

**STUDY ON ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES
OF THE LEAVES OF THE FOURTH DAY PLANT OF *Triticum aestivum*
(WHEAT GRASS)**

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(20PBC019)**

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

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

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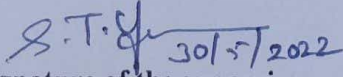
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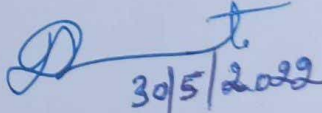
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Introduction



1. INTRODUCTION

Natural compounds derived from plants, animals, and minerals are used for treating human ailments. At present, medicinal plants are in high demand, and their popularity is growing. Plants play a crucial part in ecosystems, particularly therapeutic herbs, which have long served as a general indicator of ecosystem health. The use of medicinal plants for the treatment of ailments in humans has a long history. More than a tenth of all plant species are being used to develop products in the pharmaceutical and cosmetic industries. These plants contain bioactive chemicals that can be explored to develop medicines. Different types of seeds, roots, leaves, fruit, skin, flowers or even the whole plant may be utilized to produce therapeutic components. Medicinal plants provide low-cost health treatment to the poor and vulnerable. Traditional medicines commonly use various components of medicinal plants, such as stems, barks, leaves, flowers, and underground sections (Jamshidi-Kia *et al.*,2018).

Plants are rich sources of a wide range of secondary metabolites that are used to produce many commercial pharmaceutical medications and herbal therapies. Many secondary metabolites are being examined for their therapeutic properties, namely alkaloids, terpenoids, and phenylpropanoids (Yanqun *et al.*,2020).

Microorganisms have become the leading cause of death and disability in immune-compromised individuals, as well as the source of a variety of infectious diseases around the world, particularly in impoverished countries. Many infectious diseases including gastrointestinal tract, metabolism-related, urinary tract, skin, and surgical site infections are all caused by bacteria. In recent years, the advancement of antibiotic resistance in bacteria as a result of erroneous antibiotic use has become a major concern. Pathogenic bacteria are on the rise and have become a major source of concern in the medical community (Millat *et al.*, 2019).

Microorganisms, often known as microbes, are microscopic organisms that can only be seen under a microscope. The most prevalent are bacteria, viruses, fungi, and the protozoa group. The germs enter the host through the mouth, nose, eyes, skin, and genital openings, causing tissue damage. As a result, bacteria release poison or enzymes into the host, causing damage to the normal operation of the host organs or system. This bacterial genus is thought to be the only one that elaborates. The rise of multidrug-resistant bacteria is threatening the therapeutic efficacy of several current medications. Improved antimicrobial agents that are more active against newer and reemerging infectious illnesses are urgently needed (Rajpurohit, *et al.*, 2015).

The indiscriminate use of antibiotics has decreased infectious diseases, and bacteria are developing in the environment, becoming more virulent with each generation. To combat this global problem, now is the moment to begin research on natural and strong antimicrobials. Thus, development of antimicrobial medicines derived from natural sources with improved therapeutic benefits has become critical. This will also provide a new dimension to drug discovery research. At present, cutting-edge research is being conducted to generate natural and strong antibacterial medications for improved human health (Roy *et al.*, 2018).

Plant secondary metabolites with antimicrobial activity, implying that typical antibacterial screening procedures are likely to uncover novel compounds with mechanisms of action different from those of currently used, clinically relevant antibiotics (Joshi, 2018).

The candidate plant – *Triticum aestivum*

Wheat (*Triticum* species) is the world's most edible grain cereal-grass crop, belonging to the Gramineae (*Poaceae*) family. Wheat grass, a nutrient-rich kind of juvenile grass in the wheat family and is one of the most popular supplemental health foods consumed in the form of fresh produce, pills, frozen juice, and powder (Suriyavathana *et al.*, 2016).

The young stems of Wheat grass have been found to be effective for treating biliousness, sedative (Saha *et al.*, 2018).

Wheat grass contains eight live enzymes, 17 amino acids, and chlorophyll. It is a source of approximately all vital amino acids especially glutamic acid, aspartic acid, serine, alanine, and arginine, which are necessary to maintain our body functions. It has strong phytochemical profile and contains chlorophyll, flavonoids, apigenin, alkaloids, carbohydrates, saponins, gum and mucilage (Tufail, *et al.*, 2019).

Wheat grass juice contains the highest concentrations of vitamins A, B, C, E, and K, as well as calcium, potassium, iron, magnesium, sodium, Sulphur, and zinc. Patients with chronic diseases such as Asthma, Thyroid, Skin diseases, Atherosclerosis, Acnes and Pimples, Parkinson's disease, Joint pains, Foul odour, Menstrual disorders, TB, Constipation, Hypertension, Diabetes, Bronchitis, Insomnia, Eczema, Sterility, Obesity, and Flatulence should consider wheat grass therapy. It can also be used to treat heart issues, bladder stones, and cancer (Pannu, *et al.*, 2015).

Wheat grass contains chlorophyllin, which has bacteriostatic qualities and improves wound healing and haemoglobin and erythrocyte synthesis. Chlorophyllin aids in epithelization stabilization (Gupta, 2020).

This plant has been extensively studied in our laboratory. The antioxidant activity of the methanolic extract of Wheat grass at different stages of growth (4th, 10th, and 12th day) were compared, and found that the 4th day plant exhibits maximum activity. Further studies were carried out on the 4th-day plant and a significant anticancer activity against oral carcinoma (KB) cells was reported (Vidhya and Padma, 2007).



Fourth Day Plant of *Triticum aestivum*

In accordance with this, the present study was formulated to evaluate the effect of the leaf extract of *Triticum aestivum* against infection microbial and inflammatory conditions induced *in vitro* with the following objectives:

- To determine the anti-microbial activity effect of *Triticum aestivum* leaf extract.
- To determine the anti-inflammatory effect of *Triticum aestivum* leaf extract.

The literature collection relevant to this study was done and the review of the vast literature is presented in the following chapter.

Review of Literature



2.0. REVIEW OF LITERATURE

2.1. MEDICINAL PLANTS

Medicinal plants and plant-derived medicine are widely used in traditional cultures all over the world and they have popular in modern society as natural alternatives or supplements to synthetic chemicals. As more and more natural remedies are being commercialized, there is a need for a user-friendly but scientifically accurate reference guide to the plants and their products (Van Wyk *et al.*, 2018).

It has been estimated that in developed countries such as the United States, plant drugs constitute as much as 25% of the total drugs, while in developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is inevitable to countries such as India than the rest of the world. These countries contribute two-thirds of the plants for use in the modern system of medicine and the health care system of the rural population mainly depends on indigenous systems of medicine. Of the 2, 500,000 higher plant species on earth, more than 80,000 are medicinal plant species. India is one of the twelve biodiversity centers in the world with the presence of over 45000 different plant species (Abd El-Ghani,2016).

Medicinal plants are considered useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antimicrobial substances. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for their pharmaceutical properties (Sumathi, *et al.*, 2010).

2.2 PHYTOCONSTITUENTS

Nature has been the source of enormous medicinal agents and many modern drugs have been developed from traditional medicinal plants. The presence of diverse bioactive molecules in these medicinal plants makes them a rich source of medicine. It has been reported in the literature that about 80% of the world's population, mainly the developing countries depend on plants to meet their primary health care. Plants possess primary and secondary metabolites. Primary metabolites are peptides, carbohydrates or sugar, and lipids that are directly used in their synthesized form by plants whereas secondary metabolites are synthesized from primary

metabolites. Plant secondary metabolites such as alkaloids, flavonoids, tannins, terpenes, etc., have shown to have different properties (Tiwari *et al.*, 2019).

Secondary metabolites have been shown to possess various biological effects, which provide the scientific base for use of herbs in traditional medicine in many ancient communities. They have been described as an antibiotic, antifungal, and antiviral and therefore are able to protect plants from pathogens (Setzer 2018).

For example, flavonoids are pigmentary compounds that exist in plants. Flavonoids include flavones, flavanones, isoflavones, anthocyanins, and flavan-3-ols (catechins). Alkaloids are a group of naturally occurring nitrogenous chemical compounds found in plants, typically insoluble in water. The different organisms such as bacteria, fungi, plants, and animals are the main source of the alkaloids. Alkaloids are exerting different pharmacological actions such as antimalarial, antiasthma, anticancer, vasodilatory, antiarrhythmic, analgesic, antibacterial, and antihyperglycemic activities (Kurangi *et al.*, 2019).

2.3 PATHOGENIC MICROORGANISMS

Pathogens in hostile terms can be defined as invaders that attack host organisms. Pathogens show different types of specificity or tropism. While many can attack a wide range of hosts, others are specific to one host. The human body is a preferable host for a wide variety of pathogens as it provides a nutrient-rich, warm and moist environment. Moreover, the uniform temperature of the human body, allows the pathogen to survive and multiply itself, making it a desirable place. Emerging Infectious diseases by various pathogens had always been a matter of concern in the past and also at present. Despite the tremendous advancement in the diagnosis, treatment, and prevention procedures, these infectious diseases still remain the leading cause of death around the world, especially in developing countries (Sarmah, *et al.*, 2018)

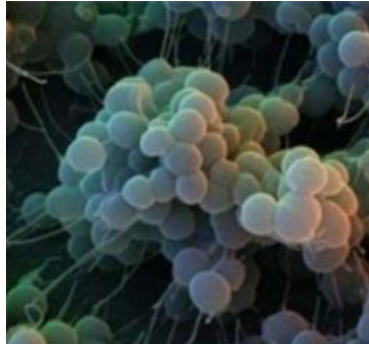
Microorganisms and the substances produced by them can be present and can grow in food and feed of animal and non-animal origin. They cause serious disease risks to people and animals, threatening their health and life. A pathogenic organism is an organism that is capable of causing diseases in a host person. The World Health Organization (WHO) listed among hazards that may be present in food as potentially harmful bacteria, viruses, toxins, parasites, and chemicals. According to the WHO, one person out of 10 people falls ill and 420,000 people die each year as a result of eating contaminated food. Foodborne diseases caused by pathogenic

bacteria can be, e.g., *Salmonellosis*, *Listeriosis*, *Escherichia coli*, *Pseudomonas*, *Staphylococcus*, *Campylobacteriosis* and *Yersiniosis* etc. (Pigłowski 2019).

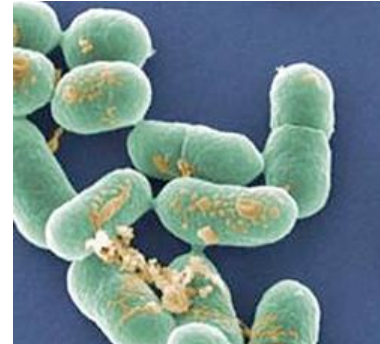
Photomicrographs of common pathogenic microorganisms



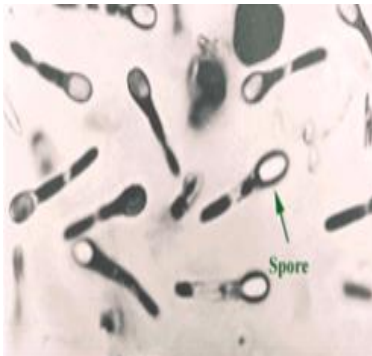
Escherichia coli



Staphylococcus aureus



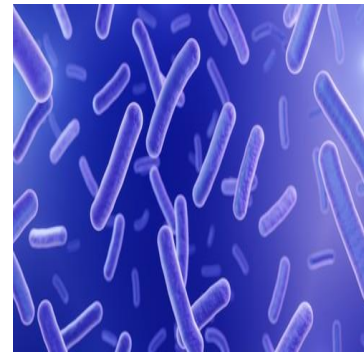
Listeria monocytogenes



Clostridium perfringens



Pseudomonas aeruginosa



Bacillus subtilis

Source: https://sphweb.bumc.bu.edu/otlt/mphmodules/ph/ph709_infectiousagents/PH709_InfectiousAgents4.html

Recently, many researchers investigated the possible utilization of some plant extracts as effective antimicrobial agents. Traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases. Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antimicrobial and antioxidant properties. The antimicrobial activities of some plant species have been widely researched. For example, the crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria. In addition, it has been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of *Escherichia coli* and other bacteria during the storage of meat, juices, and milk (Faraja *et al.*, 2018).

2.4 ANTIMICROBIAL ACTIVITY

The antimicrobial resistance rapidly emerges on a global scale and spread from one country to the other faster than previously thought. Superbugs and multidrug-resistant bacteria are endemic in many parts of the world. There is no question that the widespread use, overuse, and misuse of antimicrobials during the last 80 years have been associated with the explosion of antimicrobial resistance. On the other hand, the molecular pathways behind the emergence of antimicrobial resistance in bacteria were present since ancient times. Some of these mechanisms are the ancestors of current resistance determinants. In addition, in the presence of inhibitory and sub-inhibitory concentrations of antibiotics in natural habitats, one could assume that novel resistance mechanisms will arise against antimicrobial compounds (Christaki, *et al.*, 2020).

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, greater attention has been paid to antimicrobial activity screening, especially in plant sources. Several bioassays such as disk-diffusion, well diffusion, and broth or agar dilution are well known and commonly used, other methods such as flow cytometric and bioluminescent methods are not widely used because they require specified equipment and further evaluation for reproducibility and standardization, even if they can provide rapid results of the antimicrobial agent's effects and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism (Moore, *et al.*, 2016).

2.5 ANTI-INFLAMMATORY ACTIVITY

2.5.1 INFLAMMATION

Inflammation is part of the body's defense mechanism. It is the process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process. Inflammation can be either acute or chronic.

Acute Inflammation causes tissue damage due to trauma, microbial invasion, or noxious compounds that can induce acute inflammation. It starts rapidly, becomes severe in a short time and symptoms may last for a few days for example cellulitis or acute pneumonia. Subacute inflammation is the period between acute and chronic inflammation and may last 2 to 6 weeks.

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic

inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage. This article reviews chronic inflammation (Pahwa *et al.*, 2018)

The inflammatory response is executed in three phases. In the first phase, the vascular permeability increases, leading to the exudation of fluids from the blood into the interstitial space. The second phase is characterized by the infiltration of leukocytes from the blood into the tissue. The third phase involves the formation of granuloma and tissue repair. Inflammation is mediated by several factors like histamine, leukotrienes, nitric oxide, prostaglandins, bradykinin, cytokines, serotonin, platelet-activation factor, lipoxins, and growth factors that are released either from plasma or by the inflammatory cells (Iwalewa *et al.*, 2007).

2.5.2 INFLAMMATION AND DISEASES

Inflammation is beneficial when it occurs as a response to microbial infection or mechanical injuries. It is said to be a collection of conserved immune responses that are involved in the repair and recovery of damaged tissues. However, long-term inflammation or the dysregulation of inflammatory pathways, has the potential to damage or harm the tissues and result in the development of several chronic diseases (Kauppinen *et al.*, 2016).

In the intestine, the immune cells interact with intestinal bacteria and regulate the proinflammatory effector cells and anti-inflammatory pathways. The uncontrolled bacterial colonization, dysregulation of the homeostatic balance, disruption of the epithelial barrier, and irregular stimulation of immune effector cells may result in the onset of chronic intestinal disorders like inflammatory bowel disease, which may include Crohn's disease and ulcerative colitis. Acute enteritis is reported to be the world's second leading cause of death in developing countries. The rates of people suffering from intestinal chronic inflammatory diseases and chronic enteritis are increasing dramatically every day (Jiminez *et al.*, 2015).

Inflammation is considered to be one of the key factors in the development of several other disease conditions, like depression, diabetes, cancer, obesity, osteoporosis, cardiovascular disease, rheumatoid arthritis, Parkinson's disease, and asthma (Laveti *et al.*, 2013).

During an immune response, cytokines, the low-molecular-weight regulatory proteins, are expressed in all the phases. Fibrinogen and C-reactive protein are the principal acute phase inflammatory markers. They are implicated in coronary heart disease, atherosclerosis and other cardiovascular diseases like stroke, myocardial infarction, peripheral vascular disease and atherothrombosis (Frostedgard, 2013).

2.6 *Triticum aestivum* – THE MEDICINAL HERB

2.6.1 TAXONOMIC HIERARCHY OF *Triticum aestivum*



Fourth day Plant of *Triticum aestivum*

Kingdom: Plantae

Subkingdom : Viridiplantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Poales

Family : Poaceae

Genus : *Triticum* L.

Species : *Triticum aestivum* L.

Common name: Common wheat

2.6.2 NUTRITIONAL VALUE OF WHEAT GRASS

Wheat grass juice contains crude chlorophyll and can be taken orally and as a colon implant without toxic side effects. The world-famous scientist and grass-expert Dr. Earp Thomas have found that more than 100 elements/nutrients in fresh Wheat grass . He stated that Wheat grass is a complete food in itself and anybody can subsist on the Wheat grass alone for their whole life. Wheat grass juice provides a high level of oxygen like all green plants that contain chlorophyll. Our brain and all body tissues function at an optimal level in a highly-

oxygenated environment. Chlorophyll (Wheat grass) rebuilds the bloodstream. Many studies have shown that chlorophyll is free of any toxic reaction. The red cell count was returned to normal within a few days of the administration of chlorophyll, even in those animals which were known to be extremely anemic or low in red cell count. The world-famous Dr. Ann Wigmore says, "Wheat freshly made into drink contains chlorophyll which in turn contains synthesized sunshine, plus the electric current necessary for the revitalization of the body". A small amount of Wheat grass juice in the human diet can prevent tooth decay. Wheat grass juice is used for skin problems such as eczema or psoriasis. Wheat grass juice keeps the hair away from graying. Wheat grass juice improves digestion. Wheat grass juice is greatly used for constipation and keeping the bowels open. This is high in magnesium. (Pannu, 2015).

2.6.3 COMPOSITION OF WHEAT GRASS

The name "green blood" of Wheat grass is attributable to its high chlorophyll content which accounts for 70% of its total chemical constituents. Wheat grass juice is a rich source of Vitamins A, C, E, and B complex. It contains a plethora of minerals like calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron, and molybdenum. The various enzymes responsible for its pharmacological actions are protease, amylase, lipase, cytochrome oxidase, transhydrogenase, and superoxide dismutase (SOD). Wheat grass contains a high proportion of amino acids such as aspartic acid, glutamic acid, arginine, alanine, and serine. The major clinical utility of Wheat grass juice is due to its antioxidant action which is derived from its high content of bioflavonoids like apigenin, quercetin, and luteolin. Other compounds present, which make this grass therapeutically effective, include indole compounds, apigenin, and laetrile (Balakrishnan *et al.*, 2020).

2.6.4 TRADITIONAL AND MEDICINAL USES OF WHEAT GRASS

Wheat grass is used to cure high blood pressure, obesity, diabetes, gastritis, ulcers, asthma, anemia, and eczema. Wheat grass is used in brewing industries, ethanol production in many industries, and also is used in cosmetics industries. Wheat grass is also used in the treatment of some digestive disorders. Wheat grass is used in confectionery products such as bread, noodles, wheat gluten, and wheat straw composites. Wheat grass is used in the treatment of bone and joint diseases such as osteoarthritis and fractures and gout due to its strong anti-inflammatory effect. It is used to lower pain, swelling, and inflammation, and also it helps to stop the cutaneous and subcutaneous bleeding rapidly. It also helps to induce the natural healing process. Wheat grass is used to treat skin diseases ear and renal disorders such as acne, cuts,

boils, wounds, bites, gangrenes, and skin itching. It also acts as an inactive blood purifying agent (Gupta, 2020).

2.7 ASSAYS

2.7.1 ANTIMICROBIAL ACTIVITY

A. DISK DIFFUSION METHOD:

The plant will be evaluated for its antimicrobial potential by the disk diffusion method, wherein the zone of inhibition against the series of bacterial and fungal species will be observed.

B. BROTH DILUTION METHOD:

The plant will be evaluated for its antimicrobial potential by the broth dilution method, in which the minimum inhibitory concentration against the series of bacterial and fungal species will be observed.

C. GROWTH CURVE:

The plant will be evaluated for the antimicrobial potential by Time kill kinetics, and the duration of cell viability at different time intervals will be measured to determine the minimum inhibitory concentration for growth control.

2.7.2 ANTI-INFLAMMATORY ACTIVITY

A. PROTEIN DENATURATION ASSAY

The denaturation of proteins is one of the causes of inflammation. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins. Denaturation was induced by incubating drugs with bovine serum albumin under some experimental conditions and compared with the control. This method has been used with slight modifications as mentioned by Misso *et al.*, 2018.

B. MEMBRANE STABILIZATION TEST (HEMOLYSIS ASSAY)

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 with some modifications. The *in vitro* hemolysis assay evaluates hemoglobin release in the plasma. Stabilization of human red blood cells by hypotonicity induced membrane lysis. To examine *in vitro* anti-inflammatory action of a plant extract, the membrane stabilization technique is used widely (Yesmin *et al.*, 2020). In the hemolysis assay, human red blood cells

and test materials are co-incubated in buffers at defined pH that mimic extracellularly, early endosomal. Following a centrifugation step to pellet intact red blood cells, the amount of hemoglobin released into the medium is spectrophotometrically measured. In this model system, the erythrocyte membrane serves as a surrogate for the lipid bilayer membrane that encloses endo-lysosomal vesicles (Misso *et al.*, 2018).

C. SODIUM DICLOFENAC- Standard anti-inflammatory drug:

Diclofenac is part of the nonsteroidal anti-inflammatory category and is a weak organic acid. It is known that its main action is the ability to decrease the activity of the isoforms of the cyclooxygenase enzyme and consequent inhibition of prostaglandin synthesis. When orally used, diclofenac may present important adverse effects such as gastrointestinal bleeding, gastric ulcer, and renal and cardiac complications, among others. However, in topical formulations, these adverse effects are practically not found, and their efficacy is possibly as good as oral or intramuscular for skeletal muscle lesions (Paiva *et al.*, 2019).

The present aim is to study antimicrobial and anti-inflammatory study on the 4th day (Wheat grass plant) *Triticum aestivum*.

Methodology



3. METHODOLOGY

The present study has been designed to evaluate the antimicrobial and anti-inflammatory effects of *Triticum aestivum* leaf extract. The anti-microbial potential of *Triticum aestivum* extract was determined using disk diffusion, broth dilution, and time-kill kinetics assay. The anti-inflammatory effect of the *Triticum aestivum* extract was examined using protein denaturation, and membrane stabilization assays.

3.1 GROWING AND HARVESTING OF WHEAT GRASS

Whole wheat grains were purchased from the local market of Coimbatore, India. Wheat grains were soaked overnight and were sown in grow bag and watered regularly. On the 4th day, the Wheat grass was harvested and it was used for the study.

3.2 PREPARATION OF *TRITICUM AESTIVUM* LEAF EXTRACT

About 5g of fresh leaves were harvested and cleaned. The leaves were grounded with a mortar and pestle using 50ml of methanol. The methanolic extract of leaves was evaporated at 60°C. Then the concentration ranging from 20mg was dissolved in 5µl of Dimethylsulfoxide.

3.3 ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY TEST

3.3.1 MICROORGANISMS

The antimicrobial activity of the plant extract was tested against the following microorganisms:

1. *Escherichia coli*
2. *Pseudomonas aeruginosa*
3. *Bacillus subtilis*
4. *Staphylococcus aureus*
5. *Aspergillus niger*

These microorganisms were procured from PSG-IMS Coimbatore.

3.3.2 DISK DIFFUSION ASSAY

The widely accepted method for the evaluation of antimicrobial potential is the disc diffusion method (Millat *et al.*, 2019). In the following method, a concentration gradient was created by diffusing an antibiotic from a reliable source through the nutrient agar. The test bacteria were seeded on nutrient agar medium at dried, sterilized filter paper discs (6 mm diameter, HI-Media, China) containing the known amounts of test samples (20µg/disc).

Standard antibiotics of ciprofloxacin (5µg/disc) and blank discs were used as positive and negative control. These plates were reserved for 24 hours at a low temperature (4°C) for the maximum diffusion of the test materials to the surrounding media.

To allow optimum growth of the organisms, the plates were then incubated for 24 hours at 37°C. Microbial growth in plates was inhibited by the test materials containing antimicrobial properties and thereby a distinct, clear zone was yielded defined as a zone of inhibition. By measuring the zone of inhibition, the antimicrobial activity of the test sample was determined.

3.3.3 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory concentration (MIC) of Wheat grass extract was determined by the broth dilution method against bacterial culture.

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

3.3.4 GROWTH CURVE

The growth curve of the test cultures was compared with methanolic extract of Wheat grass and non-treated culture as control. About 1ml of methanolic extract was added to the test culture inoculated on a nutrient broth medium. Optical density was observed every 30 minutes at 540nm for both extract-treated and non-treated cultures using a UV spectrophotometer. The growth curve of the treated culture was plotted against the non-treated cultures and compared to know the significant inhibitory efficiency of Wheat grass to different test pathogens.

3.4 DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY OF *TRITICUM AESTIVUM* LEAF EXTRACT BY PROTEIN ANTI-DENATURATION AND MEMBRANE STABILIZATION ASSAYS

3.4.1 PROTEIN ANTI-DENATURATION ASSAY

The protein anti-denaturation assay was carried out based on the method proposed by Williams *et al.* (2018).

PRINCIPLE

Protein denaturation is considered one of the reasons for inflammation. This experiment is mainly done to study *in vitro* anti-inflammatory activity of test samples by protein denaturation (egg albumin) method.

REAGENTS

1. Bovine egg albumin
2. Phosphate buffer saline (pH, 6.4)
3. Standard drug – sodium diclofenac (20 μ g)
4. Plant extract

PROCEDURE

About 0.1ml of fresh chicken egg albumin was mixed with 1.9ml of phosphate-buffered saline (pH 6.4) and 1ml of the extract with varying concentrations. A similar volume of distilled water was used as a negative control. Then the mixture was incubated at 37°C in an incubator for 20 min and then heated at 70°C for 5 min. After cooling, the absorbance was measured at 660nm on the spectrophotometer. Sodium diclofenac in the final concentration of 20 μ g was used as a reference drug and similarly treated for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula;

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

3.4.2 MEMBRANE STABILIZATION ASSAY

The membrane stabilization assay was evaluated by the method of hemolysis of red blood cells based on the method proposed by Shinde *et al.*, 1989

Preparation of the suspension of erythrocytes

Fresh whole blood (3ml) collected in EDTA tubes was centrifuged at 2500 rpm for 10 rpm 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 proportion of 40% suspension with isotonic buffer solution. The reconstituted red blood cells (supernatant resuspended) were used for the analysis.

i) HEMOLYSIS INDUCED BY HEAT

PRINCIPLE

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

REAGENTS

1. Blood cell suspension
2. Phosphate buffer (pH 6.4)
3. Methanol
4. Standard drug – sodium diclofenac (20µg)
5. Extract sample

PROCEDURE

Plant extracts of increasing concentration in isotonic buffer solution were taken in centrifugation tubes. Similarly, the standard drug sodium diclofenac was taken. The negative control contained 2 ml of distilled water. A suspension of 0.1ml of red blood cells is added to each tube and mixed gently. The tubes were incubated at 54°C for 20 min in a water bath. After incubation, tubes were centrifuged at 2500 rpm for 10min at 4°C and the hemoglobin content of the supernatant was estimated using the spectrophotometer at 540nm. The percentage of inhibition of hemolysis by the extract was calculated as follows:

$$\% \text{ Inhibition of hemolysis} = (1 - \text{OD sample} / \text{OD control}) \times 100$$

Where OD sample = absorbance of the sample; OD control = absorbance of the control.

ii) HEMOLYSIS INDUCED BY HYPOTONICITY

PRINCIPLE

This assay is based on the membrane stability of human RBCs subjected to lysis induced by hypotonicity.

REAGENTS

1. Human red blood cell suspension
2. Standard drug- sodium diclofenac
3. Plant extract
4. Hypotonic buffer solution

PROCEDURE

The extract samples were prepared in distilled water at different concentrations obtained by double dilution. Sodium diclofenac at the same concentrations was used as a reference medicine. Distilled water was used as a negative control. To each tube, 0.1ml of a suspension of erythrocytes was added and then the mixtures were incubated for 1 hour at 37°C. The tubes were then centrifuged at 2500 rpm for 10 min at 4°C. The hemoglobin content of the supernatant was estimated using the spectrophotometer at 540 nm. The percentage of hemolysis was calculated assuming hemolysis produced in the presence of distilled water as 100%. The percentage inhibition of hemolysis by the extract was calculated as;

$$\% \text{ Inhibition of hemolysis} = (1 - \text{OD sample} / \text{OD control}) \times 100$$

The concentration of the extract for 50% inhibition was determined by the dose-response curve.

Results and Discussion



4. RESULT AND DISCUSSION

Plant secondary metabolites play a variety of functions such as in plant growth and developmental processes, innate immunity defense response signaling, and response to environmental stresses. In addition, plant secondary metabolites also have important functions such as repelling pests and pathogens, acting as signals for symbiosis between plants and microbes, and modifying microbial communities associated with hosts. Many plant secondary metabolites have positive beneficial effects on human health and on agriculture production, contributing significantly to the economy (Pank *et al.*, 2021).

Wheat grass, *Triticum aestivum* L, is one of the members of the Poaceae family, has high nutritional values. It contains many essential amino acids, enzymes like protease, cytochrome, amylase, lipase, vitamins, and antioxidants like flavonoids as well as phenolic compounds (Roy *et al.*, 2018).

This plant has been extensively studied in our laboratory. The antioxidant activity of the methanolic extract of Wheat grass at different stages of growth (4th, 10th, and 12th day) were compared, and found that the 4th day plant exhibits maximum activity. Further studies were carried out on the 4th-day plant and a significant anticancer activity against oral carcinoma (KB) cells was reported (Vidhya and Padma, 2007). The fourth-day plant with the optimum dose of 20µg concentration was found to be effective.

In accordance with these observations the present study was designed out to investigate the antimicrobial property and anti-inflammatory properties of the methanolic extract of the fourth-day plant of *Triticum aestivum* (Wheat grass).

4.1 DETERMINATION OF ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *Triticum aestivum* LEAF EXTRACT:

The antimicrobial activity of the leaf extract was determined using disk diffusion assay, and growth curve assay. The minimum inhibitory concentration of the antimicrobial property was also determined.

4.1.1 DETERMINATION OF ANTIMICROBIAL ACTIVITY OF THE LEAVES OF *T. aestivum* USING DISK DIFFUSION ASSAY:

The antimicrobial activity of the leaf extract was tested against four bacterial species and one fungal species at different concentrations of 5µg, 10µg, 15µg, and 20µg/disc.

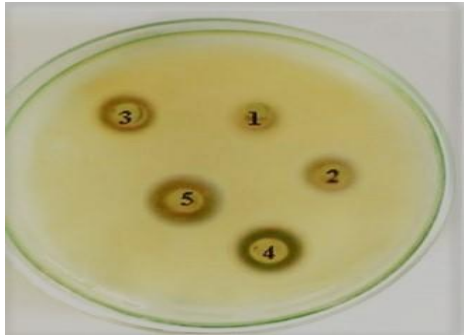
Penicillin(5µg/disc) was used as a standard antibiotic disc for the comparison. The result of antimicrobial activity is represented in Table 4.1. The results obtained showed that the methanolic extract of *Triticum aestivum* exhibited the strong zone of inhibition against the four bacterial and one fungal species at the dosage of 20µg compared to that of control, followed by 15µg, 10µg, and 5µg.

TABLE 4.1 ZONE OF INHIBITION AGAINST FIVE BACTERIAL AND ONE FUNGAL SPECIES BY THE METHANOLIC EXTRACT OF *Triticum aestivum* LEAVES

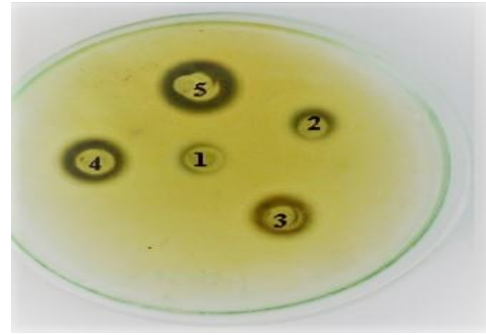
Thus a dose-dependent antimicrobial potential against both the bacterial and fungal species was observed as evident from the zone of inhibition (Figure 4.1.1 and Table 4.1).

| Organisms | The concentration of the sample (µg) | Zone of inhibition in mm |
|-------------------------------|---|---------------------------------|
| <i>Escherichia coli</i> | 5 | 0.2 |
| | 10 | 0.5 |
| | 15 | 0.9 |
| | 20 | 1.5 |
| | Control | 1.7 |
| <i>Staphylococcus aureus</i> | 5 | 0.3 |
| | 10 | 0.6 |
| | 15 | 0.8 |
| | 20 | 1.0 |
| | Control | 1.5 |
| <i>Bacillus subtilis</i> | 5 | 0.3 |
| | 10 | 0.5 |
| | 15 | 0.9 |
| | 20 | 1.3 |
| | Control | 1.5 |
| <i>Pseudomonas aeruginosa</i> | 5 | 0.2 |
| | 10 | 0.4 |
| | 15 | 0.8 |
| | 20 | 1.2 |
| | Control | 1.4 |
| <i>Aspergillus niger</i> | 5 | 0.3 |
| | 10 | 0.5 |
| | 15 | 0.9 |
| | 20 | 1.5 |
| | Control | 1.7 |

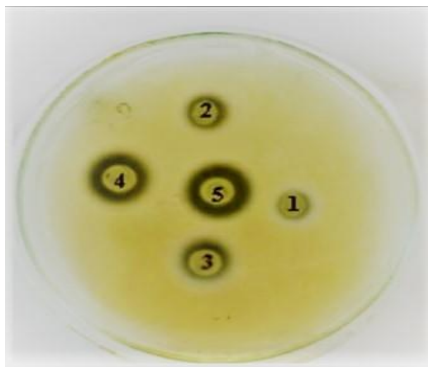
FIGURE 4.1.1 ZONE OF INHIBITION AGAINST FIVE BACTERIAL AND ONE FUNGAL SPECIES BY THE METHANOLIC EXTRACT OF *Triticum aestivum* LEAVES



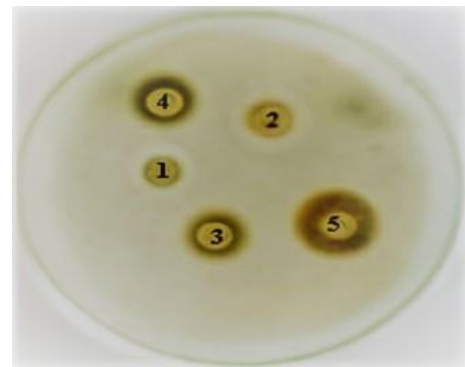
Escherichia coli



Staphylococcus aureus



Bacillus subtilis



Pseudomonas aeruginosa



Aspergillus niger

Disk 1-Control

Disk 2-5 μ g

Disk 3-10 μ g

Disk 4-15 μ g

Disk 5-20 μ g

A similar observation was reported in a study, wherein Sakha *et al.* (2018) reported that the extract from *S. aromaticum* (Clove), *P. guajava* (Guava), and *E. ganitrus* (Rudraksha) leaves showed the most promising result against *Staphylococcus aureus* with a zone of inhibition of 14mm, 16mm, and 16 mm respectively. Likewise, *S. aromaticum* (Clove), *C. longa* (Turmeric), and *P. guajava* (Guava) showed good antibacterial activity against *Escherichia coli* with a zone of inhibition of 11mm, 11mm and 10mm respectively. *A. Vulgaris* (Titepati) and *A. Indica* (Neem leaves) showed promising activity against *Pseudomonas aeruginosa* with a zone of inhibition of 11mm. *Z. armatum* (Timur) showed good results against *E. coli* with a zone of inhibition of 10mm.

Similarly in another study, Semerci *et al.* (2020) reported that the methanolic extracts of *Cedrus libani* showed maximum zone of inhibition of about 12-15 mm, 11.5-15.2 mm, and 10.5-13 mm against *Bacillus subtilis*, *S. epidermidis*, and *S. aureus*, respectively. On the other hand, no inhibitory effect against *C. Albicans*, and *S. Typhimurium* was observed for all extracts. Activities of the *Cedrus libani* towards the test microorganisms were found to be lower than those of the known antibiotics. Only the methanolic extract of *Cedrus libani* showed antibacterial activity against *E. coli* and *E. faecalis*.

Parvin *et al.* (2015) tested the extract of *C. cujete* leaves and bark against bacterial strains. Their antibacterial potency was assessed by the presence or absence of a zone of inhibition and zone diameters (mm). The chloroform fraction of both leaves and stem bark exhibited better antimicrobial activity against the tested organisms. The crude ethanol extract of stem bark exerted the lowest activity against *E. coli*. The maximum zone of inhibition was obtained for the chloroform fraction of leaves at 200 µg/disc against *E. coli*.

4.1.2 EFFECT OF METHANOLIC EXTRACT OF *Triticum aestivum* LEAF EXTRACT ON MINIMUM INHIBITORY CONCENTRATION.

The minimum inhibitory concentration of the antimicrobial potential of the methanolic leaf extract of *Triticum aestivum* was the results are shown in Figure4.1.2. The effective minimum inhibitory concentration of the methanolic extract of Wheat grass was found to be 20µg for bacterial strains and fungal strains.

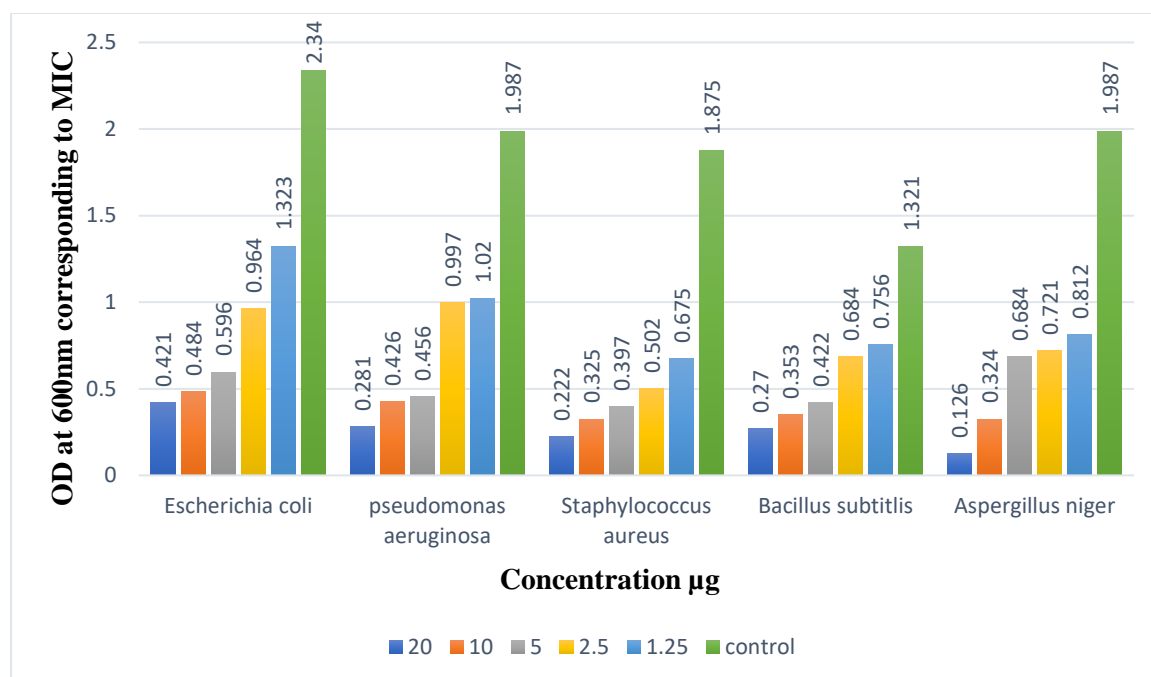


Figure 4.1.2: MINIMUM INHIBITORY CONCENTRATION AGAINST FIVE BACTERIAL AND ONE FUNGAL SPECIES BY THE METHANOLIC EXTRACT OF *Triticum aestivum* LEAVES

The results of the minimum inhibitory concentration confirmed the antimicrobial activity, wherein it is evident that the Wheat grass extracts were more potent to inhibit *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Aspergillus niger*. Among the tested microorganisms. Several studies have tested the plant extracts against the activity of microorganisms and determined their minimum inhibitory concentration.

Adeyemi *et al.* (2018) reported that the minimum inhibitory concentration was 50 mg/ml and 25 mg/ml for cold-water extract of *Chromolaena odorata* and *Phytophthorame gakarya*. On the other hand, extract of warm water had neither fungicidal nor fungistatic effect against the test organism. The ethanolic extract also showed a significant level of fungicidal activity at 50 mg/ml and 25 mg/ml.

In another study by Gonelimali *et al.* (2018), the MIC of each plant extract against the tested microorganisms revealed variability in the MIC among plant extracts, wherein the lowest MIC values (0.313, 0.625%) were exhibited by water extracts of clove and roselle against *Bacillus cereus*, respectively, as well as the ethanol extracts of clove (0.625%) against *Vibrio parahaemolyticus*.

4.1.3 EFFECT OF METHANOLIC EXTRACT OF *Triticum aestivum* LEAF EXTRACT ON GROWTH CURVE.

The growth curve of the test cultures treated with the methanolic extract of Wheat grass and untreated control was compared. In this analysis, about 1ml of methanolic extract was added to the test culture inoculated on a nutrient broth medium. Optical density was observed every 30 minutes at 540nm for both extract-treated and un-treated cultures using a UV spectrophotometer. The growth of the culture of each pathogenic species treated with the extract was compared to that of un treated control and a growth curve was desired for both the treatment groups against time. The result obtained were shown in Figure 4.1.3 (a, b,c,d,e)

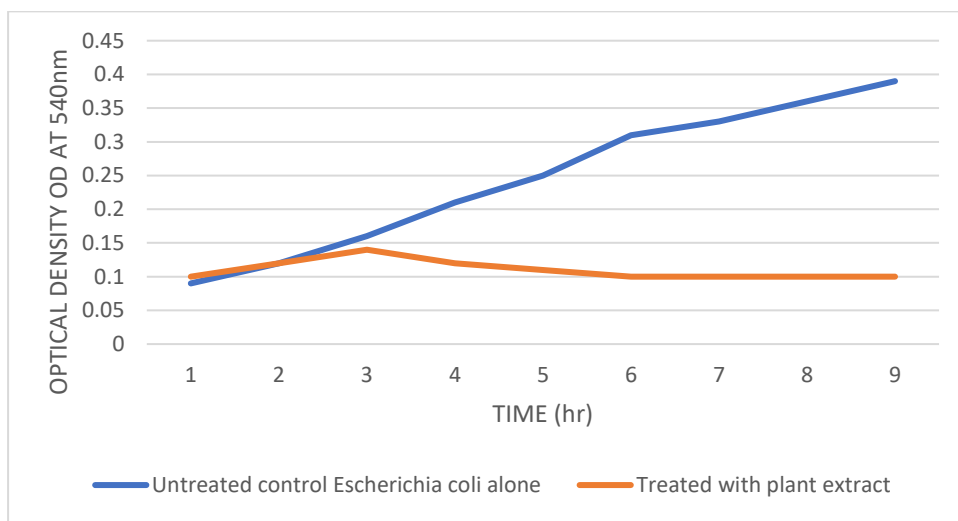


Figure 4.1.3 (a)

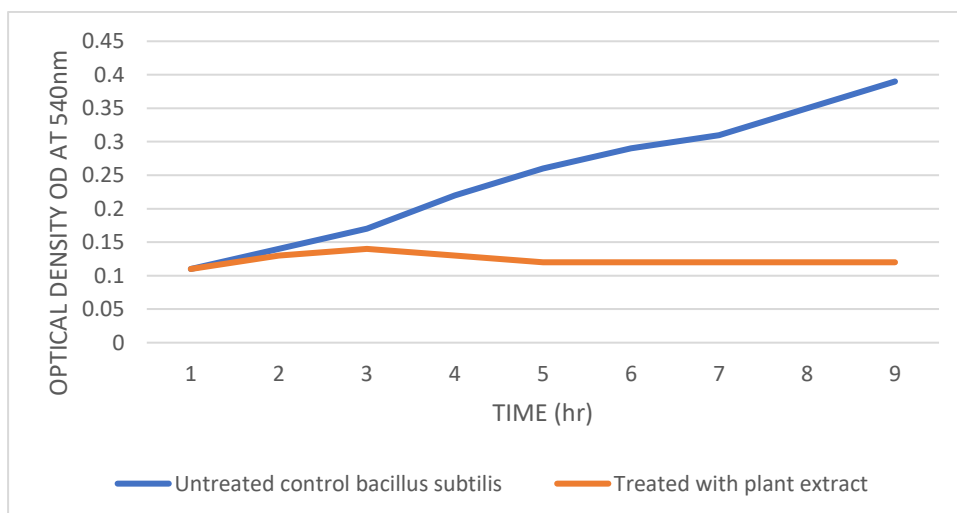


Figure 4.1.3 (b)

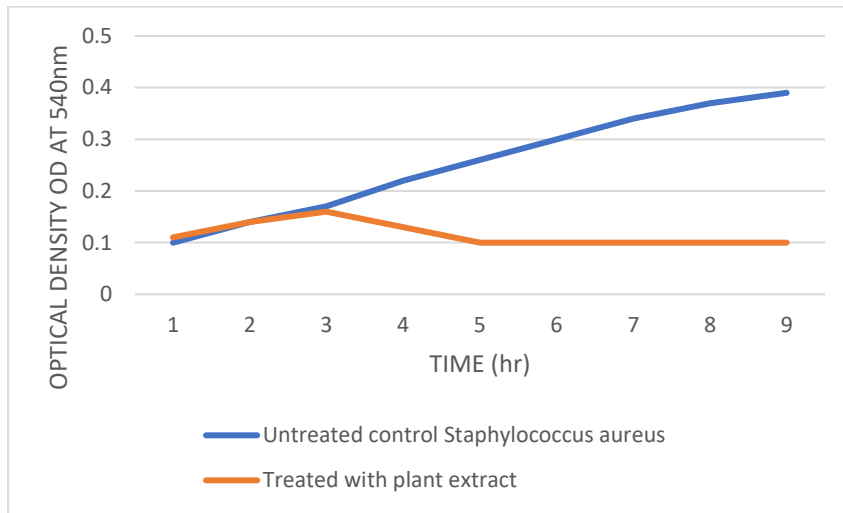


Figure 4.1.3 (c)

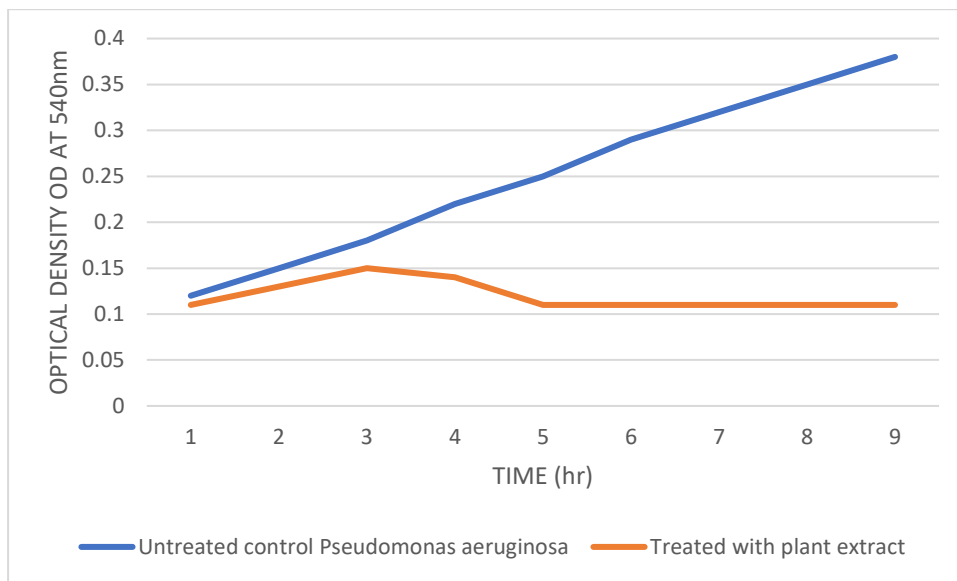


Figure 4.1.3 (d)

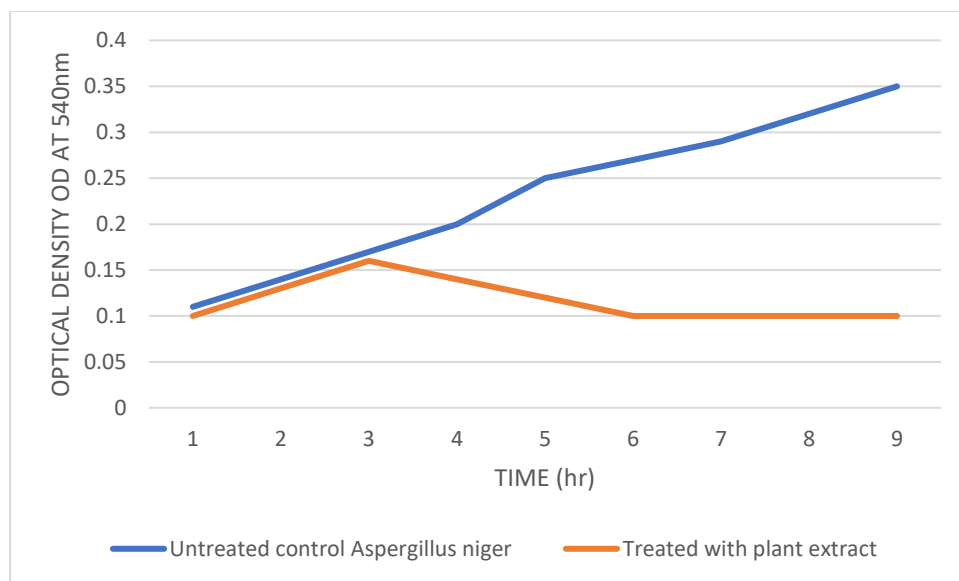


Figure 4.1.3 (e)

Figure 4.1.3: GROWTH CURVE PATHOGENIC ORGANISMS WITH EXTRACT AND WITHOUT EXTRACT

It was found that the Wheat grass extract, of the growth index of bacterial and fungal species, was decreased after a certain period of time around 5 hours and after which a stationary phase was attained.

Falcone *et al.* (2017) reported that thymol exerts a significant antimicrobial effect on each phase of the *B. cereus* growth cycle. Qais *et al.* (2019) showed a dose-dependent antimicrobial effect of *Murraya koenigii*-silver nanoparticles against methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *S. aureus*, Extended-spectrum β -lactamase-producing *E. coli*, and a sensitive *E. coli* strain was observed. The growth was monitored at different time intervals by measuring absorbance at 620 nm using a microplate reader. The treatment with nanoparticles to all the bacterial strains was given in two-fold dilution, and the wells without any amendment of *Murraya koenigii*-silver nanoparticles were taken as the control group. The extent of growth inhibition on methicillin-resistant *Staphylococcus aureus* by *Murraya koenigii*-silver nanoparticles was found to be 31.4%, 53.3%, and 85.6% at 8, 16, and 32 $\mu\text{g/ml}$, respectively, and the concentration above 32 $\mu\text{g/ml}$ was found to be inhibitory to more than 90% of the bacteria.

Shinde *et al.* (2015) Zinc oxide nanoparticles had showed maximum inhibitory activity slightly more activity against *S. aureus* than *S. Typhimurium* till 8h at a Concentration of 60 $\mu\text{g/mL}$. At 80 $\mu\text{g/ mL}$, Zinc oxide nanoparticles exhibited the complete inhibitory effect

against *S. Typhimurium*. In the case of *S. aureus*, 80 µg/mL and 100 µg/mL Zinc oxide nanoparticles showed the completely antibacterial activity. Zinc oxide nanoparticles retards the growth of both pathogens when they are present in the bacterial suspension.

4.2 DETERMINATION OF ANTI- INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *Triticum aestivum* LEAF EXTRACT :

The anti-inflammatory activity of the leaf extract was assessed using protein denaturation and erythrocyte membrane stabilization methods.

4.2.1 EFFECT OF METHANOLIC EXTRACT OF *Triticum aestivum* LEAF EXTRACT ON PROTEIN DENATURATION

Denaturation of proteins is the main cause of inflammation. As part of the investigation of the mechanism of the anti-inflammatory activity, the ability of the extract to inhibit protein denaturation was studied. This results indicated that *Triticum aestivum* extracts have maximum anti-denaturation activities compared to that of sodium diclofenac which was used as a standard anti-inflammation drug. The results of the protein denaturation test are shown in Figure 4.2.1 and Table 4.2.1.

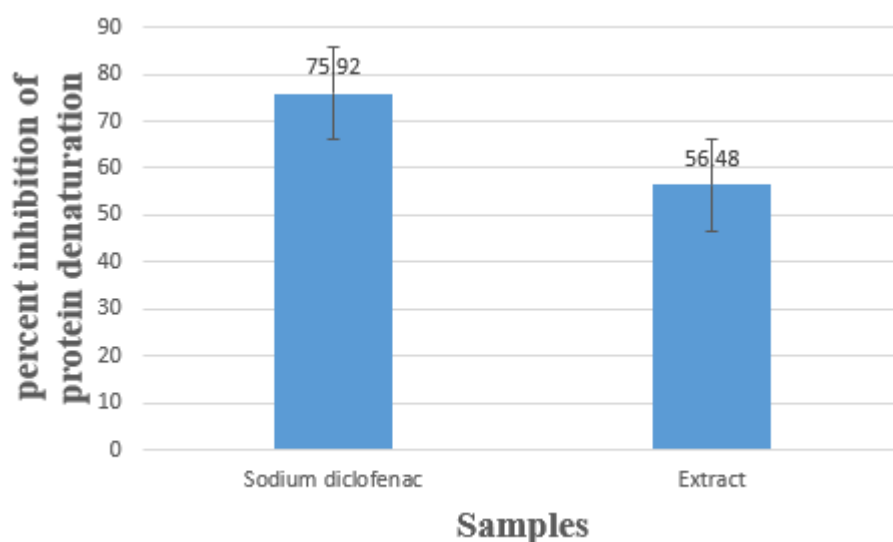


Figure 4.2.1 EFFECT OF *T. aestivum* AND SODIUM DICLOFENAC AGAINST PROTEIN DENATURATION

Table 4.2.1 PROTEIN DENATURATION ACTIVITY

| SAMPLES | PER CENT INHIBITION OF PROTEIN DENATURATION |
|-------------------|---|
| Extract | 56.48±5.78 |
| Sodium diclofenac | 75.92±8.48 |

The values are mean ±S.D. of triplicates

Kousalya *et al.* (2020) reported that at a concentration of 10 mg/ml, *Erythrina indica* have shown 33.98% of proteinase inhibitory activity and *Cardiospermum halicacabum* has shown 57.88% activity. *Mukia maderaspatana* has shown 73% of protease inhibition whereas *Delonix elata* has shown only 30% activity.

Dharmadeva *et al.* (2018) reported the anti-inflammatory activity of *F. racemosa* against the denaturation of the egg albumin. The highest inhibition rate was observed in both hot and cold-water extracts at the concentration of 1000 µg/ml. The inhibitory rate of cold-water extract and hot water extract was gradually increased with the increase in concentration while a decreasing rate of inhibition was observed in the standard drug ibuprofen.

Altundag *et al.* (2020) studied the protein-denaturation activity of *A. horridus*. Sodium diclofenac was used as a positive control from 100 to 1000 µg/mL concentrations the percentage inhibition of protein denaturation activity ranged between 32.95% and 83.53%, respectively. The maximum percent inhibition was observed at the extract concentration of 25 mg/mL which is 29.42 %.

Shaw *et al.* (2018) reported that the crude extract of both *C. caian* and *C. zeylanicum* exhibits the concentration-dependent inhibition of denaturation of protein, where the inhibition of protein denaturation increased with the concentration of extract, and the highest anti-denaturation activities were observed at 1.00 µg/ml for both plant extracts (62.31% and 49.61% respectively).

4.2.2 ERYTHROCYTE MEMBRANE STABILIZATION TEST

The anti-inflammatory activity of *Triticum aestivum* extract was assessed by its ability to inhibit albumin denaturation using membrane stabilization test. The extracts showed good inhibitory effect against hemolysis of red blood cells.

a) Hemolysis induced by heat:

The anti-inflammatory activity of *Triticum aestivum* extract was assessed by hemolysis induced by heat method using human red blood cells. The result showed the evaluation of membrane stabilizing activities of *Triticum aestivum* extract has shown good anti-hemolytic activity when compared to the reference drug, sodium diclofenac (Figure 4.2.2a. and Table 4.2.2a).

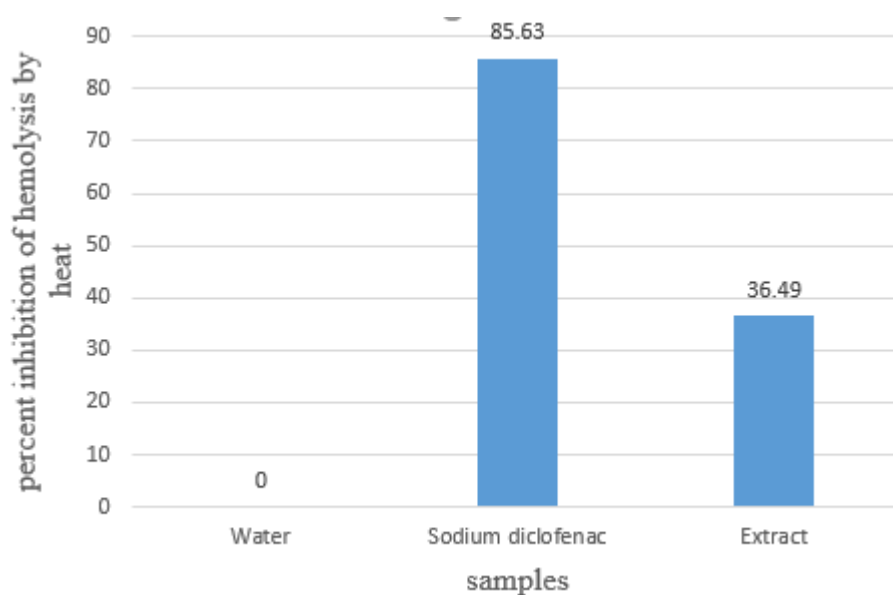


Figure 4.2.2a EFFECT OF *T. aestivum* LEAF EXTRACT AND SODIUM DICLOFENAC ON HEMOLYSIS BY HEAT

TABLE 4.2.2a HEMOLYSIS INDUCED BY HEAT

| SAMPLES | PERCENT INHIBITION OF HEMOLYSIS BY HEAT |
|-------------------|---|
| Water | 0.0±0.00 |
| Sodium diclofenac | 85.63±3.40 |
| Extract | 36.49±1.64 |

The values are mean ±S.D. of triplicate

The observations of the present study correlated with many research findings. In one such study, Anwesa *et al.*,2013 reported the *T. chebula* fruit extract demonstrated a strong stabilizing effect on the human RBC (Red Blood Cell) membrane. The membrane-stabilizing

effect of *T. chebula* fruit extract was concentration-dependent and ranged from 95.35% to 98.17%.

Yesmin *et al.*, 2020 studied the effect of ethanolic extract of *Piper Chaba* roots and ASA on heat-induced hemolysis of RBC membrane showing a high percentage of inhibition in 100 – 500 µg/ml.

b) Hemolysis induced by hypotonicity:

Human red blood cell membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced human red cell membrane lysis was taken as a measure of the anti-inflammatory activity of drugs. The anti-inflammatory activity of *Triticum aestivum* extract was also assessed by hemolysis induced by the hypotonicity method using human red blood cells. The results showed the *Triticum aestivum* extract showed good membrane stabilizing activities which are evident from the percent inhibition of hemolysis ranging 51.40% which is almost equivalent to that of the standard drug sodium diclofenac (70.48%) as shown in Figure 4.2.2.b. and Table 4.2.2.b.

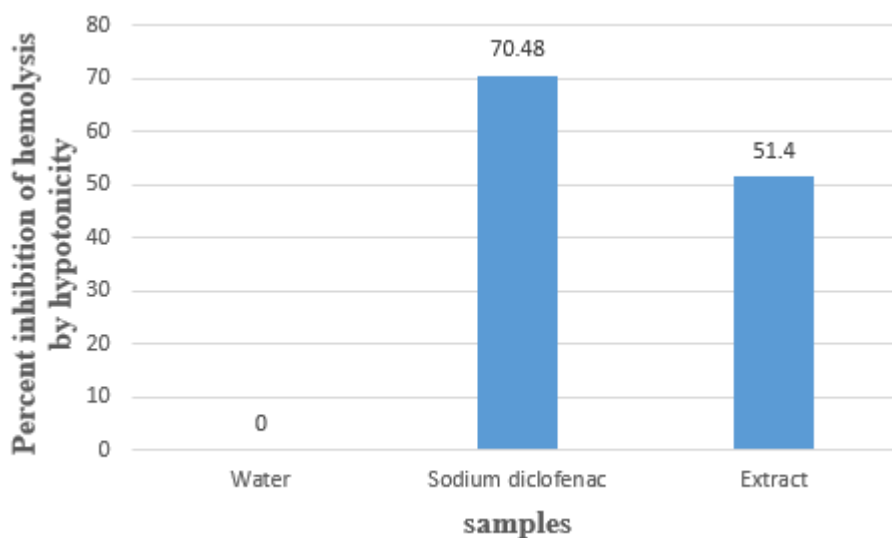


Figure 4.2.2b: EFFECT OF *T. aestivum* LEAF EXTRACT AND SODIUM DICLOFENAC ON HEMOLYSIS BY HYPOTONICITY

Table 4.2.2b: HEMOLYSIS INDUCED BY HYPOTONICITY

| SAMPLES | PERCENT INHIBITION OF HEMOLYSIS BY HYPOTONICITY |
|-------------------|---|
| Water | 0.0±0.00 |
| Sodium diclofenac | 70.48±1.34 |
| Extract | 51.40±1.04 |

The values are mean ±S.D. of triplicate

Chowdhury *et al.* (2014) studied the extract at a concentration range from 100 µg/mL to 300 µg/mL to protect the human erythrocyte membranes against lysis induced by hypotonic solution. At a concentration of 100 µg/mL, the extract inhibited 24.38% of red blood cell hemolysis as compared with 38.84% produced by Aspirin at 100 µg/mL. Since human red blood cell membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced human red blood cells membrane lysis was taken as a measure of the anti-inflammatory activity of drugs. The results obtained demonstrated that methanolic extract of leaves of *Gardenia coronaria* can significantly and dose-dependently inhibits human red blood cell membrane hemolysis.

Parvin *et al.* (2015) studied the crude ethanol extract and chloroform fractions of leaves and bark showed a concentration-dependent anti-inflammatory activity, and the protection percent increased with an increase in the concentration of the samples. At a concentration of 1.0 mg/ml, the crude ethanol extract of leaves and bark produced 53.86 ± 6.37 and 61.85 ± 5.56 % inhibition of RBC hemolysis, respectively as compared with 75.80 ± 5.04 % produced by standard drug aspirin. Likewise, chloroform fractions of leaves and bark produced 48.74 ± 0.56 and 43.55 ± 6.20 % inhibition of red blood cell hemolysis, respectively. It is clear from the data that the crude ethanol extract of leaves and bark showed a greater response than the chloroform fractions.

Chippada *et al.* (2011) reported the percentage of membrane stabilization for methanolic extracts and diclofenac sodium were done at 50, 100, 250, 500, 1000, and 2000 µg/ml. Methanolic extracts of *C. Asiatica* are effective in inhibiting the heat-induced hemolysis of human red blood cells at different concentrations (50-2000µg/ml) It showed maximum inhibition of 94.97% at 2000µg/ml.

Summary and Conclusion



5. SUMMARY AND CONCLUSION

Medicinal plants have been playing an essential role in the development of human culture. Medicinal plants are regarded as rich resources of traditional medicines and from these plants many modern medicines are produced. The secondary metabolites produced by the plants are usually responsible for the biological characteristics of plant species used throughout the world. Traditional plant medicines still enjoy a significant position in the modern-day drug industries due to the minor side effects as well as the synergistic action of the combination of compounds.

The present study was carried out to investigate the antimicrobial and anti-inflammatory properties of the leaves of fourth-day plant of *Triticum aestivum*. At first, the antimicrobial effect of *Triticum aestivum* leaf extract on was studied followed by the determination of minimum inhibitory concentration. The anti-inflammatory activity was also tested using protein denaturation and RBC hemolysis. The results obtained are summarized below.

In disk diffusion assay, the zone of inhibition was observed in methanolic extract of *Triticum aestivum* leaves using four bacterial species and one fungal species. A strong zone of inhibition against four bacterial species and one fungal species at the dosage of 20 μ g followed by 15 μ g, 10 μ g, and 5 μ g was observed.

The minimum inhibitory concentration for the antimicrobial potential of the wheat grass was determined and was found to be 20 μ g for all the tested four bacterial strains and one fungal strain. The antimicrobial activity was further confirmed by growth curve analysis wherein the Wheat grass extract inhibited the growth of bacterial and fungal species after a certain period of time of around 5 hours after which a stationary phase was attained. Following the analysis of antimicrobial activity, the plant was tested for their protective effect against inflammation using protein denaturation assay and membrane stabilization methods. The methanolic extract of *Triticum aestivum* showed good anti-denaturation activities compared to that of standard drug sodium diclofenac which infer that it can be used for the treatment of inflammation.

Thus, the research findings of the present study revealed that the leaves of the 4th day plant of wheat grass exhibit good anti-microbial and anti-inflammatory activity which can be further explored to identify a potent lead compound from the leaves that may be used to treat microbial infections and inflammatory conditions.

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