Evaluation of phytochemical constituents, antioxidant and antimicrobial activities of ethanolic extract of Tridax procumbens and Euphorbia hirta

K. Amruthashree

(20PBC003)

A Thesis submitted in

Partial fulfilment of the

Degree of Master of Science in Biochemistry

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641043

May 2022

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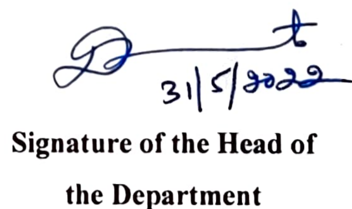

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1. INTRODUCTION

India is a country known for ancient scripts, the number system, invention of zero and Vedas Medicines in India are used by about 60 per cent of the world's population. According to the World Health Organization (WHO), around 80% of people use traditional medicine for basic health care, and over 21,000 medicinal plants have been identified worldwide (Mwitari *et al.,* 2013). Ayurveda and Kabiraji (herbal medicine) are two popular types of alternative medicine in India. Ayurvedic medicine is thought to have been practiced in India for thousands of years. It uses a variety of procedures and items to help sick people heal or feel better. Plant-based remedies are one of the things that ayurveda uses. Researchers find evidence of a traditional use of therapeutic plants in the Atharva Veda writing that is more than 3000 years (Patro, Lingaraj, 2016). Almost 95% of prescriptions were plant based in the traditional systems of Unani, Homeopathy, Ayurveda and Siddha in India.

According to Ayurveda different plant extracts had significantly contributed for remedial effects on mankind. Even till today plant materials serves as the potential sources of drugs (Mohanraj *et al.,* 2018) and has a major role in combating illness (Jhample, Sowmya *et al*.,2015). For primary health care, nearly 80% of the world’s population relies on traditional medicines, most of which involve the use of plant extracts. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries the majority of medicines are modern. Around 80% of products were of plant origin. A vast range of chemical ingredients can be found in natural substances collected from animals, plants, and microorganisms. These vast arrays of natural substances are a benefit to scientists. The traditional medical system's past laid the foundation. The crude extract of various medicinal plants is used in traditional medicine. India is well known for its practice of traditional medicine and ethnopharmacology. Due to the increased side effects of adverse drug reactions and the expense element of the contemporary system of medicine, public, academic, and government interest in traditional treatments is fast expanding. Several plants and plant parts are widely used in various parts of the world for the treatment of various ailments (Jhample *et al*., 2015).

Medicinal plants are a source of phytochemicals and are utilised to treat a variety of oxidative stress-related or other disorders due to their efficacy, low toxicity, and easy availability. From thousands of years ago the mankind has acquaintance about the benefits of

different bioactive components with therapeutic potential. Phytochemicals are produced by plants as a defence mechanism against pathogens. They are used to treat various metabolic, immunological and neurological disorders in humans in various parts of the world as a part of traditional medicine. The use of indigenous plants in commercial medicine is rising with increasing population. The antimicrobial properties of plant extracts led to increased demands.

Alkaloids, tannins, saponins, flavonoids, phenols, steroids, carotenoids, and other phytochemicals found in medicinal plants have a variety of disease-prevention properties (Barbosa *et al*., 2013). These plant-derived chemical compounds have anti-inflammatory, anti-diabetic, anti-aging, antibacterial, antiparasitic, antidepressant, anticancer, antioxidant, and wound-healing properties. The Flavonoids are the most common bioactive compounds identified in medicinal plants (Pietta PG., 2000). Antibacterial, antioxidant, anticancer and wound-healing qualities help humans avoid sickness (Cushnie TPT, Lamp AJ. 2005; Prochazkova D *et al.,* 2011; Chirumbolo *et al.,* 2012).The rich source of bioactive phytochemicals, serve as raw materials for the development and manufacture of drugs that have significant benefits for the treatment of inflammatory and infectious diseases while also being less expensive than chemically synthesized drugs with potential toxicity (Singh *et al.,* 2021). Drought, submergence, chemical pollution, UV exposure, insect and disease infection, and other unfavourable conditions cause plants to emit a variety of phytochemicals to protect themselves (Mathai K., 2000). Pathogenic bacteria's invasiveness and toxigenic characteristics have spread many infectious illnesses. To restrict the spread of illnesses, modern medicine relies on synthetic antibiotics.

In the present study, Qualitative and Quantitative analysis were carried out in different plants collected from various locations which include Tropical, Temperate and Hills/Tribal areas.

Tropical plants- *Euphorbia hirta, Tridax procumbens, Achyranthes aspera*

Temperate plants-*Passiflora foetida*

Hilly region plants- *Leucas aspera.*

Also, secondary metabolites from plants with similar properties were compared.

Euphorbia hirta (Euphorbiaceae) has been claimed as a useful folk medicine to traditional healers for the treatment of fever, pain, diarrhoea, peptic ulcers, vomiting, asthma,

bronchitis, inflammation, kidney stones, and menstrual problems and as antivenom. It can be found in all tropical countries. Because of the need to develop more effective medicines with fewer adverse effects (Rahman *et al*., 2019).

***Tridax procumbens* L.** (Asteraceae) is commonly known as coat buttons or Tridax daisy is a species of flowering plant in the daisy family. It is best known as a widespread weed and pest plant. It is native to the tropical Americas, but it has been introduced to tropical, subtropical, and mild temperate regions worldwide (Kondawar, V. B., 2019). Traditionally, Tridax procumbens has been in use in India for wound healing and as an anticoagulant, antifungal, and insect repellent. The juice extracted from the leaves is directly applied on wounds. Its leaf extracts were used for infectious skin diseases in folk medicines.

***Achyranthes aspera* Linn.** (Amaranthaceae) commonly known as Prickly Chaff flower (English) is traditionally used for treating a number of ailments. It is a small erect annual shrub found distributed throughout the tropical and subtropical regions. The plant is reported to have several medicinal properties and used as emmenagogue, purgative, diuretic, antimalarial, antihyperlipidemic, estrogenic, anti-inflammatory, antileprotic, antispasmodic, cardiotonic, antibacterial, and antiviral agents in traditional systems of medicine. It is also used as antiasthmatic, antitussive and in the treatment of snakebite, hydrophobia, urinary calculi, rabies, influenza, otorrhoea, piles, bronchitis, diarrhea, renal dropsies, gonorrhea, and abdominal pain (Bhosale *et al.,* 2012)

Passiflora foetida (Passifloraceae), known as Stinking Passion Flower, it has been widely used in traditional medicine of the state for the treatment of different types of diseases and disorders. It has rich pharmacological values particularly in mental disorder and possesses numerous bioactive compounds such as vitexin, luteolin, apigenin, etc., which may be responsible for its bacteriocidal, antidysentric, antilithic and other effects. This plant is native to the West Indies, North South America and naturalized weed in Taiwan and China.

Leucas aspera (Lamiaceae) commonly known as 'Thumbai' is one such medicinal plant which is being used traditionally as an antipyretic and insecticide. Parts of the plant are also being used for many disorders like rheumatism, psoriasis and chronic skin eruptions (Ramandeep Kaur, Naveen kumar, 2016). Leucas aspera plants are considered as traditionally important for

their medicinal properties such as Antifungal, Antioxidant, Anticancer, Phytotoxic, Antivenom, Hepatoprotective, Anti-inflammatory, Antinociceptive, Antiulcer, Antimalarial and Antidiabetic activity (Priya *et al*., 2018). *It* is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides.

The medicinal plants around the world contain many compounds with antibacterial activity (Marjorie 1999). Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds (Tomoko *et al.,* 2002). The use of botanical medicines is generally on the rise in many parts of the world (Bbosa *et al.,* 2007). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents. Numerous experiments have been carried out to screen natural products for antimicrobial property (Ateb, Erdourul. 2003; Nair, Chanda 2006; Nair *et al*., 2007; Ndhlala *et al*., 2009).

Plant extracts have many beneficial effects on health due to the great diversity of free radical scavenging molecules, such as phenolics, anthocyanins, carotenoids, and vitamins (Gunathilake *et al.,* 2018). Typical phenolics that possess antioxidant activity can play an important role in adsorbing and neutralizing free radicals (Tlili *et al*., 2013), and they are known to be mainly phenolic acids and flavonoids. These compounds have potent biological activities (Tohma *et al*., 2017; Granato *et al*., 2018; Rahman *et al*., 2018) as antioxidant, anticancer, antibacterial, antiviral, and anti-inflammatory (Tapiero *et al*., 2002).

OBJECTIVES

1. To carry out the phytochemical standardization the ethanolic extracts of the above-mentioned medicinal plants
2. To select the plants with the result of HPLC.
3. To study the free radical scavenging activity of ethanolic extract of *Tridax procumbens and Euphorbia hirta.*
4. To characterize *Tridax procumbens and Euphorbia hirta* by GC-MS and FTIR.
5. To assess the antimicrobial activity and to determine the zone of inhibition of extracts on four bacterial strains.
6. To assess the activities of Anti-inflammatory through the Protein denaturation assays and Membrane stabilization assays.

2. REVIEW OF LITERATURE

Ever since the primeval period, mankind has used the natural yields such as plant life, animals, microbes and aquatic organism, in medicine for the treatment of diseases. The practice of traditional medication has continued as a most reasonable and effortlessly accessible primary source of treatment for the humans since the prehistoric time in the effective management of disease and others various ailments (Velu *et al*., 2019). Plants have been used by humans for thousands of years to maintain their health. Medicinal plants are still the most common type of medication used around the world, particularly in tropical and economically underdeveloped regions (Cardoso *et al*., 2019). The therapeutic properties of plants gave rise to medicinal drugs made from certain plants with these benefits (Esther Salmerón-Manzano *et al.,* 2020).

Even today, hundreds of higher plants are cultivated worldwide to obtain useful substances in medicine and pharmacy. Global studies have been conducted to validate their efficacy, and some of the findings have resulted in the production of plant-based medicines. The annual global market value of medicinal plant products exceeds $100 billion (Sofowora *et al*., 2013). The therapeutic properties of plants gave rise to medicinal drugs made from certain plants with these benefits (Esther Salmerón-Manzano *et al.,* 2020).To obtain a comprehensive compilation of medicinal plants that can be used in disease prevention, original data from traditional custodians of such knowledge must be gathered (Tan *et al*., 2010).

2.1 Analysis of phyto-consistutens of plant

2.2 Antioxidant activity

2.3 Antimicrobial activity

2.4 Anti – Inflammatory potential

2.5 Plant 1. *Tridax procumbens*

2.6 Plant 2. *Euphorbia hirta*

2.7 Plant 3. *Achyranthes aspera*

2.8 Plant 4. *Leucas aspera*

2.9 Plant 5. *Passiflora foetida*

2.1 Analysis of phyto-consistutens of plant

Phytochemicals (Greek: phyton = plant) are chemical compounds found in plants that have either positive or negative health effects. Plant metabolites are not only used for food purposes but also serve as an important historical source of medicines. Plant chemicals are classified as primary and secondary metabolites. The primary constituents include common sugar, proteins, amino acids and nucleic acids. They are obtained directly by the photosynthetic process in plants, whereas, secondary constituents of plants make up the entire set of remaining plant chemicals from alkaloids, terpenoids, flavonoids, coumarins, fumarocoumarins, aromatic and aliphatic compounds (Mradu G *et al*., 2012). The richest bio reservoirs of various phytochemicals are medicinal plants used to treat various diseases and ailments. The phytochemical constituents of plants determine their medicinal properties. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and other important phytochemicals are found in various parts of plants (Junaid *et al*., 2020).

Plants are probably the best cell factories on the planet, producing over 100 000 low molecular secondary metabolites, with an estimated total number in plants exceeding 500,000. (Hoang SV *et al*., 2008).The phytochemical screening of ethanol leaf extract of *Tridax procumbens* revealed presence of carbohydrates, protein, flavonoids, saponin, tannin, steroids and absence of cardiac glycosides (Akintunde *et al*., 2017). Phytochemical analysis revealed the presence of tannins, saponins, alkaloids, carbohydrates, cardiac glycosides, flavonoids and anthraquinones in ethanolic leaves extract of *E. hirta* (Auwal *et al*., 2017).

2.2 Antioxidant activity

Free radicals are highly reactive species that are produced in the body during normal metabolic functions and are introduced from the environment. They are capable of causing tissue injury and have been linked to the pathology of a variety of human diseases. Antioxidants provide an important line of defence against radical-mediated toxicity by preventing free radical damage.

Oxidative stress is defined as a cell's redox imbalance and excessive production of free radicals, which are the most reactive molecules in the biological system. Free radicals are constantly produced and can cause extensive damage to tissues and biomolecules, resulting in a variety of chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, type 2 diabetes, and cancer (Basyal *et al*., 2021). A variety of protective antioxidants are produced by the body itself. This supply of antioxidants can be increased by some foods which are rich in antioxidants. Literature survey showed that natural antioxidants present in herbal foods become more important in older persons as more free radicals are produced by the body. Plants yield a number of antioxidants for their own protection and some of them are also useful for human beings. Antioxidants are found to be present in vegetables, fruits, spices, nuts, tea, coffee and wholegrains. Antioxidants impart the bright colours and specific flavour to fruits and vegetables. The body produces a wide range of protective antioxidants. Some antioxidant-rich foods can help to supplement this supply of antioxidants.

According to a literature review, natural antioxidants found in herbal foods become more important in older people as the body produces more free radicals. Plants produce a variety of antioxidants for their own protection, and some of them are also beneficial to humans. Vegetables, fruits, spices, nuts, tea, coffee, and wholegrains all contain antioxidants. Antioxidants give fruits and vegetables their vibrant colours and distinct flavour. Oxidative stress has been found to be the core reason to many diseases, and pursuit to find effective antioxidant drugs with fewer side effects has made Pharmacognosy an interest to modern scientific researches which bring in Genus Euphorbia of Euphorbiaceae family (Khan *et al*., 2020). The total flavonoids content and outcomes of antioxidant activity assays had very significant relativity. It indicated that flavonoids are the main components responsible for antioxidant behaviour of *Tridax procumbens* (Jing zhanget *et al*., 2012).

2.3 Antimicrobial activity

The search for biologically active compounds in natural sources has always piqued the interest of scientists looking for new sources of drugs for infectious diseases. A number of studies on the antimicrobial screening of medicinal plant extracts have been published in recent years (Kaur and Bains, 2012; Kapoor et al., 2012; Kapoor and Bansal, 2013; Sen and Batra, 2012; Baga and Bains, 2014). Several plants have been studied for antibacterial activity and have

shown promising results (Kapoor and Mishra raksha, 2013). Much research has shown that these plants are rich in a diversity of bioactive compounds found in vitro to inhibit or kill microorganisms effectively. The ethanol extracts of *Tridax procumbens* and *Euphorbia hirta* were tested against 2 Gram-positive and 2 Gram-negative bacteria using disk diffusion method. Plant extracts were more effective against Gram-positive microorganisms than they were against Gram-negative microorganisms. The bacterial strains are shown in the Table 1

Bacillus subtilis	Gram-positive bacteria
Staphylococcus aureus	Gram-positive bacteria
Escherichia coli	Gram-negative bacteria
Pseudomonas aeruginosa	Gram-negative bacteria

Table 1: Bacterial strains

Staphylococcus aureus is one of the most important bacteria causing nosocomial infections with multiple antibiotic resistances. Mostly the crude extracts have been screened, that too without Minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC), and total activity (TA) determination.

2.4 Anti – Inflammatory potential

Inflammation is a complex biological adaptive response or process that is triggered by noxious stimuli and conditions like infection and tissue injury. Inflammatory diseases are the leading cause of morbidity and mortality worldwide . It has been extensively demonstrated that oxidative stress and the inflammatory response have strong and complex interconnections. Overproduction of reactive oxygen species (ROS) predominates at the site of inflammation, causing oxidative stress and potentially damaging biomolecules such as DNA, lipids, and proteins (Basyal *et al*., 2021). Response produced due to inflammation to the tissue injury comprises of a complex collection of enzyme activation, mediator release, cell migration, tissue breakdown and repair which are designed for host protection and generally triggered in most disease circumstances.

Tridax procumbens has marked helpful effects against centrally, peripherally and inflammatory pain model (Deshmukh *et al*., 2014). 70% ethanolic arial part extracts of *T.procumbens*, ethanolic extract was effective compare to methanolic. On HPLC separation shows presence of centaurein, centaureidin and bergenin from active extract. Ethanolic extract show maximum inhibition at dose 50 µg/ml (Selvem et al., 2011). *Euphorbia hirta L.* is a well-known medicine for inflammation of respiratory tract and for asthma as it has a special reputation for causing bronchial relaxation. It can also be used as diuretic and purgative action (B. G. Rajbhoj 2020). *E. hirta*-treated mice had significantly lower levels of proinflammatory cytokines, lower levels of cell activation markers and co-stimulatory molecules, and higher levels of anti-inflammatory cytokines. In AIA animals, *E. hirta* reduced the levels of inflammatory mediators. *E. hirta* extract supplementation may be a promising treatment for arthritic and inflammatory diseases (Ahmad *et al*., 2014).

2.5 Plant 1. *Tridax procumbens*

Tridax procumbens (linn.)(Figure 1) is common weed plant. The Short-lived perennial, prostrate to ascending basil with stems up to 40-50 cm long. The plant bears daisy-like white yellow flowers toothed and generally arrowhead-shaped. Leaves are simple; the shape is ovate having acute base. Leaves petiole is 2cm long. Ovate lamina with hairs. Fruit are grey, silky, cylindrical 1.3-2.4 cm long, 0.5 -1.4 cm diameters. (Jayanti veda *et al*., 2014). The Taxonomic description was shown in Table 2.

Kingdom	Plantae
Subkingdom	Viridaeplantae
Phylum	Tracheophyta
Subphylum	Euphyllophytina
Class	Magnoliopsida
Subclass	Asteridae
Super order	Campanulanae
Order	Asterales
Family	Compositae
Subfamily	Asteroideae
Genus	Tridax
Species	procumbens - L.
Botanical name	Tridax procumbens L

Table 2: Plant 1. *Tridax procumbens* taxonomy

Vernacular names:

Marathi: Kambarmodi, Jakhamjudi & tantani,

Sanskrit: Jayanthi Veda,

Hindi: Ghamra,

English:Coat buttons,

Kannada:Jayanthi,

Bengali: Tridhara,

Telugu: Gayapaaku,

Oriva: Bishalya karani,

Tamil: Vettukaaya poondu,

Japanese: Kotobukigiku,

French: Herbe caille,

Spanish: Cadillo chisaca,

Latin: *Tridax procumbens* (L.) (Jain Ankita, Jain Amita 2012)

Figure 1: *Tridax procumbens*

Stem

Stems decumbent, producing roots at the nodes, it grows up to 50 cm tall. Stems clothed in pale hairs.

Leaves

Leaf blades 30-60 x 15-35 mm, clothed in hairs, lateral veins 2-3 on each side of the midrib. Petioles is hairy, 5-10 mm in long.

Flowers

Flowers produced in heads about 10 x 10-12 mm. Peduncles hairy,11-20 cm long. Heads surrounded by bracts, the outer bracts hairy, each bract about 7 x 4 mm, inner bracts glabrous, 7-8 mm long. Calyx (pappus) consists of barbed or fimbriate hairs 10-12 mm long. Corolla on the ray florets ligular, 9-10 x 4 mm, apex 3-lobed. Corolla of the disk florets tubular, about 5 mm long, corolla lobes about 0.5 mm long. Stamens fused to form a tube. Ovary 2-2.2 x 1 mm, densely clothed in long pale brown or golden hairs.

Fruit

Achene 1.6-2 mm long; pappus of slender, plumose bristles 5-6 mm in long, with fine spreading hairs.

Distribution

Tridax procumbens is resident to the tropical Americas and also present in tropical, subtropical, and placid temperature regions of India and worldwide. This weed got in fields, croplands, meadows in tropical climates.

Traditional Uses

Traditionally *Tridax procumbens* use as a anticoagulant, antifungal, wound healing, insect repellent, in liver infection, stomach ache and heartburn. Leave paste used topically in skin problems. Fresh leaves used directly in tissue inflammation, vesicles and cuts every were in India (Nallella Sreeamula *et al*., 2013).

2.6 Plant 2. *Euphorbia hirta*

E. hirta Linn (Figure 2) is a small annual, branched herb that can grow to 70 cm in height, purple or reddish in color with copious amounts of latex, and covered with sprout hairs. The Taxonomic description was shown in Table 3.

Kingdom	Plantae
Subkingdom	Viridaeplantae
Infrakingdom	Straptophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Infradivision	Angiosperms
Class	Magnoliopsida
Superorder	Rosanae
Order	Malpighiales
Family	Euphorbiaceae
Genus	Euphorbia
Species	hirta

Table 3: Plant 2. *Euphorbia hirta* taxonomy

Vernacular names

English – Asthuma weed

Sanskrit – Dugdhika, Kshirini, Ksheerani, Svaduparni

Hindi - Dudhi

Telugu – Reddinanabrolu

Tamil – Amanpatcharishi

Gujarat – Dudeli

Malayalam – Chittirappula, Nelapalai

Bengali – Barokheruie

Marathi – Dudhi, Mothidudhi

Malaysia – Ambin Jantin

Indonesia – Daun Biji Kcang

Philippines – Botobotonis

Thailand – Nam Nom Raatchasee

Japanese – Gelang Susu, Gendong Anak, KukonKukon, Patican.

Euphorbia hirta (Figure 2) is commonly called as Australian asthuma herb, Queensland asthuma weed, Pills bearing spurge, Cats hair, Hairy spruge, Spurge or milkweed, Garden spurge .(Uddin *et al.* , 2019).The Taxonomic description was shown in Table 4.

Figure 2: ***Euphorbia hirta***

Stem

Stems are semi-erect with several stems originating from a central tap root. Stems have a hairy surface and are filled with a milky white latex/sap.

Leaves

The leaves are opposite, biculate and simple, the stipules are linear, the leaf blade is lanceolate, oblong serrate, long elliptic, tapering, 3 – 4 cm long and 1 – 1.4 cm wide, and its margin is smoothly serrated.

Flowers

The monoecious inflorescence, an axillary or terminal cluster of flowers, is known as a cyathium, in which several cyathia are arranged in a cyme. The male and female flowers are in a pod and both appellation. The flowers are unisexual, male flowers are sessile, prophylls are linear, fringed, perianth absent and have a stamen, female flowers have a small peduncle, the perianth is fringed, the ovary is covered with tiny hairs above, 3-celled, has 3 - Styles, small and the tip is double. The flowering period is usually year round.

Fruit

The fruit is allomorphic, pistillate, elongated, 3-lobed, obtuse base covered with shoot hairs.

Seeds

Seeds are oblong, 4-sided prismatic, wrinkled and brownish pink in color, capsule 3-seeded, green and covered with fleshy spines, seeds smooth, hard mottled crustal skin with a white caruncle at the top enclosing oily endosperm**.**

Distributions

This herb is native to India but widely distributed in many parts of Africa, America, Asia, Australia and Pacific countries as tropical weed (Sood *et al*., 2005; USDA, 2009).

Traditional use

In traditional Indian medicinal systems, leaves of Euphorbia hirta used in the treatment of coryza, cough, asthma, bronchial infections, bowel complaints, helminthic infestations, wounds, kidney stones and abscesses. The aqueous extract exhibits anxiolytic, analgesic, antipyretic, and anti-inflammatory activities (Kumar *et al*., 2010).

2.7 Plant 3. *Achyranthes aspera*

Achyranthes aspera (Figure 3) is an erect plant, reaching 30 to 80 cm in height, abundantly branched and with hairy stem. The leaves are opposite, ovate, with acute or rounded apex. They are hairy and green on the upper surface, whilst smooth on the lower surface. The young leaves are silvery in colour. The spikes are very long and curved at the top. The flowers are very small, numerous, closely spaced, and green in colour. They are erect facing upward before flowering and bend down at fruiting. The fruits are small, dry, and brownish in colour. It is one-seeded, and can readily adhere to animals and clothing with their spines. Several varieties are present in Reunion, one of them being the variety sicula, characterised by a usually acuminate leaf at the top, and the variety aspersa with rounded end, apiculate or abruptly reduced into a short and narrow acumen. The Taxonomic description was shown in Table 4.

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Caryophyllanae
Order	Caryophyllales
Family	Amaranthaceae
Genus	Achyranthes
Species	aspera

Table 4: Plant 3. *Achyranthes aspera* taxonomy

Vernacular names:

English- Princkly Chaff flower

Hindi-Chirachinta,Chirchira

Kannada-Uttrani

Telugu-Antisha,Apamargamu,Uttaraene

Malayalam-Katalati

Bengali-Apang

Punjabi-Puthakanda,Kutri

Marathi-Aghada,Pandhara-aghada

Malagasy-Mahabaka

Tamil-Shiru kadaladi,Nayuruvi

Indonesia-Jarong

Africa-Grootklits,Langklitskafblom

Figure 3: ***Achyranthes aspera***

Stem

Stems are full, squarish with swollen nodes. The surfaces of the stem have longitudinal grooves. Finely hairy to hairless when older.

Leaf

Leaves are simple, opposite and petiolate. Ovate to elliptic lamina (sometimes almost circular). They are 4 to 9 cm long by 2 - 4 cm wide. The margin is entire. Both surfaces are covered in short hairs although older leaves can be smooth. Each leaf has 4 to 9 arching veins.

Inflorescence

Long upright, terminal spikes. 10 to 50 cm in length.

Flower

Flowers are scaly, very small and numerous. They are mostly green, but can contain purples and pinks. They have no petal. Calyx with 3 to 5 scarious sepals lanceolate, acute at the top. Outside, 2 spiny bracts, arched at the top and the bottom third of which is expanded in membranous wings. Flowers are pitched upwards when in bud, spread whilst flowering and then fold down to form fruits. The flowers start opening from the base of the inflorescence.

Fruit

Straw-coloured utricule, surrounded by the spiky perianth, 2.5-2.8 mm long, 1-1.5 mm wide, rounded at the base, with truncate or depressed apex; pointed downwards and pressed against the flowering stalk; indehiscent, 1-seeded, thin-walled; enclosed by persistent perianth. and bracts, detaching easily from rachis

Seed

Seed 2 to 3 mm long, 1 to 1.5 mm wide, truncate above, reddish to dark brown

and shiny, enclosed in chaffy calyx parts that remain attached.

Distribution

Achyranthes aspera is widespread through the tropics and subtropics of Europe, Africa, Asia, Australia and the Americas. It is thought to have originated from the Old Word. It occurs in open dry places at elevations up to 2000-3000 m (Nepal or Tanzania). It is often found in secondary regrowth at forest edges, in thickets, open grassland, along forest trails, in sand dunes and in seasonal swamps and dried-up watercourses (Fern, 2019; Vibrans, 2009). It grows in sandy soils, especially in the shade of trees and bushes. It is considered a weed in Mexico where it grows in disturbed areas (Vibrans, 2009). It has been reported to be invasive in some areas of Tanzania (Ruffo *et al.*, 2002). In East Java, *Achyranthes aspera* is one of the predominant species of the understorey of *Acacia nilotica* (Djufri, 2017).

Traditional use

Apamarga has been extensively used in Ayurveda as an anti-inflammatory agent besides being useful in Hemorrhoids, indigestion, cough, asthma, anemia, jaundice and snake bite. To instantly get rid of cough, read more about cough home remedies. Apamarg oil is used locally in earache. The root powder is sprinkled over the lesion in skin diseases

2.8 Plant 4. *Leucas aspera*

Leucas aspera (Figure 4) is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long; flowers white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs; calyx variable, tubular, 8-13 mm long; tube curved, contracted above the nutlets, the lower half usually glabrous and membranous, the upper half ribbed and hispid; mouth small, very oblique, not villous, the upper part produced forward; teeth small, triangular, bristle-tipped, ciliate, the upper tooth being the largest. Corolla 1 cm long; tube 5 mm long and pubescent above, annulate in the middle; upper lip 3 mm long, densely white-woolly; lower lip about twice as long, the middle lobe obviate, rounded, the lateral lobes small, subacute. Fruit nutlets, 2.5 mm long, oblong, brown, smooth, inner face angular and outer face rounded (Prajapati *et al.*, 2010). The Taxonomic description was shown in Table 5

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Leucas
Species	aspera

Table 5: Plant 4. *Leucas aspera* taxonomy

Vernacular names

Tamil: Thumbai

Sanskrit: Dronapushpi, Chitrapathrika, Chitrak-shupa

Punjabi: Guldora

Bengali: Darunaphula, Hulkasha

Gujarati: Kulnnphul

Hindi: Goma madhupati

Sindhi: Kubo

Maharashtra: Bahuphul

Bombay: Tumba

Telugu: Tunni

Figure 4: *Leucas aspera*

Stem

The stem is quadrangular, much branched, hispid or scabrid and contains a wide stele. The epidermis of the stem is covered in a thick waxy cuticle and contains few traversed stomata. Typically in younger stems the xylem tissue is radially organized and the parenchymatous pholem tissue is very narrow. As the stem ages the pholem tissue widens and can be found on both sides of the radial xylem tissue.

Leaves

Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long.

Flowers

Flowers are white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs.

Fruit

Fruit nutlets, 2.5 mm long, oblong, brown, smooth, inner face angular and outer face rounded

Distribution

Leucas aspera is commonly found throughout India and the Philippines as well as the plains of Mauritius and Java. In India and the Philippines, it is a very common weed.

Traditional uses

The plant is used in traditional medicine to cure many diseases such as cough, cold, diarrhea, and inflammatory skin disorder.

2.9 Plant 5. *Passiflora foetida*

P. foetida (Figure 5) is especially common along roadsides, around houses and sheds, along fences, and in waste areas. It requires warm, moist soil and air conditions for at least half

of the year, moderate to high soil fertility, vine support, and time off from cultivation. It may have an annual or perennial life cycle in permanently moist soils, but it is more likely to be an annual vine in areas with a long dry season. The Taxonomic description was shown in Table 6.

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Malpighiales
Family	Passifloraceae
Genus	Passiflora L.

Table 6: Plant 5. *Passiflora foetida* taxonomy

Vernacular names

Indonesia: cheplukan blungsun (Java), ermut, rajutan (Sundanese)

Malaysia: timun dendang, timun padang, timun hutan.

Philippines: kurunggut (Bikol), masaflora (Ilokano), taungon (Bisaya).

Thailand: ka thok rok (central), kra prong thong (peninsular), thao sing to (Chai Nat).

Vietnam: lac tiên.

Figure 5: ***Passiflora foetida***

Flowers

White flowers are marked with a ring of radially oriented purple streaks. Blossoms open in the morning, but close by about noon. This species is free-flowering. They have a minty fragrance.

Fruits

Red or orange, ellipsoid fruits have fleshy pulp and small, black seeds. Fruits are surrounded by finely dissected, needle-like bracts. Although young fruits are toxic and cyanogenic, ripe fruits are edible and taste like *Passiflora edulis* fruits.

Stems

Stems are round and hairy.

Leaves

Large, hairy leaves are usually tri-lobed with ovate to angular lobes. They are spirally arranged with one leaf per node. Crushed leaves produce an unpleasant odour. Leaves are cyanogenic (produces cyanide) and toxic.

Distribution

P. foetida is especially common along roadsides, around houses and sheds, along fences, and in waste areas. It requires warm, moist soil and air conditions for at least half of the year, moderate to high soil fertility, vine support, and time off from cultivation. It may have an annual

or perennial life cycle in permanently moist soils, but it is more likely to be an annual vine in areas with a long dry season. The plant grows in a variety of soils, including peats, loams, and sands, as well as soils derived from corals, volcanic debris and hilly areas.

Traditional use

Young leaves and plant tips are also edible. Dry leaves are used in tea in Vietnamese folk medicine to relieve sleeping problems, as well as treatment for itching and coughs. The *use* of Passiflora species in the popular ***medicine*** for the inflammatory diseases(Sasikala et al., 2011).

3. MATERIALS AND METHODS

This study carry out the phytochemical screening of five medicinal plants and as per the result of this analysis and HPLC results ,Tridax procumbens and Euphorbia hirta was selected for further analysis. Free radical scavenging activity was performed to analyze the Scavenging activity of the plant extracts. To assess the antimicrobial activity and to determine the zone of inhibition of extracts on four bacterial strains are *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* by Disk diffusion method. The activities of Anti-inflammatory through the Protein denaturation assays and Membrane stabilization assays. And the characterization of ethanolic extract of *Tridax procumbens* and *Euphorbia hirta* was carried out by GCMS and FTIR.

3.1 Identification and Authentication

The whole plant of all the five different medicinal plants namely ***Euphorbia hirta, Tridax procumbens, Plassiflora foetida, Achyranthes aspera and Leucas aspera*** were collected from various places of Coimbatore like Marudhamalai hills, Thondamuthur and Gobichettipalayam. The plant materials were taxonomically identified and authenticated from Agricultural University campus, Botanical Survey of India (Southern circle), Coimbatore, Tamil Nadu.

3.2 Preparation of the whole plant extracts for Phytochemical Screening

Fresh plants were collected from various places. The collected plants was washed thoroughly 2-3 times with running tap water and once with sterile distilled water and air dried in aseptic conditions. Different part of the plant were sliced into small pieces and grounded using a motar and pestle. Ten grams of five plant samples were subjected to 100 ml of different solvents like ethanol and water separately in a conical flask and it is was kept in an open orbital shaker for about 24 hours at room temperature. Then the extract was filtered using No.1 Whatman filter paper and the extract was taken in tight container and stored in a refrigeration for further use.

3.3 Qualitative phytochemical screening

The ethanolic extract of above mentioned five plants was screened for the presence of phytochemicals following the method of Khandelwal (2002).

3.3.1 Detection of Alkaloids

Mayer's test

A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chlorate and 5.0g of potassium iodide in 100ml distilled water). Formation of a cream colored precipitate indicated the presence of alkaloids.

Dragendroff's test:

A fraction of the extract was treated with Dragendroff's reagent and observed for the formation of reddish orange precipitate

3.3.2 DETECTION OF SAPONINS

Foam test

In a test tube containing about 5 ml of an extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

3.3.3 DETECTION OF TANNINS

Braymer's test

To a fraction of the extract, a few drops of 10% ferric chloride were added. The appearance of a dark green precipitate indicated the presence of tannins.

3.3.4 DETECTION OF STEROIDS

To 2.0 ml of the extract, added few drops of chloroform, mixed and 1.0 – 2.0 ml of acetic anhydride was added. Then added two drop of sulphuric acid. The appearance of red colour first, then blue and finally green appears. It indicates the presence of steroids

3.3.5 DETECTION OF FLAVONOIDS

Aqueous NaOH test

The appearance of a yellow orange color on adding 1N aqueous NaOH to the extract indicated the presence of flavonoids.

Concentrated H2SO4 test

To a small fraction of the extract, concentrated H2SO4 was added and observed for the formation of orange color.

3.3.6 DETECTION OF TERPENOIDS

Salkowski Test

A fraction of the extract was dissolved in chloroform and shaken well with an equal volume of concentrated sulphuric acid. The appearance of red color, in the chloroform layer and green fluorescence in the acid layer indicated the presence of terpenoid.

3.3.7 DETECTION OF PHLOBATANNINS

HCl test

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

3.3.8 DETECTION OF 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.

3.3.9 DETECTION OF GLYCOSIDES

Keller-Killiani test

The test solution was treated with few drops of ferric chloride solution and mixed. When conc. sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer

3.3.10 DETECTION OF PHENOLICS

Ferric chloride test

A 5% solution of $FeCl_3$ was added to a fraction of the extract and observed for the formation of deep blue color.

Lead acetate test

The reaction mixture contained a fraction of the extract and 10% lead acetate solution and observed for the formation of white precipitate.

3.3.11 DETECTION OF QUINONES

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

3.4 PREPARATION OF PLANT EXTRACTS FOR ASSAYS

Two steps were involved in the preparation of plant extracts.

3.4.1 Preparation of whole plant powder

The selected plants were collected and allowed to shade dried at room temperature for two weeks. The dried flowers were coarsely powdered by hand, then ground into a powder using an electrical blender. The powdered material was weighed and kept in air tight container.

3.4.2 Preparation of ethanolic extract

10g of the powdered plants were taken and mixed with about 100ml of ethanol in a conical flask and it is was kept in an open orbital shaker for about 5 days at room temperature. The mixture was filtered using a Whatmann No.1 filter paper and the extract was taken in 500 ml beaker. Then the mixture was suspended to rotary evaporator and a colloidal gel was obtained after evaporation and was filled in an eppendorf tube and stored in a refrigerator for further use.

3.5 FREE RADICAL SCAVENGING ACTIVITY

3.5.1 Antioxidant activity of *Tridax procumbens and Euphorbia hirta* as determined by DPPH assay

Spectrophotometric quantification of the radical scavenging ability of the extract towards DPPH free radicals was carried out by the method of Mensor et al., 2001 and its detailed procedure was given in Appendix I.

3.5.2 Determination of 2, 2′ -azino-bis-3-ethyl benzthiazoline-6-sulphonic acid(APTS) radical scavenging assay

The Procedure for ABTS (2, 2′ -azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay was based on the method proposed by (Shirwaikar et al., 2006) and its detailed procedure was given in Appendix II.

3.6 ANTIMICROBIAL ASSAY

Disc Diffusion Assay

Disk diffusion/Kibry-Bauer method was given in Appendix III

3.7 EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF THE ETHANOLIC EXTRACT OF *Tridax procumbens and Euphorbia hirta*

Inflammation is a pathophysiological response of living tissue to injuries, resulting in the accumulation of plasmatic fluid and blood cells locally. The inflammatory process is characterized by the production of various signaling molecules like prostaglandins, leukotrienes, histamine, bradykinin and platelet activating factor, as well as release of chemicals from tissues and migrating cells (Sarvankumar et al., 2011). The anti-inflammatory activity was evaluated by protein denaturation method and membrane stabilization method.

3.7.1 Protein denaturation assay

Denaturation of tissue proteins has been evidenced in inflammatory and arthritic diseases. It has also been reported that the denaturation of tissue proteins may result in the production of auto-antigens in certain arthritic diseases (Manukumar and Umesha, 2015). Compounds that

prevent protein denaturation could serve as an effective anti-inflammatory drug. Thus, the ethanolic extract of Tridax procumbens and Euphorbia hirta was tested for its inhibiting potential of protein denaturation. The protein denaturation assay was carried out following the method of Elias and Rao (1998) with some modifications and is give in Appendix IV.

Membrane stabilization test

The membrane stabilization test was evaluated by the method of hemolysis of red blood cells. This hemolysis was induced on the one hand by heat on the other hand by distilled water [29] with some modification

Preparation of the suspension of erythrocytes

Fresh whole blood (3 mL) collected from healthy volunteers in EDTA tubes was centrifuged at 2500 rpm for 10 min at 4 °C. A volume of normal saline equivalent to that of supernatant was used to dissolve the red blood cells. The volume of dissolved red blood cells obtained was measured and reconstituted in the form of a 40% v/ v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of NaH2PO4, 1.15 g of Na2HPO4 and 9 g of NaCl in 1 L of distilled water. Reconstituted red blood cells (supernatant resuspended) were used as such.

3.7.2 Heat induced hemolysis

The heat induced hemolysis assay was carried out using the method proposed by Shinde et al. (1999) and its detailed procedure was given in Appendix V.

3.8. Fourier Transform Infrared Spectral Analysis

To detect the types of chemical bonds and the functional groups that have their characteristic vibrational frequencies, Fourier Transform infrared (FTIR) spectroscopy was carried out for the extract. The light absorption by the compounds at a particular wavelength is a characteristic feature of a chemical bond. The Infrared (IR) spectrum of the extract was recorded using FTIR spectrophotometer (PerkinElmer spectrum 100, Waltham, MA) by potassium bromide (KBr, analytical grade) pellet method. The spectrum was recorded in the range of 4000 to 400 cm-1 at a resolution of 1 cm-1. The pellet was prepared by mixing 2.0mg of the leaf

sample with 200mg of KBr using an agate motor followed by pressing at a high pressure of 125 kg cm-2 in a 13mm diameter stainless steel for 1 minute using the hydraulic pellet maker.

3.9 GC-MS Spectral analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis provides qualitative and quantitative information on the components in the extract. In order to find out the active components in the ethanolic extract of Tridax procumbens and Euphorbia hirta, the same was subjected to GC-MS analysis. The area under the peak is directly proportional to the concentration of the substances in the extract. Thermo Scientific Trace Ultra version 5.0 gas chromatography (GC) instrument with Thermo Scientific DSQ II mass spectrophotometer (MS) was used. The GC was fortified with DB-35 MS capillary standard non-polar column (30m × 0.25mm × 0.25µm). The oven temperature was set to 75°C-260°C (6°C/min), with a hold of 10 min (37.51 min programme). The sample was (1µL) injected using injector A (SSL), and a constant flow of 1 mL/min of helium gas was used as carrier. The transfer line temperature of MS was set to 290°C along with ion source temperature of 230°C (electron ionization). The sample was analysed at electron energy of 70 eV (vacuum pressure- 2.21e-0.5 Torr).

3.10 High Performance Liquid Chromatography

Ion exchange chromatography (IEC) was utilized for the separation of protein from the solvent extract. The extract was loaded onto column of CM-Sepharose CL-6B equilibrated with PB which had been eluted by linear gradient of NaCl. Subsequently, HPLC was accomplished with HP Ti series 1050 liquid chromatograph fitted with photodiode array detector (DAD, HP series 1050). The fraction from the IEC was subjected to reverse phase. HPLC analysis on a C18 column equilibrated with trifluoroacetate in water (0.1%). Later, an elution gradient, acetonitrile (0.1% TFA) was applied and the absorbance was measured at 214 nm. The obtained extract along with the crude one were analysed using protein electrophoresis.

4. RESULTS AND DISCUSSION

Scientists working on disease prevention have long been interested in biologically active compounds derived from natural sources. Biological studies are required to discover additional medicinal properties of plants. Medicinal plants are sources of new drugs, and research is needed to determine the scientific evidence for plant claims. Recently, many studies on medicinal plants have been conducted. The main reason was that the synthetic drugs that humans were now using had numerous side effects that frequently resulted in serious complications. The primary screening of the compounds in plant extracts was used to develop herbal medicine, and its pharmacological activities were evaluated.

The present study to be made investigate the phytochemical screening of ***Euphorbia hirta, Tridax procumbens, Passiflora foetida, Achyranthes aspera and Leucas aspera*** **,** then two plant samples are selected according to the HPLC results and Phytochemical analysis. The in vitro antioxidant assay, antimicrobial assay and anti-inflammatory assays was carryout with the selected plants. The results of the current study and discussions were recorded and presented here.

4.1 Preliminary Phytochemical analysis

Many biological and therapeutic properties have been reported for secondary metabolites. These compounds pique the interest of pharmacists due to their therapeutic efficacy and low toxicity (Inayatullah *et al*., 2012). The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials (Das *et al*., 2010).

Anti-inflammatory plants constituents are tannin, flavonoids, glycosides and steroid. Anti-microbial plants contain saponins, glycosides, tannin and Anti-oxidant plants constituents are saponins, steroid, glycosides and tannin. The preliminary phytochemical screening of ethanolic extract of *Euphorbia hirta, Tridax procumbens, Passiflora foetida, Achyranthes aspera and Leucas aspera* was performed and the results are show in Table 7.

Table 7: Preliminary phytochemical analysis of ethanolic extract of five plants

Tests	*Achyranthus aspera*	*Passiflora foetida*	*Leucas aspera*	*Tridax procumbens*	*Euphorbia hirta*
Alkaloids	++	++	++	++	++
Saponins	++	++	++	++	++
Tannin	++	++	++	++	++
Steroid	++	+(Trace)	++	++	++
Flavonoids	++	-	++	++	++
Terpenoids	++	+(Trace)	+(Trace)	++	++
Phlobitannins	-	-	+(Trace)	++	-
Coumarins	-	-	-	-	-
Cycloglycosides	++	++	+(Trace)	+(Trace)	+(Trace)
Phenols	-	++	-	++	++
Quinone	-	-	-	-	-

+ve indicates Presence; -ve indicates Absence

The present study indicates that the qualitative phytochemical analysis of ethanolic extract of *Achranthus aspera* contains alkaloids, saponins, tannin, steroids, terpenoids and glycosides while tannins, coumarins, phenols and quinines are absent. The ethanolic extract of *Passiflora foetida* contains alkaloids, saponins, tannins, glycosides,phenols, trace amount of steroid and terpenids while flavonoids, phlobitannins, coumarins and Quinone are not present. The ethanolic extract of *Leucas aspera* contains Alkaloids, Saponins, Tannin, Steroid, Terpenoid and trace amount of Terpenoid, tannins and glycosides, while Coumarins and Quinones are absent. The ethanolic extract of *Tridax procumbens* contains alkaloids, saponins, tannin, steroids, terpenoids, phenols, glycosides and trace amount of glycoside is present. The ethanolic extract of

Euphorbia hirta contains alkaloids, saponins, tannin, steroids, terpenoids, phenols and trace amount of glycosides.

4.2 HPLC analysis of whole plants extracts

HPLC is a versatile, robust, and widely used technique for the isolation of natural products, HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture. The HPLC analysis of the ethanolic extract of *Euphorbia hirta, Tridax procumbens, Passiflora foetida, Achyranthes aspera and Leucas* was carried out using Supelco analytical-Discovery HS C18 reverse phase column (Sigma-Aldrich equipped with UV detector). The results obtained are presented in Figure 6 to 10. The HPLC spectrum of the ethanolic extract of all plants showed major and minor peaks. For the further analysis Tridax procumbens and Euphorbia hirta was taken on the bases of total number of highest peaks.

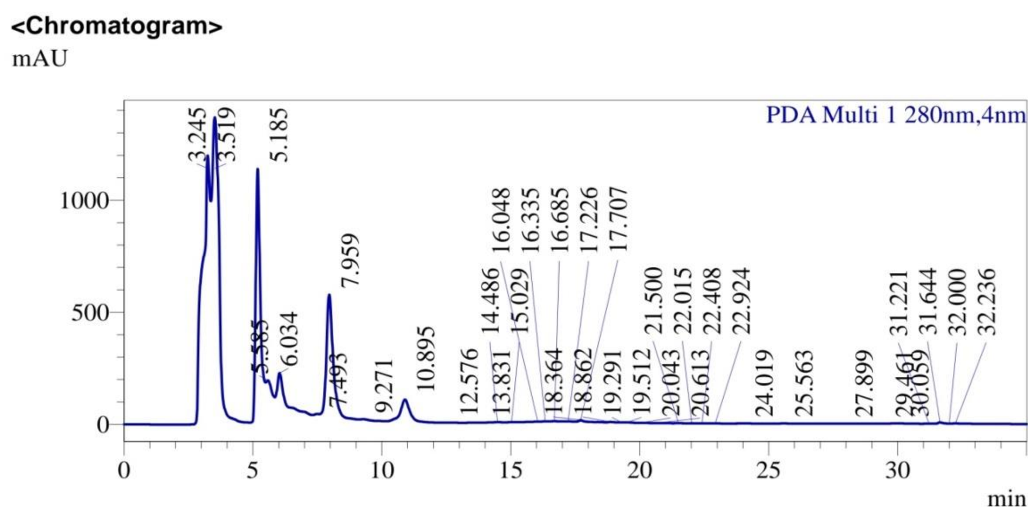


Figure 6: HPLC analysis of *Euphorbia hirta*

<Chromatogram>
mAU

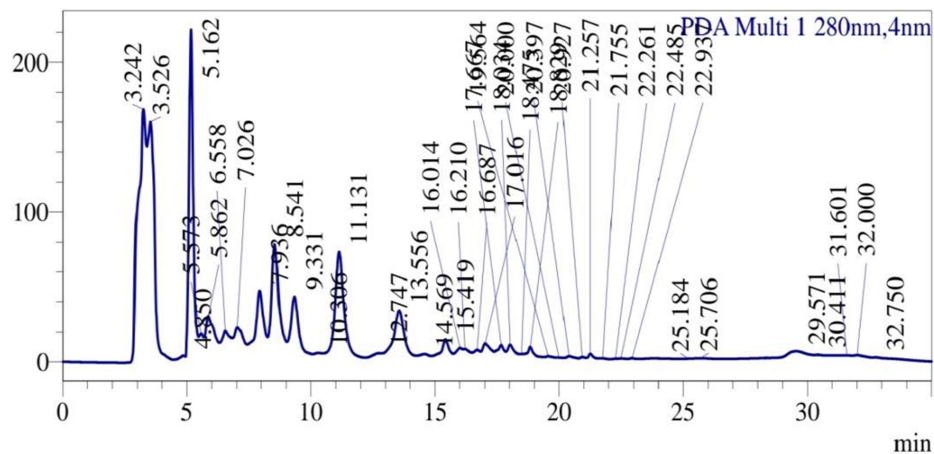


Figure 7: HPLC analysis of *Tridax procumbens*

<Chromatogram>
mAU

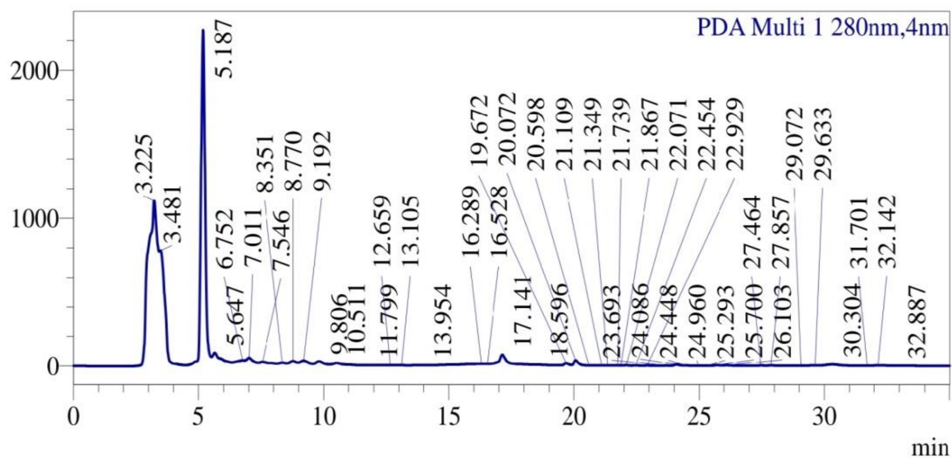


Figure 8: HPLC analysis of *Passiflora foetida*

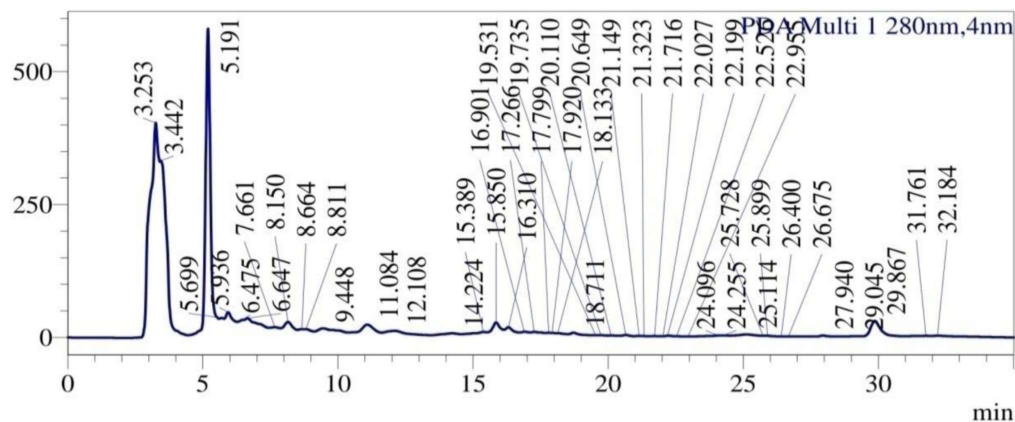


Figure 9: HPLC analysis of Achyranthus aspera

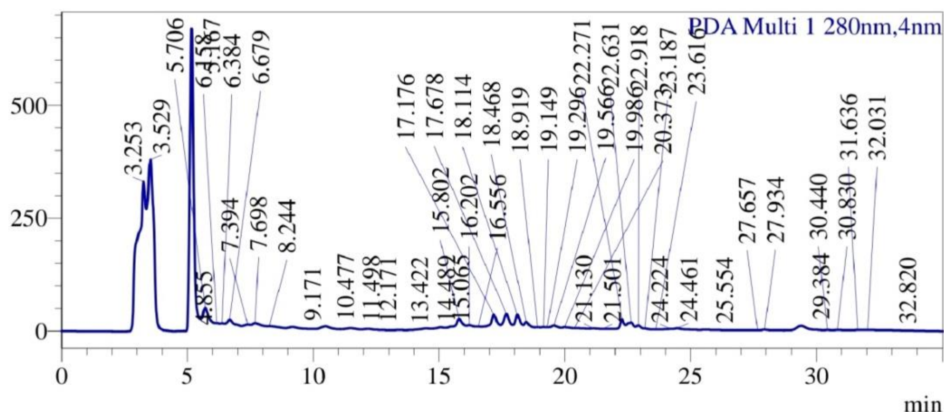


Figure 10: HPLC analysis of *Leucas aspera*

4.3 GC-MS analysis of ethanolic extract of *Tridax procumbens* and *Euphorbia hirta*

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

4.3.1 Identification of Bioactive compounds in *Tridax procumbens*

The GC/MS analysis showed 8-9 compounds majorly found in this extract which explain the therapeutic potential of the plant. The pharmacological activity of highest found Kushwaha *et al*., IJPSR, 2019; Vol. 10(5): 2492-2496. E-ISSN: 0975-8232; P-ISSN: 2320-5148. The chromatogram of the GC-MS analysis of ethanol extract of *T.procumbens* is given in Figure 11

Figure 11: GC-MS Profile of the ethanolic extract of *Tridax procumbens*

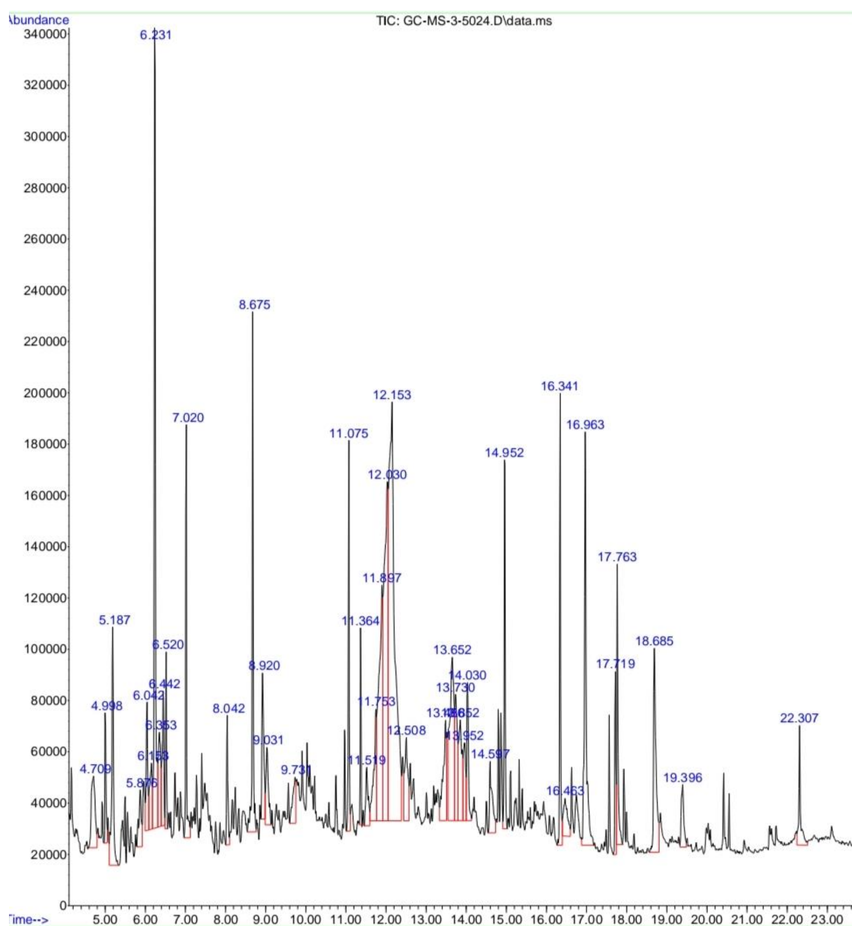


Table 8: Compounds identified in *Tridax procumbens* extract by GC-MS analysis

S.NO	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	PEAK AREA%
1.	4.709	Pentaborane(11)	C_5H_{11}	65.142	1.72
2	5.187	1S-.alpha.-Pinene	$C_{10}H_{16}$	136.23	2.58
3.	6.042	2-Hydroxyethyl butyl sulphide	$C_{12}H_{26}O_2S$	234.40	1.27
4	6.231	3-Carene	$C_{10}H_{16}$	136.23	6.72
5	6.520	D-Limonena	$C_{10}H_{16}$	136.23	1.06
6	7.020	Propane,1,1,3-triethoxy-	$C_9H_{20}O_3$	176.25	2.41
7	8.675	Napthalene	$C_{10}H_8$	128.1705	3.46
8	11.075	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta{c}pyrane-1-yl)ethanone	$C_{13}H_{18}O_2$	206.28	2.28
9	11.364	1,4-Methonoazulene,decahydro-4,8,8-trimethyl-9-methylene	$C_{15}H_{24}$	204.35	1.03
10	11.897	N1-(4-hydroxybutyl)-N3-methylguadine acetate	$C_6H_{12}O_3$	132.16	5.56
11	12.030	Thiocyanic acid,2-(2-butoxyethoxy)ethyl ester	$C_9H_{17}NO_2S$	203.3	8.18
12	12.153	Oxirane,(2,2-dimethyl	$C_7H_{14}O$	114.19	15.12

		prophyl)-			
13	16.963	Cyclohexane acetic acid	$C_8H_{14}O_2$	142.20	4.86
14	17.763	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	$C_{18}H_{30}O_2$	278.4296	2.05
15	18.685	Benzyl .beta.-d-glucoside	$C_{13}H_{16}O_7$	284.26	3.22

Identification of compounds is based on the retention time (RT), Molecular formula; molecular weight and peak area in percentage are present in Table 8. International Journal of Pharmaceutical Sciences and Research 2495 compound like Oxirane,(2,2-dimethyl prophyl)-(RetensionTime 12.153 and Peak area 15.12%) proved it the plant with pharmacological importance, detail of compounds identified from GC-MS analysis of ethanolic extract of Tridax procumbens in Table 4.2. As the reported compound are known for their very good therapeutic effects viz., antimicrobial , antiinflammatory, gene regulation activity, antiarthritic , anti-coronary, CNS depressant, antineoplastic, anti-spermatogenic and immunosuppressive activity(Kushwaha *et al*., 2019).

4.3.1 Identification of Bioactive compounds in *Euphorbia hirta*

GC-MS Chromatogram of Euphorbia hirta revealed eight peaks showing that eight different compounds were present. Identity of the active components in the extract was done by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored in the database of National Institute of Standards and Technology (NIST), were used for matching the identified components with the plant material. From there, the name, molecular weight, formula, structure and bioactivities of the compounds were ascertained. The chromatogram of the GC-MS analysis of ethanol extract of E. hirta is given in Figure 12, which clearly showed the presence of fifteen major phytochemicals at different retention time. Identification of compounds is based on the retention time (RT), Molecular formula; molecular weight and peak area in percentage are present in Table 9.

Figure 12: GC-MS Profile of the Ethanolic Extract of *Euphorbia hirta*

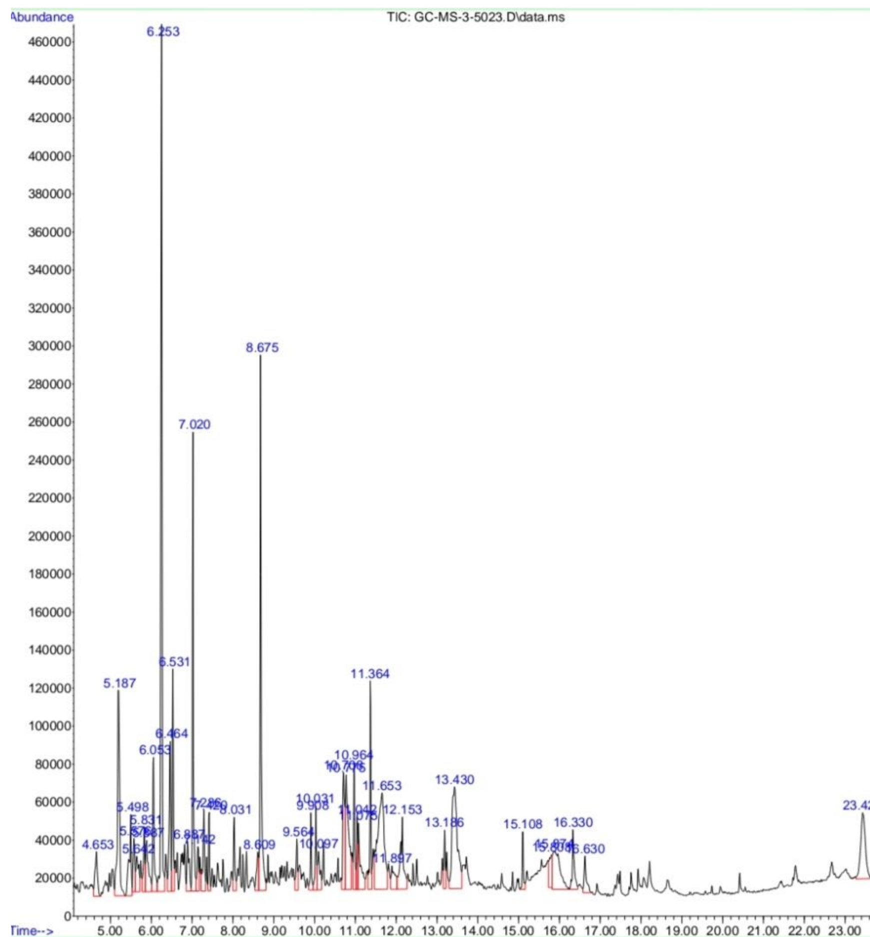


Table 9: Compounds identified in *Euphorbia hirta* extract by GC-MS analysis

S.NO	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	PEAK AREA %
1	5.4983	Deoxy-d-mannitol	$C_6H_{14}O_5$	166.17	1.88
2	5.642	Octane,2,6-dimethyl-	$C_{10}H_{18}$	138.25	1.15
3	5.831	Benzene,1-ethyl-3-methyl-	$C_{11}H_{16}$	148.24	1.10
4	5.887	2-cyclohexane-1-ol,3-methyl-	$CH_3C_6H_8OH$	112.17	1.58

5	6.053	Decane	$C_{10}H_{22}$	142.29	2.40
6	6.464	Cyclohexane,1-methyl-5-(1-methylethylene)-	$C_{10}H_{16}$	136.23	2.69
7	6.531	D-limonene	$C_{10}H_{16}$	136.23	2.82
8	6.886	Benezene,1,3-diethyl-	$C_{10}H_{14}$	134.22	1.27
9	7.020	Propane,1,1,3-triethoxy-	$C_9H_{20}O_3$	176.25	5.12
10	7.142	Benezene,2-ethyl-1,4-dimethyl	$C_{10}H_{14}$	134.22	0.89
11	7.286	Cyclohexane,1-methyl-4(1-methylethylidene)-	$C_{10}H_{18}$	138.25	1.51
12	8.031	4H-Pyrin-4-one,2,3,dihydro-3,5-dihydroxy-6methyl-	$C_6H_8O_4$	144.12	1.11
13	10.031	Napthalene,1-methyl-	$C_{14}H_{16}$	184.28	1.21
14	10.097	2-Methoxy-4-vinolyphenol	$C_9H_{10}O_2$	150.17	1.01
15	10.708	1,2,3-Benezenetriol	$C_6H_6O_3$	126.11	2.89
16	10.775	1,2,3-Benezenetriol	$C_6H_6O_3$	126.11	3.89
17	10.964	1,2,4-Methenoazulene,dcahydro-1,5,5,8a-tetramethyl	$C_{15}H_{24}$	204.3511	1.81
18	11.075	Benezene,1-chloro-4-methoxy-	$C_7H_7C_{lO}$	142.583	1.72
19	11.364	1,4-Methanoazulene,decahydro-	$C_{15}H_{24}$	204.36	2.61

		4,8,8-trimethyl-9-methylene			
20	11.653	d-Mannitol,1,4-anhydro-	$C_6H_{12}O_5$	164.16	7.38
21	12.153	Acenaphthene	$C_{12}H_8$	152.19	2.54
22	13.430	Beta-D-Ribopyranoside,methyl	$C_9H_{18}O_5$	206.24	6.40
23	23.429	Hop-22(29-en-3.beta,-ol)	$C_{30}H_{50}$	410.7	3.60

The GC-MS analysis of methanol extract of *E. hirta* confirmed the presence of palmmitic acid, aldehyde compound, diterpenes, fatty acid ester compound and ester compound. These identified compounds may be responsible for the versatility of action against several infectious diseases . GC-MS identified compound 1, 2, 3-benzenetriol is an aromatic alcohol and reported to have anticancer, antiseptic, antioxidant, antidermattic, fungicide and insecticide activity (Karki *et al*., 2019).

Phytol, a diterpene is one component of the plant, observed to have Antimicrobial, Anti-inflammatory, Anticancer, Diuretic and antiseptic activity. Eicosatrienoic acid, methyl ester the second major component of the plant may have Antimicrobial, Antiinflammatory, Anticancer, Diuretic activity. Therefore the present study validates and strengthens the candidature of Euphorbia hirta plant as a curative of multiple diseases amidst the users of traditional medicine.

GC-MS analysis have been applied by many researchers to identify the possible bioactive components present in the plant extracts and herbal preparations, which might be useful lead compounds for the development of new pharmaceutical drugs. While studying the biological activity of GC-MS found compounds it can be concluded that the plant E. hirta serve as potent source of medicine due to the presence of these phytochemicals

4.2 *In vitro* antioxidant activity of ethanolic extract of *Tridax procumbens and Euphorbia hirta*

4.2.1 DPPH radical scavenging activity

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. DPPH radical scavenging activity of Tridax procumbens and Euphorbia hirta is presented in Figure 13 and Figure 14.

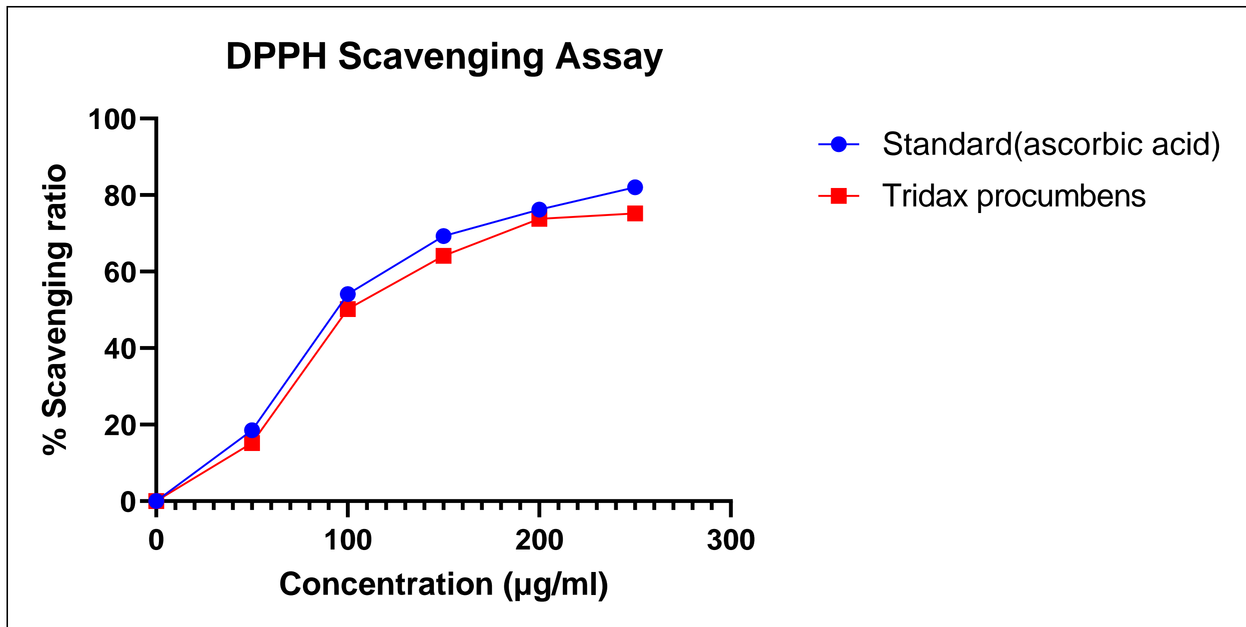


Figure 13: DPPH radical scavenging activity of Tridax procumbens

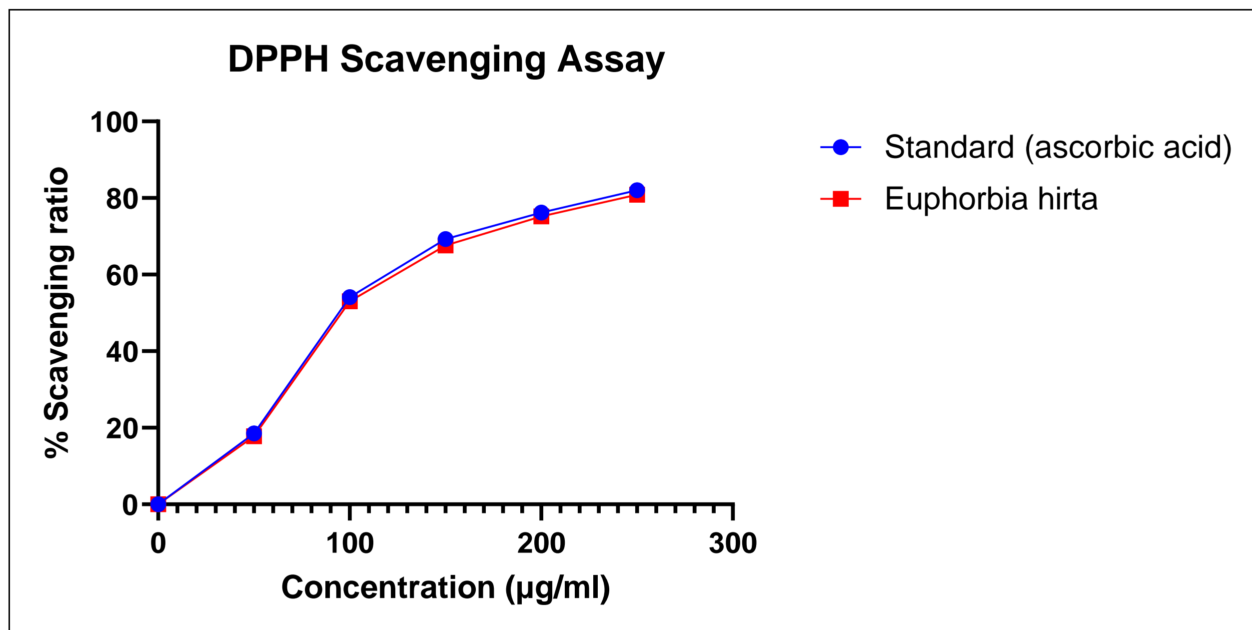


Figure 14: DPPH radical scavenging activity of Euphorbia hirta

DPPH is a stable, nitrogen centered free radical which produces deep purple colour in methanol solution. The principle of this assay is based on the reduction of purple coloured methanolic DPPH solution in the presence of hydrogen donating antioxidants by the formation of yellow coloured diphenyl-picryl hydrazine. As the absorbance decreases the more efficient, the antioxidant activity of the extract in terms of hydrogen atom donating capacity. The more antioxidant present in the extract, the more DPPH reduction will occur. The results suggested that Tridax procumbens showed effect in inhibiting DPPH, reaching up to 75.20 and 82.01% for ethanolic extract and ascorbic acid respectively at the concentration of 250µg/ml and Euphorbia hirta showed significant effect in inhibiting DPPH, reaching up to 80.89 and 82.01% for ethanolic extract and ascorbic acid respectively at the concentration of 250µg/ml respectively.

Ethanol extract of Euphorbia hirta showed greater antioxidant activity and closely to standard as ascorbic acid. The observed antioxidant of extracts may be due to the neutralization of free radicals (DPPH), either transfer of hydrogen atom or by transfer of an electron (Knezevic *et al*., 2011). The scavenging effect can be attributed to the presence of active phytoconstituents in them.

4.2.2 ABTS Scavenging Assay

ABTS scavenging activity of *Tridax procumbens* , *Euphorbia hirta* and standard as ascorbic acid are presented in Figure 15 and Figure 16. The percentage of inhibition of ethanolic extract of Tridax procumbens and ascorbic acid showed 38.20 and 42.41% at the concentration of 250µg/ml. The percentage of inhibition of ethanolic extract of Euphorbia hirta and ascorbic acid showed 41.12 and 42.41% at the concentration of 250µg/ml respectively.

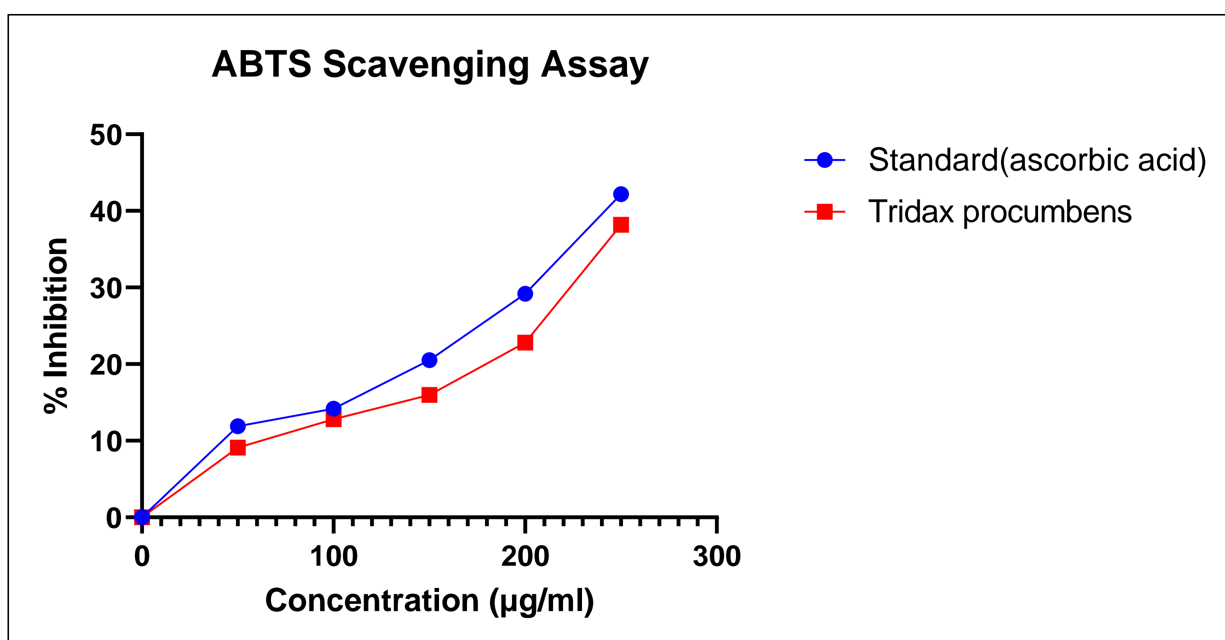


Figure 15: ABTS radical scavenging activity of *Tridax procumbens*

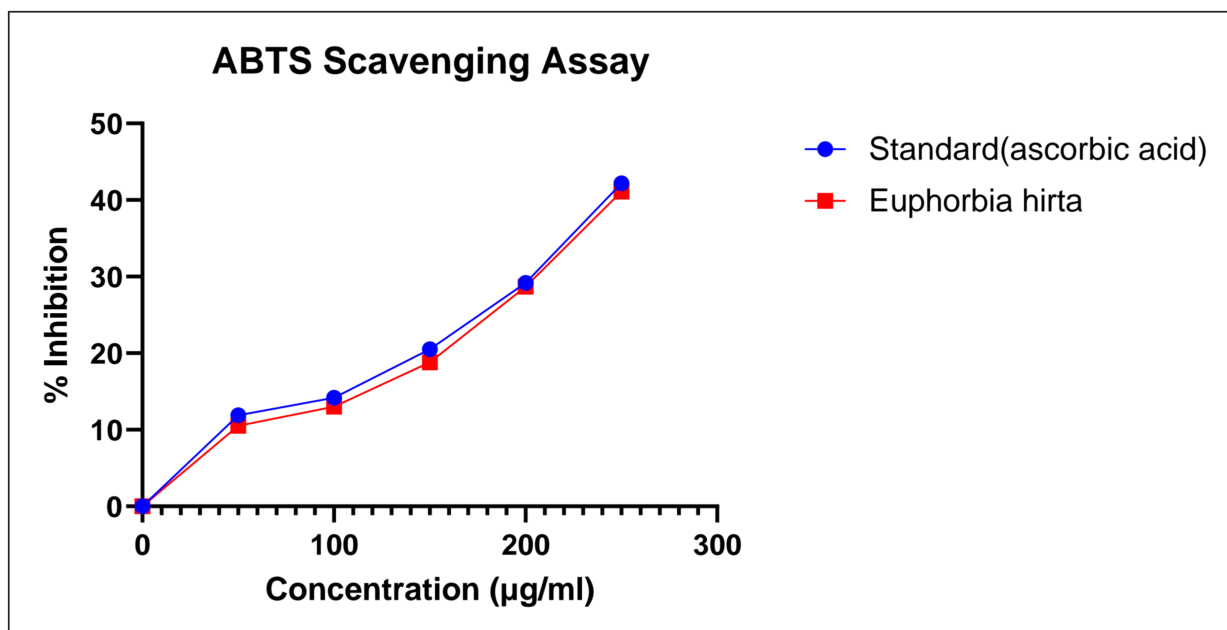


Figure 16: ABTS radical scavenging activity of *Euphorbia hirta*

Ethanolic extract of *Euphorbia hirta* showed superior antioxidant activity and is near to standard as ascorbic acid. It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to the capacity to be donors of hydrogen atoms or electrons and to capture the free radicals.

4.3 Antibacterial activity of ethanolic extract of *Tridax procumbens* and *Euphorbia hirta*

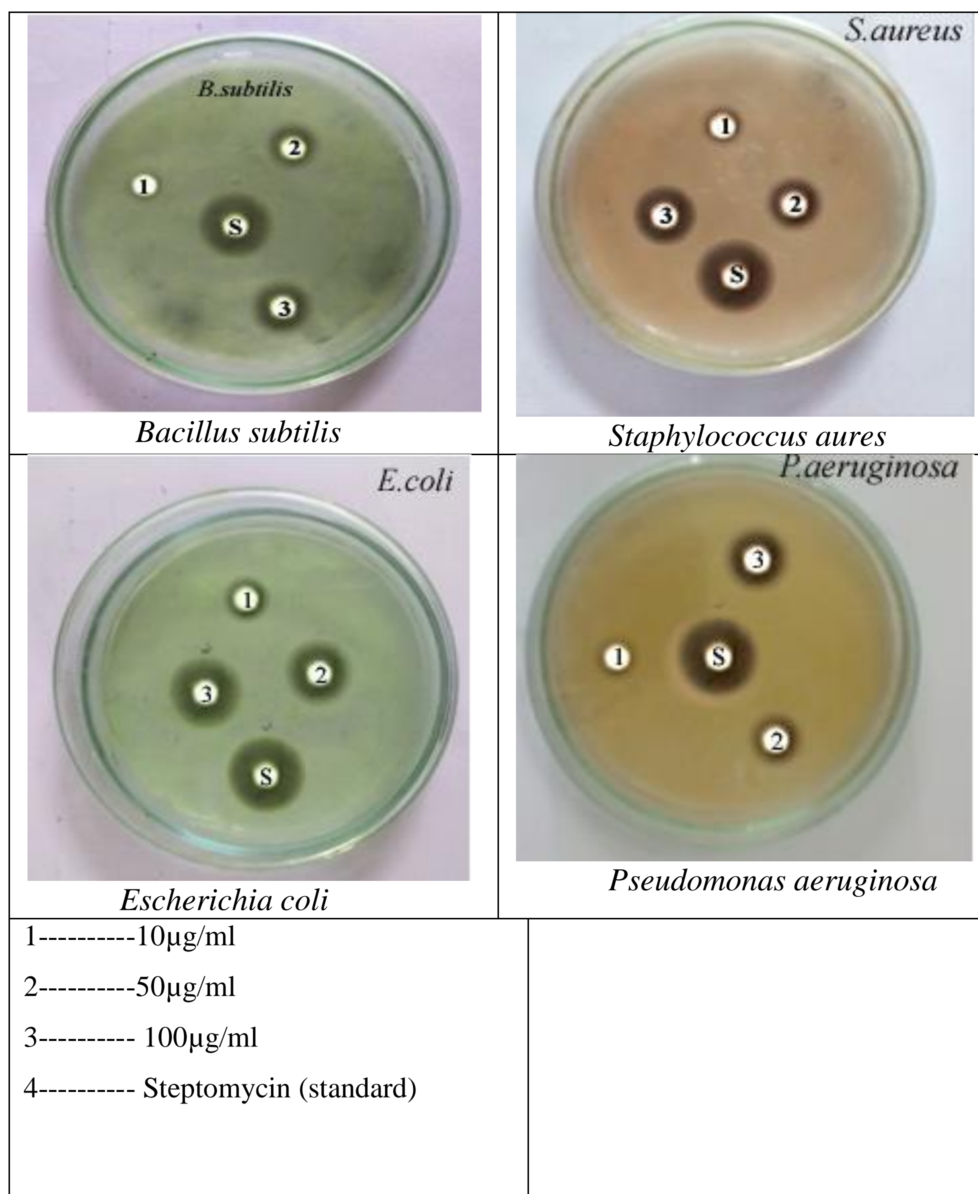
In the present study to investigate the selected pathogens include *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* was used for the assay.

4.3.1Antibacterial activity of *E.hirta*

The antibacterial activity of ethanolic extract of *E.hirta* was carried out by disc diffusion method and the results were represented in Table 10 and Figure 17. Different concentrations (10µl, 50µl and100µl) of ethanolic extract of *E.hirta* extract tested against microbes and compared with standard as streptomycin. The antimicrobial activity was greater in higher concentration while lower in lesser concentration. The highest activity of *E.hirta* extract near to the standard antibiotic as streptomycin.

Table 10: Zone of inhibition (mm) formed by the action of various concentration of ethanolic extract of *E.hirta* on various species of microbial strains

S.No	Strains	Zone of Inhibition(mm)			
		10 µg/ml	50 µg/ml	100 µg/ml	Standard
1	***B.subtilis***	1	3	8	9
2	***S.aureus***	2	4	6	8
3	***E.coli***	3	5	8	11
4	***P.aeruginosa***	1	2	4	9



Bacillus subtilis

Staphylococcus aures

Escherichia coli

Pseudomonas aeruginosa

1----------10µg/ml

2----------50µg/ml

3---------- 100µg/ml

4---------- Steptomycin (standard)

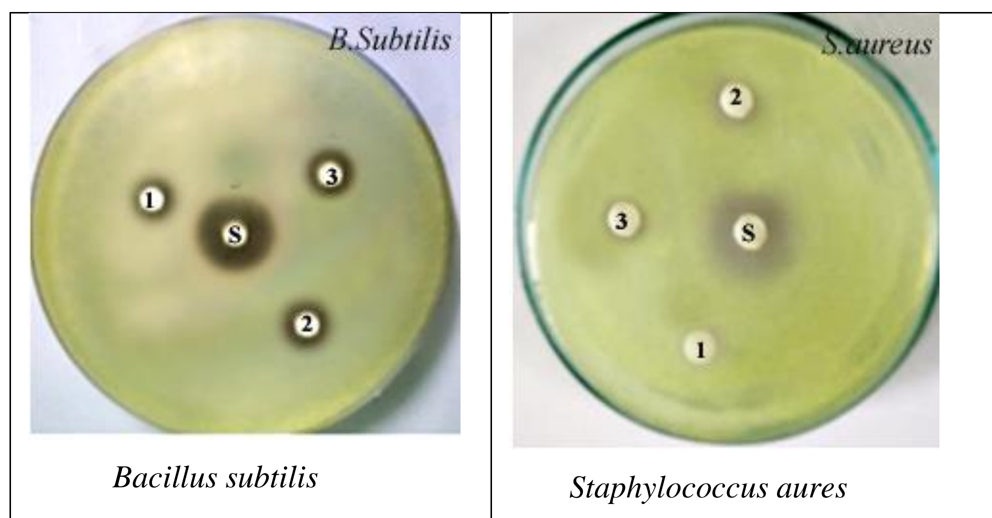
Figure 17: Antibacterial activity of ethanolic extract of *E.hirta* against Pathogens

4.3.1Antibacterial activity of *T.procumbens*

The disc diffusion method was used to test the antibacterial activity of an ethanolic extract of *T.procumbens,* and the results are shown in Table 11 and Figure 18. Different concentrations (10l, 50l, and 100l) of ethanolic extract of *T.procumbens* extract were tested against microbes and compared to streptomycin as a standard. Antimicrobial activity was higher at higher concentrations and lower at lower concentrations. The highest activity of *T.procumbens* extract is comparable to that of streptomycin.

Table 11: Zone of inhibition (mm) formed by the action of various concentration of ethanolic extract of *T.procumbens* on various species of microbial strains

S.No	Strains	Zone of Inhibition(mm)			
		10 µg/ml	50 µg/ml	100 µg/ml	Standard
1	B.subtilis	3	5	8	9
2	***S.aureus***	1	3	5	8
3	***E.coli***	2	5	6	11
4	***P.aeruginosa***	2	3	6	9



Bacillus subtilis *Staphylococcus aures*

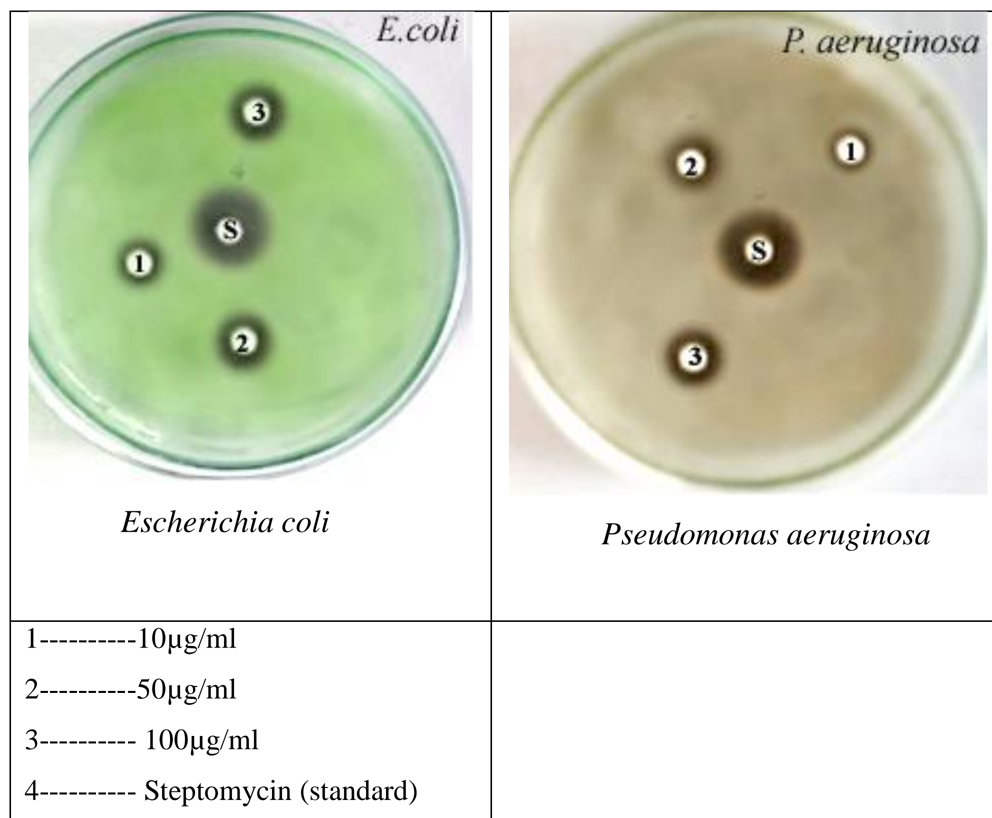


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Figure 18: Antibacterial activity of ethanolic extract of *E.hirta* against Pathogens

From the results observed in this study, it may be concluded that both ethanolic extract of *T.procumbens* and *E.hirta* possess antimicrobial activity, while comparing two plant extracts E.hirta posses high range of activity in the concentration of 100 µg/ml.

4.4 Evaluation of *in vitro* anti-inflammatory activity of the ethanolic extract of *Tridax procumbens and Euphorbia hirta*

The anti- inflammatory activity of the ethanolic extract of *Tridax procumbens* and *Euphorbia hirta* was assessed using protein denaturation method and membrane stabilization methods.

4.4.1 Protein denaturation assay

Protein denaturation is the primary cause of inflammation. The ability of the extract to inhibit protein denaturation was investigated as part of the investigation into the mechanism of

anti-inflammatory activity. The anti-inflammatory activity of *E. hirta* extract may be due to the presence of various compounds such as phytol, fatty acids, 5-HMF and others. *E. hirta* also contains various other compounds with anti-inflammatory potential such as glucosides, tannins, and flavones, which have been shown to inhibit NO production in earlier reports (Sharma *et al.*, 2014).

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and growth factors. Tridax procumbens antagonized antiepithelization and tensile strength depressing effect of dexamethasone (a known healing suppressant agent) without affecting anticontraction and antigranulation action of dexamethasone (Sneha Mundada *et al.*, 2010).

In the present study, *Euphorbia hirta* and *Tridax procumbens* extract was assayed for its in vitro anti-inflammatory activity by employing inhibition of protein denaturation method.

Figure 19: Inhibition of protein denaturation.

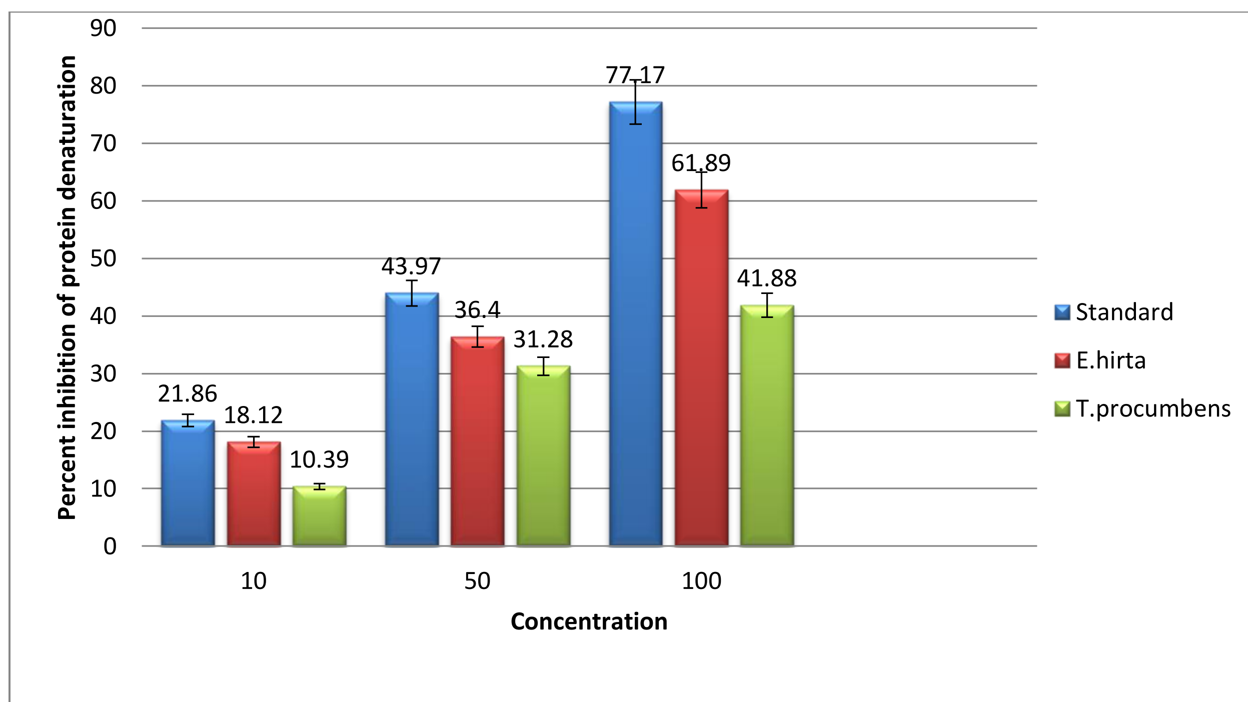

Table 12: Inhibition of Protein Denaturation of *E.hirta* and *T.procumbens*

S.No	Concentration (µg/ml)	% Inhibition of standard	% Inhibition of *E.hirta*	% Inhibition of *T.procumbens*
1	10	21.86±0.84	18.12±3.50	10.69±1.77
2	50	43.30±1.005	36.28±4.80	31.28±1.24
3	100	77.17±8.85	61.89±5.42	41.82±1.53

Values are expressed as mean ± SD

Figure: 19 and Table: 12 show the results of the protein denaturation test. Literature review reports the presence of flavonoids and alkaloids in *Euphorbia hirta* and *Tridax procumbens*. These compounds may be responsible for the anti-inflammatory activity. *In-vitro* anti-inflammatory potential was examined by adopting protein denaturation method, in which Diclofenac sodium was used as a standard. For the results of this study, ethanolic extract of *T.procumbens* and *E.hirta* effectively inhibits the protein denaturation. Pharmacological investigations clearly indicated that anti-inflammatory activity in many plants has been attributed to their flavanoid and phenol contents (Duke, J.A. 1992). Several flavanoids isolated from the medicinal plants have been discovered to possess significant anti-inflammatory activity. From the results obtained in the present study, it may be concluded that ethanolic extract of *Tridax procumbens* possesses moderate anti-inflammatory potential, which is comparable to the synthetic antiinflammatory agent Diclofenac sodium and ethanolic extract of *E.hirta.* While *Euphorbia hirta* shows great activity compared to Tridax procumbens.

4.4.2 Heat induced hemolysis

The anti-inflammatory activity of ethanolic extract of *E.hirta* and *T.procumbens* was assessed by hemolysis induced by heat method using human red blood cells. The result showed the evaluation of membrane stabilizing activities of E.hirta and T.procumbens whole plant ethanolic extract has showed good anti-hemolytic activity when compared to the reference drug, sodium diclofenac (Figure 20 and Table 13).

Figure 20: Inhibition of hemolysis induced by heat.

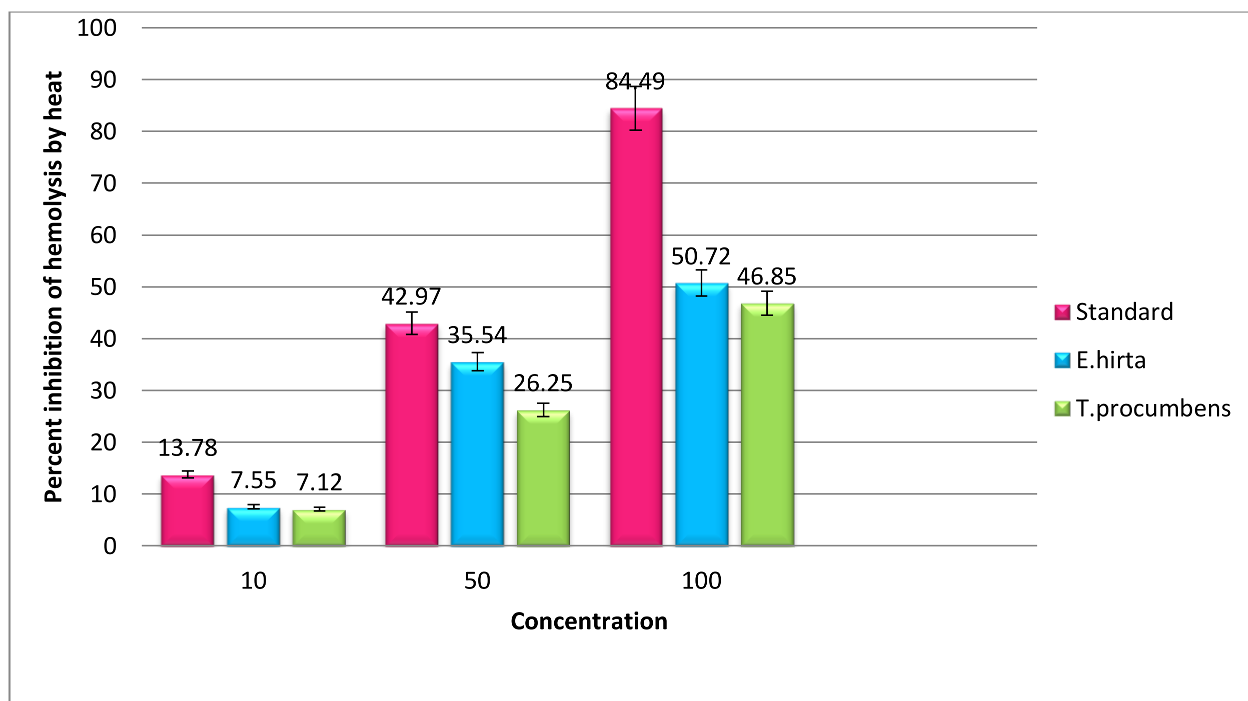

Table 13: Hemolysis induced by heat

S.No	Concentration (µg/ml)	% Inhibition of standard	% Inhibition of *E.hirta*	% Inhibition of *T.procumbens*
1	10	13.788±3.52	7.55±1.87	7.12±0.12
2	50	42.97±2.06	35.54±2.84	26.25±2.13
3	100	84.49±8.62	50.72±4.544	46.85±4.22

In this study, *E.hirta* showed a high anti- hemolytic activity compared to the reference drug, sodium diclofenac and Ethanolic extract of *T.procumbens* against heat- induced hemolysis. As a result, a dose optimization study using different concentrations of the leaf extract is required to determine the optimum dose with the greatest inhibitory effect against protein denaturation.

4.5 FT-IR analysis of the ethanolic extract of *Tridax procumbens* and *Euphorbia hirta*

FT-IR analysis was utilized to identify the functional groups corresponding to the plant extracts. The analysis was carried out in a KBr disk medium interconnected to a spectrometer with a resolution of 4 cm^{-1} between the range of 400 and 4,000 cm^{-1}. The plant extracts and composites were analyzed. The emission spectrum was recorded under a fluorescence spectrophotometer, and the corresponding functional groups identified were reported. The analysis of ethanolic extract of whole plant of *T.procumbens* and *E.hirta* were preformed in functional. However, the molecules of fundamental vibration for molecules predicted numbers of peaks were observed in Figure 21 and Figure 22.

4.5.1 FT-IR analysis of Euphorbia hirta

FT-IR analysis of plant composites revealed the functional groups corresponding to the plant extracts. Figure 21 shows the FT-IR spectrum of the composites. The observation of two alkyl halides is due to the composite formation of plant extracts (Table 14). The FT-IR spectrum of E.hirta plant extract was observed different functional groups peak with different intensity. The band observed at the frequency of 3318 cm-2 depicts the presence of O-H stretch of alcohol functional group. The peak at 2978 cm-2 and 2881 cm-2 shows C-H stretch of alkane. Similarly, absorption at 1259cm-2 and 1059cm-2 is attributed to the C = O stretch of ketones. While 2881 cm-2 and 1451 cm-2 are C-H bend of alkene. 1259 cm-2 and 1059 cm-2 depicts the presence of C-O stretch of ether.

Figure 21: FTIR analysis of ethanolic extract of *Euphorbia hirta*

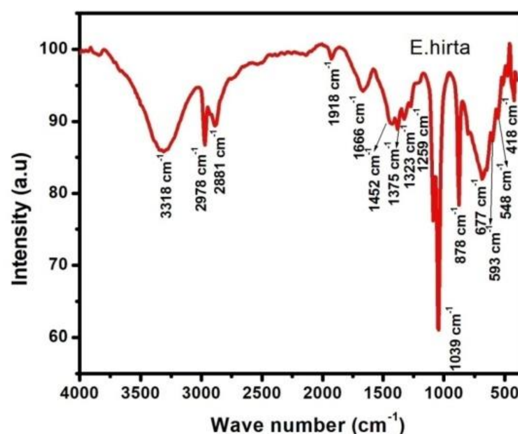

Table 14: FTIR analysis of ethanolic extract of Euphorbia hirta

S.No	Retention time(cm^{-1})	Functional group	
1	3318	O-H	Alcohol
2	2978	C-H	Alkane
3	2881	CH	Alkane
4	1918	-N=C=S	Isothiocyanate
5	1666	C=C	Alkene
6	1452	C-H	Alkane
7	1375	C-H	Alkane
8	1323	C-F	Alkyl halide
9	1259	C-O	Ether
10	1039	C-O	Ether
11	878	C-H	Alkene
12	679	C-Cl	Alkyl halide
13	593	C-Br	Alkyl halide
14	548	C-Br	Alkyl halide
15	418	C-Br	Alkyl halide

4.5.2 FT-IR analysis of *Tridax procumbens*

The broad band at 3323 cm-1 is a characteristic of the stretching vibration of hydrogen bonded hydroxyl groups of the activated carbon. The band at 2972 cm-1 refers to the presence of an aliphatic -CH stretching. The spectrum shows a pronounced band at 1668 cm-1and 1448 cm-

1, that can be assigned to the C=C stretching vibration in the structure of the activated carbon. The band at 1100–1300 cm-1 is usually found with oxidized carbons and has been assigned to C-O stretching in acid. The Figure 22 shows the results and presence of functional groups of ethanolic extract of *T.procumbens.*

Figure 22: FTIR analysis of ethanolic extract of *Tridax procumbens*

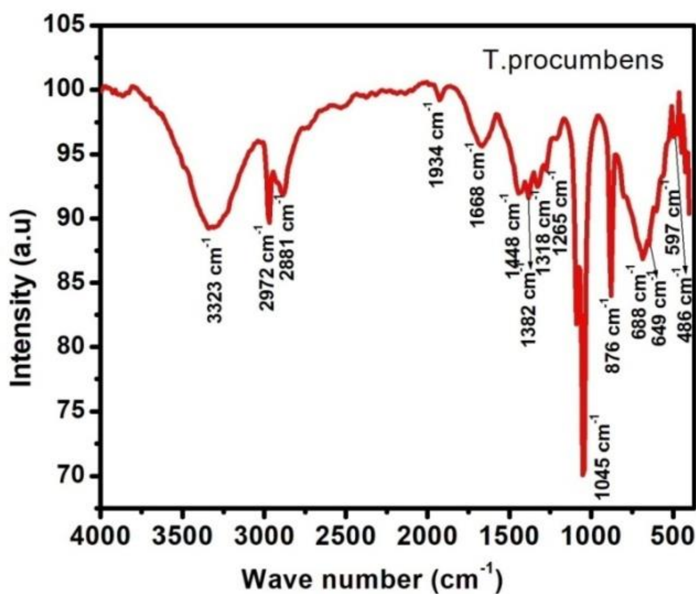

Table 15: FTIR analysis of ethanolic extract of *Tridax procumbens*

S.No	Retention time(cm^{-1})	Functional group	
1	3323	O-H	Alcohol
2	2972	C-H	Alkane
3	2881	CH	Alkane
4	1934	-N=C=S	Isothiocyanate
5	1668	C=C	Alkene
6	1448	C=C	Aromatic

7	1382	C-F	Alkyl halide
8	1318	C=O	Esters
9	1265	C-N	Amine
10	1045	C-O	Ether
11	876	-C=O	Inorganic carbonate
12	688	C-Cl	Alkyl halide
13	649	C-Br	Alkyl halide
14	597	C-Br	Alkyl halide
15	486	C-Br	Alkyl halide

In the present study, the FT-IR spectral analysis was carried out to identify the functional groups present in the plant extracts based on the peak value obtained in the infrared radiation region. The presence of these important functional groups in *T.procumbens* and *E.hirta* unveiled by the FT-IR depicts their medicinal potentials owing to the fact that the functional groups involved are present in most biologically active phytochemicals.

5. SUMMARY AND CONCLUSION

The findings of the phytochemical analysis of selected plants show high amount of tannin, flavonoids, glycosides and steroid which are used for the enhancement of antioxidant and anti-inflammatory activity of plant extact. An in vitro antioxidant, antibacterial, anti-inflammatory activity of ethanolic extract of Tridax procumbens and Euphorbia hirta was recorded and presented here

The qualitative phytochemical analysis of ethanolic extract of different plants are *Euphorbia hirta, Tridax procumbens, Passiflora foetida, Achyranthes aspera and Leucas aspera* contains the presence of alkaloids, saponins, tannin, steroids, terpenoids, phenols and trace amount of glycosides. Based upon the phytochemical screening,Number of peaks visualized in HPLC analysis of five plants and also the Literature review, two plants are selected for further studies they are "***Tridax procumbens and Euphorbia hirta***". The GC-MS analysis of Tridax procumbens and Euphorbia hirta revealed the presence of various compounds like Pentaborane, 2-Hydroxyethyl butyl sulphide, 3-Carene, Propane,1,1,3-triethoxy, Napthalene, Cyclohexane acetic acid, palmmitic acid, aldehyde compound, diterpenes, fatty acid ester compound and ester compounds. While studying the biological activity of GC-MS found compounds it can be concluded that the plant E. hirta serve as potent source of medicine due to the presence of these phytochemicals.

In vitro antioxidant activity of ethanolic extract of T.procumbens and E.hirta tests through DPPH free radical scavenging Reducing power assay and ABTS assay. Experimental evidence showed that ethanolic extract of Euphorbia hirta proved to be a high potent antioxidant activity and closely to standard as ascorbic acid while compare to Tridax procumbens.

Antibacterial study indicated that ethanolic extract of E.hirta is a potent antibacterial agent comparable to that of standard antibiotics, then T.procumbens shows moderate potent antibacterial agent.

Protein denaturation method and membrane stabilization analysis through RBC hemolysis induced by heat , the results confirmed the anti-inflammatory potential of the Euphobia hirta and Tridax procumbens. The ethanolic extract of Tridax procumbens possesses moderate anti-inflammatory potential, which is comparable to the synthetic antiinflammatory

agent Diclofenac sodium and ethanolic extract of E.hirta. While Euphorbia hirta shows great activity compared to Tridax procumbens. . To summarise, the current study found that E.hirta has modulatory effects on inflammatory conditions, which could be investigated further in order to develop a potent therapeutic agent with low toxicity and a higher therapeutic index for various diseases.

The present research work leaves the following future prospects:

- Phytochemical analysis can be performed using various spectral and chromatographic methods to isolate the chemical constituents of the Euphorbia hirta.
- An In vivo anti-inflammatory study carried out to confirm the anti-inflammatory property of the plant by using Human pancreatic cell line
- Another level of In vivo study in animal models to prove the anti-inflammatory property of combination of E.hirta and high concentration of T.procumbens.
- Discovery of novel drug of Pancreatities

BIBLIOGRAPHY

- Ahmad, Sheikh Fayaz; Attia, Sabry M.; Bakheet, Saleh A.; Ashour, Abdelkader E.; Zoheir, Khairy M. A.; Abd-Allah, Adel R. A. (2014). Anti-inflammatory effect of *Euphorbia hirta* in an adjuvant-induced arthritic murine model. Immunological Investigations, 43(3), 197–211. doi:10.3109/08820139.2013.857350
- Ahmad, W., Singh, S., & Kumar, S. (2017). Phytochemical screening and antimicrobial study of Euphorbia hirta extracts. *J Med Plants Stud*, *5*(2), 183-6.
- Akintunde, OG; Oloye, AA; Adetomiwa, AS; Subuloye, WO; Olaiya, CO (2017). *Effects of ethanol extract of <i>Tridax procumbens</i> on spermiogram and reproductive hormones in Wistar rat. Sokoto Journal of Veterinary Sciences, 15(3), 25–* . doi:10.4314/sokjvs.v15i3.4
- Al-Snafi A.E., Pharmacology and therapeutic potential of euphorbia hirta (syn: euphorbia pilulifera)- a review. IOSR Journal of Pharmacy. 2017; 7 (3) : 7-20 .
- Bahramsoltani R, Farzaei MH, Rahimi R. Medicinal plants and their natural components as future drugs for the treatment of burn wounds: An integrative review. Archives of Dermatological Research. 2014;306:601-617. DOI: 10.1007/s00403-014-1474-6
- Barbosa A, Silveira GD, de Menezes I, Neto J, Bitencurt J, Estavam CD, et al. Antidiabetic effect of the Chrysobalanus icaco L. aqueous extract in rats. Journal of Medicinal Food. 2013;16:538-543. DOI: 10.1089/jmf.2012.0084
- Bhosale UA, Yegnanarayan R, Pophale P, Somani R. Effect of aqueous extracts of Achyranthes aspera Linn. on experimental animal model for inflammation. *Anc Sci Life*. 2012;31(4):202-206. doi:10.4103/0257-7941.107362
- Cardoso, Jean Carlos; Oliveira, Maria Eduarda BS de; Cardoso, Fernanda de CI (2019). *Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. Horticultura Brasileira, 37(2), 124–132.* doi:10.1590/s0102-053620190201
- Chirumbolo S. Flavonoids in propolis acting on mast cell-mediated wound healing. Inflammopharmacology. 2012;20:99-102. DOI: 10.1007/s10787-012-0125-9
- Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005;26:343-356. DOI: 10.1016/j.ijantimicag.2005.09.002

- Das SN, Patro VJ, Dinda SC. A review: Ethnobotanical survey of genus Leucas. *Pharmacogn Rev*. 2012;6(12):100-106. doi:10.4103/0973-7847.99943
- Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *Journal of medicinal plants research*, *4*(2), 104-111.
- Dauxois, T., Ruffo, S., Arimondo, E., & Wilkens, M. (2002). Dynamics and thermodynamics of systems with long-range interactions: An introduction. In *Dynamics and thermodynamics of systems with long-range interactions* (pp. 1-19). Springer, Berlin, Heidelberg.
- Djufri, D., & Wardiah, W. (2017). The diversity of undergrowth plants on Acacia nilotica stands as food resources of banteng (Bos javanicus) in Baluran National Park, East Java, Indonesia. *Biodiversitas Journal of Biological Diversity*, *18*(1).
- Duke, J.A. (1992). Handbook of Biologically Active phytochemicals and their activities. Boca, Raton, Florida, USA: CRC Press. [ISBN:0849336708]
- Emon, N.U., Alam, S., Rudra, S. *et al.* Evaluation of pharmacological potentials of the aerial part of *Achyranthes aspera* L.: in vivo, in vitro and in silico approaches. *ADV TRADIT MED (ADTM)* 22, 141–154 (2022). https://doi.org/10.1007/s13596-020-00528-5
- Fern, J., Rohe, A., Soga, K., & Alonso, E. (Eds.). (2019). The Material Point Method for Geotechnical Engineering: A Practical Guide (1st ed.). CRC Press. https://doi.org/10.1201/9780429028090
- Ghosh P., Das P., Mukherjee R., Banik S., Karmakar S., Chatterjee S., Extraction and quantification of pigments from Indian traditional medicinal plants: A comparative study between tree, shrub, and herb. International Journal of Pharmaceutical Sciences and Research.2018;9(7):3052-9 .
- Gibson EL, Wardel J, Watts CJ. Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. Appetite. 1998;31:205-228. DOI: 10.1006/appe.1998.0180
- González-Amaro RM, Martínez-Bernal A, Basurto-Peña F, Vibrans H. Crop and non-crop productivity in a traditional maize agroecosystem of the highland of Mexico. Journal of Ethnobiology and Ethnomedicine. 2009 Dec;5(1):1-9.

- Ikewuchi, C. , Ikewuchi, J. and Ifeanacho, M. (2015) Phytochemical Composition of *Tridax procumbens* Linn Leaves: Potential as a Functional Food. *Food and Nutrition Sciences*, 6, 992-1004. doi: 10.4236/fns.2015.611103.
- Inayatullah, S., Prenzler, P. D., Obied, H. K., Rehman, A. U., & Mirza, B. (2012). Bioprospecting traditional Pakistani medicinal plants for potent antioxidants. *Food chemistry*, *132*(1), 222-229.
- Jain Ankita, Jain Amita. Tridax procumbens (L.): A weed with immense medicinal importance: A review. International Journal of Pharma and Bio Sciences. 2012; 3.(1)114-119
- Jhample, Sowmya B., et al. "Phytochemical screening and in vitro antimicrobial activity of Tridax procumbens L." *Research Journal of Life sciences, Bioinformatics, Pharmaceutical, and Chemical Sciences* 1.1 (2015): 42-51.
- Karki, S., Shrestha, K., Gautam, R., & Narayan, R. (2020). Phytochemical screening, FT-IR and GC-MS analysis of Euphorbia hirta. *Journal of Pharmacognosy and Phytochemistry*, *9*(1), 1883-1889.
- Kausar J., Muthumani D., Hedina A., S S., Anand V., Sivasamy and Anand V: review of the phytochemical and pharmacological activities of Euphorbia hirta Linn. Pharmacognosy Journal.2016;8(4):310-3 .
- Khan, S., Saleem, T., Hassan, W., Khakwani, S., Wahid, N. U. A., Bano, R., & Ahmed, A. Euphorbia Hirta: A rich source of Phytochemicals and Antioxidants.
- Khursheed, A., Jain, V., & Wani, A. R. (2022). Euphorbia hirta as a gold mine of high-value phytochemicals: A comprehensive review of its pharmacological activities and possible role against SARS-CoV-2. *Biomedical Research and Therapy*, *9*(2), 4930-4949.
- Kondawar, V. B. "A comprehensive review on phytochemistry and pharmacological use of Tridax procumbens Linn." *Journal of Pharmacognosy and Phytochemistry* 8.4 (2019): 01-10.
- Kumar S, Malhotra R, Kumar D. Euphorbia hirta: its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacogn Rev. 2010;4:58.
- Kushwaha, P., Yadav, S. S., Singh, V., & Dwivedi, L. K. (2019). Phytochemical screening and GC-MS studies of the methanolic extract of Tridax

procumbens. *International Journal of Pharmaceutical Sciences and Research*, *10*(5), 2492-2496.

- Mathai K. Nutrition in the adult years. In: Mahan LK, Escott-Stump S, editors. Krause's Food, Nutrition, and Diet Therapy. 10th ed. Philadelphia, Pa: W.B. Saunders; 2000. pp. 274-275. https://trove.nla.gov.au/version/44576287
- Mohammad A.B., Mohammad S.H., Mohammad A., Siddika R., Sultana S., Islam R.B., Euphorbia hirta Linn. a wonderful miracle plant of mediterranean region: a review. Journal of Medicinal Plant Studies. 2017; 5 (3) : 170-5 .
- Mohanraj, K., Karthikeyan, B.S., Vivek-Ananth, R.P. *et al.* IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. *Sci Rep* 8, 4329 (2018). https://doi.org/10.1038/s41598-018-22631-z
- Nallella Sreeamula et; al, Ethnobotanico medicine for common human aliments in Nalgonda and Warangal districts of Telangana Andhra Pradesh, India Annals of plant Sciences. 2013; 2(7):220-9
- Panzu P.Z., Inkoto C.L., Ngbolua K.N., Mukeba F.B., Kitadi J.M., Taba K., Review on the phytochemistry, toxicology and bioactivities of Euphorbia hirta L. A potential antisickling medicinal plant species. J. Med. Plant Herb. Ther. Res. 2020; 7 : 8-18 .
- Pascal Noel Mekam, Serena Martini, Julienne Nguefack, Davide Tagliazucchi, Emilio Stefani, Phenolic compounds profile of water and ethanol extracts of Euphorbia hirta L. leaves showing antioxidant and antifungal properties,South African Journal of Botany,Volume 127,(2019)
- Patro, Lingaraj: Medicinal Plants of India: With special reference to Odisha. International Journal of Advance Research and Innovative Ideas in Education. IJARIIE. 2. (5) 121-135 (2016) ISSN (O)-2395-4396
- Pietta PG. Flavonoids as antioxidants. Journal of Natural Products. 2000;63:1035-1042. DOI: 10.1021/np9904509
- Prajapati MS, Patel JB, Modi K, Shah MB. Leucas aspera: A review. *Pharmacogn Rev*. 2010;4(7):85-87. doi:10.4103/0973-7847.65330

- Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82:513-523. DOI: 10.1016/j.fitote.2011.01.018
- R. Priya, M. Nirmala, T. Shankar and A. Malarvizhi, R. Priya, M. Nirmala, T. Shankar and A. Malarvizhi, Phytochemical compounds of Leucas aspera L. (2018)Pharmacological Benefits of Natural Products (ISBN: 978-81-934054-2-0).
- Rahman, M. S., S. Rana, and A. A. Islam. "Antithrombotic and anti-inflammatory activities of leaf methanolic extract of Euphorbia hirta Lin." *Int J Complement Alt Med* 12.4 (2019): 154-62.
- Rajbhoj, B. G. "Phytochemical analysis and ethno medicinal use of *Calotropis procera L* and *Euphorbia hirta L."* Advances in Plant Science: 27.
- Ramandeep Kaur, Navneet Kumar. Phytochemical Composition and in vitro Antioxidant Activity of Leucas aspera Leaves. Research J. Pharm. and Tech 2016; 9(12):2217-2221. doi: 10.5958/0974-360X.2016.00448.0
- Rao N. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pacific Journal of Clinical Nutrition. 2003;12:9-22
- Rick-Leonid Ngoua-Meye-Misso, Cédric Sima-Obiang, Jean De La Croix Ndong, Joseph Privat Ondo, Felix Ovono Abessolo & Louis-Clément Obame-Engonga (2018) Phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of Lophira procera A. Chev. (Ochnaceae) medicinal plant from Gabon, Egyptian Journal of Basic and Applied Sciences, 5:1, 80-86, DOI: 10.1016/j.ejbas.2017.11.003
- Roopan, S. Mohana; Madhumitha, G. (2018). Bioorganic Phase in Natural Food: An Overview Phytochemical and Pharmacological Importance of Plant Secondary Metabolites in Modern Medicine. , 10.1007/978-3-319-74210-6(Chapter 8), 135–156. doi:10.1007/978-3-319-74210-6_8
- S.R Kethamakka, Meena S.D, Jayanti veda (Tridax procumbens) - Unnoticed Medicinal plant by Ayurveda Journal of Indian System of Medicine, 2014;2(1) 6-22
- Salmerón-Manzano, Esther, Jose A. Garrido-Cardenas, and Francisco Manzano-Agugliaro. 2020. "Worldwide Research Trends on Medicinal Plants" *International Journal of Environmental Research and Public Health* 17, no. 10: 3376. https://doi.org/10.3390/ijerph17103376

- Sasikala, V., Saravana, S., & Parimelazhagan, T. (2011). Evaluation of antioxidant potential of different parts of wild edible plant Passiflora foetida L.
- Sharma, Neelesh, Kalpa W. Samarakoon, Rajendra Gyawali, Yang-Ho Park, Sung-Jin Lee, Sung Jong Oh, Tae-Hoon Lee, and Dong Kee Jeong. "Evaluation of the antioxidant, anti-inflammatory, and anticancer activities of Euphorbia hirta ethanolic extract." *Molecules* 19, no. 9 (2014): 14567-14581.
- Singh A M, Rekha N D, Udayashankar A C, Sumana K . Phytochemical screening, antimicrobial, anti-inflammatory and anticancerous activities of ethanol and hexane extracts of Urochloa ramosa. Plant Science Today
- Sofowora, Abayomi et al. "The role and place of medicinal plants in the strategies for disease prevention." *African journal of traditional, complementary, and alternative medicines : AJTCAM* vol. 10,5 210-29. 12 Aug. 2013, doi:10.4314/ajtcam.v10i5.2
- Srivastava R., Soni N., An updated review on phytopharmacological profile of Euphorbia tithymaloides L. Journal of Pharmaceutical Innovation. 2019; 8 : 109-15 .
- Uddin, Md Shahab, Md Masum Billah, and Zannatun Nahar. "Pharmacological actions of Euphorbia hirta: A review." *International Journal of Horticulture and Food Science* 1.1 (2019): 84-89.-euphorbia names and taxonomy

APPENDICES

Free radical scavenging activity of ethanolic extract of *Tridax procumbens and Euphorbia hirta*

Appendix I

Analysis of 2, 2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) Radical Scavenging Activity of *Tridax procumbens and Euphorbia hirta*

Spectrophotometric quantification of the radical scavenging ability of the extract towards DPPH free radicals was carried out by the method of Mensor *et al*., 2001.

Principle

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant activity of plant extracts. DPPH produces violet\purple color in methanol solution and fades to shade of yellow colour in the presence of antioxidants. The assay measures the reducing ability of antioxidants towards the DPPH radical.

Reagents

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

Procedure

In a reaction mixture of 1ml, 20µl corresponding to 1mg of 1 mg of plant extracts were mixed. 0.5ml of DPPH in methanol and 0.48 ml of ethanol. After incubating at room temperature for 30 minutes. The decolourization of the purple color was measured at 520 nm. Methanol alone was used as the Reagents 29 blank and DPPH in methanol, without the extracts, was used as positive control. The radical scavenging activity was calculated as follows,

$$\text{Percent scavenging activity} = 100 - \frac{\text{A518(Sample)} - \text{A518(Blank)}}{\text{A518(Control)}} \times 100$$

Appendix II

Determination of 2, 2′ -Azino-Bis-3-Ethyl Benzthiazoline-6-Sulphonic acid(APTS) radical scavenging assay of *Tridax procumbens and Euphorbia hirta*

The Procedure for ABTS (2, 2′ -azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay was based on the method proposed by (Shirwaikar et al., 2006).

Principle

ABTS (2, 2′ -azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourization assay was employed to assess the radical scavenging effect of the whole plant extract of the plants. ABTS is a chromogen, which damages into a coloured monocation radical form (ABTS+) in the presence of oxidative agent and the ABTS+ has an absorption peak at 750nm. Antioxidants will reduce ABTS+ into its colourless form and the extent of decolourisation corresponds to the present reduction of ABTS+.

Reagents

1. Ethanol
2. ABTS solution (7Mm ABTS with 2.45 mM ammonium persulfate).

The solution was incubated at room temperature for 12- 16 hours before use.

Procedure

The Plant extracts (100µl each) were added to ABTS solution (300µl) and the final volume of each was made up to 1ml with ethanol. The absorbance was read at 745 nm and the percentage inhibition was calculated using the formula,

$$\text{Inhibition}\ (\%) = \frac{\text{A(Control)} - \text{A(Sample)}}{\text{A(Control)}} \times 100$$

Antimicrobial activity of ethanolic extract of *Tridax procumbens and Euphorbia hirta*

Appendix III

Disc Diffusion Assay

Principle

The effectiveness of antimicrobic in sensitivity testing is based on the size of the zone of inhibition that surrounds a disk that has been impregnated with a specific concencentration of the agent, the size of the inoculum, the type of medium and many other factors. Only by taking all these variables into consideration can a reliable method be worked out.

Reagents

1. Mueller-Hinton II agar

Procedure

Disk diffusion/Kibry-Bauer method was adopted in order to carry out the anti-bacterial efficacy of fabatin against the bacterial. The antibacterial activity was determined using the standard disk diffusion method on Mueller Hinton agar (MHA) medium. Small disks (10 mm) permeated with samples at different concentrations (10, 50, and 100 µg/ml) were made to be placed on an MHA agar medium and the plates were incubated at 37ºC for 24 hours. The resulting distance inhibition zones around the disks were then analysed.

Evaluation of in vitro anti-inflammatory activity of the ethanolic extract of *Tridax procumbens* and *Euphorbia hirta*

Appendix IV

Protein denaturation assay

Denaturation of tissue proteins has been evidenced in inflammatory and arthritic diseases. It has also been reported that the denaturation of tissue proteins may result in the production of auto-antigens in certain arthritic diseases (Manukumar and Umesha, 2015). Compounds that prevent protein denaturation could serve as an effective anti-inflammatory drug. Thus, the ethanolic extract of Tridax procumbens and Euphorbia hirta was tested for its inhibiting potential

of protein denaturation. The protein denaturation assay was carried out following the method of Elias and Rao (1998) with some modifications.

Reagents

1. Egg albumin solution (1.0mM)
2. Aspirin (100µg/ml) - Standard drug

Procedure

The test solution, consisting of 100,300 and 1000µl of leaf extract/aspirin, was mixed with 100µl of egg albumin solution madeup to 3ml with PBS (Phosphate Buffer Saline) at pH 6.4 and incubated at 37°C for 15 minutes. The reaction mixture was then kept in waterbath at 70°C for 5 minutes to induce denaturation. The tubes were then cooled and the turbidity was measured at 660 nm using spectrophotometer. Control tube was devoid of test solution. The percent inhibition of denaturation was calculated using the following formula,

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

Appendix V

Heat induced hemolysis Assay

The heat induced hemolysis assay was carried out using the method proposed by Shinde et al. (1999).

Reagents

1. Isosaline (0.85% NaCl, pH 7.2)
2. Aspirin (100µg/ml) - Standard drug

Procedure

The reaction mixture consisted of leaf extract (100,300 and 1000µg/ml) and 1.0ml of 10% RBC suspension. Control tube contained only saline instead of extract. Aspirin was used as the standard drug. The tubes with 2ml extract and 100µl RBC Suspension were incubated in water bath at 54°C for 20 minutes, followed by cooling under running tap water. The contents were then centrifuged at 2500 rpm for 10 minutes at 4ºC and the absorbance of the supernatant was read at 540 nm. The percent inhibition of hemolysis by the leaf extract was calculated using the following formula,

$$\text{Percent inhibition} = \frac{1 - \text{ODsample}}{\text{DOcontrol}} \text{ x } 100$$