Effect of Antioxidant Fractions of *Convolvulus arvensis* and *Fumaria officinalis* on Hematological Parameters of Rabbit

Thesis

by

Masooma Mehmood

Thesis submitted to the Lahore School of Economics

in partial fulfillment of the requirements for the degree of

MPhil Environmental Sciences and Policy

2017- 2019

Supervised by: Dr. Muhammad Nawaz Chaudhry

Co-supervisor: Dr. Naqi Hussain

Table of Contents

List of Tables

List of Figures

List of Abbreviations

1.	Fumaria Officinalis hydro alcoholic extract	FOHE
2.	Convolvulus arvensis	CA
3.	Red blood cells	RBC
4.	Packed Cell Volume	PCV
5.	Hemoglobin	Hb
6.	Monocytes	Mo
7.	Granulocytes	Gr
8.	White Blood Cells	WBC
9.	Lymphocytes	Ly
10.	Mean Corpuscular Volume	MCV
11.	Mean Corpuscular Hemoglobin	MCH
12.	Mean Corpuscular Hemoglobin Concentration	MCHC
13.	Platelets count	PLT
14.	Mean Platelet Volume	MPV
15.	Standard error of mean	SEM
16.	Total Antioxidant Capacity	TAC
17.	2,2-Diphenyl-1-picrylhydrazyl	DPPH
18.	Butylated Hydroxytoluene	BHT
19.	Remaining Methanolic Extract	RME
20.	Pet Ether	PE
21.	Tetrapleura tetraptera	TTE

ABSTRACT

Plant extracts are used extensively to treat many diseases. Many herbs extracts possess antimicrobial activity against a wide range of bacteria, yeast, molds and viruses due to phytochemical constituents. In this study extracts of *Fumaria officinalis and* **Convolvulus** *arvensis* were used. The main objectives of this study were to examine the in-vitro antioxidant and in-vivo hematological parameters of *Convolvulus arvensis* and *Fumaria officinalis*, to evaluate the effects of antioxidant fractions of two herbs on hematological parameters of rabbits, to evaluate the medicinal use and value of selected herbs. Various fractions were used to determine the antioxidant activities using Hydrogen peroxide, Phosphomolybdenum and DPPH assays. In H_2O_2 assay, methanolic extract of *F. officinalis* and *C. avensis* showed a pretty good percentage of antioxidant activity 82% to 90% at the highest concentration i.e, 1 mg/ml. Both *C. arvensis* and *F. officinalis* showed 88% of antioxidant effect through DPPH radical scavenging assay. Doses of *F. officinalis* and *C. arvensis* extract were administrated orally to two groups of rabbits along with a control group. Parameters evaluated included Hemoglobin (Hb), Red Blood Cells (RBC) and Hematocrit (PCV/Hct). The results of the hematological parameters showed that although both the herb extracts have pretty good antioxidant activity but has lowered values of hematological parameters in rabbits when compared to the control group.

CHAPTER 1

INTRODUCTION

Herbs are those parts of a plant which are mainly leafy and green in color or the flowering part like leaves and stems. They are being used in a very little amount in order to import some flavour to food and in this way they are quite different from other plants. There are a lot many herbs that are originating from mediterraean area. Extract of herbs contain antimicrobial activity which works against bacteria, viruses, yeast and molds (Martínez-Graciá et al., 2015). Herbal medicines which involve herbs, their preparations and final herbal products include all those ingredients, materials and parts of plants which are considered to have all therapeutic effects. Around 80% of the world population relies upon various traditional medicines mainly including herbal medicines which plays a vital role in diagnosing, preventing and treating many kinds of diseases and illnesses. In Spite of modifications and advancement in modern medicines, the Arab countries are still practicing traditional medicines. Increased worldwide interest has been generated regarding traditional arabic herbal medicine among many herbalists and scientific practitioners (John et al., 2015).

Herbs also consume nutritional values like Zinc, Vitamin C, Vitamin A, Magnesium, iron, Calcium, Copper, Manganese, riboflavin, niacin, pyridoxine and pantothenic acid. Different kinds of herbs exist in which the most existential one on Culinary herbs that are being discerned from vegetables as they just provide flavor rather than substance to food because they are used in a very little amount e.g. Spices (Small, 2006).

Not many plants are being used as spices and herbs both like dill seed and dill weed or seeds, coriander or leaves which are both used as herbs and spices. Also, there are some kinds of herbs which are beneficial for both medicinal and culinary purposes like those which belong to the mint family (Elansaryn & Mahmoud, 2015). Tisanes is the term which is used when some herbs can be put in boiling water in order to make herbal teas. These herbal teas are perceived to be made mainly of aromatic herbs mainly but it may not have caffeine or tannins and do not put together with milk. Mint tea and chamomile tea are one of the common examples of herbal teas. Herbal teas are majorly considered as a rich source of relaxation or sometimes it is being associated with the rituals (Dweck, 2009).

There are many studies showing that taking in herbs in any way will boost cognitive functioning. Incorporating rosemary in a diet will gradually upgrade one's psychological health. This supernatural herb is extremely helpful in combating other health problems but much beneficial for the brain functioning and memory as it fights for the factors that are

responsible for severe brain disorders and diseases like Alzheimer's. Alzheimer's disease is due to the deficiency of acetylcholine which is a chemical messenger in the brain. This drop in the level of acetylcholine is inhibited by Sage which prevents its breakdown. A study of 4 months assessed 42 individuals with Alzheimer's disease which shows that brain functioning has been immensely improved by sage extract. Many other studies also proved that sage may help in improving brain's memory in all young and old peoples (Tapsell et al., 2006).

Oregano, thyme and peppermint are some of the herbs that are stuffed with many antiseptic and antibacterial properties that are much effective in hindering acne problems like minimizing acne marks by making skin glowy, radiant and clear. Oregano has been advised to include in the diet for those people who have certain food allergies because this herb contains a large amount of antifungal and antibacterial properties which fights various food borne infections and illnesses (Korać & Khambholja, 2011).

There are herbs that are considered to cause many adverse effects. When there is inappropriate formulation and no understanding of a specific drug or plant then it will ultimately lead to many inauspicious reactions which can be lethal or threatening. In order to determine the effectiveness and safety of every plant, proper double blind clinical trials are required so it is easy to recommend them for any medical purpose. Though, many people consider herbal medicines safe to use as compared to the synthetic drugs which cause toxicity or many other harmful effects to the patient. But, this is to keep in mind that using herbal medicines without knowing their efficacy can be highly mortal or dangerous to health (Vickers, 2007).

1.1. Antioxidants and their derivatives:

Substances which provide protection to cells from various damages due to unstable molecules like free radicals are called Antioxidants. These antioxidants stabilize the unstable free radicals in order to put a stop to the damage and adverse effects that these free radicals or molecules may cause (Hamid et al., 2010).

Many chemists defined antioxidants as a group of compounds which have the potential to get oxidized as a replacement of other compounds being present. They have a wide range of uses from vulcanizing the rubber to food storage. Many biologists came forward with the benefits of antioxidants in terms of health with various publications of flavonoids, ascorbic acid, cancer, common cold and vitamins. Many scientists researched antioxidants and found out that antioxidants are the best protecting agents, used against cancer and overall health problems (Seifried et al., 2007).

Antioxidants that are present in vegetables are called dietary antioxidants such as phenolic compounds, water soluble vit-C, carotenoids and lipid soluble vit-E. Dietary antioxidants are

also responsible for the defense mechanism against oxidation process and oxidative stress. Ultimately, they provide protection against chronic diseases like heart diseases, cancer and diabetes (Podsędek, 2007).

Effectiveness of antioxidants is mainly dependent upon the oxidation/reduction potential, activation energy, rate constants, its ability to be lost or destroyed and its solubility (Brewer, 2011). Antioxidants delay the process of oxidation by hindering the formation of free radicals with the help of following mechanisms:

- By reducing the oxygen concentration
- By shattering the autoxidation chain reactions
- By chelation of metal ions through decomposing lipid peroxides
- By scavenging the species which helps in initiating peroxidation
- By preventing the formulation of peroxides (Nawar 1996).

Depending on the chemical attributes and physical features, chain breaking antioxidants are different in their effectiveness. For the effectiveness in antioxidant activity, chemical potency, solubility and availability to peroxy radicals are mainly required in these systems (Brewer, 2011).

Whether eating antioxidants are healthier for people or not is still a controversy. For a long time it has been known that diets that are rich in antioxidants are more healthier and beneficial than those that are not. Because foods that are rich in antioxidants are berries, grains, vegetables, fish, and nuts which have been consumed for alonger period of time and provide a wide range of health benefits. Foods which contain antioxidants in them are mainly high in fiber, proteins, minerals, vitamins and unsaturated fats which altogether plays a vital role in promoting health while the foods which are low in sugar and saturated fats are majorly contributes towards many common diseases.

1.2. Hematology and Hematological analysis:

Hematology is the study of blood and organs that are blood forming which may involve the diagnosis, treatment and prevention of various diseases of blood, immunologic, bone marrow and other vascular diseases. Analysis of hematology is mainly and more oftenly used for the diagnosis and treatment of animal diseases. Hematology has become more crucial and vital for recognising diseases and their treatments in small laboratory animals because, nowadays the analytical methods are much more sensitive and require smaller sample volumes (Washington & Van, 2012).

There are many other benefits of hematological analysis other than diagnosing disorders of the hematological system. They are also providing aid in the diagnosis of many organ and systemic

diseases. However, a disease can only be diagnosed through a complete blood cell (CBC) count. And the hemogram which provides valuable information regarding diagnosis, surveillance, and future development of a disease in a person (Roland, Drillich, & Iwersen, 2014).

For the prognosis of diseases, therapeutic and feed stress monitoring, hematological analysis have been considered very beneficial and helpful. Blood is the main transport system of the human body and hematological estimation usually provides all of the information of the body's response to all forms of injury. Evaluation of the degree of blood damage and diagnosing many other diseases is being carried out by hematological analysis. Physiological conditions of animals may depend upon the change in blood constituents. All the nutrients are being transported through blood to different parts of the body. This is why whenever pathogens, drugs or any other nutritional compounds affect the body it also affects the blood and thus causes potent and vigorous health and growth problems. The nutritional and clinical status of animal health can be evaluated by using blood analysis which is the rapid and easily available means of assessing. Incorporating dietary compounds have remarkable effects on the composition of blood and blood analysis is an accurate and readily available measure of nutritional value (Etim et al., 2014).

Many scientists in a joint committee formulated a hematology tests panel for studying the toxicity and safety of laboratory animals (Weingand et al., 1996). This panel mainly involves different tests such as erythrocyte count, erythrocyte morphology, platelet count, differential leukocyte count, total leukocyte count, hemoglobin concentration, mean corpuscular volume, hematocrit or packed cell volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin. Blood tests or smears are useful in analyzing reticulocyte counts and if needed and indicated bone marrow cytology should also be gathered and assessed. The joint committee suggested various tests of hemostasis which includes activated partial thromboplastin time, prothrombin time and platelet count (Washington & Van, 2012).

1.3. Profile based plants used in study:

- **Convolvulus arvensis:**

Convolvulus arvensis L. is also known as field bindweed which belongs to a family (Convolvulaceae). It is a perennial deep rooted weed and reproduces from seeds and rhizomes that are horizontal. Seeds may vary in their numbers from 25 to 300/ plant and these seeds are usable or alive for up to 50 years. When the seeds germinate the tap root which grows vertically directly changes its growth direction and grows downward. While the lateral roots show growth

in the top 30 cm of soil and then grow horizontally before turning down in order to form secondary vertical roots. Ultimately, these roots give rise to more and more lateral roots which again turn down and form verticals and the process goes on and on (Balah, 2015).

Convolvulus has an extensive root system which contains lateral roots, rhizomes and deep tap roots with which the bindweed plants can reproduce vegetatively. The depth of roots may reach to 9m and these roots can efficiently compete for issues like limited water and long periods of drought which is harmful for crops thus, the depth of roots may compete against the crops with limiter water. In a year, the plants grow 5 meters laterally which enables the infestations. Fragmented rhizomes which can easily grow into newer plants can also be the reason for spreading field bindweed (Konigsberg, 2014).

Field bindweed is basically a poisonous or toxic weed in gardens, pastures and crops that are being cultivated. It can grow in various conditions like on all soils, under full sun and full shady environment, but mainly it grows on soils which are drier, warmer and drought tolerant. In spring the stems emerge and they become flowers from May until September. Convolvulus grows fastly, suffocates young seedlings and eventually reduces the yield (Baličević et al., 2014).

Figure 1.1. Convolvulus arvensis

Field bindweed is one of the top ten world's unpleasant and worst weeds because of its abundance, economic impact and its wide range of distribution all around the world with temperate climate. This weed likewise other weeds take up water and other nutrients that are used by the desirable species otherwise (Balah, 2015).

- **Shatira Fumaria officinalis:**

It is a herbaceous plant that is found in Europe annually but its most common species has been found in Europe and Asia. These species had a very traditional role in medicines for a longer period of time. Many pharmaceuticals contain this herb in order to treat diseases of gallbladder,

gastrointestinal tract and biliary system. Fumaria is a rich source of isoquinoline alkaloids and its different structural types. This herb contains 60 species and its plants have so many benefits in traditional medicines as they have laxative, stomach, sedative, diaphoretic, blood purifying, cholagogic, anthelmintic, antiseptic and tonic properties in them (Chlebek et al., 2016).

Fumaria can also be used for the treatment of skin diseases, rashes, high blood pressure, rheumatism and conjunctivitis. In Vidal Dictionary, it has been reported that this herb plays a role in contributing bioactive compounds for the formation of various medicines like arko hard capsules, superdiet phials, actibil capsules and actisane digestion (Khamtache-Abderrahim et al., 2016).

Figure 1.2. Fumaria officinalis

RATIONALE

Medicinal plants or herbal products become more significant and their use is still growing in both veterinary and human medicines globally. Whereas antibiotic resistance is a major problem in the healthcare system due to the environmental pollution and degradation being done by pharmaceutical production. Because of the overproduction and ill usage of pharmaceuticals, their metabolites enter the environment through excretion, wastewaters, hospital wastes, sludges, leachates and sometimes through direct disposal of unused medicines which leads to severe environmental pollution that can be minimized easily. Thus, in order to overcome antibiotic resistance and the already hyped environmental pollution burden it is essential to switch ourselves from pharma products to herbal products or phytochemicals. Moreover, there are many herbs that are being investigated for many biological analyses (antioxidant, antimicrobial, antifungal, antibacterial and many more effects). Hence, this study would be helpful in determining the antioxidant effect of two selected herbs and also the hematological parameters of rabbits through the antioxidant fractions with which it would be easier to understand the medicinal use and benefits of these herbs.

CHAPTER 2
LITERATURE REVIEW

Herbs have been in use and demand even in ancient eras and are still in use to not only prevent and treat chronic diseases but also to add or improve the flavour of foods that are edible. There is less or no scientific evidence for the usage of herbs and other medicinal plants but the benefits people are getting out of these herbs has been observed and encouraged. Thus the trend and tradition of utilising such medicinal plants and herbs is still in practise even after the emergence of modern medicine. Many studies have been conducted in order to expand their understanding regarding the nutritional values of herbs by examining the molecular and cellular modes of action of several chemical compounds in herbs more comprehensively. The most advantageous actions of herbs or medicinal plants may involve antioxidant, anti inflammatory, anti thrombotic, anti hypertensive and glucose regulatory effects (Panickar, 2013).

Back then 500 years till date herbs are very essential to traditional and nontraditional kinds of medicine. The undergoing acceptance of herbs and medicinal plants is due to the fact that herbs are considered to cause very minimal side effects unlike the modern medicines. Many scientists have proved the efficiency and benefits of herbal medicines by relying on modern scientific methods and the medicine which is evidence based. These scientific methods and evidence based medicines put a clear focus on understanding the mechanism of herbal modes (Maver et al., 2015).

2.1. Herbal products/medicines preparation:

Herbs include different parts of plants which may be whole plants, its fragments or its powdered form such as leaves, flowers, stems, fruit, bark, wood, seeds, roots and rhizomes. Furthermore, herbal material also includes the gums, resins, essential oils, fresh juices, fixed oils, and the dry powders of herbs. There are several countries which process plant or herbal materials through local processes like steaming, roasting, stir baking with the help of honey, alcoholic beverages or any other materials. The final and finished herbal products are based on the herbal preparations and these herbal products include powdered form of herbal materials, extracts, tinctures and some fatty oils. These products are formed by extraction, fractionation, purification, concentration, and other physiological or biological processes. The preparation is also done by steeping or heating the herbal materials in different materials like honey, alcohol etc. The final herbal product is being made from one or the other herb. Using more than one herb is known as a ‘mixture herbal product’. Mixture herbal products and final herbal products may have excipients along with the active ingredients. The active substances that are being

added to the mixture herbal products or the finished herbal products are not considered as herbal because the active ingredients are synthetic compounds and the isolated elements from herbal materials. Several health practices and therapies of traditional medicines are using herbal medicines more frequently such as Unani, Naturopathy, Osteopathy, Ayurveda and homeopathy (Kumari & Kotecha, 2016).

2.1.1. Benefits of herbal medicines:

Medicinal plants or herbal medicines have been safer and effective to use. Hence, every year more people are going towards the medicinal plants which are considered to be safe and sound. Although these medicinal plants can potentially be toxic to health. Wherever the poisonous medicinal plants have been reported or suggested it is due to the poor identification of plants profile. Many researchers believed that plant remedies can not cause any kind of side effects and if any herb proved to be potent it is because it is being sold in a poor way, its incorrect preparation and administration by an inexperienced or untrained person. For some drug therapies and for other conventional drugs, herbal medicines may act as a booster or agonists because it is essential for effective herbal therapeutics (Nasri, 2013).

The Unani system of medicines is most particular in terms of using medicinal plants and out of many systems of medicines it has been proved very powerful in curing and managing memory problems and disorders. There are several herbs which have been assessed chemically and their efficiency have been evaluated through clinical trials. Nevertheless, the primary mechanisms of herbs are still to be assessed. Also, many studies reviewed the use of medicinal plants in preventing and treating Alzheimer's disease and memory loss by utilising conventional herbal therapies (Akram & Nawaz, 2017).

Over the past many years, consuming and using herbal medicines has been expanding around the globe. Around 50% of the United Kingdom population once in their life have consumed herbal medicines and their 100% HIV patients being admitted to hospitals are using herbal medicines. According to the World Health Organization (WHO) 65 to 80% of the developing countries population depends upon herbal medicines and is considered as a primary and major source of treatment. Brazil has the same statistic where around 66% of the population have no availability of commercial medicines. And when the access is granted, the population totally relies upon herbal medicines because of the poor medical and pharmaceutical aid and highest cost treatment through the conventional medicines (Mazzari & Prieto, 2014).

The National Center for Complementary and Integrative health revealed a survey in May 2004 that use of herbal or natural products is the most commonly used CAM therapy excluding the vitamins and other minerals (Tabish, 2008).

2.2. Antioxidants and their potential sources:

Molecules or Compounds that inhibit the free radical reactions and also inhibit cellular damage are called Antioxidants. However, the defense system of antioxidants varies from species to species but the antioxidant defense is universal. Antioxidants are present in the intracellular and extracellular environment both in the non enzymatic and enzymatic forms (Nimse & Pal, 2015).

There are many potential sources of antioxidant compounds which are found in different kinds of plant materials like fruit, leaves, vegetables, barks, roots, oil seeds, cereal crops, spices and herbs. Phenolics groups of plants and flavonoids like lignans, tanis, stilbenes, phenolic acids are majorly found in flowering tissues, leaves, and other woody parts like barks and stems. For normal growth development and defense systems against injuries and infections these flavonoids and phenolic groups are very much essential. Also, these flavonoids give plant colors which are present in fruits, leaves and flowers. They are also found as glycosylated derivatives in plants, however conjugation with organic and inorganic acids and sulfates are also known. Phenolic groups have antioxidant activity because of their redox properties which permit them to behave as reducing agents, H donors and O2 quenchers (Kähkönen et al., 1999). For health maintenance and combating diseases like cancer and coronary heart illnesses, antioxidants are extensively used in dietary supplements as vital ingredients. However, initially many research revealed that antioxidant supplements are essential in promoting health. Later, many clinical trials did not find them useful as they considered excess supplementation harmful to health. But still many researchers strongly believe in the uses of natural antioxidants in medicine and its uses are extending to the industrial uses like in cosmetics, in the preservation of food and in securing the rubber and gasoline by preventing them from degradation (Hamid et al., 2010).

In the United States, antioxidants use has expanded especially in adults. The National Health and Nutrition Examination Survey revealed that more than half of the US population consume dietary supplements, third one of them take multivitamins and more than eighth one of them uses vitamin E and C supplements (Seifried et al., 2007).

2.3. Hematological studies, Parameters and Significance:

Hematology is studying the pathophysiology of cellular elements and coagulating proteins in blood. Erythrocytes or Red blood cells, myeloids or white blood cells, immunocytes or T and B cells, platelets or thrombocytes and diseases related to them is the main focus of hematology (Washington & Van, 2012).

Hematology and hematological studies are considered to play a very important role because blood is the main transport system of the human body and evaluating the hematological profile provides information regarding body responses for every form of injury which involve toxic injuries as well. Hematology is a very practical process in diagnosing so many diseases along with the evaluation of the degree of blood damage. This is pertinent as the blood components change in regard to the physical attributes of animals. Blood constitutes different nutrients and minerals which transport to different parts of the body. That is why, anything which affects the blood will affect the whole human body badly. The blood may be affected either by drugs, pathogens or nutritions and ultimately affecting the health, maintenance, growth and reproduction of the human body (Ihedioha, Okafor & Ihedioha, 2004).

In order to investigate important status of the body, hematological changes are being used and also to evaluate the nutritional, environmental, and pathological factors. Scientists, veterinarians, and researchers found avian physiology to be massively important because of the environmental and nutritional factors (Islam et al., 2004).

Scientists observed the Aqueous extract of oleander leaf's effects on hematological parameters and pathological changes in the hearts and lungs of white rabbits living in New Zealand. Examining the rabbits through microscopes revealed that they have pneumonia in their lungs and degeneration of muscle fibres in the heart. Thus , oleander extract had very toxic effects on the heart tissues and lungs. It also induces massive and great changes in hematological indices like red blood cells, white blood cells, and platelets along with the haemoglobin concentration (Taheri et al., 2013).

2.4. Medicinal plants and their uses:

Plants have medicinal properties in their different parts such as roots, leaves, barks, fruits and seeds. A single plant contains different active ingredients in its different parts so one part of a plant can be toxic while the other part of the same plant is either harmless or beneficial. A herbaceous plant Ocimum gratissimum (Linn) belongs to the family Labiaceae which is mostly common and found in tropical Asia, India. This herb is used for treatment of paralysis and rheumatism by using it as an aromatic bath. Despite the fact that this plant is famous and considered as a herbal medicine, it also has been observed to suppress the hematopoietic system. On the contrary, due to the presence of flavonoids and saponins it also has hepatoprotective activity in rabbits. This plant extract also reduces liver enzyme activities thus, the chronic use of this herb is not advised (Effraim, Salami & Osewa, 2000).

In Western Nigeria, tetrapleura tetraptera is significantly used among men as a medicine to control birth. But it has been revealed that this plant extract emerges with toxic effects and

pathological wounds in some or the other organs. Haematological effects of ethanolic extract of tetrapleura tetraptera (TTE) fruit in male rabbits has been observed. The rabbits are being administered by the TTE extract and the venous blood samples of rabbits showed that TTE extracts caused a massive reduction in RBC and WBC count (Odesanmi, Lawal, & Ojokuku, 2010).

- **Fumaria officinalis**

This herb is also known as an earth smoke medicinal plant which plays a significant role in empirical medicine in various countries. Fumaria is being used for dermatological problems such as eczema, scabies or milk crust but very few studies support its dermatological use. While on the other hand it is beneficial to treat problems related to the biliary system. Many studies have been done, a number of empirical and clinical reports and animal experimentation have been published so far. In Germany, it has been found that fumaria have the ability to fight against diseases of gallbladder and biliary systems along with gastrointestinal tract (Hentschel, Dressler, & Hahn, 1995).

Fumitory also contain carbohydrates, flavonoids, alkaloids, glycosides, terpenoids, phytosterols, proteins, amino acids, fixed oils, steroids, tannins, saponins, phenolic groups and several other chemical constituents (Al-Snafi, 2020).

Antioxidant activities of fumaria capreolata and fumaria bastardii with their alkaloid extracts have been assessed by calculating their reducing capacity and their ability to inhibit peroxidation and DPPH radical scavenging activity. Both of the plant extracts have powerful total antioxidant activity. However, bastardii extract is more powerful than the other species of fumaria. Moreover, both the plant extracts have a very potent reducing power and DPPH free radical scavenging at specific doses (Chibane, 2007).

For the treatment of skin diseases, hypertension, rheumatism and infections Fumaria has been used significantly. It is used in the form of tea for treating hepatic and gallbladder diseases in the North of Portugal. While in Italy this herb is used as a respiratory stimulant, hypertensive, cholagogue, antispasmodic and anti arteriosclerosis. In Cyprus, the plant has been used against constipation, liver detoxification and hypertension. People of Morocco use it for the treatment of hypertension and cardiovascular diseases (Al-Snafi, 2020).

Alcoholic extract of Fumaria Officinalis in rabbits have been administered in order to investigate its haematological effects. Fumaria Officinalis is an annual herb from the genus Fumaria which is used in herbal medicine for a longer period of time and is widespread in treating and combating many diseases. FOHE is Fumaria Officinalis hydro alcoholic extract

which minimizes haematological activity in rabbits and also should permit its consideration in managing anaemia and other immunity dependent disorders (Khoshvaghti et al., 2014).

- **Convolvulus arvensis:**

It is an annual herb which is considered as a weed and commonly found in Asia and Europe. It is also called a perennial climber and is used for so many purposes. Its roots and resins are laxative, purgative, diuretic and cholagogue while its flowers are also laxative which are used as tea infusion which also helps in treating fever and wounds. On the other hand its leaves are very beneficial in minimizing menstrual pains or cramps. On testing this herb on rabbits it showed immunostimulant effects and also cytotoxic effects for cancerous cells on humans. In 50% aqueous ethanol, CA extract had a remarkable antioxidant activity when measured by TEAC, FRAP and ORAC assays. It also showed protective effects against degradation of lipids in the food models. Thus CA is consumed as a source of antioxidants in many food industries (Azman et al., 2015).

Convolvulus is famous as it contains alkaloids which are the compounds that have anticancer properties but at high doses it may prove to be toxic. When CA extract administered orally it inhibits tumour growth but at a certain dose. Tumour growth inhibited by 70% at the highest dose of extract. It also inhibits angiogenesis in embryos of chicks, makes lymphocyte survival more possible and improves yeast phagocytosis. It did not kill the whole tumour cells in culture; this is why it needs further study to state whether it works as an anticancer agent or not (Meng, et al., 2002).

The members of this herb family have cytotoxic activity on some of the tumour lines. Water extract of Convolvulus which has higher molecular weight has powerful stimulating and antiangiogenesis effects. It has been seen that the presence of Lipophilic glycosides as a non polar component when extracted by chloroform showed the highest degree of cytotoxic properties (Sadeghi-Aliabadi, Ghasemi, & Kohi, 2009).

Convolvulus have been used in the restoration of fertility of agriculture lands which is subjected to use of chemicals and pesticides extensively. Eradication of chromium, cadmium, and copper from the soil is very important. Potential accumulation of Convolvulus plants with Cadmium II, chromium VI and copper II has been observed by using an agar based medium. The data of the field revealed that convolvulus is an active plant for the phytoremediation of soils that are being contaminated with Cd II, Cr VI and Cu II. Moreover, CA has also been considered as a possible Cr hyperaccumulator plant species because of chromium concentration in the dry leaf tissues (Gardea-Torresdey et al., 2004).

OBJECTIVES OF THE STUDY

The main objectives of this study are:

- To examine the in-vitro antioxidant and in-vivo hematological parameters of wild herbs i.e Convolvulus arvensis and Fumaria officinalis.
- To evaluate the effects of antioxidant fractions of two herbs on hematological parameters of rabbits such as RBC, Hemoglobin and Hct.
- To evaluate the medicinal use and value of selected herbs

CHAPTER 3

METHODOLOGY

A descriptive methodology was adopted for the purpose of completion of this research. All the information and data regarding the specific herbs, hematological parameters and antioxidant activity were collected through available literature from articles, books, and documents in order to carry out Secondary data collection. While for primary data all lab tests and treatment were conducted in the Pakistan Council of Scientific and Industrial Research (PCSIR) lab located in Ferozepur road lahore. The lab tests were included in vitro-antioxidant activity of plants or wild herbs along with hematological parameters of rabbits in order to analyze the effects of wild herbs on hematology of rabbits. Also, evaluating the medicinal use of selected wild herbs. Methodology adopted for this study is illustrated in figure 3.1.:

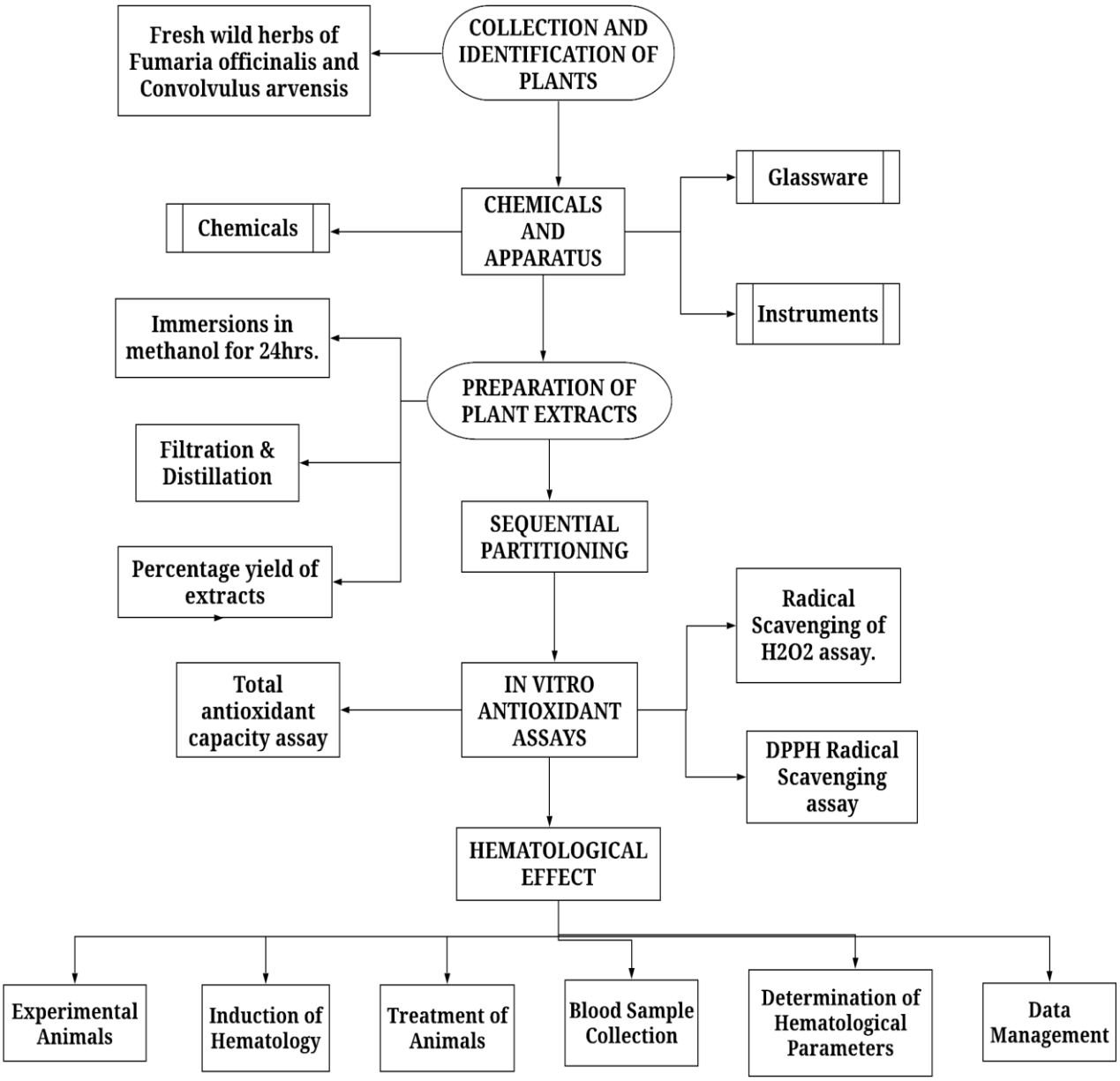

Figure 3.1. Methodology flowchart

3.1. Collection and Authentication of Plant Material:

Fresh wild herbs of *Fumaria officinalis* and *Convolvulus arvensis* were collected from the botanical garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Lahore. The herbs were taxonomically identified and authenticated by Prof. Dr. M Zaheer Botanist of Botany Department, Government College University Lahore. The authenticated wild herbs were used for the preparation of extracts.

3.2. Chemicals and Apparatus:

Chemicals:

- Ethanol
- Methanol
- Sulphuric acid
- Sodium di-hydrogen phosphate
- Distilled water
- Ammonium molybdate
- Hydrogen peroxide
- Butylated hydroxytoluene (BHT)
- Ascorbic acid
- Petroleum ether
- Chloroform
- Ethyl acetate
- Potassium di-hydrogen phosphate
- Di-potassium hydrogen phosphate
- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- Phenyl hydrazine

Glassware:

- Beakers (50 mL, 100 mL)
- Measuring Flasks (50 mL, 100 mL)
- Test tubes (20 mL)
- Pipettes (2 mL,5 mL,10 mL)
- Round bottom flasks (500 mL)
- Measuring cylinder (100 mL)
- Cuvettes (10 mL)
- Vails (20 mL)
- Separating funnel

Instruments:

- Water bath
- Weighing balance (Japan)
- Rotary evaporator (Japan)
- pH-meter
- Ultra-violet/ Visible (UV/VIS) spectrophotometer

- Sysmex hematology analyzer

3.3. Preparation of the Extracts:

The collected samples were cleaned and put under the shaded area. After a few days, these herbs were air dried, crushed and ground into fine powder with the help of mortar and pestle.

3.3.1. Immersion in Methanol:

The sample powder was passed through sieve no. 40 and stored in an airtight box. The powdered form of these herbs were cleaned and used for the extraction purpose. 500 grams of powdered form of each herb were immersed in an aqueous methanol (1:15 ratio) in different flasks and placed in a shaded area for 48 hours.

3.3.2. Filtration and Distillation:

After the completion of this process, the solvents were filtered through Whatman filter paper no.1. These extracts were prepared by vacuum distillation using a rotary evaporator at 56 ℃ to reduce the volume of methanol to dryness. The concentrated extracts were kept in a desiccator for 5h to remove the excessive moisture. The crude extracts were packed in an airtight round bottom flask for further studies.

3.3.3. Percentage yield of extract:

(a) Fumaria officinalis:

Percentage yield of the crude extract were determined as following formula:

Amount of herb taken = 500g

Weight of extract after dryness = 6.02g

% extract = 6.02/500*100

= 1.20%

(b) Convolvulus arvensis:

Percentage yield of the crude extract were determined as following formula:

Amount of herb taken = 500g

Weight of extract after dryness = 6.9g

% extract = 6.9/500*100

= 1.38 %

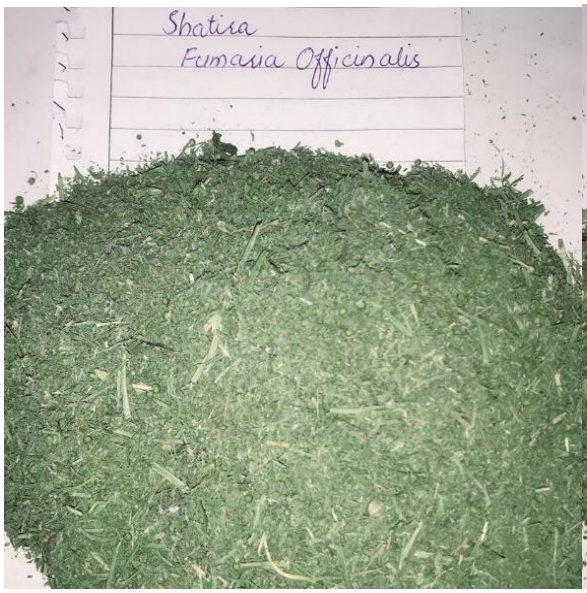

Figure 3.2. Powder form of *F. officinalis*

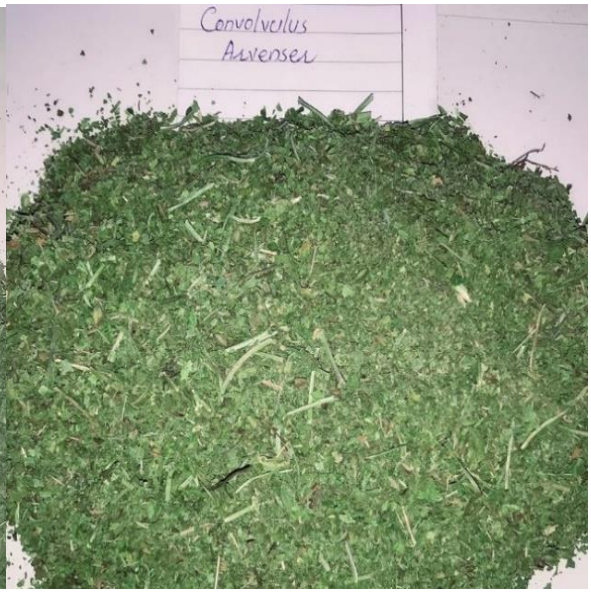

Figure 3.3. Powder form of *C. arvensis*

Figure 3.4. Crude extract of *F. officinalis*

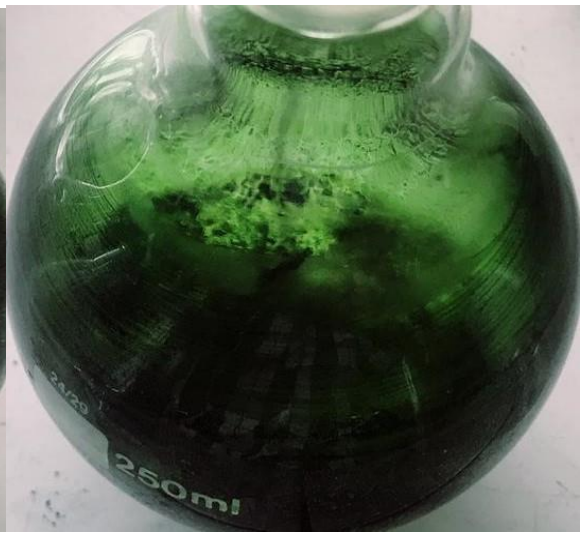

Figure 3.5. Crude extract of *C. arvensis*

3.4. Sequential Partitioning:

The crude extracts were suspended in water in a separating funnel and then extracted with various solvents with increasing polarity such as petroleum ether, chloroform, ethyl acetate. The mixture was mixed thoroughly and aqueous layers are separated from organic solvents for different species separately. The organic fractions were concentrated to dryness under vacuum with a rotary evaporator at 56 ℃. The dried fractions were packed in airtight round bottom flasks for further studies.

3.5. In-vitro Antioxidant Assays:

The fractions of extracts of Fumaria officinalis and Convolvulus arvensis were used for the evaluation of in-vitro antioxidant assays.

3.5.1. Radical scavenging of hydrogen peroxide assay:

The free radical scavenging activity using hydrogen peroxide (H2O2) of plant extracts was determined by the modified method of Dehpour. The principle is based upon the capacity of the extracts to decompose the hydrogen peroxide to water or the intensity of the color of H_2O_2 solution decreased to light color by the addition of antioxidant or phytocompound.

Reagents:

- 6% H2O2 diluted with water
- 0.1 M phosphate buffer maintained the pH-7.4
- Ethanol
- Ascorbic acid

Instrument:

- Ultraviolet-visible (UV/VIS) spectrophotometer

Reagent preparation:

- 6% hydrogen peroxide was prepared by dissolving 3 mL of H2O2 in 7 mL of distilled water.
- 0.1 M phosphate buffer was prepared by dissolving 4.325 g of di-potassium hydrogen phosphate and 3.425 g of potassium di-hydrogen phosphate in 500 mL of distilled water. pH buffer was maintained at 7.4.

Procedure:

The extracts solution of Fumaria officinalis and Convolvulus arvensis were prepared separately by dissolving 15 mg of each crude extract in 10 mL of ethanol and obtained a stock solution of 1 mg/1 mL ratio. 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL of stock solution was taken in separate vials and 3.8 mL of 0.1 M phosphate buffer was added separately to each vial. To these vials 0.2 mL of 6% hydrogen peroxide solution was added separately. The mixture without sample was used as blank. Vials were closed with Al foil for 10 minutes. After 10 minutes, the absorbance of the reaction mixture was calculated at 230 nm by using the UV-Visible spectrophotometer. Ascorbic acid was used as standard for this activity. The percentage inhibition of H_2O_2 were calculated by using the formula:

Percentage inhibition = $Absorption\ of\ blank - Absortion\ of\ sampleAbsorption\ of\ sample \times 100$

Figure 3.6. Scavenging activity of H2O2 assay

3.5.2. DPPH radical scavenging assay:

This is a very popular method as far as natural products antioxidant potential is to be studied. DPPH· (2, 2-diphenyl-1-picrylhydrazyl) free radical is stable when it is in powdered form in a bottle but when it's out of the bottle and mixed with methanol solution it produces chain reactions thereby, we need to do the assay in which antioxidants donate H^+ to DPPH to stabilize it so if the extract solution has antioxidant, it will donate hydrogen to DPPH free radical. When the DPPH molecule delocalized it showed purple or violet in color with maximum absorption band of around 517nm. So, when the hydrogen atom reacts with DPPH it stabilizes it and the reduced form of DPPH is made with the change in its original color which is violet but on stabilizing its color changes into light yellow to transparent depending upon the antioxidant activity. Thus, antioxidant activity can be evaluated by analyzing the decrease or increase of absorbance.

Reagents:

- Ethanol
- 0.1 mM DPPH diluted with ethanol
- Butylated hydroxytoluene (BHT)

Instrument:

- Ultra-violet/ visible (UV/VIS) spectrophotometer

Reagent preparation:

- 0.1 mM DPPH solutions were prepared by dissolving 4 mg of DPPH reagent in 100 mL of ethanol.

Procedure:

Take 10 mg crude extracts of Fumaria officinalis and Convolvulus arvensis were dissolved in 10 mL ethanol to get a stock solution of 1 mg/1 mL ratio. 125 ppm, 250 ppm, 500 ppm and 1000 ppm of the stock solution was taken in separate vials. 3 mL of 0.1 mM DPPH solution was added separately in each vial. The reaction mixture without sample was used as blank. The sample solutions and blank solutions were put in a dark area for 30 minutes. After 30 minutes, the absorbance of the reaction mixture was measured at 517 nm using the UV-Visible spectrophotometer. Butylated hydroxytoluene (BHT) was used as standard. The percentage inhibition of DPPH radical scavenging assay was calculated by using the formula:

Percentage inhibition = $Absorption\ of\ blank - Absortion\ of\ sampleAbsorption\ of\ sample \times 100$

Figure 3.7. DPPH radical scavenging assay

3.5.3. Total antioxidant capacity assay:

The total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity through the formation of phosphomolybdenum complexes. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity can be calculated by the method described by Prieto et al. (1999).

Reagents:

- Ammonium molybdate

- Sulphuric acid
- Sodium di-hydrogen phosphate
- Distilled water
- Methanol
- Butylated hydroxytoluene (BHT)
- Instrument:
- Ultra-violet/ visible (UV/VIS) spectrophotometer

Preparation of molybdate reagent solution:

1.06 g sodium di-hydrogen phosphate, 0.496 g of ammonium molybdate and 3.34 mL of sulphuric acid were dissolved in 20 mL of distilled water and made up to 100 mL by adding distilled water.

Procedure:

The extracts solution of Fumaria officinalis and Convolvulus arvensis were prepared separately by dissolving 10 mg of each crude extract in 10 mL of methanol to get a stock solution of 1 mg/1 mL ratio. 500 ppm and 1000 ppm of the stock solution was taken in separate test tubes and maintained the volume to 2 mL of each test tube. 8 mL of molybdate reagent was added in each test tube. These test tubes were capped and incubated in a boiling water bath at 95 ℃ for 90 minutes. A typical blank solution contained 2 mL of methanol and 8 mL of reagent solution was incubated under the same condition as the rest of the sample. After incubation, these test tubes were normalized to room temperature for 10-15 minutes. The absorbance of the reaction mixture was measured at 695 nm using the UV-Visible spectrophotometer. Butylated hydroxytoluene (BHT) was used as standard.

Figure 3.8. Total antioxidant capacity assay

3.6. Hematological Effect:

3.6.1. Experimental animals:

Male healthy rabbits 3–4-week-old with a weight of 700-800g were used in this study. Animals were bred in the animal house of University of Veterinary and Animal Sciences, Lahore.

The rabbits were kept in polypropylene cages, maintained under standard laboratory conditions of 12-hour of light and dark cycles, at temperature of 25 ± 2ºC and 35-60% humidity. Normal diets were purchased from local vendors commercially and rabbits were fed. All the animals had free access to water and food. For handling experimental animals proper ethical guidelines and procedures were followed. Animals were kept in the laboratory environment for a week and then divided into groups. Three groups were made with each group comprising 3 rabbits i.e. Group I, II, III. Group I named as the control group, Group II is the STZ induced group and the animals in group III were administered with the methanolic extract orally at two days interval for 7 days. Intragastric gavage technique was used for extract administration. Before initiating the first oral administration, blood from both groups of rabbits were taken. On the 7th day at the end of the experimental period, the oral administration repeated. Rabbits were observed to have any signs of illness or change in their behaviour or mortality.

Figure 3.9. Experimental rabbits

3.6.2. Induction of hematology:

Streptozotocin was induced in the rabbit to produce the required hematological state throughout the experimental week. It was dissolved in 0.1 M phenylhydrazine at 4.5 pH, and was injected intraperitoneally.

3.6.3. Treatment of animals:

Rabbits were divided into three groups

- Group I (C): Control group
- Group II (STZ) : Induced with STZ (70 mg/kg, intraperitoneal way)
- Group III (TR): Treated rabbits (200 mg/kg, oral gavage)

3.6.4. Blood sample collection:

In the start of experiments blood samples were collected and then from the central auricular artery and marginal ear vein for the determination of hematological parameters on the 7th day. Firstly, the ear was sterilized through swabbing with 80% ethanol. Bleeding was boosted by slightly milking the ear from the body. Blood of nearly 4ml was drawn into the bottles comprising anticoagulant (EDTA) which were shaken and taken for hematological parameter assessment. The animals were euthanized by using chloroform on the 7th day.

Figure 3.10. Blood Sample Collection

3.6.5. Determination of hematological parameters:

The CBC was done on an automated hematology analyzer with a use of well mixed whole blood to which EDTA was added to stop clotting. The hematological parameters comprising White Blood Cells (WBC) count, Lymphocytes (Ly), Monocytes (Mo), Granulocytes (Gr), Hemoglobin (Hb), Red Blood Cells (RBC) count, Hematocrit (PCV/Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets count (PLT), Mean Platelet Volume (MPV) were measured by Sysmex hematology analyzer.

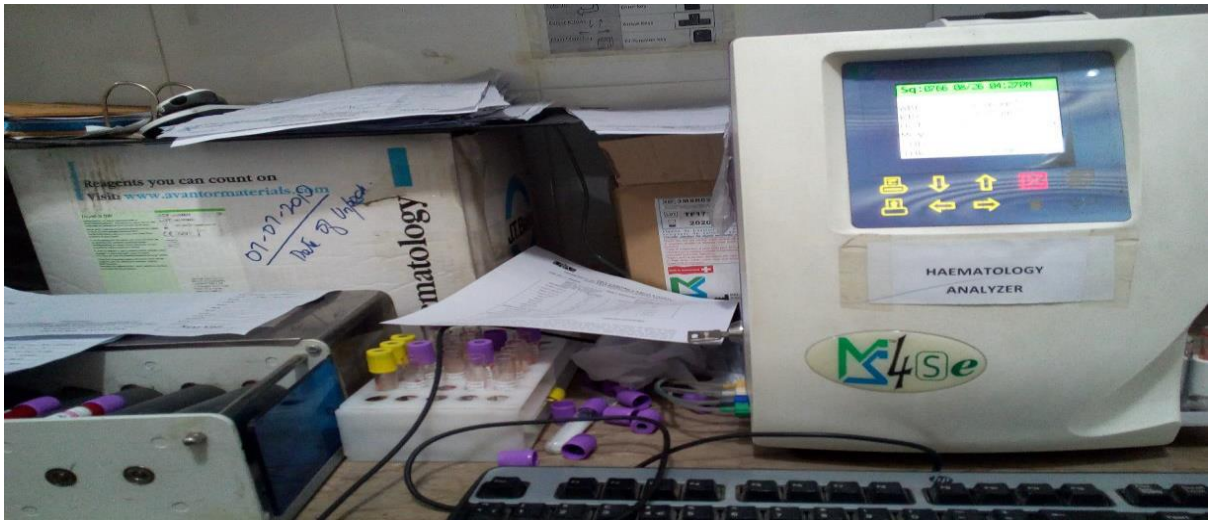

Figure 3.11. Sysmex hematology analyzer

3.6.6. Data management:

Data on various hematological parameters were collected on day zero and then on the seventh day of the experiment. Both the values were then compared for the two dose levels. The results were then tabulated on Excel program. For analysis results were expressed as mean ± standard error of mean (SEM). Difference among two groups were assessed and their statistical significance by using one way analysis of variance (ANOVA) which was followed by Turkey's tests in order to separate the means and attain the definite significant differences among different groups.

CHAPTER 4

RESULTS

4. In-Vitro Antioxidant Assays:

4.1. Radical scavenging of hydrogen peroxide assay:

- ***Fumaria officinalis*:**

The extracts derived from *Fumaria officinalis* exhibited various degrees of antioxidant capacity. The scavenging effects of our extracts on hydrogen peroxide (H_2O_2) radical are shown in Table-4.1. The extract of *Fumaria officinalis* was prepared with methanol and their fractions like petroleum ether, chloroform, ethyl acetate and remaining methanolic extract. Maximum scavenging activity was found at concentration of 1 mg/mL while the minimum scavenging activity was found at 0.2 mg/ml. The extraction solution and their fractions determined the different percentage inhibition of H_2O_2 scavenging effect shown in Table-4.1 and Graph-(Figure 4.1 & 4.2).

Table 4.1: Scavenging activity of H_2O_2 assay of *Fumaria officinalis*

Concentration (mg/ml)	%age inhibition of H_2O_2 activity					
	MCE	PE	CF	EA	RME	AA
0.2	44.51%	57.84%	52.20%	48.09%	48.70%	70%
0.4	52.10%	65.09%	60.78%	56.24%	59.18%	74%
0.6	61.20%	70.08%	68.09%	62.09%	65.71%	79%
0.8	70.87%	74.17%	74.82%	71.50%	72.16%	83%
1	82.08%	81.89%	82.04%	79.91%	80.09%	90%

*MCE= Methanolic Crude Extract, AA= Ascorbic Acid, PE= Pet Ether, CF= Chloroform, EA= Ethyl Acetate, RME= Remaining Methanolic Extract

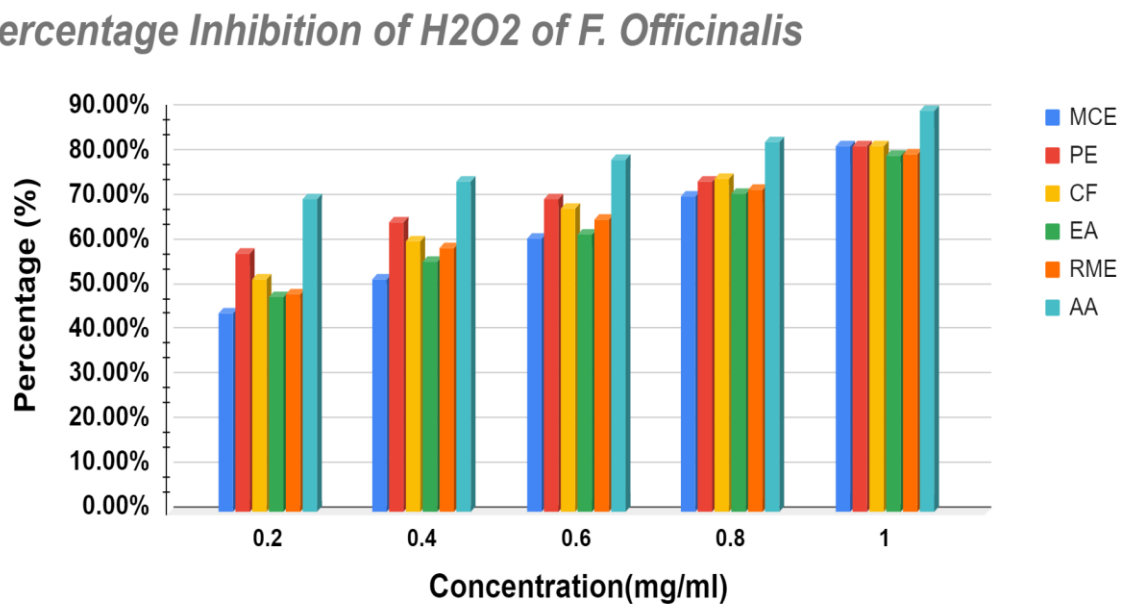

Figure 4.1. Percentage inhibition of H_2O_2 of *Fumaria officinalis*

- ***Convolvulus arvensis*:**

The extracts derived from *C. arvensis* exhibited various degrees of antioxidant capacity. The scavenging effects of our extracts on hydrogen peroxide (H_2O_2) radical are shown in Table-4.2. The extract of *C. arvensis* was prepared with methanol and their fractions like petroleum ether, chloroform, ethyl acetate and remaining methanolic extract. Maximum scavenging activity was found at concentration of 1 mg/mL while the minimum scavenging activity was found at 0.2mg/ml. The extraction solution and their fractions determined the different %inhibition of H_2O_2 scavenging effect shown in the Table-4.2 and Graph (figure 4.3 & 4.4).

Table 4.2. Scavenging activity of H_2O_2 assay of *Convolvulus arvensis*

Concentration (mg/ml)	%age inhibition of H_2O_2 activity					
	MCE	PE	CF	EA	RME	AA
0.2	44.35%	46.29%	52.34%	52.87%	39.82%	70%
0.4	56.18%	52.45%	63.31%	61.13%	48.78%	74%
0.6	64.14%	60.49%	69.01%	69.03%	58.09%	79%
0.8	72.94%	71.09%	76.30%	76.27%	67.18%	83%
1	83.52%	80.18%	82.01%	80.29%	80.82%	90%

*MCE= Methanolic Crude Extract, AA= Ascorbic Acid, PE= Pet Ether, CF= Chloroform, EA= Ethyl Acetate, RME= Remaining Methanolic Extract

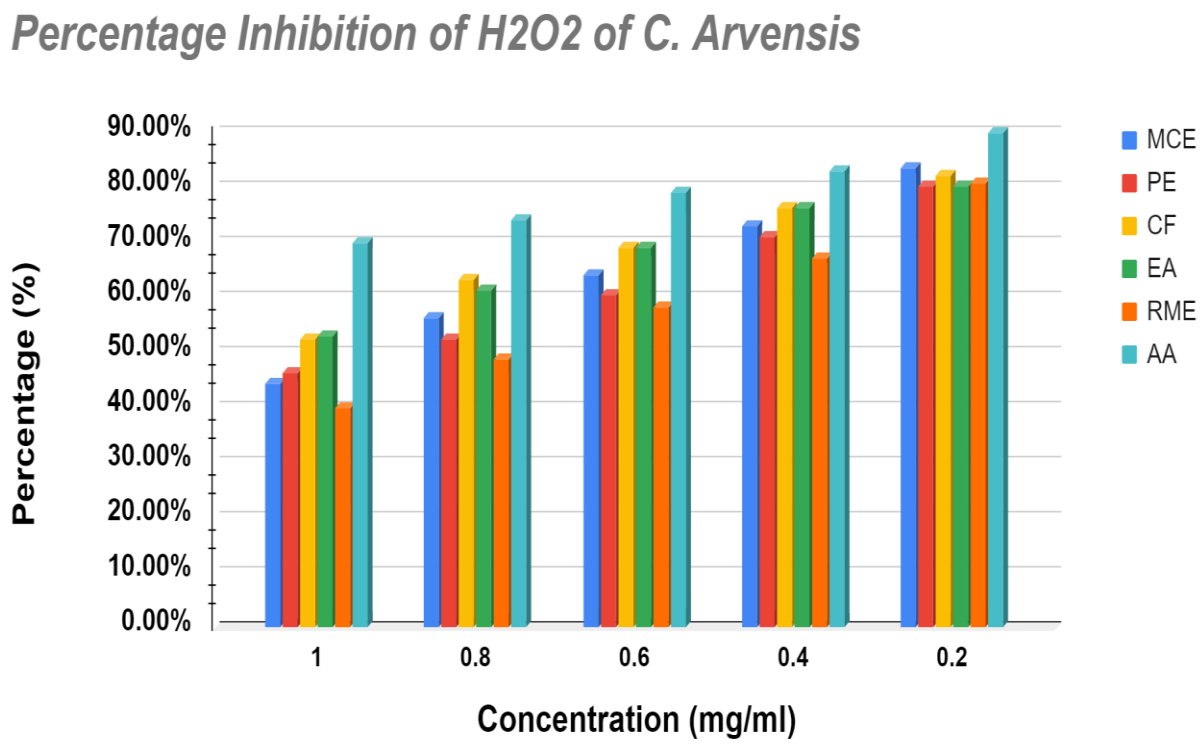

Figure 4.2. Percentage inhibition of H_2O_2 of *Convolvulus arvensis*

4.2. DPPH radical scavenging assay:

The scavenging effects of extracts of *Fumaria officinalis* and *Convolvulus arvensis* on DPPH radicals are shown in Table 4.3 and 4.4 respectively. These extracts were prepared with methanol and their fractions like petroleum ether, chloroform, ethyl acetate and remaining methanolic extract. Maximum scavenging activity was found at concentration of 1 mg/mL while the minimum scavenging activity was found at 0.125 mg/mL for each extract. The extraction solution and their fractions determined the different % inhibition of DPPH scavenging effect shown in Table (4.3 and 4.4) and Graph (figure 4.3 & 4.4).

Table 4.3: DPPH radical scavenging assay of *Fumaria officinalis*

Concentration (mg/ml)	%age inhibition of DPPH activity					
	MCE	PE	CF	EA	RME	BHT
0.125	53.06%	49.89%	49.05%	58.87%	42.02%	72.48%
0.25	61.94%	61.42%	58.78%	71.09%	53.31%	79.89%
0.5	68.13%	68.04%	66.03%	76.24%	59.13%	85.48%
1	72.20%	71.95%	70.44%	80.06%	65.09%	88.35%

*DPPH= 2,2-Diphenyl-1-picrylhydrazyl, BHT= Butylated Hydroxytoluene, MCE= Methanolic Crude Extract, PE= Pet Ether, CF= Chloroform, EA= Ethyl Acetate, RME= Remaining Methanolic Extract

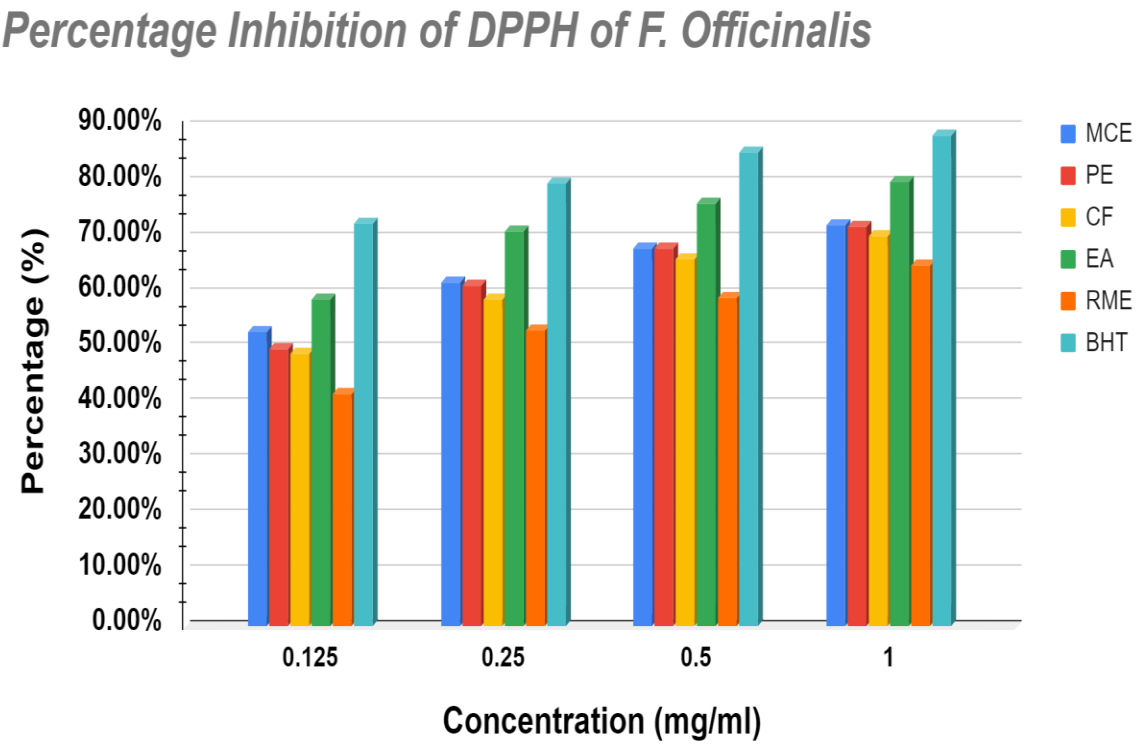

Figure 4.3. Percentage inhibition of DPPH of *Fumaria officinalis*

- ***Convolvulus arvensis*:**

Table 4.4. DPPH radical scavenging assay of *Convolvulus arvensis*

Concentration (mg/ml)	%age inhibition of DPPH activity					
	MCE	PE	CF	EA	RME	BHT
0.125	61.54%	53.10%	50.21%	53.45%	44.01%	72.48%
0.25	72.29%	61.95%	59.03%	62.08%	51.87%	79.89%
0.5	77.45%	68.43%	64.12%	65.89%	57.05%	85.48%
1	80.19%	72.02%	68.55%	71.01%	63.12%	88.35%

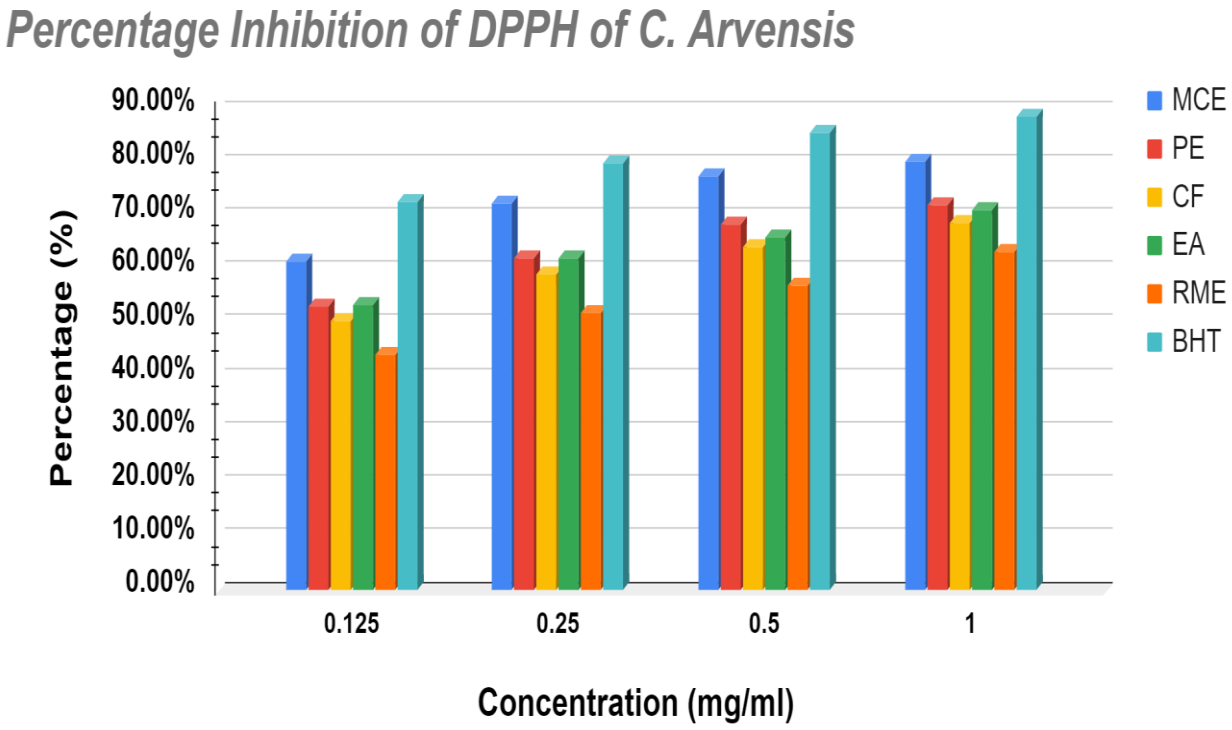

Figure 4.4. Percentage inhibition of DPPH of *Convolvulus arvensis*

4.3. Total antioxidant capacity assay:

The reducing capacity of antioxidants was coined in a single measure as "Total Antioxidant Capacity (TAC)". Butylated hydroxytoluene (BHT) was used as standard and showed a curve in between absorbance and concentration. Maximum absorbance of standard curve was found at concentration of 125ppm while the minimum absorbance was found at 1000ppm for each extract. The curve shown in figure 4.5 below:

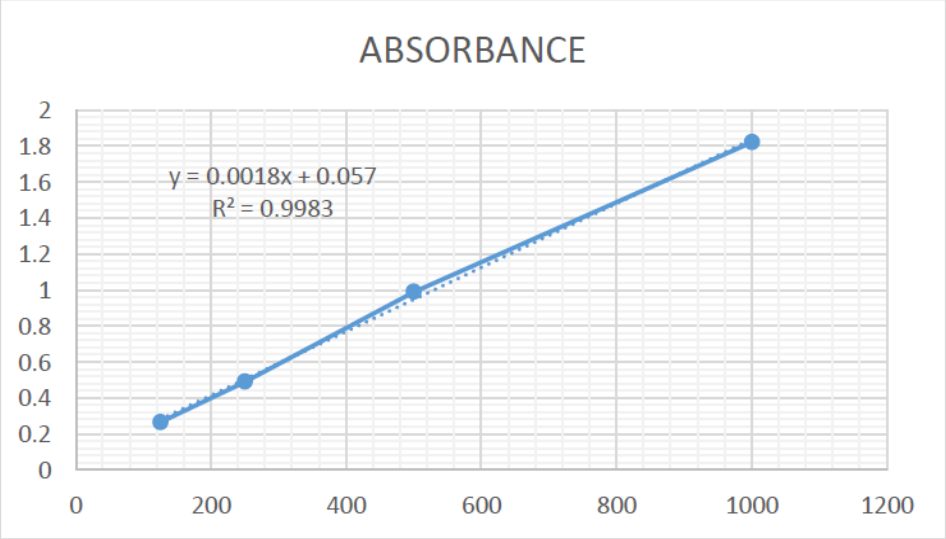

Figure 4.5. Absorbance of total antioxidant assay

According to the above standard curve, an equation can show the scavenging effects of extracts of *Fumaria officinalis* and *Convolvulus arvensis* on phosphomolybdenum assay are shown in Table 4.5 and 4.6 respectively. These extracts were prepared with methanol and their fractions like petroleum ether, chloroform, ethyl acetate and remaining methanolic extract. These fractions were determined by mgBHTE/g of each extract as compared with the standard curve equation as measured shown in Graph (fig 4.22 and 4.23).

- ***Fumaria officinalis*:**

Table 4.5 Total antioxidant capacity assay of *Fumaria officinalis*

Fractions of extract	mg BHTE/g of *F. officinalis*
MCE	246.02±09.31
PE	193.89±06.23
CF	279.43±10.61
EA	368.15±12.33
RME	259.04±09.77

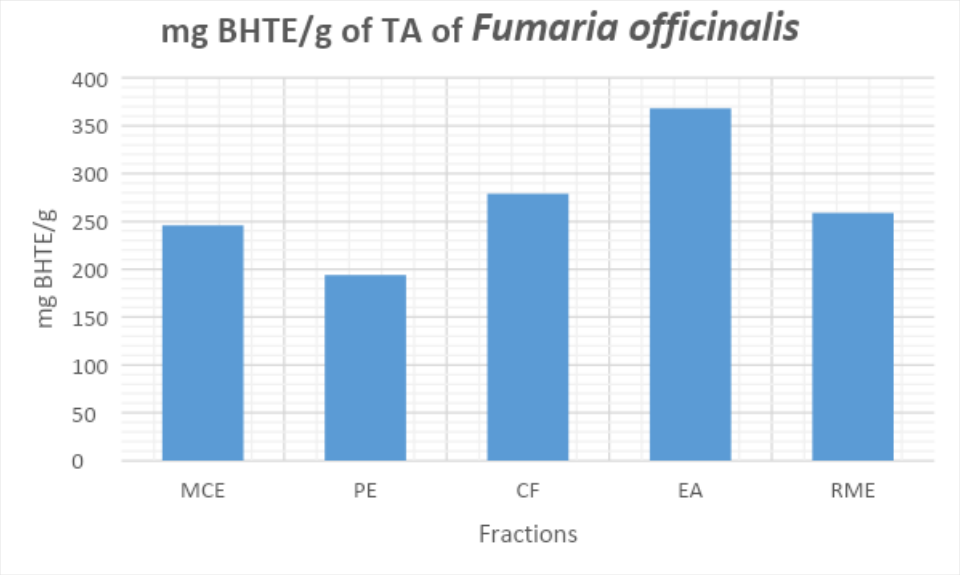

Figure 4.6. Total antioxidant assay of *Fumaria officinalis*

- ***Convolvulus arvensis*:**

Table 4.6 Total antioxidant capacity assay of *Convolvulus arvensis*

Fractions of extract	mg BHTE/g of *C. arvensis*
MCE	258.10±10.16
PE	162.67±08.72
CF	248.07±09.97
EA	373.87±12.53
RME	275.29±11.47

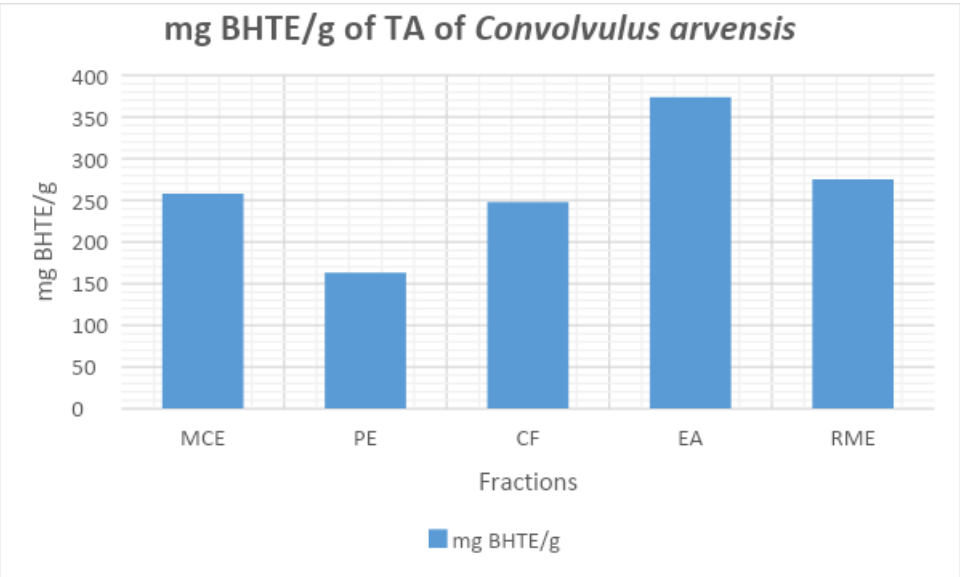

Figure 4.7. Total antioxidant activity of *Convolvulus arvensis*

4.4. Hematological parameters:

Generally, during the 7 days of this experimental study, the levels of hematological parameters did not change significantly among the normal male rabbits in the control groups. Three parameters were discussed in this study such as Packed Cell Volume/ Hematocrit (PCV/Hct), Hemoglobin (Hb) and Red Blood Cells (RBC). These three parameters were treated with methanolic extracts of convolvulus arvensis and fumaria officinalis.

4.4.1. Effects of methanolic extract of *Convolvulus arvensis* on hematological parameters in male rabbits

The methanolic extract of *C. arvensis* induced changes in total and differential hemoglobin, packed cell volume/hematocrit and red blood cells count in normal male rabbits. Zero and seven days after administration of the extract of C. arvensis at the dose levels of 200 mg/kg, there was a significant decrease in levels of total RBC, PCV/Hct and Hb ($p < 0.05$). The fractions of methanolic extract such as ethyl acetate, chloroform and petroleum ether are also treated on these parameters and show significant decrease in levels of these parameters ($p < 0.05$). Table 4.7 showed the determined values of these groups on zero and seven days as below:

Table 4.7: Effects of methanolic extract of *C. arvensis* on hematological parameters in male rabbits

Groups	PCV/Hct		Hb		RBC	
	0 day	7th day	0 day	7th day	0 day	7th day
Control	40.2±0.31	40.1±0.32	10.9±0.15	10.3±0.14	5.8±0.24	5.5±0.22
Phenyl hydrazine	39.2±0.28	27.1±0.12	10.2±0.13	8.2±0.12	5.4±0.21	4.2±0.19
Ethyl acetate	34.7±0.23	25.3±0.19	10.3±0.15	6.9±0.13	5.6±0.21	3.6±0.16
Chloroform	33.8±0.24	22.8±0.18	10.5±0.17	6.7±0.14	5.3±0.23	3.2±0.18
Methanolic crude extract	35.7±0.21	22.5±0.18	10.4±0.18	6.8±0.13	5.2±0.21	2.9±0.19
Petroleum ether	35.1±0.23	23.2±0.18	10.3±0.18	6.1±0.12	5.5±0.17	2.8±0.11

4.4.2. Effects of methanolic extract of *F. officinalis* on hematological parameters in male rabbits

The methanolic extract of *F. officinalis* induced changes in total and differential hemoglobin, packed cell volume/hematocrit and red blood cells count in normal male rabbits. Zero and seven days after administration of this extract at the dose levels of 200 mg/kg, there was a significant decrease in levels of total RBC, PCV/Hct and Hb ($p < 0.05$). The fractions of methanolic extract such as ethyl acetate, chloroform and petroleum ether are also treated on these parameters and show significant decrease in levels of these parameters ($p < 0.05$). Table 4.8. showed the determined values of these groups on zero and seven days as below:

Table 4. 8. Effects of methanolic extract of *Fumaria officinalis* on hematological parameters in male rabbits

Groups	PCV/Hct		Hb		RBC	
	0 day	7th day	0 day	7th day	0 day	7th day
Control	40.2±0.31	40.1±0.32	10.9±0.15	10.3±0.14	5.8±0.24	5.5±0.22
Phenyl hydrazine	39.2±0.28	27.1±0.12	10.2±0.13	8.2±0.12	5.4±0.21	4.2±0.19
Ethyl acetate	33.5±0.21	24.2±0.17	10.4±0.16	7.3±0.12	5.5±0.11	3.4±0.19
Chloroform	35.1±0.19	23.8±0.14	10.5±0.16	7.1±0.12	5.7±0.21	3.1±0.15
Methanolic crude extract	34.8±0.17	23.1±0.12	10.3±0.15	6.9±0.14	5.4±0.19	2.6±0.13
Petroleum ether	34.7±0.16	22.8±0.13	10.1±0.13	6.8±0.12	5.3±0.18	2.4±0.12

CHAPTER 5
DISCUSSION

Medicinal plants are used worldwide for the treatment and prevention of several diseases and its utilisation is increasing with time. It has been revealed that plant derived medications are broadly accepted and provide easy access for treating and managing various illnesses through self-medication. Herbal remedies or herbal products are being scientifically investigated till date due to significant benefits of medicinal plants to the healthcare system. These medical plants also have antimicrobial, antimalarial, anticancer and antioxidant properties in them which leads to its further scientific experimentation. Herbs which have antioxidant compounds in them are quite efficient in inhibiting the oxidation chemical reactions (which are believed to be toxic for human health). Hence, in order to assess the antioxidant activity of herbs, different assays or protocols have been in practice by many scientists and researchers for a longer period of time. Antioxidant activity assays may be generally categorized as hydrogen atom transfer based assay or single electron transfer based assays. Majority of HAT assays are kinetics based and include a scheme of competitive reaction wherein antioxidant and substrate compete for free radicals thermally produced through the decomposition of azo compounds. SET assays measure the capacity of an antioxidant in the reduction of an oxidant which on its reduced form or reduction changes color. SET assays are much convenient and easier than HAT assays. SET assays like H2O2, DPPH, and Total antioxidant activity were selected to examine the reduction capacity. These methods are involved in the mechanism of a single electron transfer system.

In H_2O_2 assay, methanolic extract *F. officinalis* showed a pretty good percentage of antioxidant activity 82% to 90% at the highest concentration i.e, 1 mg/ml. F. Maiza-Benabdesselam and his co-worker investigated the antioxidant activities of the alkaloid extracts of *Fumaria capreolata* and *Fumaria bastardii* through evaluating their antioxidant potential by antioxidant assays. Both plant extracts showed strong total antioxidant activity, however, extract of *Fumaria bastardii* was more vigorous than *Fumaria capreolata* (Chibane, 2007). Consequently, species of Fumaria plant extracts had impactful reducing power and pretty good DPPH free radical scavenging activity which means that this plant has some antioxidant compounds in them which play a vital role in the healthcare system and would help in combating several illnesses.

A study has been conducted which evaluated the antioxidant activity of the ethanolic extracts of *Convolvulus arvensis*. To provide prevention against degradation of lipids in muscle foods *C. arvensis* showed strong antioxidant effects. Hence, it is indicated that *C. arvensis* extract

can be used as a natural food antioxidant and helps in nurturing healthcare (Azman et.al, 2015). Table 4.2 also proved *C. arvensis* to have good antioxidant potential as it showed 90% of antioxidant activity with H_2O_2 free radical scavenging protocol with 1 mg/ml concentration which revealed that this herb has antioxidant compounds in it.

DPPH was used as a stable free radical donor to evaluate free radical scavenging effects. Table 4.4 showed that C. arvensis showed 88% of antioxidant effect through DPPH. Likewise, a study has been carried out in which a 50% aqueous solution of ethanol C. arvensis showed remarkable antioxidant effects which is assessed through FRAP, TEAC and ORAC protocols. It also helps in retaining redness of meat which confirmed that it could be utilised by the food industries as an enriched source of antioxidants (Azman et.al, 2015).

F. officinalis showed 79% to 90% antioxidant effects through H_2O_2 radical scavenging assay at the highest concentration i.e, 1 mg/ml which is greater than that of DPPH free radical scavenging which showed 72% to 88% of antioxidant effects. This slight difference is because there are a lot of analytical methods that are available for the assessment of antioxidant activity. These assays or protocols have different kinds of oxidants, reaction mechanisms, probes, chemicals, species, reaction conditions and even they differ from each other in expressing or computing results. Every assay is different in terms of pH, frequency, reaction time, solvents and standard compounds. Hence, the results of the same plant species from different protocols of in vitro antioxidant assay will always be differ (Magalhães et. al, 2007).

Total antioxidant capacity of these extracts also showed different antioxidant effects. The fractions of methanolic extracts of these plants showed values on the basis of mgBHTE/g, ethyl acetate showed greater values than other fractions such as petroleum ether, methanolic extract, chloroform and remaining methanolic extract. *C. arvensis* (373.87±12.53) showed a bit higher value of total antioxidant activity as compared to *F. officinalis* which showed (368.15±12.33). Abbas and his co-workers carried out a research in which they took fifteen weeds (*C. arvensis* is one of them) and evaluated their phytochemical potential. The results of this experiment showed that these weeds have Alkaloids, saponins, glycosides, terpenoids, anthraquinine, steroids, flavonoids and tannins in them. Out of all alkaloids and tannins were the greatest in amount which proved them to be medically significant and important (Abbas et al., 2012). Antioxidant potential of plant extracts mainly depend upon the phenolic compounds that are present in the plant species. Because of the fact that phenolic groups have higher redox properties which helps in inhibiting oxidation and thus leads to higher antioxidant activity. Consequently, antioxidant properties are directly proportional to the phenolic compounds present in the plant species.

The evaluation of hematological parameters containing the red cells (erythrocytes), white cells (leucocytes) and the platelets (thrombocytes) and factors that related to them give data on necrosis, inflammation and other several visceral organs infections and some other stress factors. This plays a very crucial role in nutrition, pathological and physiological rank of an organism. A significant decrease in WBC, PCV/Hct and Hemoglobin of treated rabbits were recorded against the extracts of *F. officinalis* and *C. arvensis*. Methanolic extracts of *F. officinalis* and *C. arvensis* may bring inhibition of RBC development that reduces the RBC counts and leads to a reduction in Hb content and PCV/Hct. The depletion in RBC count and Hb content can be attributed to defective hemopoiesis. Further discussed the decrease of (2.9×1012/L), (2.6×1012/L), RBC for *C. arvensis* and *F. officinalis* treated rabbits respectively. The decrease of (6.8g/dL) and (6.9g/dL) Hb for *C. arvensis* and *F. officinalis* treated rabbits respectively. And decrease (22.5%) and (23.1%) PCV/Hct for *C. arvensis* and *F. officinalis* treated rabbits against PCV/Hct of the control rabbits. Fall in Hb content, RBC count and PCV/Hct can be interrelated with induction of anemia in experimental animals after exposure to toxic complexes. The reduction in Hb content, RBC count and PCV/Hct can be correlated with paling of animals, weakness and morbidity.

CONCLUSION

Medicinal plants have been used to cure different diseases for centuries. These are used because of their cost effectiveness, lesser side effects and ease of usage. In this study, it was aimed to examine the in-vitro antioxidant and in-vivo hematological parameters of wild herbs such as *Fumaria officinalis* and *Convolvulus arvensis*. In order to obtain these herbal fractions or partitions, methanolic extracts of these herbs were prepared. These fractions were used for antioxidant activities using Hydrogen peroxide, Phosphomolybdenum and DPPH assays. In H2O2 assay, methanolic extract *F. officinalis* and *C. avensis* showed a pretty good percentage of antioxidant activity 82% to 90% at the highest concentration i.e, 1 mg/ml. Both *C. arvensis* and *F. officinalis* showed 88% of antioxidant effect through DPPH radical scavenging assay. The slight difference in the values of two protocols is because these assays or protocols have different kinds of oxidants, reaction mechanisms, probes, chemicals, species, reaction conditions. Antioxidant potential of plant extracts mainly depend upon the phenolic compounds that are present in the plant species. Because these phenolic groups have higher redox properties which helps in inhibiting oxidation and thus leads to higher antioxidant activity. Consequently, antioxidant properties are directly proportional to the phenolic compounds present in the plant species. Further, these fractions were investigated for their effects on hematological parameters of rabbits such as RBC, Hemoglobin and Hct. It was concluded that *F. officinalis* and *C. arvensis* showed pretty good antioxidant activity but has lowered values of hematological parameters in rabbits when compared to the control group. There is very little or no research has been done on the antioxidant activity of these two herbs and their antioxidant effect on hematological parameters of rabbits is still not known. As there is no research which has been carried out on in vitro antioxidant effects of these two herbs thus, this study can be a guideline for further evaluation of biological activities.

REFERENCES

Martínez-Graciá, C., González-Bermúdez, C. A., Cabellero-Valcárcel, A. M., Santaella-Pascual, M., & Frontela-Saseta, C. (2015). Use of herbs and spices for food preservation: advantages and limitations. *Current opinion in food science*, *6*, 38-43.

John, L. J., & Shantakumari, N. (2015). Herbal medicines use during pregnancy: a review from the Middle East. *Oman medical journal*, *30*(4), 229.

Small, E. (2006). *Culinary herbs*. NRC Research Press.

Elansary, H. O., & Mahmoud, E. A. (2015). Egyptian herbal tea infusions' antioxidants and their antiproliferative and cytotoxic activities against cancer cells. *Natural product research*, *29*(5), 474-479.

Dweck, A. C. (2009). The internal and external use of medicinal plants. *Clinics in dermatology*, *27*(2), 148-158.

Tapsell, L. C., Hemphill, I., Cobiac, L., Sullivan, D. R., Fenech, M., Patch, C. S., ... & Fazio, V. A. (2006). Health benefits of herbs and spices: the past, the present, the future.

Korać, R. R., & Khambholja, K. M. (2011). Potential of herbs in skin protection from ultraviolet radiation. *Pharmacognosy reviews*, *5*(10), 164.

Vickers, A. J. (2007). Which botanicals or other unconventional anticancer agents should we take to clinical trial?. *Journal of the Society for Integrative Oncology*, *5*(3), 125.

Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of pure and applied chemistry*, *4*(8), 142-151.

Seifried, H. E., Anderson, D. E., Fisher, E. I., & Milner, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. *The Journal of nutritional biochemistry*, *18*(9), 567-579.

Podsędek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT-Food Science and Technology*, *40*(1), 1-11.

Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive reviews in food science and food safety*, *10*(4), 221-247.

Washington, I. M., & Van Hoosier, G. (2012). Clinical biochemistry and hematology. In *The laboratory rabbit, guinea pig, hamster, and other rodents* (pp. 57-116). Academic Press.

Roland, L., Drillich, M., & Iwersen, M. (2014). Hematology as a diagnostic tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation*, *26*(5), 592-598.

Etim, N. N., Enyenihi, G. E., Akpabio, U., & Offiong, E. E. (2014). Effects of nutrition on haematology of rabbits: a review. *European Scientific Journal*, *10*(3).

Balah, M. A. (2015). Allelopathic effects of Bindweed (Convolvulus arvensis L.) root exudates on plants and soil Microflora. *Egyptian journal of desert research*, *65*(1), 31-46.

Konigsberg, E. R. (2014). *Factors involved in the success and establishment of the field bindweed gall mite Aceria malherbae Nuzzaci (Acari: eriophyidae)* (Doctoral dissertation, Montana State University-Bozeman, College of Agriculture).

Baličević, R., Ravlić, M., Knežević, M., & Serezlija, I. (2014). Allelopathic effect of field bindweed (Convolvulus arvensis L.) water extracts on germination and initial growth of maize. *The Journal of Animal & Plant Sciences*, *24*(6), 1844-1848.

Chlebek, J., Novák, Z., Kassemová, D., Šafratová, M., Kostelník, J., Malý, L., ... & Kuneš, J. (2016). Isoquinoline alkaloids from Fumaria officinalis L. and their biological activities related to Alzheimer's disease. *Chemistry & biodiversity*, *13*(1), 91-99.
Khamtache-Abderrahim, S., Lequart-Pillon, M., Gontier, E., Gaillard, I., Pilard, S., Mathiron, D., ... & Maiza-Benabdesselam, F. (2016). Isoquinoline alkaloid fractions of Fumaria officinalis: Characterization and evaluation of their antioxidant and antibacterial activities. *Industrial Crops and Products*, *94*, 1001-1008.
S Panickar, K. (2013). Beneficial effects of herbs, spices and medicinal plants on the metabolic syndrome, brain and cognitive function. *Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents)*, *13*(1), 13-29.
Maver, T., Maver, U., Stana Kleinschek, K., Smrke, D. M., & Kreft, S. (2015). A review of herbal medicines in wound healing. *International Journal of Dermatology*, *54*(7), 740-751.
Kumari, R., & Kotecha, M. (2016). A review on the Standardization of herbal medicines. *Int J Pharm Sci Res*, *7*(2), 97-106.
Nasri, H. (2013). Toxicity and safety of medicinal plants. *Journal of HerbMed Pharmacology*, *2*.
Akram, M., & Nawaz, A. (2017). Effects of medicinal plants on Alzheimer's disease and memory deficits. *Neural Regeneration Research*, *12*(4), 660.
Mazzari, A. L., & Prieto, J. M. (2014). Herbal medicines in Brazil: pharmacokinetic profile and potential herb-drug interactions. *Frontiers in pharmacology*, *5*, 162.
Tabish, S. A. (2008). Complementary and alternative healthcare: is it evidence-based?. International journal of health sciences, 2(1), V.
Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *Rsc Advances*, *5*(35), 27986-28006.
Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry*, *47*(10), 3954-3962.
Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of pure and applied chemistry*, *4*(8), 142-151.
Ihedioha, J. I., Okafor, C., & Ihedioha, T. E. (2004). The haematological profile of the Sprague-Dawley outbred albino rat in Nsukka, Nigeria. *Animal Research International*, *1*(2), 125-132.
Islam, M. S., Lucky, N. S., Islam, M. R., Ahad, A., Das, B. R., Rahman, M. M., & Siddiui, M. S. I. (2004). Haematological parameters of Fayoumi, Assil and local chickens reared in Sylhet region in Bangladesh. *Int. J. Poult. Sci*, *3*(2), 144-147.
Taheri, S., Solati, A., Moradi, P., Tavassoly, A., & Yadi, J. (2013). Toxic effects of Nerium oleander aqueous leaf extract on haematological parameters and histopathological changes of the lungs and heart in rabbits. *Comparative clinical pathology*, *22*(6), 1189-1193.
Odesanmi, S. O., Lawal, R. A., & Ojokuku, S. A. (2010). Haematological effects of ethanolic fruit extract of Tetrapleura tetraptera in male Dutch white rabbits. *Research Journal of Medicinal Plant*, *4*(4), 213-217.

Effraim, K. D., Salami, H. A., & Osewa, T. S. (2000). The effects of aqueous leaf extract of Ocimum gratissium on haematological and biochemical parameters in rabbits. *African Journal of biomedical research*, *3*(3), 175-179.
Hentschel, C., Dressler, S., & Hahn, E. G. (1995). Fumaria officinalis (Fumitory)--clinical applications. *Fortschritte der Medizin*, *113*(19), 291-292.
Al-Snafi, A. E. (2020). Constituents and pharmacology of Fumaria officinalis-A review. *IOSR Journal of Pharmacy*, *10*(1), 17-25.
Chibane, M. (2007). Antioxidant activities of alkaloid extracts of two Algerian species of Fumaria: Fumaria capreolata and Fumaria bastardii, Records of Natural Products.
Al-Snafi, A. E. (2020). Constituents and pharmacology of Fumaria officinalis-A review. *IOSR Journal of Pharmacy*, *10*(1), 17-25.
Khoshvaghti, A., Derakhshanian, S., Zamanzadeh, A., & Sarvestani, A. A. (2014). The effects of Fumaria officinalis hydroalcoholic extracts on haematological profile of New Zealand rabbits. *Comparative Clinical Pathology*, *23*(4), 869-873.
Azman, N. A. M., Gallego, M. G., Juliá, L., Fajari, L., & Almajano, M. (2015). The effect of Convolvulus arvensis dried extract as a potential antioxidant in food models. *Antioxidants*, *4*(1), 170-184.
Meng, X. L., Riordan, N. H., Casciari, J. J., Zhu, Y., Zhong, J., González, M. J., ... & Riordan, H. D. (2002). Effects of a high molecular mass Convolvulus arvensis extract on tumor growth and angiogenesis. *Puerto Rico health sciences journal*, *21*(4).
Sadeghi-Aliabadi, H., Ghasemi, N., & Kohi, M. (2009). Cytotoxic effect of Convolvulus arvensis extracts on human cancerous cell line. *Research in Pharmaceutical Sciences*, *3*(1), 31-34.
Gardea-Torresdey, J. L., Peralta-Videa, J. R., Montes, M., De la Rosa, G., & Corral-Diaz, B. (2004). Bioaccumulation of cadmium, chromium and copper by Convolvulus arvensis L.: impact on plant growth and uptake of nutritional elements. *Bioresource technology*, *92*(3), 229-235.
Chibane, M. (2007). Antioxidant activities of alkaloid extracts of two Algerian species of Fumaria: Fumaria capreolata and Fumaria bastardii.
Azman, N. A. M., Gallego, M. G., Juliá, L., Fajari, L., & Almajano, M. (2015). The effect of Convolvulus arvensis dried extract as a potential antioxidant in food models. *Antioxidants*, *4*(1), 170-184.
Magalhães, L. M., Segundo, M. A., Reis, S., & Lima, J. L. (2008). Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica chimica acta*, *613*(1), 1-19.
Abbas, M. N., Rana, S. A., Shahid, M., Rana, N., Mahmood-ul-Hassan, M., & Hussain, M. (2012). Chemical evaluation of weed seeds mixed with wheat grains at harvest. *The Journal of Animal & Plant Sciences*, *22*(2), 283-288.