

ANTIBACTERIAL, ANTIOXIDANT ACTIVITIES AND PREPARATION OF ANTIBACTERIAL STRIP FROM THE BARK EXTRACT OF *PHYLLANTHUS EMBLICA L.*

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

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APRIL-2021



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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **UBAGARA BLESSCY A (37230091)** and **SHERIN SANGEETHA S (37230081)** who carried out the project entitled **“ANTIBACTERIAL, ANTIOXIDANT ACTIVITIES AND PREPARATION OF ANTIBACTERIAL STRIP FROM THE BARK EXTRACT OF *PHYLLANTHUS EMBLICA L.*”** under my supervision from December 2020 to March 2021.

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ABSTRACT

Medicinal plants have been used as a source of medicine to alleviate diseases. *Phyllanthus emblica* L. is a tropical and subtropical tree, which is Amla, also known as Indian Gooseberry, is a small fruit-bearing deciduous tree of the family Phyllanthaceae, that is indigenous to India. "*Phyllanthus emblica* L. (Amla) is a very special fruit and rich in antioxidants that help in fighting free radicals in the body that are effective in reducing the cell damage which reduces the free radicals that can cause cancer and inflammation disease in the body". ROS-mediated oxidative stress is considered the most important causative factor in the pathology of ALD. The ROS contribute to liver damage through a variety of mechanisms including inactivation of antioxidant enzymes, depletion of reduced glutathione, alteration of the breakdown of fat molecules, and lipid peroxidation. Herbal extracts exhibit a protective mechanism against oxidative stress by enhancing antioxidant enzyme activities and averting GSH depletion. The study aimed to evaluate the antioxidant and antibacterial activities of methanolic and aqueous extracts of barks of *Phyllanthus emblica* L. Antioxidant assays such as DPPH• radical, superoxide radical, Phosphomolybdenum reduction, and Fe 3+ reducing power , Hydroxyl radical scavenging assays were carried out for evaluating antioxidant activities. Antibacterial activity was carried out by the well diffusion method. Anti-inflammatory activity test was carried out by the human blood. GCMS analysis was carried out to identify the active compounds present in the extract. It also showed antioxidant compounds such as flavones and Hexadecanoic acid.

Keywords: Antioxidant, Antibacterial, DPPH radical, Phosphomolybdenum reduction, Superoxide radical, GCMS.

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LIST OF SYMBOLS AND ABBREVIATIONS

DPPH	- 2, 2-diphenyl-1-picrylhydrazyl
H₂SO₄	- Sulfuric acid or Hydrogen sulphate
K₃Fe(CN₆)	- Potassium ferricyanide
mM	- millimolar
µg/mL	- microgram / millilitre
W/V	- weight/volume
UV-Vis	- Ultra violet – Visible spectrophotometer
pH	- Potential of Hydrogen
µL	- microliter
µM	- micro molar
°C	- Degree Celsius
nm	- nanometer
FeSO₄	- Ferrous sulphate
H₂O₂	- Hydrogen peroxide
NBT	- Nitro blue tetrazolium dye
EDTA	- Ethylene diamine tetra acetic acid
SNP	- Sodium nitro prusside
rpm	- revolutions per minute
GAE	- Gallic acid equivalent
QE	- Quercetin equivalent
DMSO	- Dimethyl sulphuric acid

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CHAPTER 1

INTRODUCTION

1.1. HISTORY OF USE OF TRADITIONAL MEDICINAL PLANTS

Before the introduction of chemical medicines, human beings relied on the healing properties of medicinal plants. Medicinal plants has a significant role in treating various diseases. In India, plants have been used for its medicinal properties long before the ancient times. Also among old civic establishments, India has been known to be rich archive of therapeutic plants. Restorative plants are the "spine" of conventional medication, which implies more than 3.3 billion individuals in the less evolved nations use therapeutic plants consistently. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences.

On definition, traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet medical care needs. Albeit current medication may exist one next to the other with such conventional practice, home grown prescriptions have frequently kept up their prominence for verifiable what's more, social reasons. Such items have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged.

1.1.1 The role of herbal medicines in traditional healing

The pharmacological treatment of disease began long ago with the use of herbs (Schulz *et al.*, 2001). Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose.

1.1.2 Traditional Indian medicine

Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment.

1.1.3 Traditional Chinese medicine

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12 000 items used by traditional healers, about 500 are in common use (Li *et al.*, 2000). Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy.

Traditional Chinese medicine is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market.

1.1.4 Traditional Japanese medicine

Many herbal remedies found their way from China into the Japanese systems of traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century (Saito *et al.*, 2000)

1.2 TRADITIONAL USES OF *PHYLLANTHUS EMBLICA L.*

In traditional Indian medicine, dried and fresh fruits of the plant are used. According to belief in Indian mythology, amla is the first tree to be created in the universe. The binomial name of amla is *Phyllanthus emblica L.* It belongs to the family Phyllanthaceae. *Phyllanthus emblica L.* is a slow-growing, deciduous tree famous for its edible fruit. It grows best in areas where the annual daytime temperature are within the range 20-29°C, but can tolerate 14-35°C. Amla can also be called as emblica or Indian gooseberry. Indian gooseberry is a highly nutritious and immunity developing fruit which contains vitamin-C, amino acids and minerals in it. *Phyllanthus emblica L.* is rich in polyphenols and hydrolysable tannin derived compounds that act as antioxidants.

The fruits of amla are widely used for preparing ayurvedic medicines. It has a useful position in degenerative sicknesses like cancer, diabetes, liver treatment, ulcer, anemia, coronary heart disease and is also an essential constituent in hepatoprotective formulation. Amla is also used in treating common cold, fever and prevents from ulcer and dyspepsia.

1.3 USES OF *PHYLLANTHUS EMBLICA L.* BARK

Bark of *Phyllanthus emblica L.* possess strong antioxidant and radical scavenging activities. The bark, roots, leaves and immature fruits of amla are highly valued as a source of tannins. The dry stem bark contains 8-20% tannin. The bark of twigs is usually richer, containing 12-24% tannin. Quickly dried bark contains much more tannin than slowly dried bark.

Being a rich source of tannin (21–33%), the bark of *Phyllanthus emblica L.* is one of the main raw materials used for tannin extract production in China. Most of the pharmacological studies, focused on PE fruit, while the other parts of *Phyllanthus emblica L.* particularly, bark, is yet to study. Bark of *Phyllanthus emblica L.* has wound healing activity. The tannin of the bark belongs to the group of proanthocyanidins giving positive leucodelphinidin on hydrolysis. *Phyllanthus emblica L.* extracts have been demonstrated to have potent tumor repressive properties against a number of cancer types both *in vitro* and *in vivo*.

1.3.1 Other uses

Amla is used in the preparation of inks, acts as a rich ingredient in hair oils and shampoos. Hair fall is a common issue for both men and women and it is caused due to poor dietary habits, pollution, stress, lack of nutrition, hereditary, etc. As amla is rich in carotene, iron and antioxidants it not only rejuvenates the hair but also prevents baldness, hair fall and treats dandruff.

The high tannin content of Indian gooseberry fruit serves as a mordant for fixing dyes in fabrics.

1.4 PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF. PHYLLANTHUS EMBLICA L.

Phytochemical screening is very important in identifying new sources of remedial and industrially essential compounds like alkaloids, tannins, steroids, flavonoids, phenols, glycosides, terpenoids and saponins. Phytochemicals are substances produced by plants and these substances have biological activity. They can be found in fruits, vegetables, plants, grains, beans etc. The phytochemicals present in a medicinal plant can determine the level of potency it has against a disease.

Phyllanthus emblica L. has been used for the anti-inflammatory and anti-pyretic treatments by the rural population. *Phyllanthus emblica* L. has been used for the treatment of several disorders such as the Scurvy, Cancer and Heart diseases. Gram positive microorganisms like *Staphylococcus aureus* gram negative microbes like *Pseudomonas aeruginosa*, and *Escherichia coli* were used and antimicrobial activity of the concentrated extracts was evaluated by the diameter zone of inhibition against the above microorganisms. Plant extracts were active against both gram positive, gram negative Bacteria. The above observation indicates that, *P. emblica* has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

1.4.1 Cancer preventive effects

Phyllanthus emblica L. is the best treatment for ailment traditional ayurvedic medicine.

There is some little scientific investigation that deals with the chemopreventive

activity of *Phyllanthus emblica* L. in laboratory and experimental studies. Only a few studies have speculated the chemopreventive effects of *P.emblica* against liver cancer.

1.5 ANTI-DIABETIC ACTIVITY OF QUERCETIN EXTRACTED FROM *PHYLLANTHUS EMBLICA* L.

The Flavonoids are naturally occurring compounds widely distributed as secondary metabolites in the plant kingdom. It has beneficial clinical properties, such as anti-inflammatory, cardioprotective, antibacterial, and anticancer activities. Quercetin is one of the flavonoids found in the fruits of medicinal plants, such as *Phyllanthus emblica* L.

1.6 ANTIMICROBIAL AGENT OF *PHYLLANTHUS EMBLICA* L.

Aqueous extracts of *Phyllanthus emblica* L. showed positive results against human pathogens, including bacteria, viruses, and fungi. Activity become stronger against gram-positive bacteria.

1.7 THERAPEUTIC POTENTIAL OF *PHYLLANTHUS EMBLICA* L.

1.7.1 Hypopolipidemic

The silent epidemic of ischemic heart disease, type 2 diabetes mellitus (T2DM), hypertension and stroke is been currently faced in India. Indian people develop both diabetes and ischemic heart disease a decade earlier compared to whites. A scientific scrutiny should be undertaken to corroborate the recent evidence that certain medicinal plants possess hypoglycemic, lipid-lowering and immunomodulating properties on account of their rich flavonoid and other glucose-lowering active constituents.

1.7.2 Dermoprotective

Clinical and laboratory studies have identified the benefits of an array of natural ingredients for skin care over the last 20 years. Consequently, these ingredients and compounds are today being developed not only for anti-aging effects, but also for dermatologic disorders. *E.officinalis* extract is known to provide protection for human

dermal fibroblasts against oxidative stress due to its potential antioxidant properties. Hence it is supposed to be essential for natural skin care. *E.officinalis* extract works effectively in mitigative, therapeutic and cosmetic applications through control of collagen metabolism. Thus, *emblica* extract is remarkably better natural active to ascorbic acid, with promising cosmeceutical benefits against photo-aging.

1.7.3 Cardioprotective

The main etiologic factor in atherogenesis is ox-LDL (oxidized Low-density lipoprotein), and antioxidants are accepted as effective treatment for atherosclerosis. Results suggest that *Phyllanthus emblica L.* is effective in inhibiting the progress of atherosclerosis by reducing oxidation injury or by inhibiting ox-LDL-induced vascular smooth muscle cell proliferation, which may be promising mechanisms for treating atherosclerosis.

The effects of the fruit juice obtained from *E. officinalis* on myocardial dysfunction in diabetic rats were explored by Patel *et al.*. Treatment with the fruit juice not only prevented the streptozotocin-induced loss of body weight, increases in water and food intake, increases in serum glucose levels and disturbed lipid profile, but also an increase in serum LDH and creatinine kinase-MB levels, and increased myocardial hypertrophy and cardiomyopathy. There was a decrease in antioxidant enzyme levels (in SOD, reduced GSH and CAT) in diabetic hearts, which could be improved by treatment with fruit juice. Hence, *E. officinalis* fruit juice may be beneficial for the treatment of myocardial damage associated with type 1 diabetes mellitus.

1.7.4 Gastroprotective

Phyllanthus emblica L., besides its food value, can be used as a gastroprotective agent in non-steroidal anti-inflammatory drug-induced gastropathy. It has been suggested that the antioxidative property of *Phyllanthus emblica L.* is the key to its therapeutic effect. Additionally, pretreatment with the butanol extract of the water fraction of *Phyllanthus emblica L.* fruits exhibited increased secretion of gastric mucus and hexosamine in the indomethacin-induced ulceration of rats. The ulcer protective potential of methanolic extract of *E. officinalis* was also assessed in different acute gastric ulcer models in rats. The results showed that *E. officinalis* had

a significant ulcer protective as well as healing effects which might be due to its effects both on offensive and defensive mucosal factors.

1.7.5 Cerebroprotective

There are very limited therapeutic options for treating multi-factorial ailments and disorders of cognition such as Alzheimer's disease. However, certain plant-derived agents, including, for example, galantamine and rivastigmine are finding an application in modern medicine. These plants are used both in herbal as well as conventional medicine and offer benefits that pharmaceutical drugs lack.

The upregulated complement system plays a damaging role in disorders of the central nervous system (CNS). The classical along with the alternate pathways are two major pathways activated in neuroinflammatory disorders such as Alzheimer's disease, multiple sclerosis, traumatic brain injury, spinal cord injury, HIV-associated dementia and Parkinson's disease. Numerous active ingredients of herbal origin are found to have complement-inhibitory activity. These herbal ingredients along with other anti-inflammatory roles might be useful in treating neuroinflammation associated with CNS disorders. Several stress-induced diseases, including the process of aging, may be related to accumulation of oxidative free radicals in different tissues.

CHAPTER 2

REVIEW OF LITERATURE

2.1 PHYTOCHEMISTRY, PHARMACOLOGY AND MEDICINAL PROPERTIES OF *PHYLLANTHUS EMBLICA* L.

Bhakta Prasad *et al.*, 2014 has reported that all pieces of the plant are utilized for restorative purposes, particularly the natural product, which has been utilized in Ayurveda as a strong Rasayana (rejuvenator). *P. emblica* contains phytochemicals including fixed oils, phosphatides, essential oils, tannins, minerals, vitamins, amino acids, fatty acids, glycosides, etc. and there are various pharmaceutical potential of *P. emblica* including antimicrobial, antioxidant, anti-inflammatory, analgesic and antipyretic, adaptogenic, hepatoprotective, antitumor and antiulcerogenic activities either in combined formulation or *P.emblica* alone.

2.2 ANTICANCER PROPERTY

Michael Witcher *et al.*, 2015 has investigated that there is a wealth of information emanating from both *in vitro* and *in vivo* studies indicating fruit extract of the *Phyllanthus emblica* L. tree, commonly referred to as Indian Gooseberries, has potent anticancer properties. The bioactivity in this extract is thought to be principally mediated by polyphenols, especially tannins and flavonoids. It remains unclear how polyphenols from *Phyllanthus emblica* L. can incorporate both cancer-preventative and antitumor properties.

2.3 ANTIOXIDANT ACTIVITY

Liu, X *et al.*, 2008 has evaluated that the antioxidant activities are performed by *in vitro* experiments using scavenging assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, hydroxyl radicals and superoxide anion, chelating ability of ferrous ion, reducing power, and inhibition capability of Fe (II)-induced lipid peroxidation. Total phenolic content was ranged from 81.5 to 120.9 mg gallic acid equivalents (GAE)/g and the flavanoid content was varied from 20.3 to 38.7 mg quercetin equivalents (QE)/g

Prabhu Srinivasan *et al.*, 2018 has assessed the molecular interactions of the ligands, quercetin, gallic acid, and metformin with various diabetes mellitus-related

protein targets, such as glycogen phosphorylase and peroxisome proliferator-activated receptor gamma were assessed. It was revealed that quercetin possesses good binding affinity to both targets. Quercetin is a major constituent of methanolic extracts of *Phyllanthus emblica* L. fruit.

2.4 ANTIBACTERIAL ACTIVITY

D A Dhale *et al.*, 2011 has reported that plant extracts were dynamic against both gram positive and gram negative microscopic organisms.. The antimicrobial activities of the extracts at different doses (10mg and 20mg/ml) of the leaves and fruits were evaluated against some common pathogenic bacteria using agar disc diffusion method. Gram positive microorganisms like *Staphylococcus aureus*, gram negative microscopic organisms like *Pseudomonas aeruginosa*, and *Escherichia coli* were used and antimicrobial activity of the concentrated extracts was evaluated by the diameter zone of inhibition against the above microorganisms. The above observation indicates that, *P. emblica* has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

2.5 IDENTIFICATION OF VOLATILE COMPONENTS IN *PHYLLANTHUS EMBLICA* L.

The volatile components and *in vitro* antimicrobial activities of Emblica (*Phyllanthus emblica* L.) essential oils (EOs) obtained by hydrodistillation (HD-EO) and supercritical fluid extraction (SFE-EO) were investigated. The compositions of volatile compounds in these oils were tentatively determined by gas chromatography-mass spectrometry. The antimicrobial activities of these two extracts were investigated with microbiological tests against Gram-positive and Gram-negative bacteria and three pathogenic fungi. The main components of both oils were β -caryophyllene, β -bourbonene, 1-octen-3-ol, thymol, and methyleugenol.

2.6 CHEMICAL CONSTITUENTS OF *PHYLLANTHUS EMBLICA* L.

Phytochemical investigations on *Phyllanthus emblica* L. have resulted in the isolation of the two new flavonoids, kaempferol-3-O- α -L-(6"-methyl)-rhamnopyranoside and kaempferol-3-O- α -L-(6"-ethyl)-rhamnopyranoside. Their structures were determined on the basis of extensive spectroscopic studies including 2D-NMR experiments.

Emblica officinalis is a small to medium sized deciduous tree belonging to family Euphorbiaceae and 8-18 meters stature with meager light dim bark shedding in little slight unpredictable chips, leaves are basic, subsessile, firmly set along the branchlets, light green resembling pinnate leaves; blossoms are greenish yellow, in axillary fascicles, unisexual, guys various on short thin pedicels, females few, subsessile, ovary 3-celled; organic products globose, beefy, light yellow with six dark vertical wrinkles encasing six trigonous seeds in 2-cultivated 3 crustaceous cocci1 found all through India, the ocean coast regions and on slope slants up to 200 meters, also cultivated in plains. They are helpful in vitiated states of tridosha, diabetes, hack, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, fart, hyperacidity, peptic ulcer, erysipelas, skin sicknesses, uncleanliness, haematogenesis, irritations, weakness, gauntness, hepatopathy, jaundice, strangury, loose bowels, diarrhea, hemorrhages, leucorrhoea, menorrhagia, heart problems, irregular fevers and grayness of hair.

2.7. ANTIDIABETIC

Akhtar *et al.*, 2011 evaluated the anti-hyperglycemic and lipid-lowering properties of *E. officinalis* fruit in normal as well as diabetic human volunteers. The results indicated a significant decrease in fasting and 2 h post-prandial blood glucose levels on the 21st day in both normal and diabetic subjects receiving 1, 2 or 3 g *E. officinalis* powder per day as compared with their baseline values. Significant decreases were also observed in TC and triglycerides in both normal and diabetic volunteers on day 21 that were given either 2 or 3 g *E. officinalis* powder per day. Both normal and diabetic volunteers receiving 2 or 3 g *E. officinalis* powder significantly improved high-density lipoprotein-cholesterol and lowered low-density lipoprotein-cholesterol levels.

2.8 ANTIDERMATITIS

Draeos *et al.*, 2010 in a double-blind study examined the skin lightening ability of a topical formulation containing kojic acid, *E. officinalis* extract, and glycolic acid compared with prescription generic hydroquinone cream 4%. Study results demonstrated efficacy parity between the study product and hydroquinone 4%. Thus,

this novel skin lightening preparation is an alternative to hydroquinone 4% for participants with mild to moderate facial dyschromia.

2.9 PLANT PROFILE

Botanical name: *Phyllanthus emblica* L.

Synonym : *Phyllanthus mairei* H.Lêv. *Emblica officinalis* Gaertn.

Family name : Phyllanthaceae

Taxonomical Classification:

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Euphorbiales
- Family: Euphorbiaceae
- Genus: *Phyllanthus*
- Species: *Phyllanthus emblica*

Vernacular names:

English	: Gooseberry, Emblica, Myrobalan
Tamil	: Nellikai
Hindi	: Amla
Sanskrit	: Amalaki, Dhatriphala
Malayalam	: Nelli
Telugu	: Rasi usiri
Oriya	: Anlaa
Punjabi	: Aula

Description:

Phyllanthus emblica L. is a small to medium sized tree with curved trunk and spreading branches. It reaches a height of upto 18m upon maturity and it has simple, light green, pennet leaves. It has greenish yellow flowers. The flowers are pollinated by bees and the plant is not self-fertile. The fruit is nearly spherical, light greenish yellow and hard on appearance, with 6 vertical stripes or furrows. The taste of Indian gooseberry is sour, bitter and astringent, and it is quite fibrous.

Fruits:

The fruit is an extremely rich source of ascorbic acid, 100g of juice containing 600-1300mg, sometimes even more. The tannins of the fruit belong to the group of gallotannins and ellagitannins, giving on hydrolysis large amounts of gallic acid, small amount of ellagic acid and glucose. The tannin in the fruit prevents or retards the oxidation of the vitamin, so that the fruits can be preserved in salt solution or as dry powder while still maintaining their antiscorbutic value. Many of the medicinal applications of the fruits can be ascribed to the presence of ascorbic acid and to the astringent action of the tannins. Fruit extracts has shown antioxidant and antitumor activities in in-vitro and animal tests. Phyllemblin has also been isolated from the amla fruits; It potentiates the action of adrenaline, has a mild depressant action on the central nervous system and has spasmolytic properties. Emblica fruits are also a richer source of pectin.

Leaf:

Leaf extracts have shown inhibitory activity on human leukocytes and platelets, which at least partly confirms their anti-inflammatory and antipyretic properties. Leaves of emblica may yield 22-28% tannin.

Habitat and Cultivation:

It is a potential crop which grows in marginal soils and various kinds of degraded lands such as salt-affected soils, dry and semi-dry regions. Found wild and cultivated in all parts of India, especially the south, to an altitude of 1500 m. Although it is native to India, also grows in tropical and subtropical regions of Pakistan, Uzbekistan, Sri Lanka, South East Asia, China, Malaysia, Indonesia, Peninsula, Burma and Ceylon.

CHAPTER 3

AIM AND SCOPE

AIM:

To investigate antibacterial, antioxidant activities and preparation of antibacterial strip from the bark extract of *Phyllanthus emblica L.*

SCOPE:

Phyllanthus emblica L. has been used as a medicinal plant for many centuries, as it has proved its fighting capabilities against numerous diseases. As there is no detailed work so far done in the Amla bark extract, different tests were performed (phytochemical, antibacterial, anti-inflammatory) to know the potential of bark of *Phyllanthus emblica L.* for human welfare.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Collection of bark and preparation of extract

The *Phyllanthus emblica* barks were gathered at Guindy, Chennai, Tamilnadu, India. The barks were hacked into little pieces. Around 50 g of bark was absorbed methanol for 72 h and the supernatant was sifted by channel paper. At that point the supernatant was consolidated in a hot plate at 50°C, which yields ruddy earthy coloured sticky concentrate. Around 25 g of bark was absorbed refined water and bubbled in cooker. The rosy earthy coloured supernatant fluid was sifted by channel paper, dense in a hot plate at 50°C, which yields earthy sticky concentrate and stored in a refrigerator for further analysis.



Figure 4.1.1: Methanolic and Aqueous bark extract of *Phyllanthus emblica* L.

4.1.2 Chemicals and reagents

Antioxidant activity:

1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, Trichloroacetic acid (TCA), sodium phosphate, ammonium molybdate, sulphuric acid, phosphate buffer, potassium ferricyanide, FeCl_3 , HCl , H_2SO_4 , H_2O_2 , FeSO_4 , Salicylic acid, Ethylene

diamine tetra acetic acid (EDTA), Nitro blue tetrazolium dye (NBT), Riboflavin, superoxide buffer.

Antibacterial activity:

Peptone, NaCl, yeast extract, Agar, Dimethyl sulphoxide (DMSO), Tetracyclin.

Anti-inflammatory:

Saline, antihemolytic buffer.

4.1.3 Equipments

- Weighing Balance
- Centrifuge
- Incubator
- Autoclave
- Refrigerator
- UV- spectrophotometer
- Laminar airflow chamber
- UV illuminator

4.2 METHODS

4.2.1 *In vitro* antioxidant assays

4.2.1.1 DPPH radical scavenging assay

The cell reinforcement action of methanolic bark concentrate of *Phyllanthus emblica* L. was estimated based on stable DPPH free extreme decrease strategy. One mL of 0.1 mM DPPH arrangement in methanol was blended in with 1 mL of different fixations (20-120µg/mL) of bark extricate. The blend was then permitted to represent 30 minutes at room temperature. 1 mL methanol and 1 ml of DPPH arrangement was utilized as the control. The abatement in absorbance was estimated utilizing UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH radical inhibition} = [\text{Control} - \text{Sample} / \text{Control}] \times 100$$

4.2.1.2 Ferric (Fe^{3+}) reducing power assay

The reducing power of methanolic bark extract of *Phyllanthus emblica* L. was determined by Fe^{3+} reduction method. One mL of bark extract of different concentrations (20-120 $\mu\text{g/mL}$) was mixed with 1 mL of phosphate buffer (pH 6.6 - 0.2M) and 1 mL of 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$] solution. The mixtures were then incubated at 50°C in water bath for 20 min. 500 μL of 10% (w/v) trichloro acetic acid was added to each mixture. Then 100 μL of 0.1% (w/v) freshly prepared $FeCl_3$ solution was added and the absorbance was measured at 700nm in UV-Vis spectrophotometer. The percentage of reduction was calculated as:

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

4.2.1.3 Phosphomolybdenum reduction assay

The antioxidant capacity of methanolic bark extract of *Phyllanthus emblica* L. The bark extract with concentrations ranging from (20-120 $\mu\text{g/mL}$) was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm in UV-Vis spectrophotometer. The percentage of reduction was calculated as:

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

4.2.1.4 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extractives was determined by the method of (Halliwell *et al.*, 1989). The scavenging activity of hydroxyl radicals was measured with Fenton reaction. 1 mL of bark extract of different concentrations (20-120 $\mu\text{g/mL}$) was mixed with 1 mL of $FeSO_4$ (6 mM) and 1 mL of H_2O_2 (2.5 μM). The mixtures were then incubated at room temperature for 10 min. 1 mL salicylic acid solution (6mM) was added to each mixture. The absorbance was measured at 500 nm in UV-Vis spectrophotometer. The percentage of Inhibition was calculated as:

$$\% \text{ Inhibition of OH radical} = [\text{Control} - \text{Sample} / \text{Control}] \times 100$$

4.2.1.5 Superoxide radical scavenging assay

Superoxide radical scavenging activity was generated from the photo reduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)¹¹. Various concentrations of bark extract (20 - 120 µg/mL) and 1 mL superoxide buffer were added in each test tube. 200 µL of riboflavin (1.5 mM), 100 µL of EDTA (12 mM) and 150 µL of NBT (50 mM) reagents were added and kept in UV illuminator for 90 seconds to incubate. Absorbance was measured at 590 nm in UV-Vis spectrophotometer. The percentage of inhibition was calculated as:

$$\% \text{ Inhibition of superoxide radical} = [\text{Control} - \text{Sample} / \text{Control}] \times 100$$

4.2.2 Antibacterial activity

4.2.2.1 Microorganisms

Microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* were stored in a refrigerator at the microbiology laboratory and used for the antimicrobial study.

4.2.2.2 Aseptic conditions

The aseptic chamber which consists of wooden box (1.3m × 1.6m × 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from lamp).

4.2.2.3 Agar well diffusion method

Nutrient broth agar medium prepared according to the standard methods (peptone- 0.5 g, yeast extract-0.3 g, NaCl-0.5 g, distilled water- 100mL, agar- 2 g). The required amount of ingredients was weighed and suspended in the required volume of 100 mL of distilled water and afterward autoclaved at 15 lbs and 121 C for 15 min. The hot medium was poured in sterile petriplates which were kept in the aseptic laminar chamber. The medium was permitted to cement for 15 min.

Antibacterial activity of methanolic and aqueous extract of *Phyllanthus emblica L.* was done utilizing agar well dissemination strategy. The set supplement agar in the petriplates was vaccinated by apportioning the inoculum utilizing disinfected q-tips

which are recently inundated in the inoculum containing test cylinder and spread equitably onto 17 the cemented agar medium. Five wells were made in each plate with the assistance of a clean well-drill of 8 mm breadth. The root tuber extract was then poured into each well containing 250, 375, and 500 µg/mL concentrations. Every one of the plates with extract stacked wells were hatched at 37°C for 24 h and the antibacterial movement was surveyed by estimating the breadth of the hindrance zone conformed to the well. Antibiotic medication (tetracycline) (25 µg) was utilized as the positive control.

4.2.3 Qualitative phytochemical analysis

The different qualitative chemical tests were performed for establishing the profile of methanol extract of bark of *Phyllanthus emblica L.* for its synthetic composition. The accompanying tests were performed to recognize different phytoconstituents present in the methanol extract of *Phyllanthus emblica L.*

1. Detection of alkaloids

Solvents free extract (200 µl) was stirred with few mL of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloids reagents as follows.

A. Hager's test

To a few mL of filtrate, a few mL of Hager's reagents (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicates the test position.

2. Detection of glycosides

The extract (200 µl) was dissolved in 100 µl of methanol. Few drops of SNP (sodium nitro prusside) was mixed with few drops of pyridine solution. Few drops of conc. H₂SO₄ added further. Red brown colour indicates the presence of glycosides.

3. Detection of saponins

A. Foam test

The extract (200 µl) was diluted with distilled water and made upto 5 mL. The suspension was shaken in a graduated cylinder for 15 min. A 0.5 cm layer of foam indicated the presence of saponins.

4. Detection of phenolic compounds

A. Ferric chloride test

The extract (200 µl) was dissolved with few drops of neutral (200 µl) ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

B. Detection of Flavonoid

The extract (200 µl) and few drops of diluted NaOH solution was added and mixed well. Lemon yellow colour indicated the presence of flavonoids compounds.

C. Test for Terpenoids

The extract (200 µl) and few drops of methanol, 1 mL of chloroform was added and mixed well. Add a little of conc. H_2SO_4 was carefully added to form a reddish brown layer.

D. Detection of Steroids

The extract (200 µl) and few drops of acetic anhydride was poured followed by few drops of conc. H_2SO_4 added along the sides of the test tube carefully. A mixture of various shades of colour showed the presence of steroids.

5. Detection of tannins

The extract (200 µl) and few drops of H_2O was dissolved with few drops of lead acetate solution. A white precipitate indicates the presence of tannins.

4.2.4 Quantitative phytochemical analysis

- Determination of total Phenols

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

- Determination of total Flavonoids

Five hundred μL of methanolic and aqueous extract of *Phyllanthus emblica* L. (1 mg/mL) was mixed with 1 mL of methanol and 1 mL of 5% (w/v) sodium nitrite solution. Then, 1 mL of 10% (w/v) aluminium chloride solution was added, shaken well, followed by 100 μL of 1 M NaOH solution was added. The absorbance was measured at 510 nm and the result was expressed as ($\mu\text{g}/\text{mg}$ of extract) quercetin equivalent, which is a common reference compound.

4.2.5 Anti-inflammatory activity

First step to perform anti-inflammatory activity is to prepare saline solution and blood saline. Saline solution is prepared by dissolving 0.9 g NaCl in 100 ml distilled water. Blood saline preparation is done by adding 1ml of human blood in 9 ml of saline solution. After adding, centrifuge the solution for 5 minutes at 3000 rpm. After centrifuging remove the supernatant and add 10 ml of saline and centrifuge it again for 5 minutes at 3000 rpm. After centrifugation remove the supernatant, collect the pellet and add 10 ml of saline. Now the blood saline is prepared. Further to proceed with anti-inflammatory test various concentrations of bark extract (20-120 was combined with 2 ml of anti-haemolytic buffer solution was added to make up the total volume followed by 200 μL of blood saline. Incubate at 54°C for 20 minutes and the absorbance was measured at 560 nm using UV-Vis spectrophotometer. The percentage of inhibition was calculated as:

$$\% \text{ of inhibition} = [\text{Control} - \text{Sample} / \text{Control}] \times 100$$

4.2.6 Thin layer chromatography

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups.

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

4.2.7 Gas chromatography–Mass Spectrometry (GC–MS)

Gas Chromatography: A Shimadzu GC-2010 Plus gas chromatography was equipped with a straight deactivated 2 mm direct injector linear and a 15m Alltech EC-5 column (250 μ I.D., 0.25 μ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2 minutes, then ramp at 20°C per minute to 450°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

Mass Spectrum: A direct connection with capillary column metal quadupole mass filter prerod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of

5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan.

4.2.8 Preparation of antibacterial strip

The method to be described is based on the observation that the diffusion of the *Phyllanthus emblica L.* bark extract from impregnated gada cloth onto the underlying medium is achieved in 12 h time. Experiments were performed to show the inhibition zone of the period of contact of the antibiotic strip with the medium. The concentration of bark extract used to impregnate into gada cloth was 100 µg/mL.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 ANTIOXIDANT ACTIVITY

5.1.1 DPPH radical scavenging activity

DPPH (α , α -diphenyl- β -picrylhydrazyl) is a stable free radical scavenging activity was assessed according to (Blois et al., 1958) which makes it an accurate and economical method for radical scavenging activity of antioxidants. The DPPH has nitrogen atom whose odd electron gets reduced by the hydrogen atom present in the antioxidant correspondingly hydrazine. DPPH is widely used antioxidant assay because it can measure both lipid soluble and water soluble antioxidants and the results are highly reproducible when compared to other assays. The maximum DPPH radical scavenging activity of methanol and aqueous extracts were 84.04% and 84.92% at 120 $\mu\text{g/mL}$ concentration (Table 5.1.1) and their IC_{50} were 15.78 $\mu\text{g/mL}$ (methanol) and 32.70 $\mu\text{g/mL}$ (aqueous) concentration.

Table 5.1.1: DPPH radical scavenging activity of methanol and aqueous extract of *Phyllanthus emblica* L.

S. NO	Concentration $\mu\text{g/mL}$	% OF INHIBITION	
		METHANOL Extract	AQUEOUS Extract
1	20	63.39	25.79
2	40	82.16	61.15
3	60	82.63	70.14
4	80	83.05	72.75
5	100	84.04	77.10
6	120	84.04	84.92

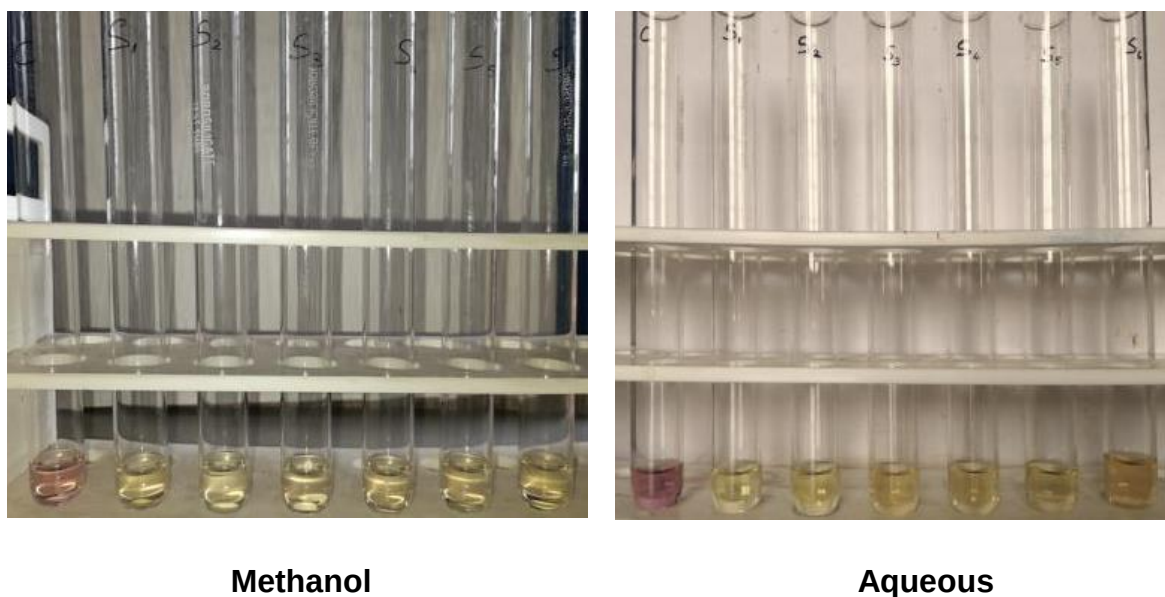
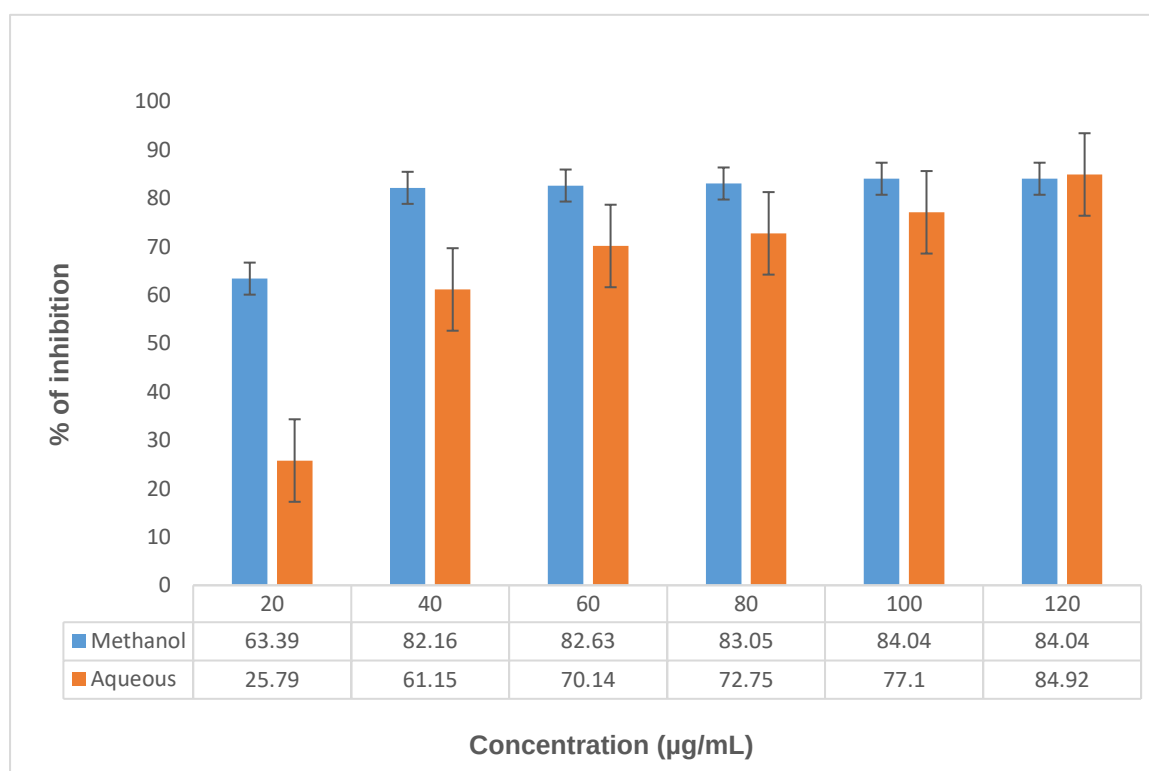


Figure 5.1.1: DPPH radical scavenging activity of methanol and aqueous extract of *Phyllanthus emblica L.*



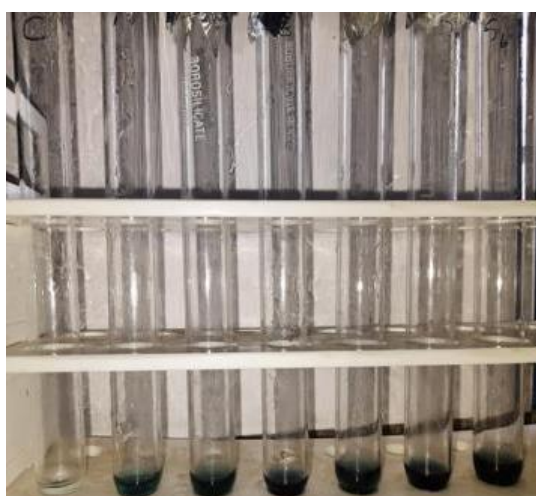
Graph 5.1.1: DPPH radical scavenging activity of methanolic and aqueous extract of *Phyllanthus emblica L.*

5.1.2 Phosphomolybdenum reduction activity

This assay allows quantitative estimation of rate of reduction among oxidant, antioxidant and phosphomolybdenum ligand. In this method, MO^{6+} reduced to MO^{5+} . The maximum phosphomolybdenum reduction activity of Methanol and Aqueous extract were 98.6% and 78.97% at 120 $\mu\text{g/mL}$ concentration and their RC_{50} were 10.79 $\mu\text{g/mL}$ (methanol) and 33.8 $\mu\text{g/mL}$ (aqueous) concentration. Phosphomolybdenum assay involves thermally generating auto oxidation during the prolonged incubation period at a higher temperature (Table 5.1.2).

Table 5.1.2: Phosphomolybdenum reduction activity of methanolic and aqueous extract of *Phyllanthus emblica L.*

S. NO	Concentration ($\mu\text{g/mL}$)	% OF INHIBITION	
		METHANOLIC Extract	AQUEOUS Extract
1	20	92.61	32.33
2	40	95.94	59.18
3	60	97.27	60.78
4	80	98.06	66.23
5	100	98.18	74.79
6	120	98.60	78.97

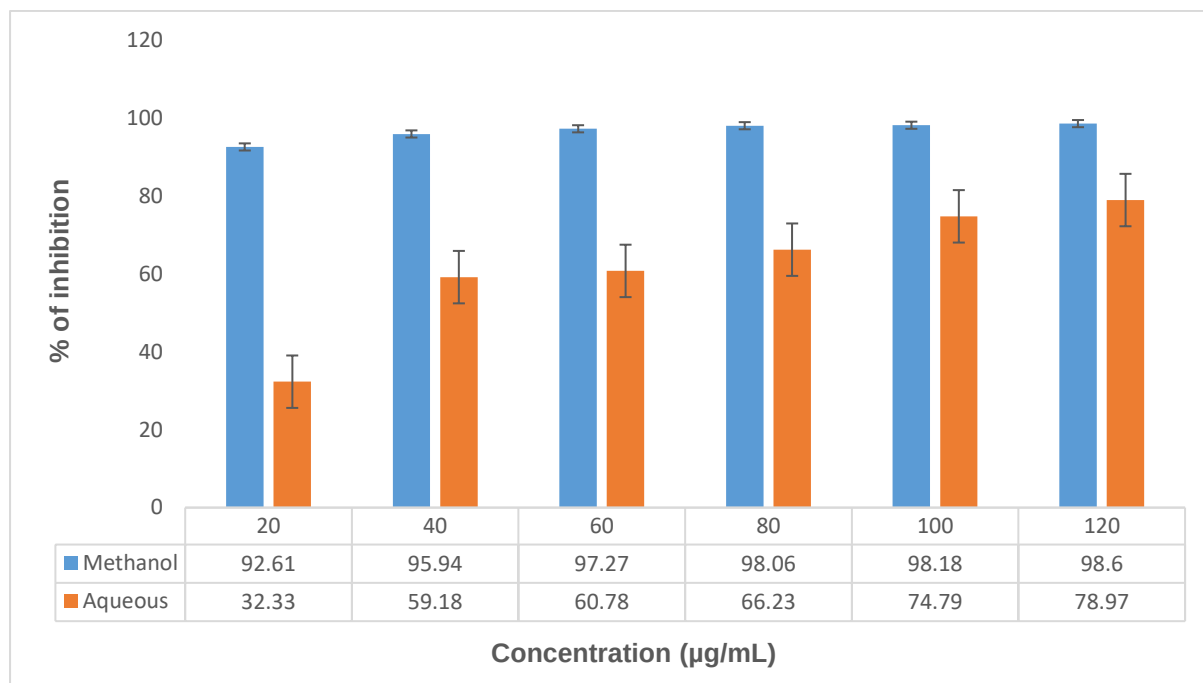


Methanol



Aqueous

Figure 5.1.2: Phosphomolybdenum reduction activity of methanolic and aqueous extract of *Phyllanthus emblica* L.



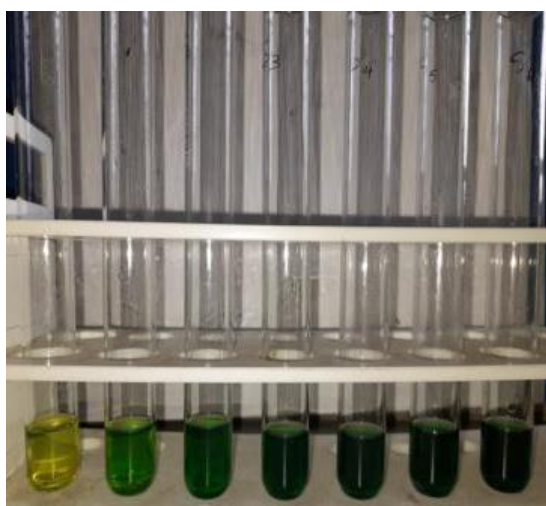
Graph 5.1.2: Phosphomolybdenum reduction activity of methanolic and aqueous extract of *Phyllanthus emblica* L.

5.1.3 Ferric (Fe^{3+}) reducing power activity

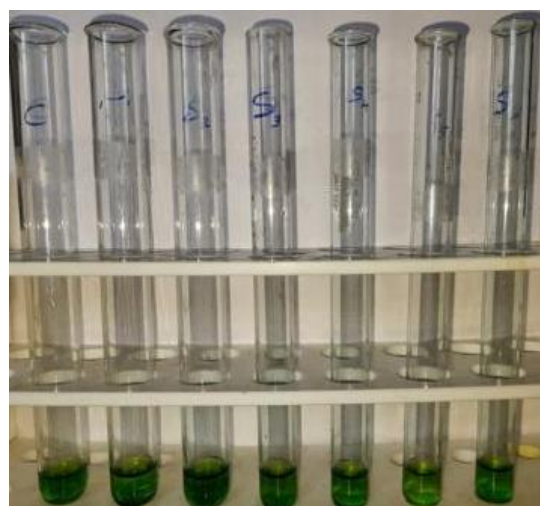
The reducing power assay was carried out by the reduction of Fe^{3+} and Fe^{2+} from colourless ferric complex. The maximum Fe reduction activity of methanol and aqueous extract were 99.42% and 65.14% at 120 µg/mL Concentration and their RC_{50} were 10.27 µg/mL (methanol) and 51.08 µg/mL (aqueous) concentration. Also in this assay, higher absorbance of the reaction mixture indicates higher reduction potential and a significant indicator of its potential antioxidant activity. The antioxidants such as phenolic acids and flavonoids were present and showed the reducing capacity in a concentration dependant manner (Table 5.1.3).

Table 5.1.3: Ferric (Fe^{3+}) reducing power activity of methanolic and aqueous extract of *Phyllanthus emblica* L.

S. NO	Concentration ($\mu\text{g/mL}$)	% OF INHIBITION	
		METHANOLIC Extract	AQUEOUS Extract
1	20	97.29	21.93
2	40	98.66	42.70
3	60	98.94	58.73
4	80	99.22	61.19
5	100	99.32	64.35
6	120	99.42	65.14

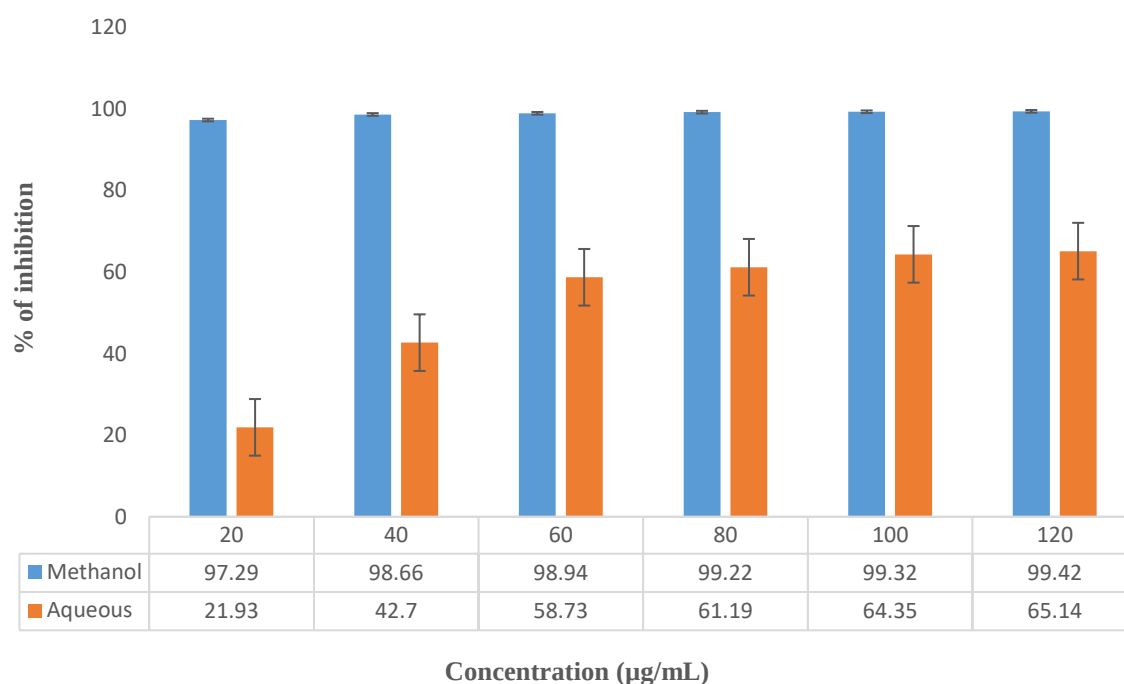


Methanol



Aqueous

Figure 5.1.3: Ferric (Fe^{3+}) reducing power activity of methanolic and aqueous extract of *Phyllanthus emblica* L.



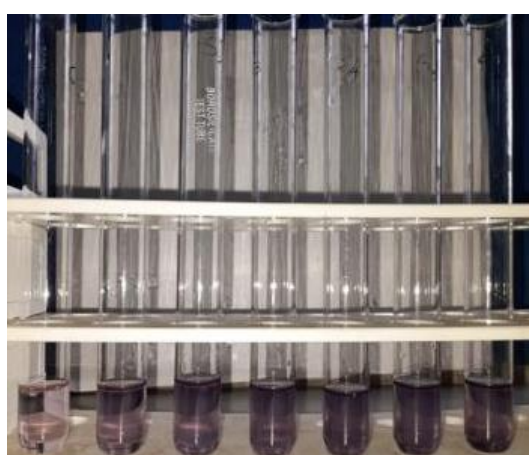
Graph 5.1.3: Ferric reducing power activity of methanolic and aqueous extract of *Phyllanthus emblica L.*

5.1.4 Hydroxyl radical scavenging activity

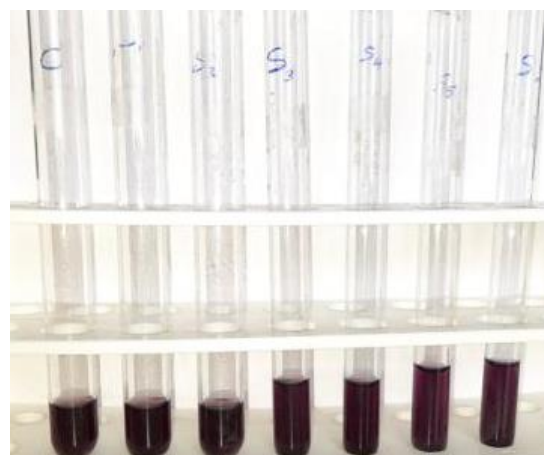
Hydroxyl radicals generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress originated disease generation of hydroxyl radicals is crucial for the irreversible damage inflicted by oxidative stress. The maximum hydroxyl radical scavenging activity of methanolic and aqueous extract were 43.96% and 40.07% at 120 µg/mL concentration and their IC₅₀ were 136.49 µg/mL (methanol) and 149.74 µg/mL (aqueous) concentration was shown in the (Table 5.1.4).

Table 5.1.4: Hydroxyl radical scavenging activity of methanolic and aqueous extract of *Phyllanthus emblica L.*

S. NO	Concentration ($\mu\text{g/mL}$)	% OF INHIBITION	
		METHANOLIC Extract	AQUEOUS Extract
1	20	6.23	5.52
2	40	10.62	9.34
3	60	13.92	12.71
4	80	20.15	16.77
5	100	36.99	35.84
6	120	43.96	40.07

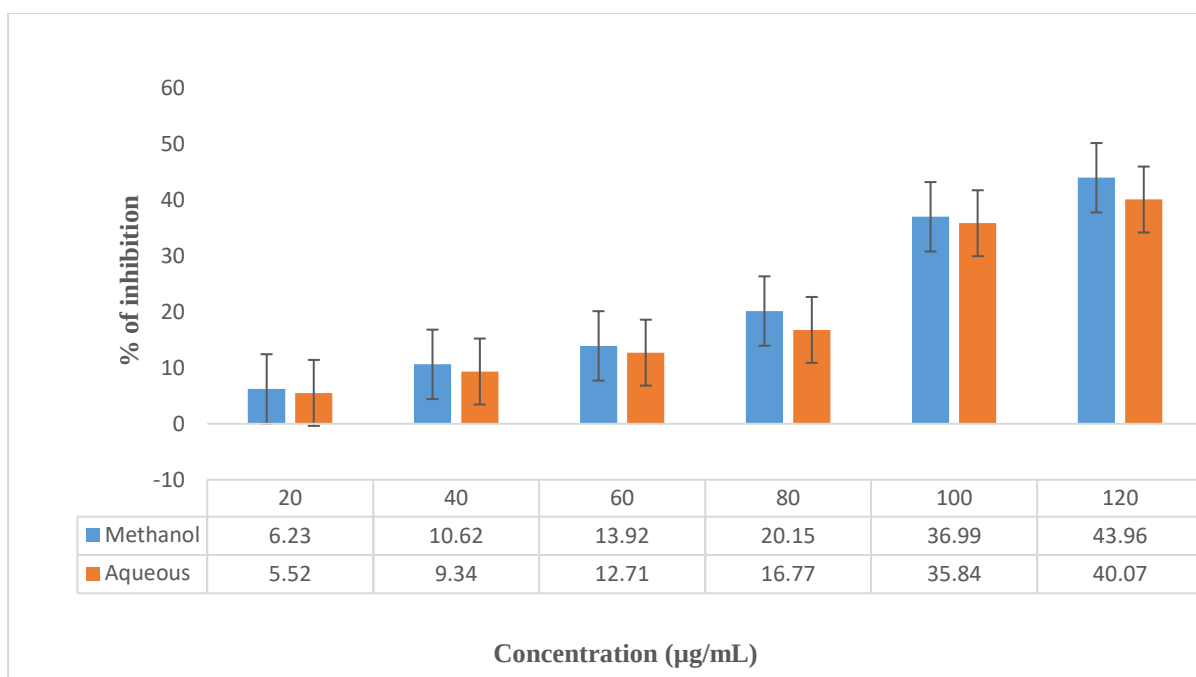


Methanol



Aqueous

Figure 5.1.4: Hydroxyl radical scavenging activity of methanolic and aqueous extract of *Phyllanthus emblica L.*



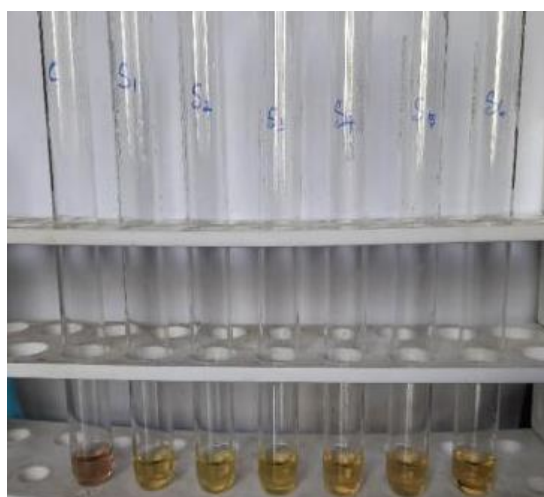
Graph 5.1.4: Hydroxyl radical activity of methanolic and aqueous extract of *Phyllanthus emblica L.*

5.1.5 Superoxide radical scavenging activity

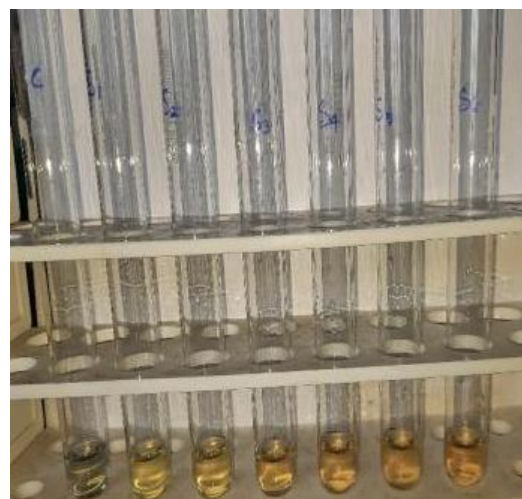
Superoxide anion is also very harmful of cell parts and their belongings can be amplified in light of the fact that it produces different sorts of free revolutionaries and oxidizing specialists. Flavonoids are powerful cell reinforcements, essentially on the grounds that they search superoxide anions. Superoxide anions got from broken up oxygen by the riboflavin-light-NBT framework will decrease NBT in this framework. The maximum superoxide radical scavenging activity of methanolic and aqueous extract were 68.14% and 60.4% at 120 µg/mL concentration and their IC₅₀ were 53 µg/mL (methanol) (Table 5.1.5) 88.61 µg/mL (aqueous) concentration.

Table 5.1.5: Superoxide radical scavenging activity of methanolic and ethanol extract of *Phyllanthus emblica L.*

S. NO	Concentration ($\mu\text{g/mL}$)	% OF INHIBITION	
		METHANOLIC Extract	AQUEOUS Extract
1	20	37.16	18.81
2	40	46.46	23.25
3	60	56.63	25.25
4	80	59.29	40.59
5	100	63.71	56.43
6	120	68.14	60.40

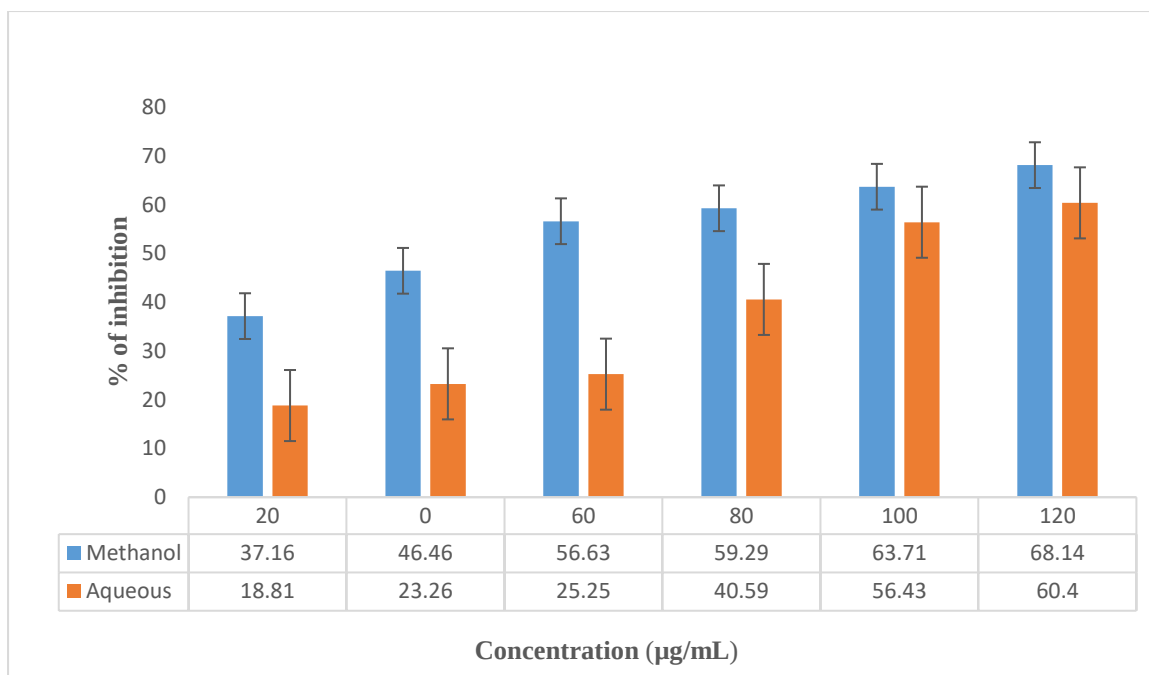


Methanol



Aqueous

Figure 5.1.5: Superoxide radical scavenging activity of methanolic and ethanol extract of *Phyllanthus emblica L.*



Graph 5.1.5: Superoxide radical scavenging activity of methanolic and aqueous extract of *Phyllanthus emblica L.*

5.2 ANTIBACTERIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD

The methanolic and aqueous extract of bark of *Phyllanthus emblica L.* was tested for its antibacterial activity against Gram-positive microorganisms, for example, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*. The methanolic and aqueous extract of bark of *Phyllanthus emblica L.* inhibited the growth of microorganism with values ranging between (250, 375, 500 µg/mL). The methanolic extract of bark of *Phyllanthus emblica L.* showed maximum zone of inhibition of 24 mm at 500 µg/mL against *Escherichia coli* followed by maximum 22 mm at 500 µg/mL *Staphylococcus aureus*. This specificity of activity might be because of the affectability of the test compounds is related with various cell divider construction of microorganisms, while the cell dividers of microbes contain murein. The aqueous extract of bark of *Phyllanthus emblica L.* showed no results.

Table 5.2: Antibacterial activity of methanolic extract of bark of *Phyllanthus emblica L.*

S.NO	Organism	ZONE OF INHIBITION (nm)			
		250 µg/mL	375 µg/mL	500 µg/mL	Standard (Tetracycline)
1	<i>Staphylococcus aureus</i>	15	20	22	35
2	<i>Pseudomonas aeruginosa</i>	12	15	20	37
3	<i>Escherichia coli</i>	15	19	24	36
4	<i>Enterococcus faecalis</i>	14	17	20	39



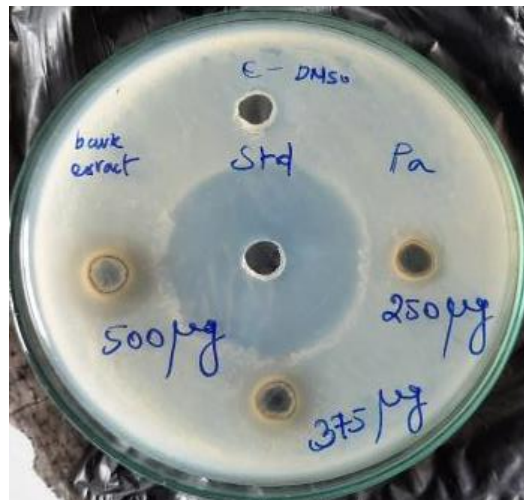
Enterococcus faecalis



Staphylococcus aureus



Escherichia coli



Pseudomonas aeruginosa

Figure 5.2: Antibacterial activity of methanolic extract of bark of *Phyllanthus emblica* L.

Table 5.2: Antibacterial activity of aqueous extract of bark of *Phyllanthus emblica* L.

S.NO	Organism	ZONE OF INHIBITION (nm)			
		250 µg/mL	375 µg/mL	500 µg/mL	Standard (Tetracycline)
1	<i>Staphylococcus aureus</i>	—	—	—	34
2	<i>Pseudomonas Aeruginosa</i>	—	—	—	38
3	<i>Escherichia coli</i>	—	—	—	41
4	<i>Enterococcus faecalis</i>	—	—	—	33



Pseudomonas aeruginosa



Staphylococcus aureus



Escherichia coli



Enterococcus faecalis

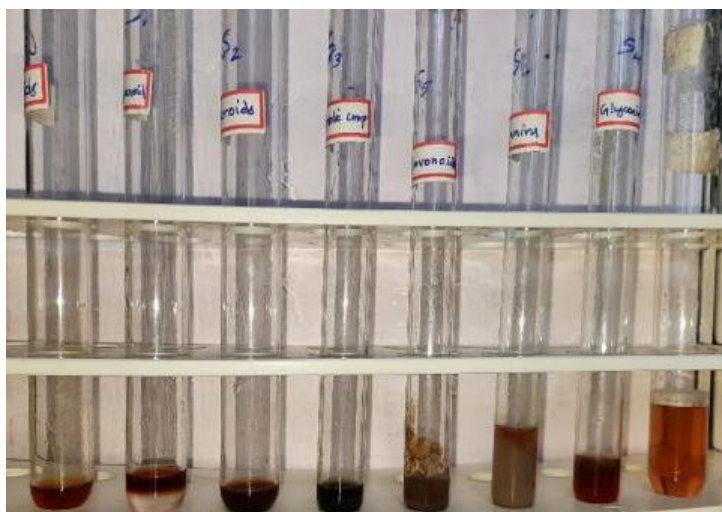
Figure 5.2: Antibacterial activity of aqueous extract of bark of *Phyllanthus emblica* L.

5.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF METHANOLIC AND AQUEOUS EXTRACT OF BARK OF *PHYLLANTHUS EMBLICA L.*

The phytochemical analysis was carried out according to the standard procedures. The results of methanolic extract of bark of *Phyllanthus emblica L.* showed the presence of alkaloids, terpenoids, flavonoids, phenols, steroids, glycosides, tannins, saponins were given in the (Table 5.3).

Table 5.3: Qualitative phytochemical analysis of methanolic and aqueous extract of bark of *Phyllanthus emblica L.*

S. No	Phytoconstituents	Chemical reagents	Methanolic extract	Aqueous Extract
1	Alkaloids	Hager's reagent	+	+
2	Terpenoids	CHCl ₃ + conc. H ₂ SO ₄	+	+
3	Flavonoids	NaOH solution	++	-
4	Phenols	FeCl ₃ solution	++	+
5	Steroids	Acetic anhydride + conc. H ₂ SO ₄	+	-
6	Glycosides	SNP + Pyridine solution + conc. H ₂ SO ₄	+	+
7	Saponins	Foam test	+	+
8	Tannins	H ₂ O + Lead acetate solution	+	+



Methanol



Aqueous

Figure 5.3: Qualitative phytochemical analysis of methanolic and aqueous extract of bark of *Phyllanthus emblica* L.

5.4 QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF METHANOLIC AND AQUEOUS EXTRACT OF BARK OF *PHYLLANTHUS EMBLICA* L.

Phenolic are the most wide spread optional metabolite in plant realm. These different gatherings of mixtures have gotten a lot of consideration as likely regular cancer

prevention agent as far as their capacity to go about as both effective revolutionary foragers and metal chelator. It has been accounted for that the cancer prevention agent action of phenol is chiefly because of 35 their redox properties, hydrogen contributors and singlet oxygen quenchers (Rice-Evans *et al.*, 1995). Therefore, in the present study, total phenolic content present in the extract was estimated using modified Folin-ciocalteau method and flavonoid content was estimated by AlCl_3 method. The phenolic content of methanolic and aqueous extract of bark of *Phyllanthus emblica* L. was expressed as gallic acid equivalent. The flavonoid content of methanolic and aqueous extract of bark of *Phyllanthus emblica* L. was expressed as quercetin equivalent. The total phenolic contents of methanolic and aqueous extract were 192.76 $\mu\text{g/mL}$ and 100.73 $\mu\text{g/mL}$ of GAE and the total flavonoid content of methanolic and aqueous extract were 115.30 $\mu\text{g/mL}$ and 14.41 $\mu\text{g/mL}$ of QE were shown in the (Table 5.4).

Table 5.4: Quantitative phytochemical analysis of methanolic and aqueous extract of bark of *Phyllanthus emblica* L.

S. NO	Phytochemicals	AMOUNT ($\mu\text{g/mL}$)	
		Methanolic Extract	Aqueous Extract
1	Total Phenols	192.76	100.73
2	Total Flavonoids	115.30	14.41



Aqueous



Methanol

Figure 5.4: Quantitative phytochemical analysis of methanolic and aqueous extract of bark of *Phyllanthus emblica* L.

5.5 ANTI-INFLAMMATORY ACTIVITY

The maximum anti-inflammatory activity of methanolic and aqueous extract were at 72.98% and 57.39% at 120 µg/mL concentration and their IC₅₀ were 77.07 µg/mL (methanol) and IC₅₀ was 104.55 µg/mL (aqueous) concentration (Table 5.5).

Table 5.5: Anti-inflammatory activity of methanolic and aqueous extract of bark of *Phyllanthus emblica* L.

S. NO	Concentration (µg/mL)	% OF INHIBITION	
		METHANOLIC Extract	AQUEOUS Extract
1	20	30.71	3.49
2	40	36.19	6.13
3	60	47.26	13.46
4	80	51.90	30.94
5	100	52.74	44.79
6	120	72.98	57.39

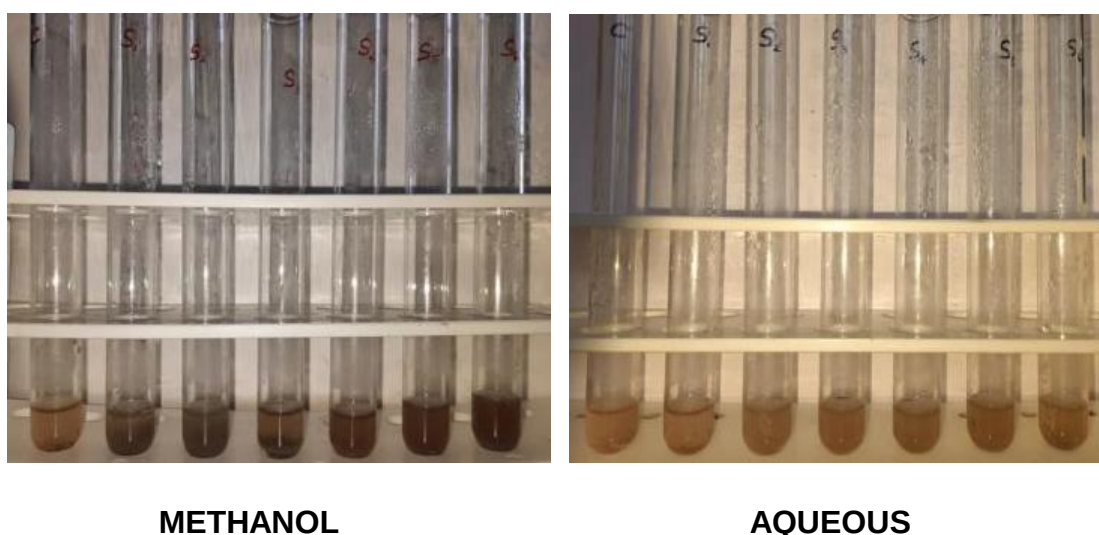
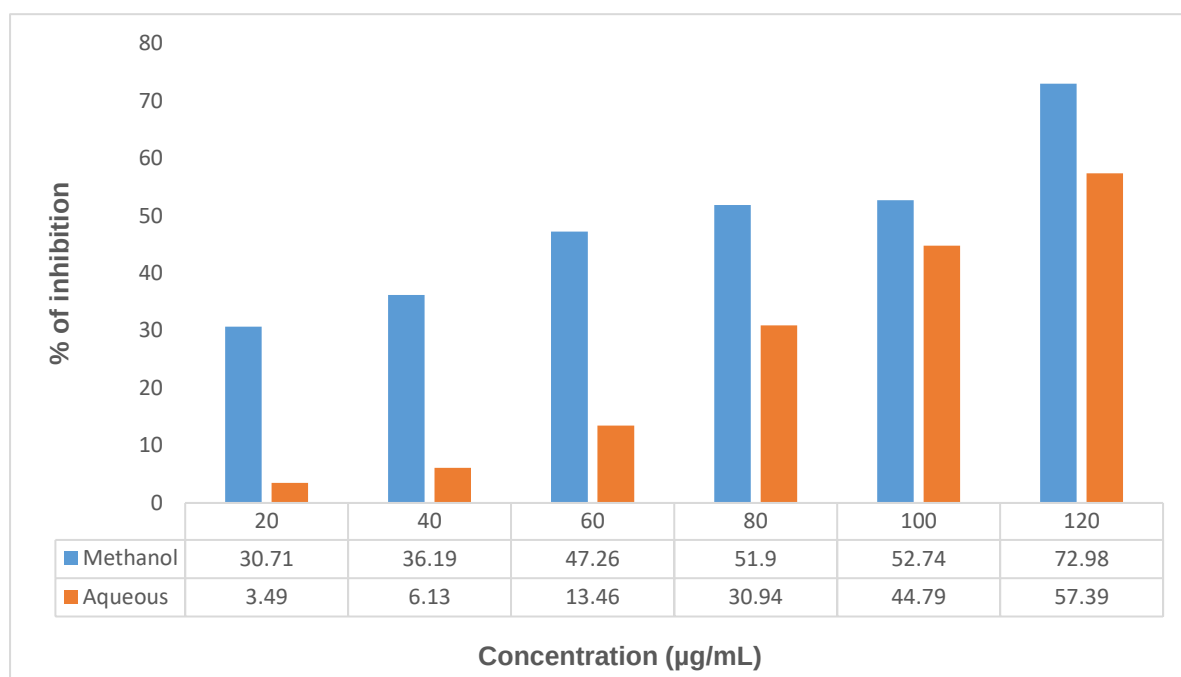


Figure 5.5: Anti-inflammatory activity of methanolic and aqueous extract of bark of *Phyllanthus emblica* L.



Graph 5.5: Anti-inflammatory activity of methanolic and aqueous extract of bark of *Phyllanthus emblica L.*

5.6 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) was carried out for the methanolic bark extract of *Phyllanthus emblica L.* Merck TLC aluminium sheet, silica gel 60 F254 (20×20 cm), preloaded plate. The extracts were spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram for the methanolic bark extract were developed in a mixture of methanol solvent mixture. The spots were visualized under UV light at 254 nm. The R_f (Retention factor) values of the coloured spots were recorded.

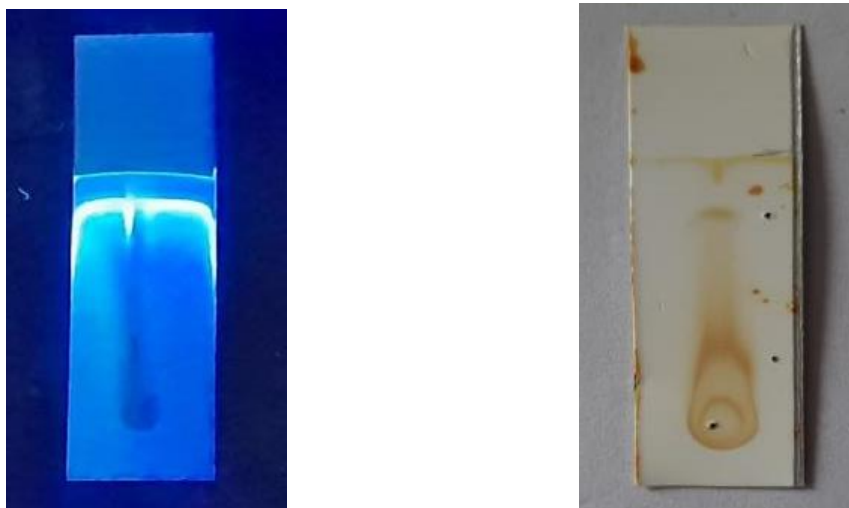


Figure 5.6: Thin layer chromatography

Distanced travelled by the Solvent front = 3

First spot } Distanced travelled by the Solute = 0.8
 Second spot } = 2.4

Retention factor (Rf) = $\frac{\text{Distanced travelled by the solute}}{\text{Distanced travelled by the solvent}}$

$$= 0.8 / 3 = 0.267$$

$$= 2.4 / 3 = 0.8$$

5.7 GC-MS ANALYSIS

GC-MS analysis of Methanolic extract of bark of *Phyllanthus emblica* L. was shown in the (Table 5.7) and the chromatogram was repeated in the (Figure 5.6). Antioxidant compounds.

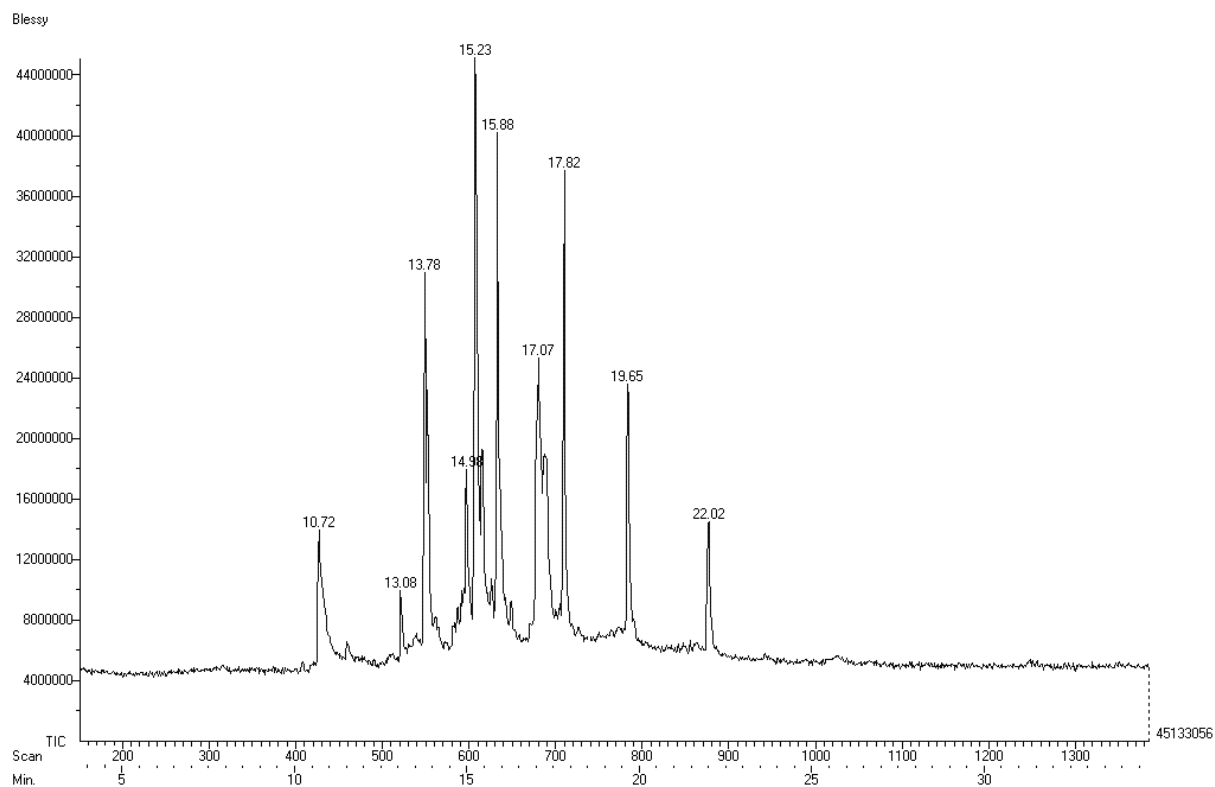
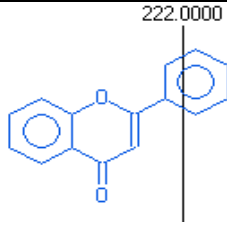
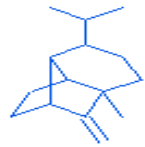


Figure 5.7: Gas chromatogram of methanolic extract of bark of *Phyllanthus emblica* L.

Table 5.7: Gas chromatogram of methanolic extract of bark of *Phyllanthus emblica* L.

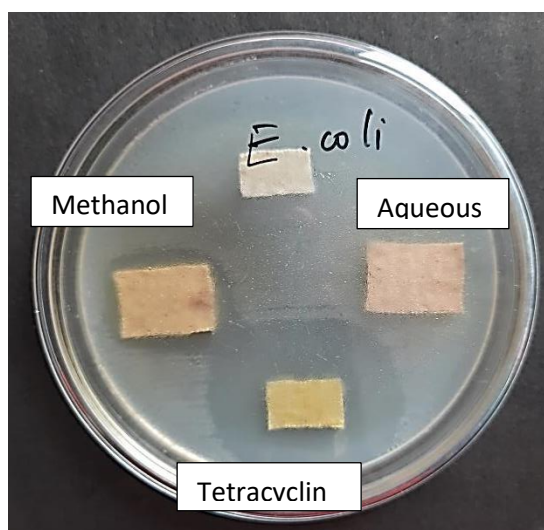
S.NO	Compound Name	RT	Compound structure	Mol. Weight (g/mol)	Mol. Formula
1	9-Hexacosene	22.02		364.7	C ₂₆ H ₅₂
2	Cyclotetracosane	19.65		336.6	C ₂₄ H ₄₈

3	1-Docosene	17.82		308.6	C ₂₂ H ₄₄
4	10-Octadecenoic acid, methyl ester	17.07		296.5	C ₁₉ H ₃₆ O ₂
5	1-Eicosene	15.88		280.5	C ₂₀ H ₄₀
6	n-Hexadecanoic acid	15.23		256.42	C ₁₆ H ₃₂ O ₂
7	Dodecanoic acid, 11-oxo-, methyl ester	14.98		228.33	C ₁₃ H ₂₄ O ₃
8	Flavone	13.78		222.24	C ₁₅ H ₁₀ O ₂
9	4,7-Methanoazulene, decahydro-1,4,9,9-tetramethyl-	13.08		206.37	C ₁₅ H ₂₆
10	Isosativene	10.72		204.35	C ₁₅ H ₂₄

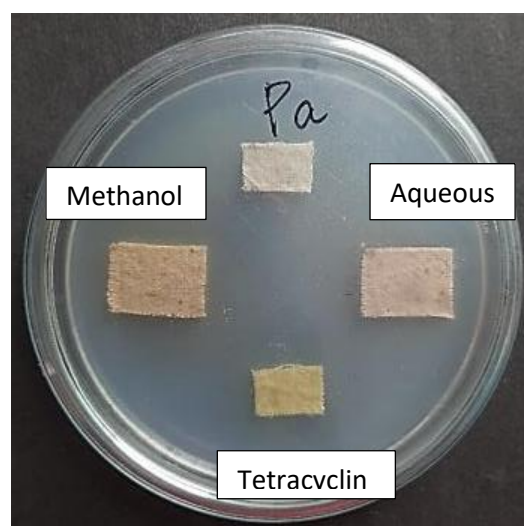
5.8 ANTIBACTERIAL STRIP

Table 5.8: Antibacterial strip

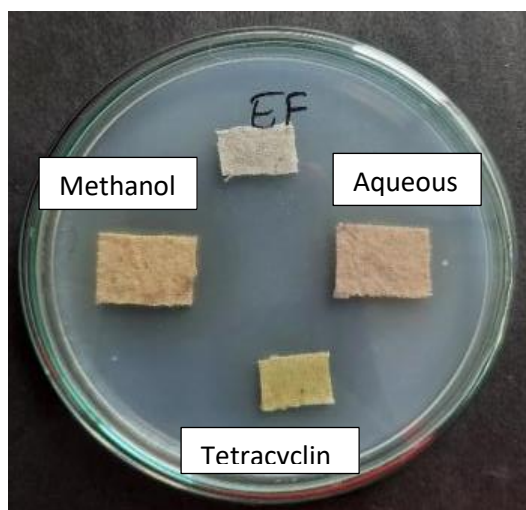
S. NO	Organisms	ZONE OF INHIBITION			
		Control DMSO 100 µg/mL	Aqueous extract 100 µg/mL	Methanolic extract 100 µg/mL	Standard Tetracyclin 100 µg/mL
1	Staphylococcus aureus	—	—	20	34
2	Pseudomonas aeruginosa	—	—	22	32
3	Enterococcus faecalis	—	—	21	28
4	Escherichia coli	—	—	23	35



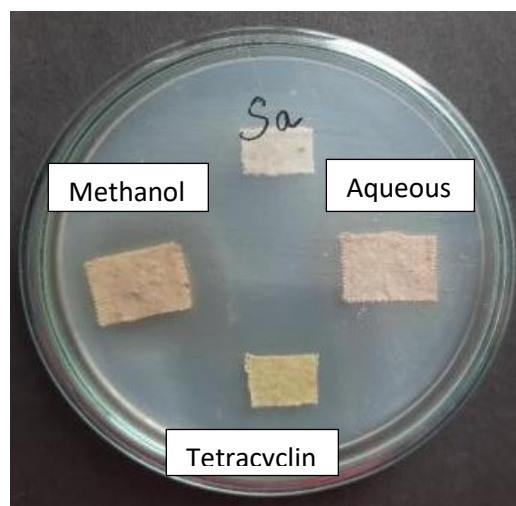
Escherichia coli



Pseudomonas aeruginosa



Enterococcus faecalis



Staphylococcus aureus

Figure 5.8: Antibacterial strip

The results are shown in (Figure 5.8) Results were obtained and compared with standard antibiotics tetracycline. The concentration of antibiotic solutions used for impregnation of the gada cloth strips should be kept as low as is compatible with clear results. The strip showed the maximum zone of inhibition of methanolic extract of 23 mm at 100 $\mu\text{g/mL}$ concentration against *Escherichia coli*.

CHAPTER 6

SUMMARY AND CONCLUSION

- The maximum DPPH radical scavenging activity of methanol and aqueous extracts were 84.04% and 84.92% at 120 $\mu\text{g/mL}$ concentration and their IC_{50} were 15.78 $\mu\text{g/mL}$ (methanol) and 32.70 $\mu\text{g/mL}$ (aqueous) concentration.
- The maximum phosphomolybdenum reduction activity of Methanol and Aqueous extract were 98.6% and 78.97% at 120 $\mu\text{g/mL}$ concentration and their RC_{50} were 10.79 $\mu\text{g/mL}$ (methanol) and 33.8 $\mu\text{g/mL}$ (aqueous) concentration.
- The maximum Fe reduction activity of methanol and aqueous extract were 99.42% and 65.14% at 120 $\mu\text{g/mL}$ Concentration and their RC_{50} were 10.27 $\mu\text{g/mL}$ (methanol) and 51.08 $\mu\text{g/mL}$ (aqueous) concentration.
- The maximum hydroxyl radical scavenging activity of methanolic and aqueous extract were 43.96% and 6.91% at 120 $\mu\text{g/mL}$ concentration and their IC_{50} were 136.49 $\mu\text{g/mL}$ (methanol) and 149.74 $\mu\text{g/mL}$ (aqueous) concentration.
- The maximum superoxide radical scavenging activity of methanolic and aqueous extract were 68.14% and 60.4% at 120 $\mu\text{g/mL}$ concentration and their IC_{50} were 53 $\mu\text{g/mL}$ (methanol) 88.61 $\mu\text{g/mL}$ (aqueous) concentration.
- The phytochemical analysis of methanolic and aqueous concentrate of bark of *Phyllanthus emblica L.* showed the presence of terpenoids, flavonoids, phenolic mixtures, glycosides and saponins.
- The total phenolic contents of methanolic and aqueous extract were 192.76 $\mu\text{g/mL}$ and 100.73 $\mu\text{g/mL}$ of GAE and the total flavonoid content of methanolic and aqueous extract were 115.30 $\mu\text{g/mL}$ and 14.41 $\mu\text{g/mL}$ of QE
- The maximum anti-inflammatory activity of methanolic and aqueous extract were at 72.98% and 57.39% at 120 $\mu\text{g/mL}$ concentration and their IC_{50} were 77.07 $\mu\text{g/mL}$ (methanol) and IC_{50} was 104.55 $\mu\text{g/mL}$ (aqueous) concentration.

The results of the present study indicate that methanolic extract of bark of *Phyllanthus emblica L.* have significant radical scavenging activities and antibacterial activities. As an outcome of its antibacterial activity, bark of *Phyllanthus emblica L.* are regarded as a very promising antibiotic candidate for the treatment of Gram-positive infections.

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