adults had been residing with diabetes in 2014, growing from 4.7% to 8.5% in person population (Daimari *et al.*, (2019)).

The incidence of degenerative strategies is correlated in molecular biology with the lifestyles of a surplus of unfastened radicals, selling oxidative strategies which are dangerous to the body. Plants excessive content material of compounds with antioxidant residences capable of seize unfastened radicals (carotenoid, phenolic, Slavonic, anthocyanin derivatives, unsaturated fatty acids, vitamins, enzymes and cofactors) has inspired hobby in the use of them in prophylactic and healing phototherapy. Oxidative strain is a highly new concept, broadly utilized in clinical sciences with inside the past 3 decades. It takes an energetic component with inside the body structure of very not unusual place diseases, like diabetes, excessive blood pressure, preeclampsia, atherosclerosis, acute renal failure (Munteanu *et al.*, (2021)).

OBJECTIVES

The purpose of this study is to collect the entire plant of the following five medicinal plants namely *Amaranthus spinosus*, *Acalypha indica*, *Clitoria ternate*, *Amaranthus viridis*, and *Ocimum basilium*.

- To prepare ethanolic extracts of the plants.
- To investigate the phytochemical screening of the plants.
- To perform HPLC of the plants.
- To conduct GC-MS analyses of the plant extract.
- To test the antidiabetic activity from the ethanolic extract of *Amaranthus spinosus* and *Acalypha indica*.
- To test the antioxidant activity from the ethanolic extract of *Amaranthus spinosus* and *Acalypha indica*.
- To test the antimicrobial activity from the ethanolic extract of *Amaranthus spinosus* and *Acalypha indica*.

Review of Literature

2. REVIEW OF LITERATURE

Medicinal plant has started to consider an essential source in treating/stopping a numerous type of disease. Each Plant includes numerous of vital elements that may be utilized in clinical field, and may also be concerned in the Improvement of various types of drugs. A lot of undeveloped countries or even developed countries are using herbal medicine to preserve human wellbeing, Private fitness condition, and treating certain type of Diseases (Mohammed *et al.*, 2019).

In health system, the application of various plants as medicinal Plants has Attained key significance all around the world. The Medicinal plants are not only used for the remedy of sicknesses of people and vegetation however there are also important supply of substances that hold the good Fitness and situations of frame. For primary health care, approximately 2/3 of the population of Global relays on the use of medicinal plants for herbal medicine. The Higher adaptability and compatibility of Medicinal plants with the human frame which has much less side-effects and higher cultural acceptability which provides great Significance to Medicinal plants in medicinal field. It has been demonstrated that the maximum usage of drugs has plant extracts as an active ingredient. Many of them have bioactive issue which are extracted from plants as an energetic ingredient. Through Numerous researches, the medicinal activities of medicinal plant have been discovered from the study of traditional and therapeutic cures. The most common knowledge of man and his claims and believes have made loads of development in the sector of science and technology (Ur Rehman *et al.*, 2021).

The review of literature pertaining to the present study Phytochemical characterization of *Acalypha indica* and *Amaranthus spinosus*.

2.1 Phytochemical analysis.

2.2 Antidiabetic activity.

2.3 Anti-microbial activity.

2.4 Antioxidant activity.

2.5 *Acalypha indica*.

2.6 *Amaranthus spinosus*.

2.7 *clitoria ternate*.

2.8 *Ocimum basilium*.

2.9 *Amaranthus viridis*.

2.10 HPLC.

2.11 GCMS.

2.1 PHYTOCHEMICAL ANALYSIS

Phytochemicals (Greek: phyton = plant) are chemical substances obviously found in the plant attributing to positive or negative fitness effects. Medicinal plants are utilized for different diseases and ailments are the richest bio reservoirs of numerous phytochemicals. The medicinal houses of the plant life are decided through the phytochemical constituents. (Shaikh et al., 2020). Medicinal plants are currently in extensive significance view because of their unique attributes as an enormous supply of healing phytochemicals that could end in the development of novel drugs. The overwhelming majority of the phytochemicals from plant sources, for instance, flavonoids and phenolics are accounted for to possess positive effect on health and cancer prevention. Modern Mediterranean and DASH (Dietary Approaches to prevent Hypertension) integrate a wealthy diet of phytochemicals from fruit and vegetable sources. High content material of flavonoids and phenolics in medicinal plants has been related with their antioxidant exercises that play a task within the prevention of the event of age-related disease, especially cause by oxidative pressure. With respect to the gainful phytochemicals in medicinal plants, the research on medicinal plants especially is as significant because the exploration on ordinary medication. (Khattak *et al.*, 2020)

2.2 ANTIDIABETIC ACTIVITY

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin action, insulin secretion or both. Diabetic complications might be linked to alteration within the body's antioxidant defense system, increased oxidative stress, and dyslipidemia (Kifle *et al.*, 2022). There are two major types of diabetes, type 1 (insulin-dependent DM) and type 2 (non-insulin-dependent DM). Type I diabetes Occurs when the human immune system destroys pancreatic beta cells. Pancreatic β -cells are involved in insulin secretion. Insulin levels can be effectively controlled by continuous

injections at timely doses. Elevated post-prandial blood glucose is one of the earliest disease markers in predicting subsequent microvascular and macrovascular complications that can progress to fully symptomatic type 2 diabetes (T2DM). Widely recognized. Type 2 diabetes accounts for about 90% of diabetic cases and usually begins with as insulin resistance until the pancreas slowly loses its ability to produce insulin (Daimari *et al.*, 2019).

Diabetes mellitus can lead acute complications such as increased alcohol concentration and ketoacidosis. Its etiology is unclear but appears to be precipitated by Ketoacidosis-like factors, particularly those leading to Dehydration. Diabetes also leads to chronic complications such as kidney failure, retinopathy, cardiovascular disorders and foot ulcers. Patients with diabetes have an increased frequency of cardiovascular, cerebrovascular, peripheral, and atherosclerotic cardiovascular diseases. There is no effective cure for diabetes, but it can be controlled with oral antidiabetic drugs, insulin, and dietary modification. Medicinal plants may provide alternative management. Affordability, accessibility, cost, tolerability and compromised efficacy are some of the limitations of current conventional antidiabetic agents. African herbal medicines commonly used in the treatment of diabetes and provide an alternative therapy (Kifle *et al.*, 2022).

2.3 ANTIOXIDANT ACTIVITY

Oxidation is one amongst the foremost necessary processes that turn out free radicals in food, chemicals and even in living Systems. Free radicals have a crucial role within the processes of food spoilage, chemical materials degradation and also contribute to additional human disorders in people in general. Active oxygen and specifically, free radicals are considered to induce aerophilic injury in bio molecules. They're believed to cause depletion of system antioxidants, modification in organic phenomenon and induce abnormal proteins. Oxidants square measure currently thought-about to be concerned in a variety of aspects of burned injury and tissue repairs. Oxygen free radicals contribute to additional tissue injury in the events following skin injury and square measure proverbial to impair healing method. Antioxidants, on the opposite hand, significantly forestall tissue injury that stimulates wound healing method, there square measure accessible artificial Antioxidants like Butylated radical anisole (BHA), Butylated radical toluene's (BHT), tertiary butylated Hydroquinone and acid esters, however are suspected to cause or prompt negative health effects. Hence, Strong restrictions are placed on their application and there's a trend to substitute them with naturally Occurring antioxidants (Barku *et al.*, 2020).

It takes an active part in the physiology of quite common diseases, like diabetes, high pressure level, toxemia of pregnancy, induration of the arteries, acute renal disorder, Alzheimer's and Parkinson's. The cells, through metabolizing oxygen, produce reactive species of oxygen (ROS), that are undoubtedly harmful. Beneath traditional circumstances, the speed and amplitude of oxidizer formation is balanced by the speed of their removal. However, loss of balance between pro-oxidants and antioxidants leads to aerobic stress. High levels of ROS in Biological cells have an outsized impact on their functioning, resulting in deficient cell operation, aging, or diseases (Munteanu *et al.*, 2021).

There has been increasing interest within the therapeutic potentials of medicative plants as antioxidants in reducing such radical elicited tissue injury since it's the assumption that the inhibitor property is that the main causative factor to the therapeutic advantage of several medicative plants. As a result, several vegetables, fruits and lots of different plant species have already been exploited commercially either as inhibitor additives or as nutritional supplements or investigated within the rummage around for novel antioxidants. It's been mentioned that the inhibitor activity of plants can be because of their synthetic resin compounds like flavonoids which are a group of Polyphenolic compounds with famous properties that embody radical scavenging, inhibition of hydrolytic and Oxidative enzymes and anti-inflammatory action. Some evidences counsel that the biological actions of those Compounds are associated with their inhibitor activity. Medicinal Plants with inhibitor or free radical-scavenging activity so will play a big role in healing of wound and within the correction of the many human disorders (Barku *et al.*, 2013).

2.4 ANTIMICROBIAL ACTIVITY

Since their discovery, antibiotics are indispensable within the treatment of microbial infections (especially bacterial infections) and have helped in extending the typical life Expectancy. Globally, antibiotics have a significant useful impact, including the reduction of morbidity and deathrate caused by bacterial infections in humans, particularly in the developing countries with limited public health infrastructure. Antibiotics have however been overused and misused within the general population, leading to a rise in Antibiotic resistance rates among several microorganisms. Hence in recent years, antibiotic resistance has been recognized as a serious global health threat. Consequently, efforts are made to counteract the antibiotic resistance menace, while exploring alternative sources of antimicrobial agents, such as Medicinal plants (Owusu *et al.*, 2021).

Extracts isolated from medicinal plants are reported exhibit various biological activities like antimicrobial, anti-inflammatory, and antioxidant activities. The Antimicrobial compounds from medicinal plants may inhibit the expansion of bacteria, fungi, Viruses, and protozoa by different mechanisms than those of presently used antimicrobials and may have a big clinical value within the treatment of resistant microbial strains .Some of those active compounds show both intrinsic antibacterial activity and antibiotic Resistance-modifying activities and a few of them, while not effective as antibiotics by themselves, when combined with antibiotics, can help overcome antibiotic resistance in bacteria. Chemically complex compounds have great therapeutic potential as they have fewer side effects compared to synthetic drugs and also low chances of developing resistance. Bacteria may develop resistance to medicinal plants treatment if just one Active ingredient with a selected target is involved, a condition almost like an antibiotic (Vaou *et al.*, 2021).

2.5 ACALYPHA INDICA

Acalypha indica may be a traditional medicinal plant, Well-known by older generations in many countries, particularly in Africa and Asia. It grows well in most parts of west and south of Africa Northeast, including Somalia, Ethiopia and other Regions. The plant also can be found in most wet, Tropical and temperate countries in Asia, Europe and both North and South American regions. It grows as a weed in bushes, backyards, alongside roads and other places like home and crop premises. Many international manuscripts on *Acalypha indica* were published from Indian region because this plant features a close connection with Ayurveda, Siddha and Unani Medicinal practices executed by older Indian (Chekuri *et al.*,2020).

Figure.1 *Acalypha indica*



The root of this plant is treating as a stimulant, harsh and difficult purgative. The leaves Extract of the plant reduces mutagenicity in *E. coli*. The leaves square measure laxative whereas a moisturizer for the diagnosis of facial disfunction. The leaves of the plant are often employed in the diagnosing of jaundice, Piles and conjointly outwardly skin eruptions, ring worms. Leaf be use like medication an equivalent as associate in nursing antipyretic, it be a tonic used for protozoal infection conjointly fever. It is to apply within anthrax, blood disease, at the side of antidysentery, anti-diabetic and Hansen's disease supposed for the elimination of abdominal obstacle and they also are useful within the treatment of respiratory disease. The leaves of the plant contain too be report toward seize the prevalence of contraceptive action. The extract of the origin is to be able to condense the blood glucose stage via half-hour. Leaves possess anti-periodic with laxative properties and also the leaf extract are often applied to insect bites. Root is of use throughout agitation, heart condition and retain excretions. The 50% ethyl alcohol extracts of pods reveal the anti-fertility activity in feminine unusual person rats. Cough are often simply Cured by creating burnt pods with very little salt at the side of honey 3-4 times. Roots square measure worn throughout the cure of Antipyretic, diabetes, useful in coffer criticism, gorge plight, eyes defect at the side of the diagnosing of viscus Disorder, stiff state of affairs, ulcer, wound, boils what is more numerous crust malady. The extract of the foundation bark among alcohol be capable of exist use meant for backward fever (Anoop *et al.*, 2018).

Table 1. Taxonomic Classification of *Acalypha indica*

Kingdom	Plantae
Unranked	Angiosperms
Unranked	Eudicots
Order	Malpighias
Family	Euphorbiaceous
Genus	<i>Acalypha</i>
Species	<i>A. indica</i>

The fresh *Acalypha indica* plant contains a wide variety of nutrients like carbohydrates, proteins, vitamins, and lipids. They set to prepare its documentation with elaborated observation of essential and non-essential serious metals content as a district of the flavored standardization preparation. *Acalypha indica* has high iron content, followed by copper, nickel zinc, chromium which are useful for patients with mineral deficiency problems (Mondal *et al.*, 2021).

2.6 AMARANTHUS SPINOSIS

There's an excellent interest in *Amaranthus*, a third-millennium tropical food plant. The *Amaranthus* (L.) belongs to the Amaranthaceae, Caryophyllales, and includes dicotyledonous annual plants and consists of roughly 70 species, which may be classified into grain and vegetable amaranths. Most of the amaranth species are native to America and only 15 species originate from Asia, Africa, Australia, and Europe. The plant is adapted to grow under different agro-climatic conditions and reported to be heat, drought, and pest tolerant. *Amaranthus spp.* are often found in subtropical, tropical, and temperate climate zones round the world. The crop demonstrated its capacity to cultivate both as a grain and leafy in those areas and seasons where other crops are unable to flourish (Ahmad, *et al.*, 2018).

Figure.2 *Amaranthus spinosus*



<https://images.app.goo.gl/Rpf11stVqmwbokek6>

This plant is employed as food in several tropical countries, particularly social group and mass people. This plant has been reportable to possess some pharmacological properties. It's utilized in ancient medicines are terribly effective, have lower facet effects, area unit

accessible and affordable with low value. It's used as antipyretic, laxative, stomachic, medicinal drug traditionally. The seed of it's used as a poultice for broken bones and also the root of its accustomed treat Gonorrhoea. It's been reportable in trendy scientific analysis that it's anti-depressant activity, anti-diabetic properties, medicament activity, immunomodulatory activity, medication property, anti-hyperlipidemic, spermatogenic activity, anti-malarial and result on hematology (AL Mamun *et al.*, 2021).

In traditional Indian medicine, the tribes of Kerala used the thorny leaves of *A.spinous* to cure jaundice, Stomach problems, and rheumatic pain and to prevent malaria. The tribes of Sikkim used the leaves of the tree as a diuretic and in anemia as well as in stomach problems, especially in indigestion and peptic ulcers. The leaves are also used to treat hemorrhoids and leprosy. They believe that the poultice of leaves is useful in burns and abscesses to drain pus. The roots are used by them in eczema, gonorrhoea and menstruation. In Manipur, cooked *A.spinous* is eaten by patients with diabetes and high blood pressure (Basu *et al.*, 2019).

Table.2 Taxonomic classification *A. spinous*

Kingdom	Plantae
Subkingdom	Viridiaeplantae
Phylum	Magnoliophyte
Subphylum	Euphyllophytina
Division	Magnoliophyte
Class	Magnoliopsida
Subclass	Caryophyllidae
Order	Caryophyllales
Suborder	Chenopodiineae
Family	Amaranthaceae
Genus	<i>Amaranthus</i>
Species	<i>Spinous</i>

Currently, the pharmacological actions of *A. spinous* have been studied by many researchers. The plant was found to have several pharmacological activities including anti-

hyperlipidemic, anti-diabetic, antitumor, immunomodulatory properties. The pharmacological activities of *A. spinous* are known in the literature, Study Is needed to explore other pharmacological properties, if any, of the different parts of the factory. Because it contains a large amount of nutrients, *A. spinous* has excellent nutritional value and used as a green vegetable. Effect of environment and cultural practices on productivity and its nutritional profile should be thoroughly studied. Also, more work crop treatment is necessary because treatment affects the concentration and nutrients bioavailable. There is little scientific work regarding the nutritional properties of *A. spinous* studies in this direction are needed (Basu *et al.*, 2019).

2.7 CLITORIA TERNATEA

Clitoria ternatea L. (CT), Fabaceae could be an alright familiar Ayurvedic Medicine used for various ailments. Indian systems of drugs significantly In writing, the roots, seeds and leaves of CT have long been wide Used as a brain tonic and is believed to market memory and intel-Ligence genus *Clitoria ternatea* has witnessed a medical specialty and Toxicological analysis of those claims inform to some vital Therapeutic advantages of this ancient drug that are highlighted In this review. CT has been used as Associate in nursing ingredient in ‘MedhyaRasayana’ a rejuvenating direction used for treatment of medicine disorders and regarded to strengthen a person’s intellect. In ancient systems of drugs transmit-Ted orally or in writing (esp. Ayurveda) varied therapeutic effects have been attributed to roots, leaves and seeds of CT. A number of bioactive secondary metabolites and medical specialty activities of the plant is reportable (Mukherjee *et al.*, 2008).

Figure.3 *Clitoria ternatea*



<https://images.app.goo.gl/9bdTFK5kvryvDo8n9>

Table. 3 Taxonomic classification of *Clitoria ternatea*

Kingdom	Plantae
Subkingdom	Viridaeplanta
Division	Tracheophyta
Subdivision	Spermatophytina
Superorder	Rosanae
Family	Fabaceae
Genus	Clitoria

The plant showed many pharmacological effects including antioxidant, hypolipidemic, anticancer, anti-inflammatory, analgesic, antipyretic, antidiabetic, CNS, antimicrobial, gastro-intestinal antiparasitic, Insecticidal and many other pharmacological effects. This Review will highlight the chemical constituents and Pharmacological effects of *Clitoria ternatea* (Snafi *et al.*, 2016).

2.8 OCIMUM BASILICUM

Ocimum basilicum, commonly known as basil, is naturally distributed in the East Anatolia region in Turkey. The leaves of these plants are used in traditional cuisine as spices. Basil, which is a popular culinary Herb, and its essential oils have been used extensively for many years in flavoring of meats and sausages. Basil Oil has also found a wide application in perfumery, as well as in dental and oral products. In addition, because public nowadays prefers natural food additives, naturally derived antimicrobial agents such as basil, are becoming more important in antimicrobial packaging as they present a perceived lower risk to consumers (Kaya *et al.*,2016).

Figure .4 *Ocimum basilium*



<https://images.app.goo.gl/Gs8KTM7o98ZDsdBc6>

2.9 AMARANTHUS VIRIDIS

Amaranth viridis. Annual herb, upright, 10 ~ 75 (100) cm handle; slender, branched, angular, bare Leaves; Glabrous, long stems, 1 cm, deltoid thin layer The flowers are diamond-shaped and oval in size, measuring 2-7 x 1.5-5.5 cm. Green, axillary or terminal, often panicles, 2.5 tonnes Length 12 cm, width 25 mm. Bracts and preventatives become oval Make lanceolate-egg-shaped, whitish, pale or reddish awns, Prophylactic drug shorter than perianth (1mm); perianth, Male flower, elongated oval, pointed, dented, 1.5 mm, The female flower grows long and thin and spatula, Eventually 1.25 to 1.75 mm, midribs are green and thick Moreover. Capitals 2 to 3, short, upright. Capsule sub sphere, 1.25 to 1.5 mm. Seed, 1-1.25 mm, round, compressed, dark brown to black, mesh-like. Blooming summer autumn. Whole decoction plant is used to stop dysentery and inflammation. The plant possess anti-diabetic, Antihyperlipidemic, antioxidants, antiproliferative and antifungal lectin Plants are emollients and anthelmintics (Duke). Root juice is used for the treatment of inflammation during urination (Reyad *et al.*, 2015).

Figure .5 *Amaranthus viridis*



<https://images.app.goo.gl/7nFnXWPdtBkanh3v6>

2.10 HPLC

HPLC is a versatile, robust and widely used technology for separating natural products. HPLC is a chromatography technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify, and purify components. Mixture. Among the various analytical methods, this method is now becoming the main choice for fingerprint research for quality control of herbs. Natural products are often isolated after evaluating relatively coarse extracts with a biological assay to fully characterize their properties. The resolution of HPLC is ideal for rapid processing of such multi-Component samples, both analytical and preparative scales. Several authors describe the use of HPLC for the characterization and quantification of secondary metabolites, primarily phenolic compounds, steroids, flavonoids, and alkaloids in plant extracts (Bologn *et al.*, 2014).

2.11 GCMS

Chromatography is a term used to describe separation techniques the mobile phase carrying the mixture moves Contact with a stationary phase that selectively absorbs. That also affects it with its basic role as an analytical technique for quality control and standardization of Phyto therapeutics. The principle of standardized gas chromatography in phytotherapy is adsorption and distribution. Gas within a family of chromatographic methods Chromatography (GC) is one of the most widely used techniques. It first described in 1952 by James and Martin, One of the most important tools for separating volatiles link. Gas chromatography mass spectrometry, dash Very compatible technology, most systems with

general methods for quantification Identification purpose. Unknown organic compound complex mixtures, interpretation and also, by comparing the spectrum with the reference spectrum Gas Chromatography-Mass Spectrometry (GC.MS) has established a solid position as an important technique in recent years. Platform for profiling secondary metabolites of both plants and non-vegetable seeds (Sermakkani *et al.*, 2011).

Gas chromatography has a very wide range of uses. However, its first and most important application of Is the separation and analysis of mixtures of several components of Such as essential oils, Hydrocarbons and solvents. Using the flame ionization detector and the electron capture detector (very sensitive), gas chromatography can quantify substances present at very low concentrations. This means that the second most important area of the application is in pollutant investigation, Forensic work, and general trace analysis. Due to its simplicity, Sensitivity, and effectiveness in separating the components of the mixture, gas Chromatography is one of the most important tools in chemistry. Widely used for quantitative and qualitative analysis of mixtures, purification of compounds, and determination of thermochemical constants such as solution and evaporation heating, vapor pressure, activity coefficient. (Padma *et al.*, 2019).

Materials and Methods

3. METHODS AND MATERIALS

The materials and methods pertaining to the study of phytochemical analysis and characterization of *Amaranthus spinosus* and *Acalypha indica* is discussed under the following headings.

Global estimates assume that more than three-quarters of the world population cannot afford allopathic products, and thus have to rely upon the use of traditional medicines, most of which are derived from Plants. Scientific research has identified only about 1% of the Known medicinal plant species (folk, ethnic, auxiliary medicine) is recognized as having therapeutic value of for humans. The World Health Organization encourages, promotes and facilitates the effective use of herbal medicines for Health programs in developing countries. In addition, the demand for herbal medicine has reached a new high level in west as well (Dhar *et al.*, 2002)

3.1 IDENTIFICATION AND AUTHENTICATION

Whole plant of fresh *Amaranthus spinosus*, *Acalypha indica*, *Ocimum basilium*, *Cilitoria ternate*, *Amaranthus viridis* were collected from several locations. These samples are free from disease. The plants were identified and authenticated by Botanical Survey of India Coimbatore.

3.2 PLANT EXTRACT PREPARATION FOR PHYTOCHEMICAL SCREENING

Plants are collected from several locations. The plants were properly washed 2-3 times with running tap water and once with sterile distilled water before being air dried under aseptic conditions. Using a mortar and pestle, different parts of the plant were chopped into small pieces and pulverised. A conical flask containing ten grams of five plant samples was filled with 100ml of ethanol and left at room temperature for around 24 hours in an open orbital shaker. The extract was then filtered using Whatman NO.1 filter paper before placed in a air tight container and kept refrigerated for later use.

3.3 PHYTOCHEMICAL SCREENING IN QUALITATIVE CONDITIONS

The presence of phytochemicals in the ethanolic extract of the five plants were determined using the Khandelwal method (2002).

3.3.1 Test for alkaloids (Mayer's test)

To the 1ml of extract, 1 ml of Mayer's reagent (Potassium iodide Solution) was added. Formation of whitish yellow or cream coloured Precipitate indicates the presence of alkaloids.

3.3.2 Test for steroids (Liebermann Burchard test)

To the 1ml of extract, 2ml of chloroform and 2ml of acetic acid and 2ml of concentrated sulphuric acid were added. Formation of violet to blue or green colour indicates the presence of steroids.

3.3.3 Test for terpenoids

To the 1 ml of extract, 2ml of acetic acid and 1ml of sulphuric acid were added. Formation of blue green ring indicates the presence of Terpenoids.

3.3.4 Test for flavonoids

To the 1 ml of extract, 1ml of concentrated sulphuric acid were added an orange colouration indicates the presence of flavonoids.

3.3.5 Test for saponins (Froth test)

To the 1 ml of extract, 5 ml of distilled water was added and Shaked Vigorously. Formation of froth indicates the presence of Saponins.

3.3.6 Test for phenols (Ferric Chloride test)

To the 1ml of extract, add 5-6 drops of 3% Ferric chloride solution and observe for the formation of deep blue color.

3.3.7 Test for tannins (Ferric chloride test)

To the 1ml of extract, 1ml of 1% ferric chloride solution was added formation of blue, black or brownish green colour indicates the presence of tannins.

3.3.8 Test for cardiac glycosides (Keller killiani test)

To the 1ml of extract, add 5ml of distilled water and evaporate it to dryness. Then to the Sample add 2ml of glacial acetic acid containing trace amount of ferric chloride solution. Then add 1ml of concentrated Sulphuric acid to the sides of the tube. Formation of brown ring underlayer with blue colour indicates presence of cardiac glycosides.

3.3.9 Test for Coumarins

Take 1ml of extract and add 1.5ml of 10% NaOH, then observe for the formation of yellow colour which indicates the presence of coumarins.

3.3.10 Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or Colouration).

3.3.11 Test for phlobatannins (HCl test)

To 2ml of extract, added dilute HCl and observed for red precipitate that indicates the presence of phlobatannins.

3.4 PLANT EXTRACT PREPARATIONS FOR ASSAYS

The preparation of plant extracts required two processes.

3.4.1 Whole plant powder preparation

The chosen plants were collected and shade dried for weeks at room temperature. The dried flowers were roughly mashed by hand before being blended into a powder with an electric blender. Weighed powdered material was prepared in an airtight container.

3.4.2 Ethanolic extract preparation

10g of powdered plants were combined with roughly 100ml of ethanol and maintained for about 5 days at room temperature in an open orbital shaker. A whatman No.1 filter paper was used to filter the mixture, and the extract was collected in a 500ml beaker. The mixture was then suspended in a Rotary evaporator, and the following evaporation, a colloidal gel was formed, which was then poured in an eppendorf tube and kept in the refridgerator for later use.

3.5 Characterization of *Amaranthus spinosus* and *Acalypha indica*

3.5.1 High Performance Liquid Chromatography (HPLC)

The protein was separated from the solvent extract using ion exchange chromatography (IEC). The extract was put onto a CM-Sepharose CL-6B column that had been equilibrated with PB and eluted with a linear NaCl gradient. Following that, HPLC was performed using an HP Ti series 1050 liquid chromatograph with a photodiode array detector (DAD, HP series 1050). Reverse phase was applied to the portion from the IEC. Using a C18 column equilibrated with trifluoroacetate in water, HPLC analysis was performed (0.1 percent TFA). The absorbance was measured at 214 nm after an elution gradient of acetonitrile (0.1 percent TFA) was applied. Protein electrophoresis was used to examine the produced extract as well as the crude extract.

3.5.2 SPECTRAL ANALYSIS WITH GC-MS

The components in the extract can be identified qualitatively and quantitatively using gas chromatography-mass spectroscopy (GC-MS) analysis. The ethanolic extracts of *Amaranthus spinosus* and *Acalypha indica* were subjected to GC-MS analysis to determine the active components. The concentration of the chemicals in the extract is exactly proportional to the area under the peak. The equipment was utilized was a Thermo Scientific Trace Ultra version 5.0 gas chromatography (GC) system with Thermo scientific DSQ2 mass spectrophotometer (MS). A DB-35 MS capillary standard non polar column (30m,0.25mm) was added to the GC. The oven was preheated to 75 -260 degree with a 10-minute hold (37.51 min programme). The sample (IL) was injected using injector A(SSL), and the carrier was helium gas at a rate of 1mL/min. MS transfer line temperature was set to 290 degree C, with a 230 degree C ion source temperature (electric ionization). The material was examined at a 70 Ev electron energy level (vacuum pressure-2.21e-0.5 Torr).

3.6 IN VITRO ALPHA AMYLASE INHIBITION STUDY (Subramanian et al., 2008)

Procedure

Alpha -amylase method, the enzyme solution was prepared by dissolving alpha -amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5mg/ml. 1ml of the extract

of various concentrations (250,500,750,1000µg/ml) and 1ml of enzyme solutions were mixed together and incubated at 25 c for 10minutes.

After incubation, 1ml of starch (0.5%) solution was added to mixture and further incubated at 25 c for 10 minutes. The reaction was then stopped by adding 2ml of dinitro salicylic acid (DNS, color reagent), heating the reaction mixture in a boiling water bath (5minutes). After cooling, the absorbance was measured calorimetrically at 565nm.The inhibition percentage was calculated using the given formula,

$$\text{Percent inhibition} = \frac{\text{Abs Control} - \text{Abs sample} \times 100}{\text{Abs control}}$$

Where Abs control is the absorbance of the control reaction (containing all reagents expect the test sample) and Abs sample is the absorbance of the test sample.

3.7 NON-ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN METHOD (Daksha et al., 2021)

Procedure

Glucose (2 percent), haemoglobin (0.06 percent) and gentamycin (0.2percent) solutions were prepared in phosphate buffer 0.01 M, PH 7.4. 1 ml each of the above solutions was mixed and 1ml of the ethanol extract of varying concentrations was added to it, respectively.

The reaction mixture was incubated in dark at room temperature for 72hrs and then the degree of glycosylation of haemoglobin was measured calorimetrically at 520nm. Metformin was used as a standard drug for the assay and percentage inhibition was calculated using the formula,

$$\text{Percent inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance control} \times 100}{\text{Absorbance sample}}$$

Where Abs control is the absorbance of the control reaction (containing all reagents expect the test sample) and Abs sample is the absorbance of the test sample.

Evaluation of free radical scavenging of plant extracts

The free radical quenching ability of the ethanol extract of *Amaranthus spinosus* and *Acalypha indica* against a battery of free radicals namely DPPH, ABTS, H₂O₂ and hydroxyl radicals and their ability to inhibit nitric oxide and superoxide generation along with the chelating and reducing activity were tested. The procedure for each of the above assays are explained as follows

3.8 DPPH radical scavenging assay

The scavenging ability of the *Amaranthus spinosus* and *Acalypha indica* towards the relatively stable free radical DPPH was measured by the method of Mensor et al. (2001).

Principle

DPPH is a stable free radical containing an odd electron in its structure, which is responsible for the absorbance at 515-517nm. In the presence of antioxidants, DPPH radical can accept an electron donated by antioxidants thereby converting it to diphenyl picryl hydrazine. The degree of discoloration from deep violet to light yellow was measured at 515nm, which indicates the scavenging effect of the extracts.

Reagents

1. DPPH – (2, 2-diphenyl-2-picryl hydroxyl hydrate) (0.3mM in methanol)
2. Methanol

Procedure

The combination of the plant extracts of different concentrations were added with 0.5mL of 0.3mM DPPH and made up to 1.0mL with methanol. The mixture was allowed to react at room temperature for 30 minutes. DPPH solution with methanol was used as positive control and methanol alone served as blank. After 30 minutes of incubation, the discoloration from deep violet to yellow color was measured at 515nm.

The per cent scavenging activity was calculated by the following formula

$$\% \text{ Scavenging activity} = (A(\text{Control}) - A(\text{Sample})) / (A(\text{Control})) \times 100$$

3.9 ABTS radical scavenging assay

The antioxidant effect of the ethanol extract of *Amaranthus spinosus* and *Acalypha indica* were determined using ABTS radical cation decolorization assay according to the method of Shirwaikar et al. (2006).

Reagents

1. ABTS – 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
2. Ammonium persulfate
3. Ethanol
4. The ABTS radical cations (ABTS⁺) were produced by reacting ABTS (7mM) with aqueous solution of ammonium persulfate (2.45mM).

Procedure

The mixture was kept in dark for 12 – 16 hours at room temperature before use. The combination of all the plant extracts was added to 0.3mL of ABTS solution and the final volume was made up to 1.0mL with ethanol. The absorbance (A) was read at 745nm and the percentage inhibition was calculated as follows

$$\% \text{ Scavenging activity} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})}$$

3.10 DISK DIFFUSION ASSAY

The widely accepted method for the evaluation of antimicrobial potential is the disc diffusion method (millat et al., 2019). In the following method, a concentration gradient was created by diffusing an antibiotic from a reliable source through the nutrient agar. The test bacteria were seeded on nutrient agar medium at dried, sterilized filter paper discs (6 mm diameter, HI-Media, China) containing the known amounts of test samples (20µg/disc). Standard antibiotics of ciprofloxacin (5µg/disc) and blank discs were used as a positive and negative control. These plates were reserved for 24 hours at a low temperature (4°C) for the maximum diffusion of the test materials to the surrounding media. To allow optimum growth of the organisms, the plates were then incubated for 24 hours at 37°C. Microbial growth in plates was inhibited by the test materials containing antimicrobial properties and thereby a

distinct, clear zone was yielded defined as a zone of inhibition. By measuring the zone of inhibition, the antimicrobial activity of the test sample was determined.

3.11 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory concentration (MIC) of *Amaranthus spinosus* and *Acalypha indica* extract was determined by the broth dilution method against bacterial culture.

MICs are the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. 100µL of nutrients broth for bacteria and SDB for fungal was distributed from the 1st to the 6th well of a 96-well polypropylene microtiter plate. The dilutions of leaves formulation were added to the 2nd test well of each microtiter line, and then 100µL of scalar dilution was transferred from the 2nd to the 6th well. The 1st well was considered as growth control because no leaf extract was added. We then added 100µL of a bacterial suspension to each well. Plates were incubated at 37°C for 18 h. Then the plates were read at 600nm for growth using a Varioskan plate reader.

Results and Discussion

4. RESULT AND DISSCUSSION

Plant being the main source of various medicines, plays an important and significant role in the health of both plants and humans. A variety of herbs and plants are known for curative as well as therapeutic aids. In the medical system, the use of various plants as medicinal plants has become central worldwide, with in both plants and humans. Medicinal plant is not only used to treat diseases of humans and plants, but is also a source of important materials to keep the body healthy and in good condition. In primary health care, about two-thirds of the population worldwide depends on the medicinal plants. Better adaptability and compatibility of medicinal plants with human body i.e. Have few side effects, and are highly culturally receptive, which gives medicinal plant great importance in the medical field. It has been proven in that most of the medicines used have plant extract as the main ingredient. Many of them have bioactive ingredients that are extracted as active ingredients from plants. Through different studies, medical activities of medicinal plants have been discovered from traditional and therapeutic treatment studies (rehman *et al.*, 2021).

4.1 The present study was taken to investigate the phytochemical analysis of *amaranthus viridis*, *amaranthus spinosus*, *clitoria ternata*, *acalypha indica* and *ocimum basilium*.

Table.4 Phytochemical consituent for ethanolic extract of *amaranthus viridis*, *amaranthus spinosus*, *clitoria ternata*, *acalypha indica* and *ocimum basilium*.

Tests	<i>Amaranthus viridis</i>	<i>Amaranthus spinosus</i>	<i>Clitoria ternata</i>	<i>Acalypha indica</i>	<i>Ocimum basilium</i>
Alkaloids	++	+	-	++	+
Saponins	++	++	++	++	+
Tannins	++	++	+	+	+
Steroids	+	-	++	++	++
Flavoids	+	++	+	-	+
Terpenoids	+	-	+	++	++

Phlobitannins	-	++	++	+	-
Coumarins	++	-	+	+	++
Cycloglycosides	+	++	+	++	+
Phenols	+	++	+	++	+
Quione	+	-	-	+	-

+ =presence of the phytochemical constituent, - = absent

Based up on the highest phytochemical constituents, I have selected two plants namely *amaranthus spinosus* and *acalypha indica*. Phytochemical analysis of five medicinal plants were tested. The results revealed the presence of medically active compounds such as alkaloids, saponins, tannins, steroids, flavonoids, terpenoids, phlobitannins, coumarins, cycloglycosides, phenols and quinone.

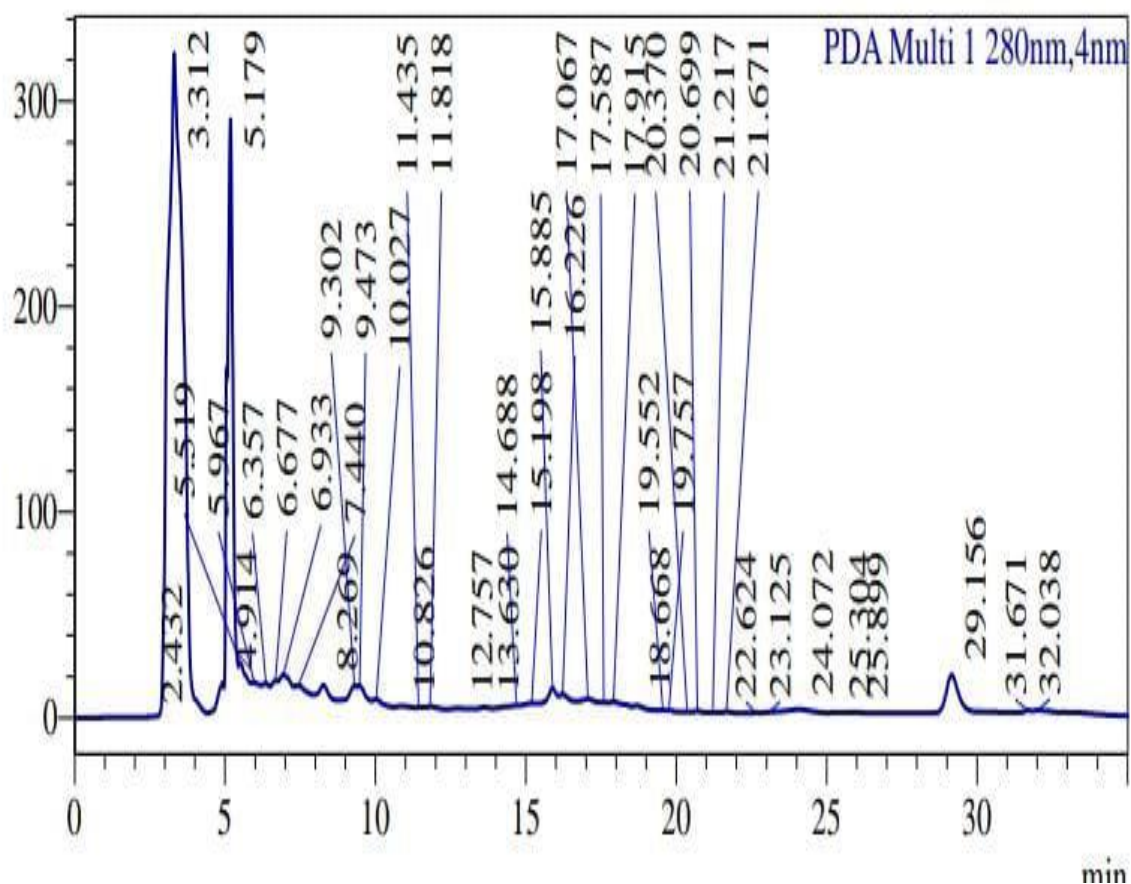
(yadav *et al.*,2011) phytochemical analysis performed on aqueous extract of *bryophyllum pinnatum* revealed the presence of components known to have medicinal as well as physiological activity. Analysis of plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They include organisms such as anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protection, and endothelial function enhancement, as well as angiogenesis and inhibition of cell proliferation activity.

(wadood *et al.*,2013) *punica granatum* and *fagonia cretica* selected as sources of secondary metabolites. Alkaloids, flavonoids, terpenoids, tannins, and reducing sugars. Medicinal plants play an important role in preventing various diseases. Antidiuretic, anti-inflammatory, anti-analgesic, anti- the anti-cancer, anti-virus, anti-malaria, antibacterial and anti-fungal activity of medicinal plants is due to the presence of the above secondary metabolites. Medicinal plants are used to discover and test secondary plant substances that are very useful in the production of new drugs.

4.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

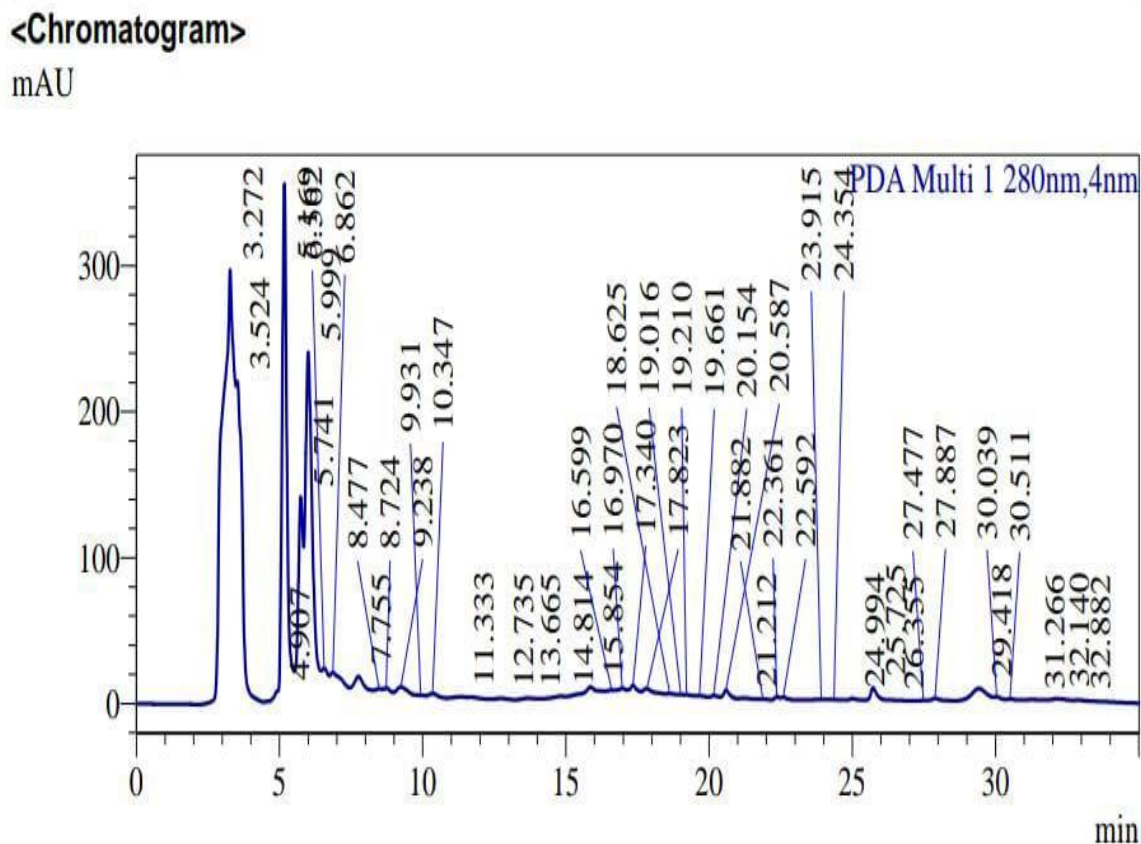
High performance liquid chromatography (HPLC) is today the leading technique for chemical analysis and related applications, with an ability to separate, analyze, and/or purify virtually any sample.

Figure.6 HPLC analysis of *Amaranthus spinosus*



In HPLC, separated active compound present in ethanolic extract of *Amaranthus spinosus*. The amounts of active compound present in root, leaf and stem extracts were present respectively, number of peaks and retention time indicates the number of plant compound present in it. In *Amaranthus spinosus* 41 peaks were present and in those 2 major peaks indicates the number of phytoconstituent were present in it. According to qualitative result it contains alkaloids, flavonoids, saponins, tannins, steroids, cycloglycosides and phenols.

Figure.7 HPLC analysis of *acalypha indica*



In HPLC, separated active compound present in ethanolic extract of *acalypha indica*. The amounts of active compound present in the root, leaf and stem extracts, the number of peaks and retention time indicates the number of plant compound present in it. In *acalypha indica* 46 peaks were present and in those 5 major peaks indicates the number of phytoconstituent were present in it. According to qualitative result it contains alkaloids, flavonoids, saponin, tannin, steroids, terpenoids, coumarins, cycloglycosides and phenols.

4.3 IN VITRO A AMYLASE INHIBITION STUDY

Amylase is one of the most important industrial enzymes with a variety of uses, including the conversion of starch to α -amylase are starch degrading enzymes that catalyzes the hydrolysis of the internal α -1,4-o-glycosidic bonds of polysaccharides while retaining the α -anomeric arrangement of low molecular weight products such as glucose, maltose, and maltotriose units (mobini *et al.*,2012).

Figure.8 In Vitro α Amylase inhibition assay of ethanolic extract of *Amaranthus spinosus*

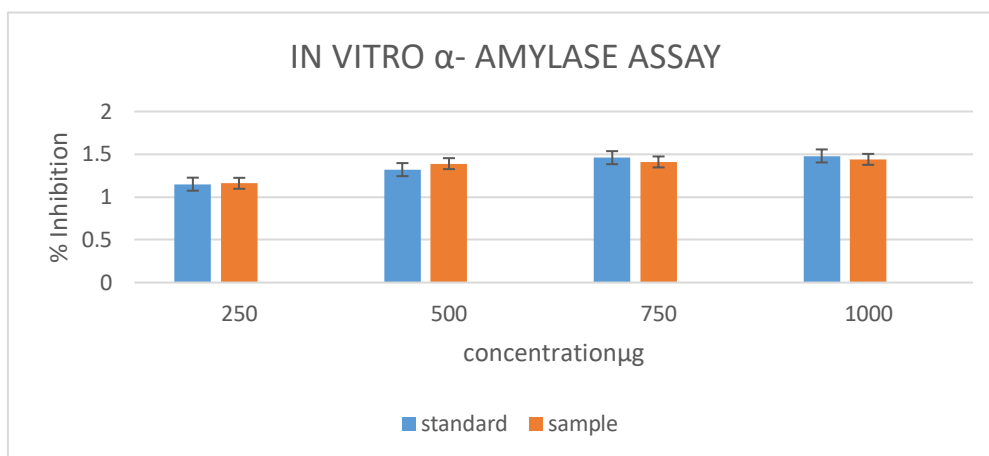
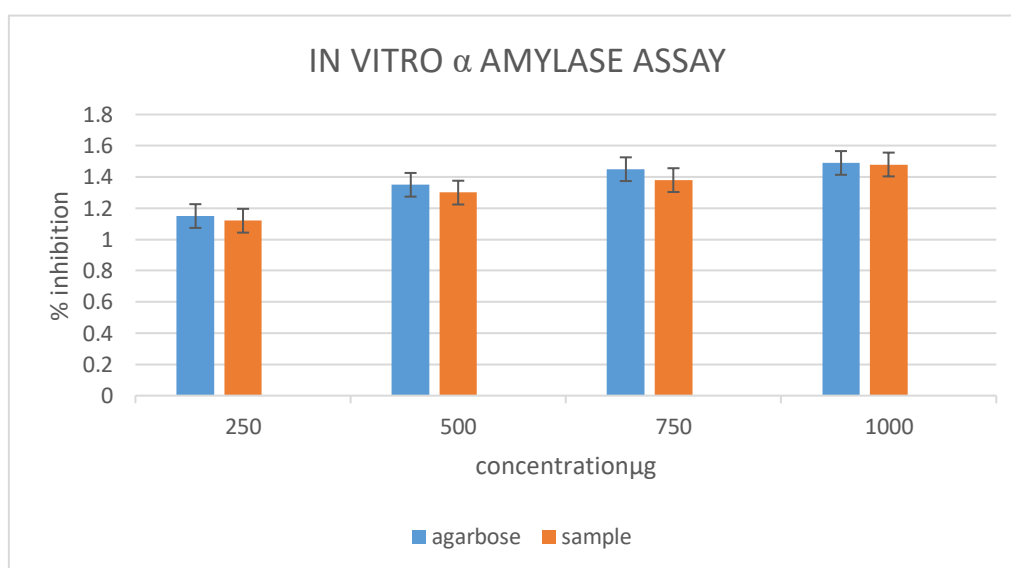


Figure.9 In vitro α amylase inhibition assay for ethanolic extract of *Acalypha indica*



The α - amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates and potentially useful in control of obesity and diabetes. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase of starch. Out of two tested spices samples *Acalypha indica* exhibited much α -amylase in vitro inhibitory activity whereas *Amaranthus spinosus* exhibited low activity.

(Dastard *et al.*,2015) The suppression activities of the extracts obtained from *t. Polium*, *t. Orientalea*, *t. Oliverianum* are investigated on the alpha amylase enzyme. In between three species of plants studied were found to be inhibitory concentration-dependent effects on amylase activity. Strongest activity (at concentration: 25 mg / ml) demonstrated by hac extract of polium. (97.77%) *t. Orientale* extract showed weaker activity. Maybe because it's too expensive. The concentration of the extract is certainly confirmed derived from a real substance to change the enzyme.

4.4 NON-ENZYMATIC GLYCOSYLATION OF HEMOGLOBIN METHOD

Non-enzymatic glycation products are a group of complexes, heterogeneous compounds that accumulate in the plasma and tissues of diabetes and renal failure. There is evidence that these compounds may be involved in the etiology of chronic complications associated with diabetes and renal failure. Therefore, there are two important points in measuring non-enzymatic glycation products. First, measurements of early glycation products can be used to estimate a subject's glucose exposure levels and previous metabolic controls. On the other hand, measurement of intermediate and late products of saccharification reaction is a valuable tool for verifying the relationship between saccharification products and tissue modification (Lapolla, *et al.*,2005).

Figure.10 Non enzymatic glycosylation of Haemoglobin assay for *Amaranthu spinosus*

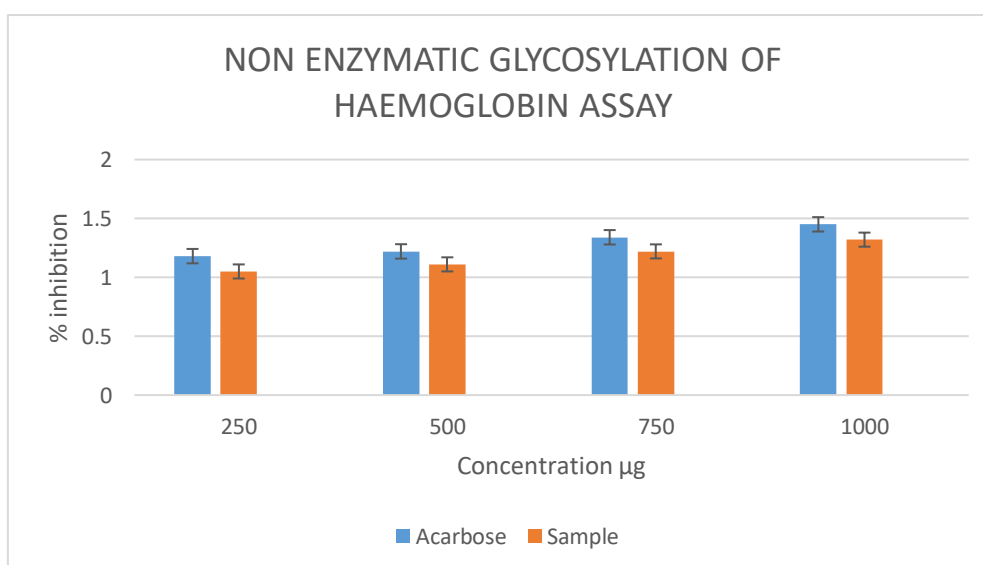
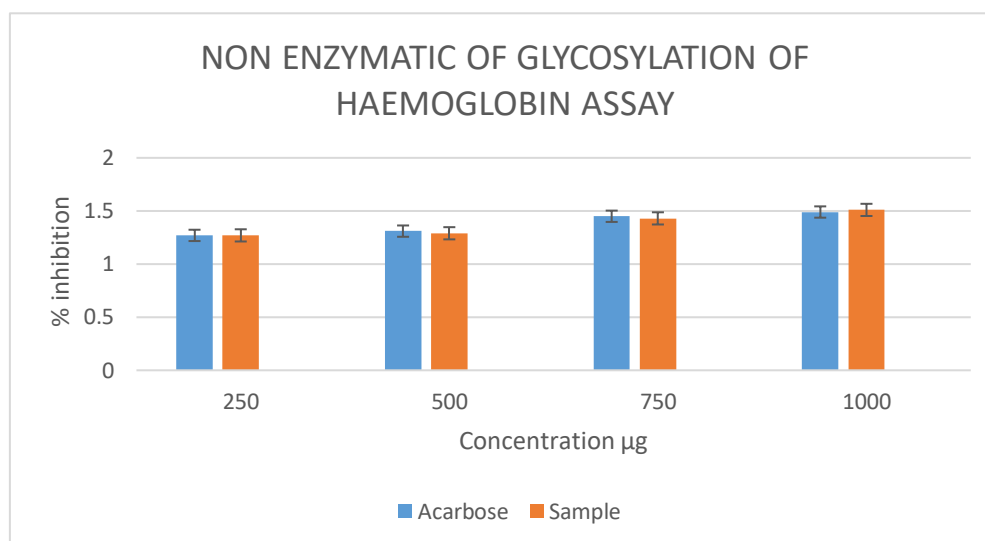


Figure.11 Non enzymatic glycosylation of Haemoglobin assay for *Acalypha indica*



Percentage inhibition of Non enzymatic of Glycosylation of Haemoglobin was measured at various concentrations of ethonolic extract of *Amaranthus spinosus* and *Acalypha indica*. *Acalypha indica* shows strongest activity where *Amaranthus spinosus* shows weaker activity.

(Chauhan.*et al.*,2019) The results of hemoglobin in vitro glycosylation and H₂O₂ free radical scavenger activity assay clearly show that the polyherbal formulation has significant anti-diabetic and antioxidant activity. It has been observed that various extracts of polyherbal formulations inhibit the formation of saccharified end products. Further identification and characterization of bioactive compounds involved in such activities and further investigation in vivo are needed. Free radicals play an important role in the development of degenerative diseases such as diabetes, so supplementation with antioxidants can overcome diabetic complications. This polyherbal formulation helps to treat diabetic patients to maintain their blood glucose levels.

4.5 DPPH RADICAL SCAVENGING ASSAY

Antioxidants continue to be considered an important dietary supplement of many health benefits. 1,1 -diphenyl -2 -pic ryl-hydrazyl (DPPH) is a stable, unpaired free radical valence electron on the atom of the nitrogen bridge DPPH radical removal is the basis of the popular DPPH antioxidant assay. Oxygen, light, PH of the reaction mixture has the ability to affect the absorbance of DPPH (sharma *et al.*, 2009).

Figure.12 DPPH Scavenging Assay for ethanolic extract of *Amaranthus spinosus*

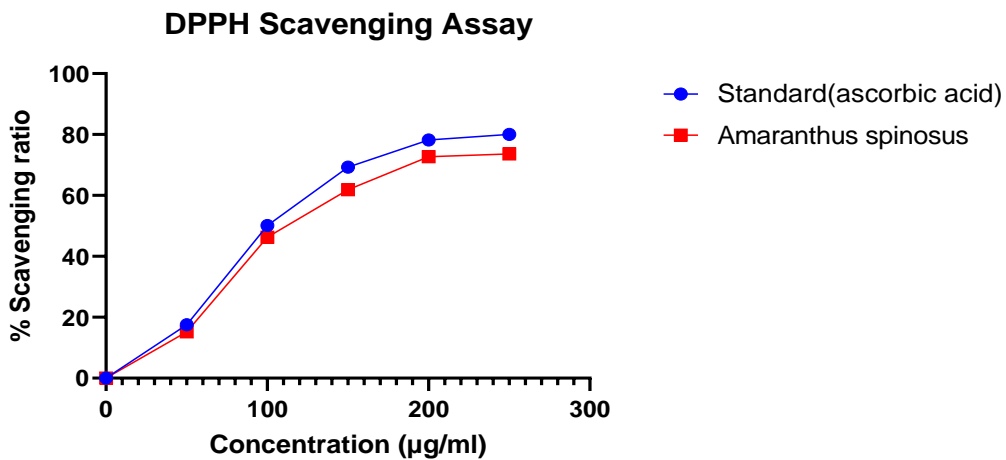
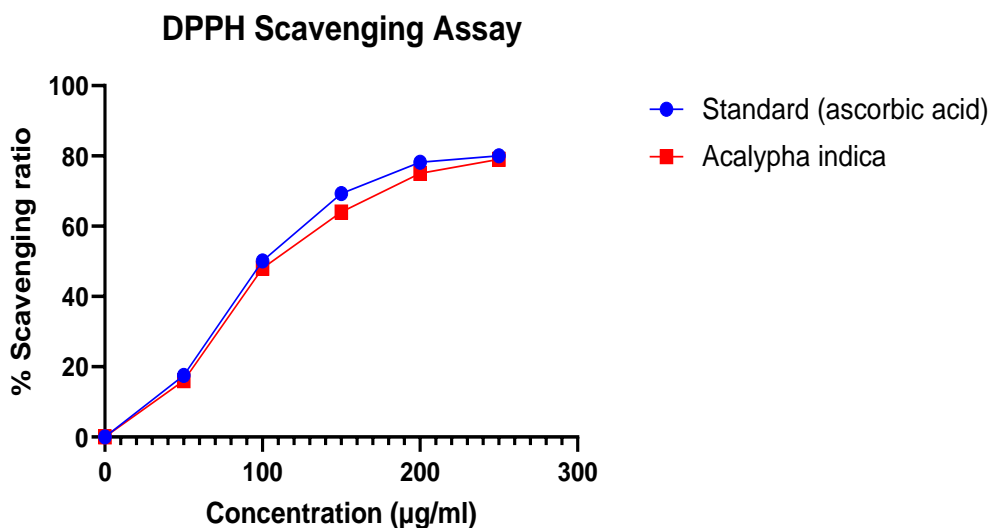


Figure.13 DPPH Scavenging Assay for ethanolic extract of *Acalypha indica*



The results obtained from the ethanolic extract of *Acalypha indica* exhibited the best antioxidant activity. *Amaranthus spinosus* were less active using the DPPH method did not show a good antioxidant activity compared to *Acalypha indica*.

(jadid *et al.*,2017) The antioxidant activity measurement was revealed that the *p. Retrofractum* of methanol extract has higher activity than ascorbic acid. Several reports have shown that methanolic extracts of medicinal plants have excellent pharmaceutical activity. Recent evidence suggested that methanol extract from *P.retrofractum* fruit may mediate mast

cell stabilization. Consistent with the results of the methanol extract, the antioxidant activity of ethyl acetate extracts also increased in a concentration-dependent manner, even higher than that of ascorbic acid. Ethyl acetate extract has been used to extract several phenolic and nitrogenous compounds.

4.6 ABTS RADICAL SCAVENGING ASSAY

Generation of ABTS [2,2- azinobis -(3- ethyl- benzothiazoline- 6- sulfonic acid)] radical cation forms the basis for spectrophotometry methods that have been applied to the measurement of total antioxidant activity of pure substances aqueous mixtures and beverages. The original ABTS assay was based on the activation of metmyoglobin by hydrogen peroxide in the presence of ABTS to generate radical cations, in the presence or absence of antioxidants (pellegrini *et al.*, 2018).

Figure.14 ABTS Scavenging Assay for ethanolic extract of *Amaranthus spinosus*

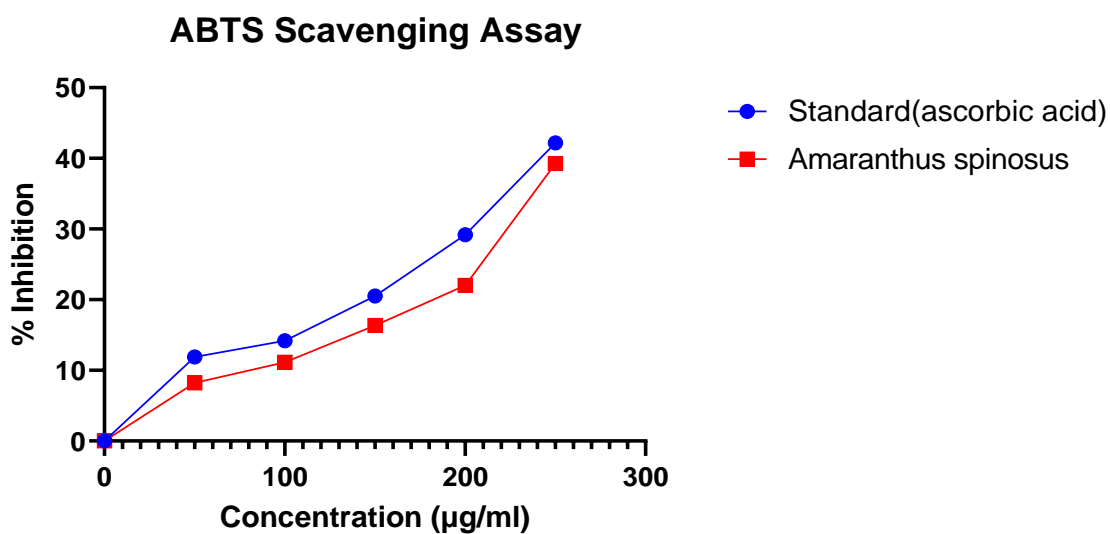
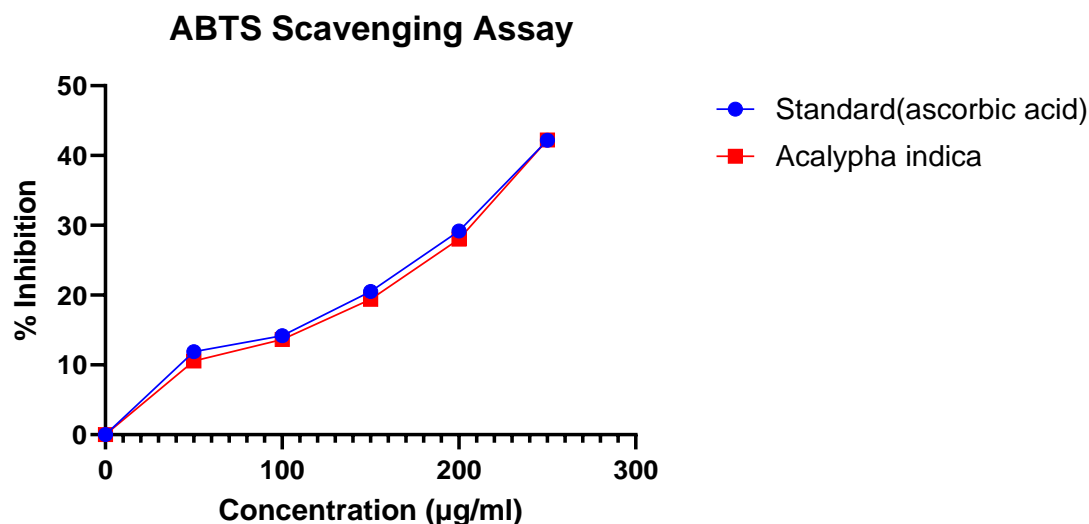


Figure.15 Scavenging Assay for ethanolic extract of *Acalypha indica*



The result obtained from the ethonolic extract of *Acalypha indica* shows the strong radical scavenger, where *Amaranthus spinosus* shows less radical scavenger activity compared to *Acalypha indica*.

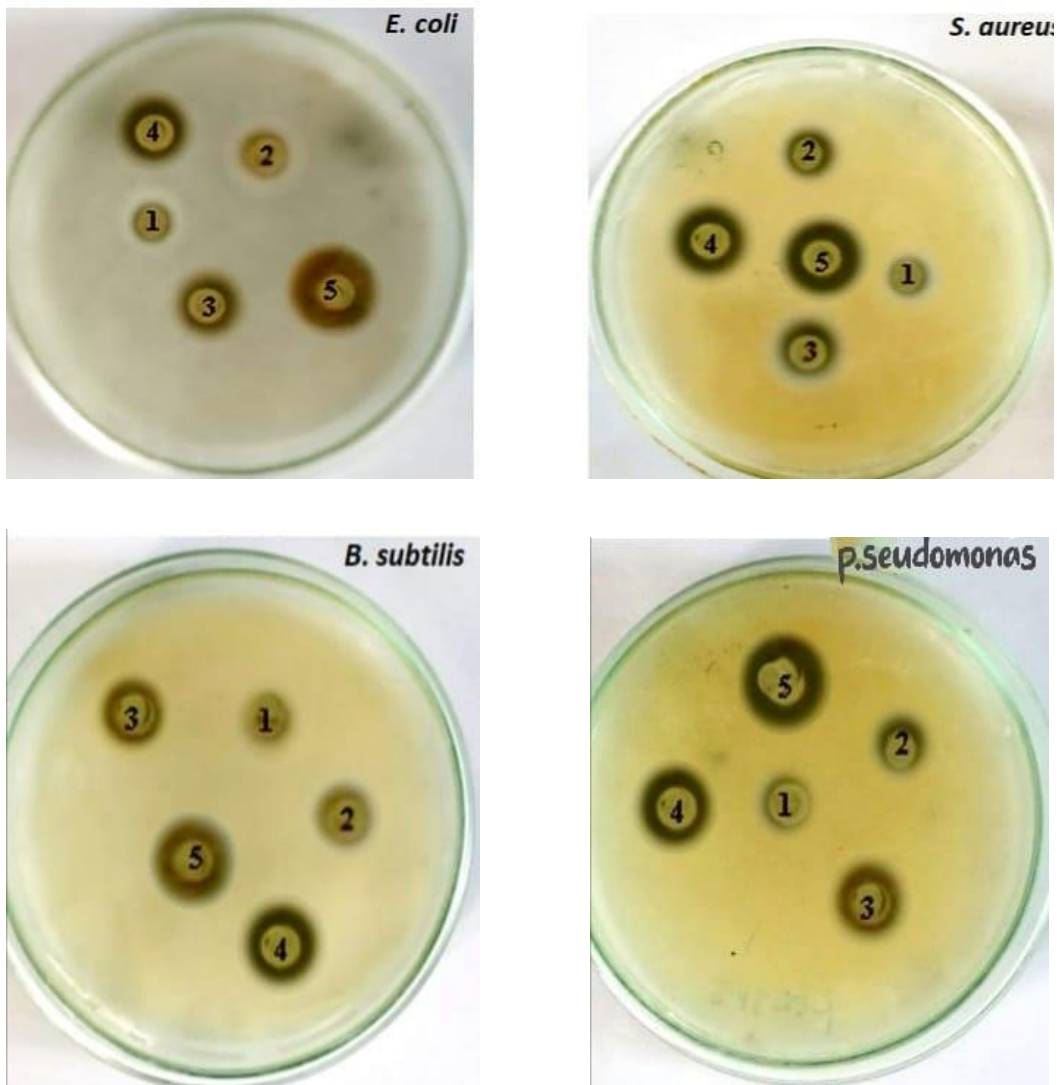
(smarth *et al.*,2008) the extracts of *m. Piperita*, *a. Vasica* and *b. Campestris* extracts showed the highest antioxidant activity. Stimulants the methanol solution of these plant extracts was strong radical scavenger, indicating that active compounds of different polarity would be present in these plants. The antioxidant activity of these plants might be due to their flavonoid and phenol levels.

(dudonne *et al.*,2009) in the ABTS assay, values range from 0.15 to 99.80%, which represents more variability than the DPPH assay about 665 times. Oak extract had the best antioxidant capacity (99.80% of abts inhibition), followed by pine extract (commercial and commercial 83.68% and 76.71%) aqueous extract), cinnamon extract (64.88%) and clove extract (*eugenia caryophyllus clovis*) (46.68%). As observed by DPPH assay, sage extract showed lowest antioxidant capacity (0.15%).

4.7 DISC DIFFUSION METHOD

Disc diffusion methods have a great popularity in busy clinical microbiology laboratories because of their relative simplicity and ability to easily test multiple antimicrobial agents on each bacterial isolate (Biemer *et al.*, 2018)

Figure 16. Zone of inhibition against four bacterial species by the ethanolic extract of *Amaranthus spinosus* and *Acalypha indica*



Control -1

Disc 2-20 μ g (*Amaranthus spinosus*)

Disc 3-40 μ g (*Amaranthus spinosus*)

Disc 4-20 μ g (*Acalypha indica*)

Disc 5-40 μ g (*Acalypha indica*)

Table.5 Zone of inhibition for ethanolic extract of *Amaranthus spinosus*

Organism	Control	Concentration µg	Zone of inhibition
<i>Escherichia coli</i>	0.02	20	1.2
		40	1.5
<i>Pseudomonas aeruginosa</i>	1.1	20	1.3
		40	1.7
<i>Bacillus subtilis</i>	1.4	20	1.4
		40	1.6
<i>Staphylococcus aureus</i>	1.5	20	1.5
		40	1.7

Thus, the results in the present study show that *Amaranthus spinosus* of ethanolic extract exhibited a significant antimicrobial against most of the tested microorganism.

Table.6 Zone of inhibition for ethanolic extract of *Acalypha indica*

Organism	Control	Concentration µg	Zone of inhibition
<i>Escherichia coli</i>	0.02	20	0.6
		40	0.99
<i>Pseudomonas aeruginosa</i>	1.1	20	0.5
		40	1.1
<i>Bacillus subtilis</i>	1.4	20	0.8
		40	1.2
<i>Staphylococcus aureus</i>	1.5	20	0.6
		40	1.1

The results in the present study show that ethanolic extract of *Acalypha indica* exhibited a significant antimicrobial against most of the tested microorganism.

(Bakht *et al.*,2011) the disk diffusion susceptibility assay was used to -investigate the antibacterial activity. Using six different solvents extracted samples from *e. Alba* against *b. Subtilis*. The data indicated that *b. Subtilis* was susceptible to all the six different solvents extracted from *e. Alba*. The data suggested that petroleum ether, di chloromethane, ethyl acetate, butanol and water extracted samples at all levels inhibited the growth of *b. Subtilis*.

4.8 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The antibacterial effect of compounds is often explained in terms of minimum inhibitory concentration (mic), the lowest concentration of the compound capable of inhibiting the growth of the challenging organism. Diffusion assay, in which the drug is applied to a well or paper disc in the center of the agar plate seeded with test microorganisms are not suitable for testing essential oils, as the components are partitioned the agar according to the affinity with water (markham, *et al.*, 2019).

Ethanolic extract of *Amaranthus spinosus* and *Acalypha indica* were subjected to the determination of minimum inhibitory concentration, Lower MIC values were exhibited by the ethanolic extracts (40µg/ml) against most of the bacterial strains.

Table.7 Minimum Inhibitory Concentration for ethanolic extract of *Amaranthus spinosus*

Organism	control	40µg/ml	20µg/ml	10µg/ml	5 µg/ml	2.5 µg/ml	1.25 µg/ml
<i>Escherichia coli</i>	+	-	-	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	-	-	+	+	+	+
<i>Bacillus subtilis</i>	+	-	-	+	+	+	+
<i>Staphylococcus aureus</i>	+	-	-	+	+	+	+

Table.8 Mnimum Inhibitory Concentration of ethanolic extract of *Acalypha indica*

Organism	Control	40µg/ml	20µg/ml	10µg/ml	5 µg/ml	2.5 µg/ml	1.25 µg/ml
<i>Escherichia coli</i>	+	-	-	-	+	+	+
<i>Pseudomonas aeruginosa</i>	+	-	-	-	+	+	+
<i>Bacillus subtilis</i>	+	-	-	-	+	+	+
<i>Staphylococcus aureus</i>	+	-	-	-	+	+	+

The results of MIC assay confirmed the findings of antimicrobial assays, where it was reported that methanol extracts were more potent inhibitors of the microorganism tested as that *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*.

4.9 GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

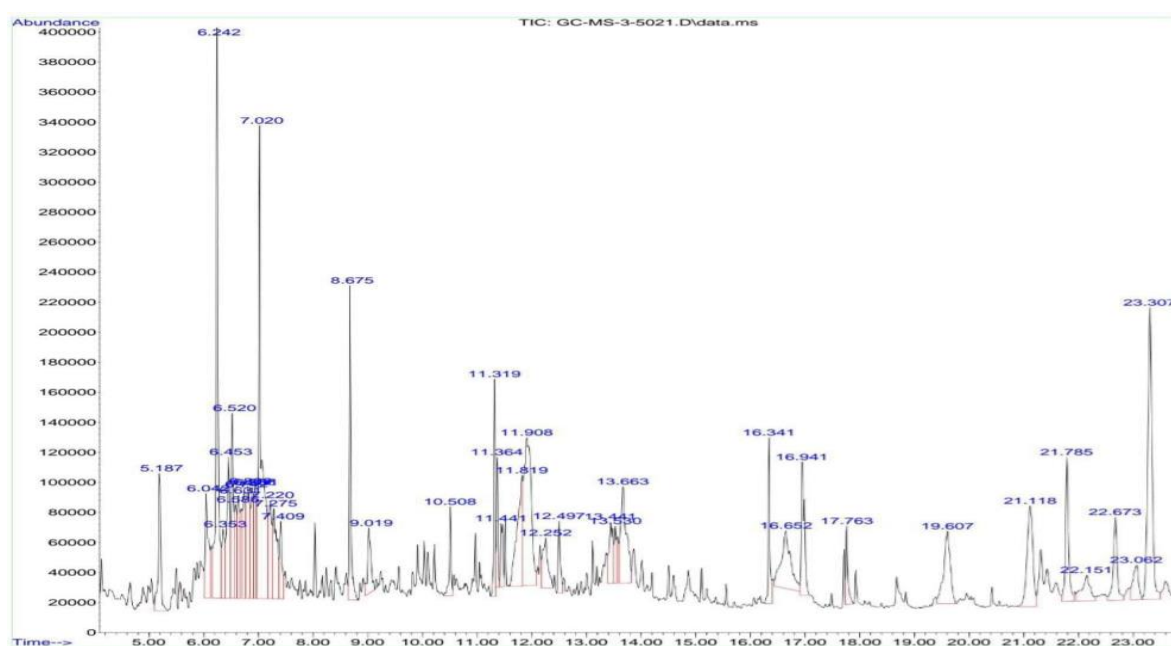


Figure.17 GC-MS CHROMATOGRAM OF *Amaranthus spinosus*

Table.9 Physical properties of bioactive compounds in *Amaranthus spinosus*

S.no	R/t	Predicted compounds by NIST database	Molecular formula	Molecular weight	Peak area %
1	5.187	1R-alpha-pinene	C10H16	136.23	2.13
2	6.042	Acetic acid,(ethylthio)	C4H8O2S	180.3	2.00
3	6.242	3-carene	C10H16	136.24	7.46
4	7.020	Propane,1,1,3-triethoxy	C9H20O3	176.25	9.01
5	7.409	2-decanone	C10H20O	156.2652	1.11
6	9.019	2-Furancarboxaldehyde,5-(hydroxymethyl)	C6H6O3	126.11	1.32
7	11.319	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	C6H13NO3	147.17	1.83
8	11.364	1,4-methanoazulene, decahydro-4,8,8-trimethyl 1-9-methylene	C15H24	204.36	1.11
9	11.819	Ethanol,2(1-methyleoxy)-,acetate	C5H10O3	118.13	3.60
10	11.908	Oxirane,(2,2-dimethylpropyl)-	C7H14O	162.66	2.69
11	13.441	Octyl thioglycolate	C10H20O2S	204.330	1.46
12	13.530	D-glycerol-1-gluco-heptose	C7H14O7	210.18	0.92
13	16.652	Alpha,-Amyrin	C30H50O	426.7	3.34
14	21.118	Urs-12-en-3-ol,acetate,(3,beta.)-	C32H52O2	468.8	3.64
15	22.151	Tetrasiloxane, decamethyl	C10H30O3Si	310.6854	1.30

Table.10 Physical properties of bioactive compounds in *Acalypha indica*

S.no.	R/t	Name of the compound predicted by NIST database	Molecular formula	Mw	Peak area%
1	4.653	-2-amino-3 methyl-11-butanol	C5H13NO	103.16	0.95
2	5.198	1s-.alpha.-pinene	C10H16	136.23	1.57
3	6.053	DI-arabinitol	C5H12O5	152.14	0.73
4	6.253	3-carene			3.86
5	7.020	Propane,1,2,3-triethoxy	C9H20O3	134.17	4.23
6	8.042	4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144.12	1.35
7	8.275	2,5-pyrrolidinedione,1-methyl-	C5H7N0	113.1146	0.52
8	8.675	Naphthalene			1.68
9	9.019	2-furancarboxaldehyde,5-(hydroxyl methyl)-	C6H6O3	126.11	1.18
10	11.364	1,4-methanoazolene, decahydro-4,8,8-trimethyl-9-methylene-	C15H24	204.36	0.78
11	11.964	N1-(4-hydroxybutyl)-n3-methylguanidine acetate	C6H15N3O	145.21	6.38
12	12.286	Beta-d-glucopyranose,1,6-anhydro-	C6H10O5	162.14	1.09
13	13.630	Ethyl.alpha.-d-glucopyranoside			7.54
14	14.030	Myo-inositol,4-c-methyl-pivaloin	C7H14O6	194.18	3.90

15	14.419	N-serylserine	C6h12n2o	192.1699	21.85
16	14.597	4-0-methylmannose	C7H14O6	194.18	23.44
17	14.863	Cyclohexanone,3-(4-hydroxybutyl)-2-methyl-			1.03
18	17.718	9,12-octadecadienoic acid	C18H32O	280.445	0.69
19	17.774	9,12,15-octadecatrienoic acid,(z,z,z)-	C18H32O2	280.4455	3.86
20	21.607	Non anionic acid 9-(3-hexenylidene-cyclopropylidene)-,2-hydroxy-1-(hydroxymethyl)ethyl ester,(z,z,z)-	C24H48O6	432.6	1.18

In the present study 20 compounds from the ethanolic whole plant extract of *Acalypha indica* were identified by Gas-Chromatography-Mass spectrometry(GC-MS) analysis.chemical identification of the plant constituents was based on their retention time(RT), molecular weight,molecular formula by computer search.The result revealed the presence of medicinally significant constituents in the plants studied.Therefore the ethanolic extract from plant could be seen as a good source for usin drugs. According to the GC-MS analysis *Acalpha indica* shows more number of predicted compounds compared to *Amaranthus spinosus*.

Summary and conclusion

5. SUMMARY AND CONCLUSION

Herbal medicine is the use of plants for medicinal and therapeutic purpose for curing of diseases and improve human health. Plants use as food and in traditional medicine are more likely to yeild pharmalogically active compounds. The medicinal properties of plants have been investigated in the recent scientific development throughtout the world,due to their potent therapeutic efficacy ,no side effects and economic viability. Medicinal plants are serving as raw material for drugs which are effective and reasonable health care of people. Plants are also rich diety sources of biomolecules,vitamins and minerals which are crucial for maintaining the healthy body. It has been scientifically estabilised that every part of the plants has medicinal properties include flower,root and stem, leafs,fruits,seed and Whole plant.The present study was able to identify the ‘Phytochemical characterazation of *Amaranthus spinosus* and *Acalypha indica*’.

Since long *Amaranthus spinosus* and *Acalypha indica* plant extracts used for the treatment of different diseases.In traditional and other system of medicines uses of *Amaranthus spinosus* and *Acalypha indica* helped patients to get rid diseases.

Priliminary phytochemicals screenings of *amaranthus viridis*, *amaranthus spinosus*, *clitoria ternata*, *acalypha indica* and *ocimum basilium* of whole plant ethanol were performed. It gave positive tests for alkaloids, flavoinds, tannins, saponnins, phenols. This study shows these plants have a potency for use in pharmaceutical bioactive components for therapeutic drugs.

HPLC analysis revealed that whole plant ethonolic extract of *Amaranthus spinosus* and *Acalypha indica* have high number of bio active compounds present in it.According to the qualitative result it contains alkaloids,flavonoids,saponins, tannins, steroids, terpenoids and phenols.

The suppression activities of *Amaranthus spinosus* and *Acalypha indica* of ethonolic extract are investigated on the alpha amylase enzyme. Out of two tested spices samples *Acalypha indica* exhibited much α -amylase in vitro inhibitory activity whereas *Amaranthus spinosus* exhibited low activity.

The results of hemoglobin in vitro glycosylation and free radical scavenger activity of *Acalypha indica* shows strongest activity where *Amaranthus spinosus* shows weaker activity.

The antioxidant activity measurement at various concentrations was revealed that the *Acalypha indica* of ethanol extract has higher activity than ascorbic acid compared to *Amaranthus spinosus*.

The disc diffusion susceptibility assay was used to investigate the antibacterial activity for ethanolic extract of *Acalypha indica* it has the ability to inhibit some pathogens compared to the ethanolic extract of *Amaranthus spinosus*.

The results of MIC assay confirmed the findings of antimicrobial assays, where it was reported that ethanolic extracts of *Acalypha indica* were more potent inhibitors of the microorganism tested as that *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* compared to the ethanolic extract of *Amaranthus spinosus*.

The plant extracts were subjected to GC-MS study for the identification of medicinal properties, According to the GC-MS analysis 20 predicted compounds by NIST database and eight major peaks are identified from the ethanolic whole plant extract of *Acalypha indica* and *Amaranthus spinosus* shows 15 predicted compounds and five major peaks. Chemical identification of the plant constituents was conducted based on their retention time (RT), molecular formula and molecular weight. The importance of the study is due to the biological activity of some of these compounds. The present study reveals the presence of predicted compounds in *Acalypha indica* is much higher than the *Amaranthus spinosus*.

Based on the results of this study, it is possible to conclude that *Acalypha indica* consists of numerous important biological activities including antioxidant, antidiabetic and antimicrobial activities compared to *Amaranthus spinosus*. The whole plant of *Acalypha indica* have been used in conventional medicine and traditional medicine for decades and the studies done yet have authenticated the medical practices.

RECOMMENDATIONS FOR FUTURE STUDIES

- However, more clinical and pathological studies is needed to be investigate the unexploited potential of the plant.
- Pharmacological activities of *Acalypha indica* will have to be confirmed in different species of animals. More experiments are required in this directions.
- Seasonal variation studies on the pharmacological activities of different parts of *Amaranthus spinosus* must be carried out to know season wise maximum activity.
- There is a dearth of scientific work related to nutraceutical properties of *A. spinosus* studies in this direction are needed.

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