Phytochemical Quantification, Antioxidant and Antibacterial Potential of Leaves and Stem of Convolvulus arvensis

SUPERIOR UNIVERSITY

Thesis Submitted to
The Superior University Lahore
In Partial Fulfillment of the
Requirement for the Degree of
MS Botany

By
MUHAMMAD ZAHEER IQBAL
Roll No. SU92-MSBNW-F22-004

Session: 2022-2024

Faculty of Sciences

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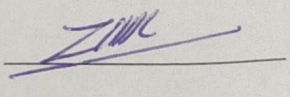
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LIST OF ABBREVIATIONS

C. arvensis	*Convolvulus arvensis*
TPC	Total phenolic content
TFC	Total flavonoid content
BMI	Body Mass Index
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
mg.GAE.g-1	milligrams of Gallic acid equivalents per gram
ADA	Albumin Denaturation Assay:
MBC	minimum bactericidal concentration
AB	Antibiotic
AMR	Antimicrobial resistance
FDA	Food and Drug Administration
ZOI	Zone of inhibition

ABSTRACT

Extrapolating resistance of microorganisms to synthetic drugs is a worldwide problem and can be minimized with the use of natural products. Recently, the population have reverted to natural products-based medicine. Natural products offer better efficacy and efficiency in diseases management with trifling side effects. The phytochemicals derived from natural sources are bioactive and are widely used as medicine. It is assessed that the 50 -75% of the people throughout globe use the traditional medicine in health care and medications. The present study aims to investigate the chemical composition via qualitatively and quantitatively, antioxidant activity, antimicrobial and anti-inflammatory potential of extracts from *C. arvensis* leaves and stem. Initially, stem and leaves extract obtained using suitable solvent. Polyphenols, flavonoids, alkaloids, steroids, saponin are abundant in plant extracts. Such components was investigated in the extracts using standard spectrometric and chemical methods both qualitatively and quantitatively wherever required. The antioxidant activity of stem and leave extract examined through ABTS and DPPH assay. The antimicrobial potential of *C. arvensis* extracts was evaluated against four bacterial species *E. coli, P. aeruginosa* (gram-negative) while the gram-positive B. *cereus and S. aureus.* The in-vitro anti-inflammatory activity was explored through spectrometry-based protein denaturation method. The outcomes indicated that the plant leaves are rich source of various phytochemicals and the quantitative analysis of TPC and TFC showed that the leaves methanolic extract contain higher value that are 180.4 ± 0.11 mg Gallic acid equivalent and 168 ± 0.09 mg Quercetin acid equivalent respectively. The leaves extract also exhibited the higher DPPH and ABTS value 295±0.3 and 187.0±3 respectively as compare to the stem extract. The plant leaves sample also showed higher Zone of inhibition against various bacterial strain *E. coli* (23.5± 0.12 mm) *P. aeruginosa* (20.2± 0.10 mm) while the gram positive *B. cereus* (14.9± 0.09) and *S. aureus* (17.2 ± 0.12 mm) respectively. It unblemished from ZOI value the *Convolvulus arvensis* leaves are supplementary effective against used the bacterial strain.

Key words: *Convolvulus arvensis*, phytochemicals, flavonoids, phenolic compounds, antioxidant, anti-inflammatory, antimicrobial

CHAPTER 1

INTRODUCTION

Medicine derived from the plants is the earliest type of treatment that man has discovered. It was the foundation of many ancient civilizations, and it is still the kind of medicine that is used in the majority of countries throughout the globe today. The plant demonstrated a diverse array of pharmacological actions, including antioxidant, antimicrobial, cardiovascular, anticancer, central nervous, immunological, analgesic, anti-inflammatory and antipyretic attributes, amongst numerous additional pharmacological effects (1). The pharmaceuticals are currently focusing on natural phytochemicals, derived from the plant source. Such medications are excellent bioactive source and have many traditional. The *C. arvensis* known as bindweed is a climber shrubs that grows in wild area with other plants in the European, United States and South Asian region. The *C. arvensis* used as wound healing agent, laxative, fever reducer and curing mumps by the folk inhalers. It also used to as anti-spasmodic, anti-hemorrhagic and anti-angiogenetic agents due to presence of natural phytochemicals (2).

The *C. arvensis* shrub traditionally used as relief flu and cough, treat painful joints, swelling and inflammation. In literature, it is reported that these suburbs contain various major phytochemicals, such as steroids, carbohydrates, coumarins and lipids. The phytochemical investigation of this shrub has been limited for the detection of alkaloids, flavonoids, saponin and other important bioactive chemicals. The biological activities of flavonoids and phenolic compound comprise protection against the microbes, inflammatory allergies, hepatoxins, viruses and free radical scavenging capabilities. Various species of *C. arvensis* have discovered having medicinal significance includes those which may reduce and may be eliminated the various symptoms such as memory loss, fever, sleeplessness hair loss and heart diseases (3). Many modern nutraceutical products still have plants at their core. Plants as a protective mechanism against environmental stressors and other elements including insect infestations, wounds, and diseases produce many secondary metabolites. Since the beginning of medicine, various secondary metabolites from plant have been used employed for a variety of therapeutic objectives. For many years, medicinal plants served as a major source of information for pharmacological research and medication development. Plants compose a large number of physiologically active compounds

through a process known as secondary metabolism (4). These substances are employed in a wide range of medical applications, as pharmaceutical ingredients that are active as well as models for compounds that may be developed in the future. In human different illness are associated to the accumulation with free radicals. The antioxidant scavenges free radicals and reduces their effect. Therefore, search of the naturally existence antioxidant of herbs origin is most imperative. Consequently, this study should be performed to investigate the significance bioactive and their role in biological activities (5). The aim present study was undertaken to investigate chemical composition via qualitatively and quantitatively, antioxidant activity, antibacterial potentials and anti-inflammatory capabilities of *C. arvensis* leaves and stem extract. Composition of phenol, flavonoids, tannin and other phytochemicals are determined qualitatively and quantitatively from the ethanolic and aqueous extract of stem, leaves of *C. arvensis*, and evaluated their anti-inflammatory, antimicrobial and antioxidant activities. Furthermore, it revealed strong hope to development of additional novel chemotherapeutic mediators from plants, that in the future might be serve in production of the synthetically reworded therapeutic agents. Supplementary studies are suggested to explore different parts of plants significance which frequently occurring in nature. Field bindweed also known as a *C. arvensis* have a history of used traditional by folk medicinal healer. They are supposed to possess numerous health-enhancing stuffs, comprising antidiabetic, antibacterial, laxative and antioxidant properties. Conversely, despite their extensive usage, here is an insufficiency of inclusive scientific research to authenticate these privileges and exploring the possible applications of field bindweed extracts in recent medicine and therapeutic purposes.

1.1 *CONVOLVULUS ARVENSIS*

Field bindweed or *Convolvulus arvensis*, is bindweeds species in *Convolvulaceae* instinctive to Asia and Europe. It is climbing and rhizomatous or creeping perennial herbaceous plant through stems upward to the 0.5 to 2 m in the length. It is frequently originate at the ground level through small pink and white flowers. Typical names for this plant include European bindweed, smaller bindweed, withy wind (in baskets willow crops), creeping jenny, perennial morning glory, small-flowered morning glory, and possession vine (6).

1.1.1 DISTRIBUTION AND OCCURRENCE OF *CONVOLVULUS ARVENSIS*

Herbaceous greenery weed *C. arvensis* usually occurs in regions having mild tropical to Mediterranean climates. It is a member of the Convolvulaceae family that referred to locally is "Leli." The plant may grow carpets that are up to five centimeters thick above the ground, while its stems can reach up to 2 meters in length (7).

1.1.2 TAXONOMY

Carl Linnaeus originally described field bindweed within Species Plantarum in 1753. Many subspecies, variations, among synonymous taxa were found and reported as allegedly new species in the nations of China, Russia, Ethiopia, and Morocco throughout the next centuries. When botanists discovered the plant in the Mexican Republic, Chile, and the western part of California, they identified novel species and forms even though the plant may not be native to these regions (8).

Jacques Denys Choisy reduced several synonyms to ten variants of *Convolvulus arvensis* in the ninth book of Augustin of Pyramus de Candolle's-Prodromus, which was published in 1845. However, he also identified some species that are additionally reduced as synonyms of *C. arvensis.* Over time, the appropriate authorities lost recognition for most, if not all, of these breeds and variations (9).

1.1.3 SCIENTIFIC CLASSIFICATION

Convolvulus arvensis, subspecies Spermatophyta, is a member of the kingdom Plantae, subkingdom Tracheophyta. It belongs to the following groups: order Solanales, family *Convolvulaceae*, genus Convolvulus, class Magnoliopsida, subclass Asteridae, division Magnoliophyta, and species arvensis (10) .

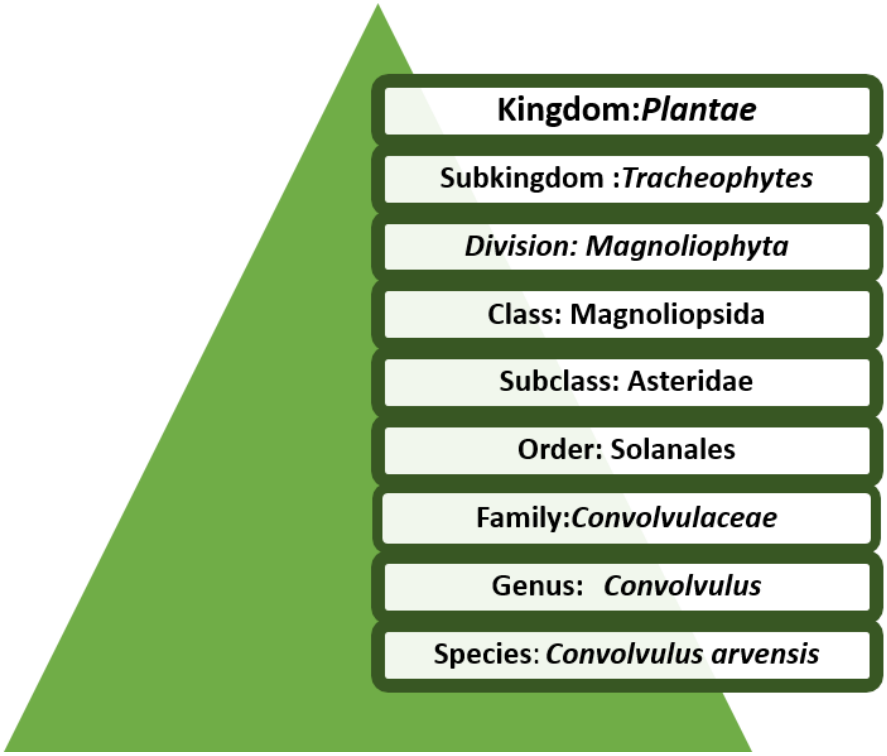


Figure 1.1. Taxonomic distribution of *C. arvensis* plant

1.1.4 DESCRIPTION OF *C. ARVENSIS*

Perennial vine *Convolvulus arvensis* usually reaches a height of one meter (3.3 ft). When the outside vines are cut off, or in the spring, the vine resprouts from its woody rhizomes.

With a 1-3 cm (0.39-1.18 in) petiole, the leaves that are spirally arranged are straight to arrowhead-shaped, 2-4 cm (0.79-1.97 in) long, and alternating. The trumpet-shaped blooms have a length of 1-3.5 cm (0.39-0.98 in), are white or shades of pink and have five radial stripes that are somewhat darker pink (11). Midsummer is flowering time (June to September in the UK) when funnel-shaped, white to light pink blooms start to appear. Flowers are borne on tiny bracts and are about 0.75-1 in (1.9-2.5 cm) across. Round, light brown fruits measure 0.125 inch (3.2 mm) in width. Every fruit has two or more seeds. There are four seeds that can survive in soil for years after being consumed by birds. The stems ascend by counterclockwise twisting around the stems of neighboring plants (12).



Figure 1.2 Leaves, stem and flower physiology of *C. arvensis*

1.2 PHYTOCHEMICAL CONSTITUENTS OF *C. ARVENSIS*

A number of the alkaloids found in bindweed are poisonous to mice, including pseudotropine, as well as smaller concentrations of tropine, tropinone, or meso-cuscohygrine. It is also said that the plant's psychedelic qualities stem from the alkaloids. Its leaves, which include glycosides, are traditionally used as a laxative, diuretic, and to alleviate hypertension. The most prevalent flavonoids found in the aerial sections of *C. arvensis* are quercetin, the compound rutin and kaempferol; these compounds have antimicrobial, anti-inflammatory, and cancer-fighting abilities (13) . Field bindweed, or *C arvensis*, is a plant with a wide range of phytochemicals that support both its varied biological activity and possible medical use. Key components found in this plant include glycosides, alkaloids, phenolic acids, flavonoids, terpenoids, and tannins. Alkaloids with attainable therapeutic benefits include convolvine and calystegines (14).

1.2.1 ALKALOIDS

1.2.1.1 CONVOLVINE

Alkaloids, which frequently function as neurotransmitters and communication systems, have been shown to be the most efficient against inhibitors of metabolic processes. They are being used to treat a wide range of illnesses, such as diabetes, cancer, allergies, inflammation, and many more. The endogenous alkaloid through numerous potential medicinal solicitations is called convolvine (15).

1.2.1.2 CALYSTEGINE

Calystegines were polyhydroxylated alkaloids that bind to and inhibit the group of enzyme which are in charge of cleaving glycosidic bonds among carbohydrates. This limitation can slow down the metabolism of sugar, which is why calystegines are useful in the treating of diabetes alongside possibly obesity. The capacity of calystegines to modulate glycosidase activity implies that they may possess neuroprotective qualities.

These alkaloids, known as nortropane alkaloid compounds, possess the capacity to obstruct glycosidases, among the enzymes implicated in the breakdown complicated carbohydrates. In the realm of diabetes research, calystegines are attracting attention due to their ability to manage blood sugar levels. Their possible anti-obesity actions are further suggested by their involvement in blocking the metabolism of carbohydrates (16).

1.2.3 FLAVONOIDS

1.2.3.1 RUTIN

Rutin may have anti-inflammatory and antioxidant properties. Additionally, it could provide some defense against illnesses like cancer. Rutin is widely used for a variety of conditions, including exercise-induced respiratory infections, wrinkled skin, and autism; however, none of these use is well supported by research (17).

1.2.3.2 QUERCETIN

Recognized for its potent antioxidant qualities, In addition to lowering blood pressure as well as inflammation, quercetin could possibly possess anticancer properties. It is beneficial in addressing the symptoms of allergies since it has antiviral properties and lowers histamine production (18).

1.2.4 PHENOLIC SUBSTANCES

1.1.4.1 CHLOROGENIC ACID

Products phenolic and polyphenolic, by themselves or in conjunction with vitamins like C and E, can act as antioxidants to shield different bodily tissues from oxidative damage. The most prevalent antioxidants in diets high in fruits and vegetables are polyphenols (19) .

1.2.5 TERPENOIDS

Plant sterols that resemble cholesterol in structure are called phytosterols. They efficiently decrease blood levels of cholesterol by competing with cholesterol from food for assimilation in the digestive tract. since of this characteristic, phytosterols are good for cardiovascular wellness since they lower the probability of atherosclerosis (20).

1.2.6 GLYCOSIDES

1.2.6.1 CALYSTEGINS

The alystegins function as glycosides that block glycosidases in spite of their job as alkaloids. This exercise can aid in the treatment of diabetes and is essential for controlling blood sugar levels. Since calystegins affect the metabolism of carbohydrates, they may also be useful in neurological protection and anti-obesity therapies (21).

1.2.7 ADDITIONAL SUBSTANCES

1.2.7.1 RESINS

Complex combinations of both volatile as well as non-volatile terpenoid substances make up resins. They can strengthen the plant's defenses against infections and have antibacterial qualities (22) .

1.2.7.2 TANNINS:

Tannins are abrasive polyphenolic chemicals that constrict tissues and stop bleeding, hence can aid in the healing of wounds. Tannins are helpful in the treatment of infections since they also have antibacterial properties (23).

The phytochemical analysis of the *C. arvensis* aerial section of the plant showed the presence of rutin in the portion containing ethyl acetate along with stigma-sterol within hexane fraction. subsequently a phytochemical analysis of C. arvensis' aerial parts, a number of rr-alkanes along with n-alkanols, as well as a-amyrin, This includes campesterol, stigmasterol, along with 8-sitosterol, were isolated and identified (21).

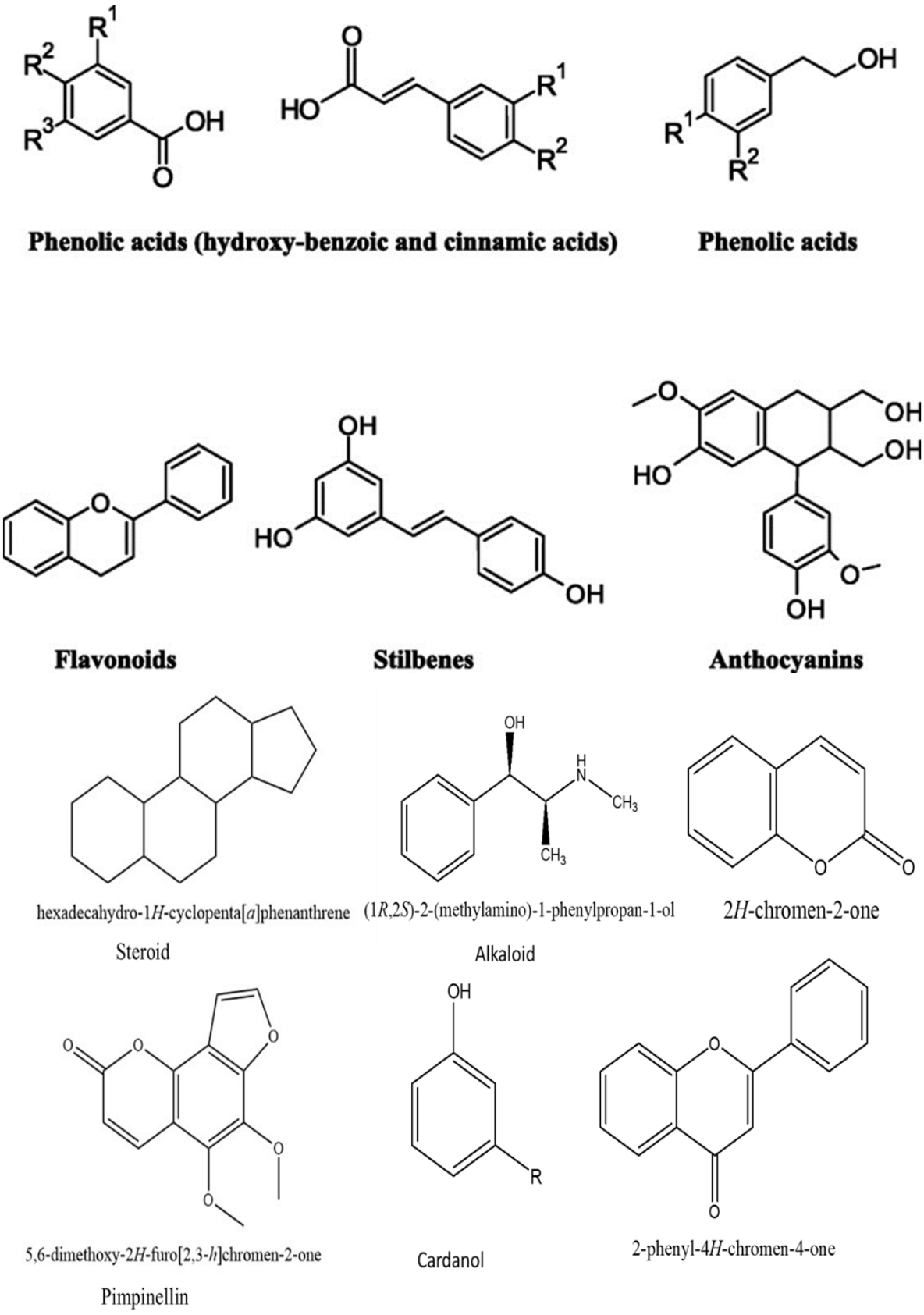


Figure 1.3 Illustrating the structure of some chemical constituent present in *C. arvensis*

A broad class of chemicals known as phenolic compounds is present in plants and is distinguished by the presence of one or more hydroxyl groups joined to an aromatic ring. Multiple phenolic units are present in polyphenols, a subclass of phenolic chemicals that are well-known for their antioxidant qualities. Phenolic acids, tannins, lignans, stilbenes, and flavonoids are among the main varieties of phenolic chemicals. Hydroxybenzoic acids include protocatechuic acid and gallic acid. These molecules are highly polar due to the presence of carboxyl and hydroxyl groups. Ferulic acid and caffeic acid are both hydroxycinnamic acids. Both caffeic and ferulic acids are hydroxycinnamic acids. Significant polarity is another characteristic of these compounds. Phenolic acids, which are derived from benzoic and cinnamic acids, are found in beans. Ferulic acid is the most prevalent phenolic acid, followed in order by gallic, vanillic, p-hydroxybenzoic, and p-coumaric acids (24).

1.3 FOLK MEDICINAL IMPORTANT OF *C. ARVENSIS*

Beginning as early from the 1730s, the herb was reportedly utilized in traditional medicinal systems. *C. arvensis* aerial portions were employed as a diuretic, wound healer, antispasmodic, anti-hemorrhagic, and to cure jaundice and parasites (46–50). Furthermore, it was utilized as a diuretic and for skin conditions including dandruff, a parasitic condition, and spider bites. The *C. arvensis* had also been traditionally used as a decoction to alleviate discomfort, inflammation, and aching joints in cases of cough and flu (3)

There are several uses for this plant. The root as well as resin have laxative, purgative, a urinary retention and cholagogue properties. The leaf is potentially useful throughout the menstrual cycle, while the flower are laxative and may be utilized in addition to tea infusion, as well as to cure fever along with wounds. Conversely, Meng et al. demonstrated that, during non-toxic concentrations, the widely used *C. arvensis* extract exhibited over 50% reduction of tumor formation activity, suggesting that it may be a viable anti-cancer drug. When examined on rabbits, also displayed an immune-stimulant impact. It was also shown that the plant had detrimental impacts on human malignant cells (25).

Due to its wide range of medicinal benefits, *C. arvensis*, often known as field bindweed, is highly valued in traditional as well as folk medicine. This herb is especially well-known for its strong anti-inflammatory properties, which make them

an effective natural treatment for pain relief and swelling reduction. Applying crushed leaves for a poultice to wounds including inflammations can help heal them locally. Plant extracts that concentrated form are also employed, based on the particular medical requirement (26).

C. arvensis has several advantages, but it should be used carefully because excessive amounts of the plant can be poisonous. To guarantee safety and effectiveness while taking the plant medicinally, it is best to speak with a healthcare professional before using it. Accurate identification is essential to prevent confusion with related species (27).

1.4 PHARMACOLOGICAL ATTRIBUTES OF *C. ARVENSIS*

C. arvensis has been shown to have therapeutic value in a few preclinical studies. The major primary biological agents in plants, such as lipids, proteins, along with carbohydrates, in addition to the additional metabolites such as polyphenols and terpenoids, are what give them their therapeutic properties (28). Medicinal plants contain a bounty in antimicrobial compounds with a variety of therapeutic uses. Diffusion as well as broth dilution procedures were used to assess the antibacterial properties of stem against *S. aureus* as well as Penicillium species. The extracts could be able to stop germs and fungi from growing. Long-lasting antibiotics become less effective against dangerous bacteria owing to the potential developing resistance to pharmaceuticals in these microorganisms due to the extensive use of antibacterial drugs, which ultimately results in risks to the global community's health. Therefore, new antibiotics need to be developed in order to prevent the spread of bacteria that are evolving (28).

1.4.1 CYTOTOXIC EFFECT

A refined bindweed extract is used to boost immune system performance, prevent blood vessel formation, and stop the growth for tumor cells. During chicken chorioallantoin its membranes, the elevated molecular weight extracts reduced angiogenesis about 73%, while in mice, it reduced tumor development by 77% given a dosage of 14 mg (60). Human Beings tumor cells was used to test the cytotoxic properties of hydroalcoholic, ethyl acetate, and chloroform extracts containing the arial portions of *C. arvensis*. The cultivated cells were treated with varying doses of the extracts and left for a full 72 hours of incubation. By employing the MTT technique, cell survival was assessed. Of all the extracts, chloroform extract had the

maximum cytotoxic impact (IC50 of 15 μg/ml), while hydro alcoholic together with ethyl acetate extracts had lower cytotoxic effects against Hela. The cells were inhibited with a pure extract of bindweed (IC50 values of 25 around 65μg/ml, accordingly). PGM protein purified from water-based extract enhanced lymphocyte function and prevented tumor development and vasculature in chick embryos (29).

C. arvensis aerial parts have been extracted employing 80% water-based ethanol and fragmented using petroleum-based ether, the solvent chloroform ethyl acetate (ETA), and the n-but. The resulting mixtures were then tested on mouse bone marrow, alongside measurements made for overall white blood cell-counts, chromosomal-aberrations (CA), and mitotic index (MI) for two different doses in each portion—200 as well 400 mg/kg. Employing three doses of necrosis factor alpha (TNF-α) in cultivated media—25μg/ml, 50μg/ml, with 100μg/ml—each fractions, these in vitro studies evaluated the cultured life expectancy of human rhabdomyosarcoma and human conventional lymphocytes. The chloroform along with n-butanol fractions, at doses of between 200 and 400 mg/kg, dramatically reduced MI and raised CA, according to the results. Only the petroleum ether in large dosages produced notable results. The large dosage had the opposite effect from the low dosage's ethyl acetate portion, which raised MI and lowered CA. The in vitro investigation revealed that all fractions' cultivated cell viability was inhibited as concentrations rose, and that TNF-α levels fell along with them (30).

1.4.2 ANTIOXIDANT EFFECT

Various methods have been used to assess the *C. arvensis* antioxidant activity. The antioxidant activity was determined by measuring the oxygen radical's absorbance capacity ferric reduction antioxidant power, and Trolox equivalents (TE)/g DW. The results were 1.62 mmol TE/g DW, 1.71 mmol TE/g DW, and 2.11 nmol TE/g DW, respectively. *C. arvensis* containing gelatin-based films shown a potent antioxidant effect during halting the lipid breakdown of muscle meal in an early trial. *C. arvensis* extracts can therefore be utilized as a herbal antioxidant. The *C. arvensis* aerial components were extracted, and then further fractionated to provide antioxidant-rich fraction. Three assays were conducted on varying doses of methanolic extract the ethyl acetate portion: reducing power, nitric oxide scavenging activity, as well as antioxidant assays using the DPPH technique. In every model, each component demonstrated a dose-dependent ability to scavenge free radicals. The extract made

from methanol and associated ethyl acetate portion were shown to have IC50 values of 131.03 ± 2.46 with 43.21 ± 4.45 µg/ml, correspondingly, in the DPPH model, whereas L-ascorbic acid along the rutin had IC50 values of 6.537 ± 0.235 -5.437 ± 0.206 µg/ml, correspondingly. The extract made from methanol and associated ethyl acetate component had IC50 values of 130.12 ± 2.46 respectively 57.5 ± 4.45 µg/ml in terms of Nitric oxide scavenger activity, whereas 21.06 ± 0.953 and the fractions had strong reducing power when concentration increased. The ethyl acetate antioxidant potential was within norms, although it demonstrated superior free radical scavenging and excellent reducing power as compared to the methanolic extract (31). The plant contains abundant in antioxidants, including phenolic acids as well as flavonoids, which lower stress levels and neutralized free radicals. The antioxidant's action aids in preventing cellular damage that advancing general well-being.

C. arvensis capacity of eliminating free radicals also aids in shielding the body against a number of illnesses linked to oxidative stress, such as cancers and cardiovascular conditions (32).

1.4.3 ANTIMICROBIAL ACTIVITY

Antimicrobial activities of *C. arvensis* have been established against a range of diseases by bacteria and fungi. Plant extracts can be used to treat infections, especially wound infections, since they have the potential to inhibit the development of certain microorganisms. This antibacterial effect is believed to be caused by alkaloids and saponins, two instances of bioactive substances. Around 250 varieties of blooming plants of the genus Convolvulus are extensively dispersed throughout Pakistan. Its leaf extract has been used in traditional medicine to treat a variety of illnesses. The current investigation attempts to assess the phytochemical content and antibacterial activity of the whole plant. The *C. arvensis* methanolic extract had a flavonoid concentration of 12.9 µg quercetin equivalent (QE)/mg, while the total phenolic contents were determined to be 96 µg of gallic acid equivalent (GAE) per mg extract. The total antioxidant capacity of the methanolic extract was determined by measuring 26.03 µg glutamic ascorbic acid equivalent/mg extract, with a scavenging potential of 27.12%. The plant was least active against *E. coli* and most active against *S. aureus* (33).

1.4.4 DIURETIC CHARACTERISTICS

The *C. arvensis* has diuretic potential that aid in lowering the body's accumulation of fluid. They go by the name "water pills" occasionally. The majority of diuretics aid

the kidneys in eliminating water and salt from the urine. As a result, less fluid passes through both veins and artery walls.

1.4.5 GASTROINTESTINAL ALLEVIATION

Good evidence suggests that *C. arvensis* may assist to speed up or improve stomach emptying which makes it ideal for gastroparesis patients. In conclusion, herbs seems to affect the stomach the most and can hasten the process of gastric emptying. It is therefore a helpful natural medication for encouraging regularity as well as comfort in the digestive system (34).

1.4.6 RESPIRATORY ACTIVITY

Primarily *C. arvensis* was used for the treatment of respiratory ailments including coughs and colds. The expectorant qualities of the plant aid in releasing mucus throughout the respiratory system, relieving congestion and making breathing easier (35) .

1.4.7 ANTICANCER ACTIVITY

According to preliminary research, *C. arvensis* may possess anticancer capabilities. Plant extracts exhibit the ability to stop some cancer cells form growing; this may be achieved by preventing cell division and inducing apoptosis, or programmed cell death. To completely comprehend its anticancer effects and methods of action, additional study is necessary.

1.4.8 ANALGESIC CAPABILITIES

Analgesic properties of the plant aid in pain relief. This is presumably because of its sedative along with anti-inflammatory qualities, which combine to lessen pain perception and ease people with a variety of uncomfortable ailments. Conclusively, *C. arvensis* has a significant plant in herbal medicine due to its many pharmacological properties. Its promise as an alternative medicine for a range of illnesses is highlighted by its anti-inflammatory, antioxidant, antibacterial, wound-healing, urine stimulant gastrointestinal, pulmonary, anticancer, as well as analgesic qualities. However, cautious use and expert advice are advised due to its possible toxicity in high dosages. Even if there are a plethora of medications used to treat inflammatory illnesses, the current selection of these medications does not address the issue of their effective therapy and relapses, which can occur often after the medication is stopped.

Examine the gel that contains *C. arvensis* anti-inflammatory properties in cases of aseptic arthritis brought on by carrageenan.

Studies were conducted on sexually mature male white rats that weighed between 155 and 180 g at the beginning of the experiment. The animals' right hind paw was treated with a 5% gel containing *C. arvensis* extract and a 5% gel containing ibuprofen one hour before to the injections of phlogin and following each the volume of the paw measured. Using a plethysmometer, the volume of the animals' paws was measured. Results: Using a gel containing *C. arvensis* extract showed a more pronounced anti-exudative action. Thus, following 1, 2.3, and 4 hours from the start of the carrageenan activity, the rat paws' volume rose in comparison to the starting by 45.5, 51.9, 59.7, and 51.9%, respectively. When *C. arvensis* extract-containing gel was applied, the exudation process's development was nearly twice as inhibited as it was in the control group. In conclusion: In aseptic arthritis caused by carrageenan, the gel containing the extract of C. arvensis has an expressed anti-exudative activity (36).

1.5 PROBLEM STATEMENT

Field bindweed also known as a *C. arvensis* have a history of used traditional by folk medicinal healer. They are supposed to possess numerous health-enhancing stuffs, comprising antidiabetic, antibacterial, laxative and antioxidant properties (20). Conversely, despite their extensive usage, here is an insufficiency of inclusive scientific research to authenticate these privileges and exploring the possible applications of field bindweed extracts in recent medicine and therapeutic purposes.

1.6 FUTURE PERSPECTIVES

Composition of phenol, flavonoids, tannin and other phytochemicals are determined qualitatively and quantitatively from the methanolic and aqueous extract of stem and leaves of *C. arvensis* and evaluated their anti-inflammatory, antimicrobial and antioxidant activities. Furthermore, it revealed strong hope to development of additional novel chemotherapeutic mediators from plants, that in the future might be serve in production of the synthetically reworded therapeutic agents. Supplementary studies are suggested to explore different parts of plants significance which frequently occurring in nature.

1.7 AIM AND OBJECTIVES

Natural herbs are growing more important in the sphere of medicine and therapy, according to literature. Plants that contain a variety of bioactive chemicals have the potential to cure illnesses.

The aims and objectives of our research are as follows:

- Extraction of aqueous and methanolic extract of stem and leaf of *C. arvensis.*
- Qualitative and quantitative phytochemical screening of aqueous and methanolic extract of stem and leaf of *C. arvensis* through various method for significant identification of bioactive compounds
- Investigate the antioxidant capability of plant extract through DPPH assay
- Explore antibacterial activity of plant extracts against selected Gram-negative and Gram-positive bacteria.

CHAPTER 2

LITERATURE REVIEW

Al-Snafi et al., (2016) stated that the phytochemical analyses revealed that *C. arvensis* contained phenolic compounds, alkaloids, cammonia resin, dihydroxy nicotine, beta-methyl esculetin sucrose, puranol, sucrose, reducing sugars, starch, mucilage, steroids, resin, tannins, and unsaturated fats sterols/triterpenes. Previous research on pharmacology demonstrated *Convolvulus scammonia*, had purgative, vasorelaxant, antiplatelet collection, anticancer, as well as cellular protective effects, whereas *C. arvensis* possess cytotoxic, which antioxidant, vasorelaxant, hepato-protective immune-stimulant antibacterial, antidiarrheal, and the diuretic effects. The components and pharmacological consequences of *C. scammonia* and *C. arvensis* will be highlighted in this study (31) .

Angel Lalrindiki et al., (2022) stated that a plant that proliferates and contends with different plants for nutrients, light, humidity, and place is called a weed. These are fragrant annual plants. They invade vast woodland regions, empty lands, and pastures. Secondary metabolites called phytochemicals are known to affect a range of plant functions. The methanol extracted extract from the leaves was subjected to a qualitative and quantitative phytochemical evaluation, which reported an abundance of flavonoids, the compounds phenols alkaloids, the compound quinines glycosides from cardiac tissue, terpenoids, as well as tannins, with the exception of saponin followed by anthraquinones. On the other hand, quantifying on the extract showed that its flavonoid content was 27.8±0.665 mg/g, its phenol content was 8.6±0.846 mg/g, and its alkaloid content was 2.06±0.73 mg/g (37).

Ahmad Mohammad Salamatullah (2022) demonstrated that the traditional phytotherapy makes use of the medicinal herb *C. arvensis*, which belongs within Convolvulaceae family. The aim of this study was to evaluate the chemical composition, antioxidants and antibacterial capabilities of *C. arvensis* essential oils. GC-MS was used to analyse the chemical composition in order to meet this goal. To measure antioxidant power, TAC, FRAP, and DPPH tests were used. Using disc diffusion among minimum-inhibitory concentrations (MICs) experiments, the antibacterial power was assessed against clinically significant pathogenic microbes (*E. coli, S. aureus. K. pneumoniae and S. pneumoniae*,) and fungus (*A. niger, A. flavus and C. albicans*).

The yield of extracted oil (EO) from *C. arvensis* was found to be 0.34 percent of total weight of leaves, with the highest concentrations found as cuprenne (34%) followed by thymol (20%), 16% himachalene and the 10% longifolene. EOCA had significant antioxidant benefits; the overall antioxidant capacity was found to508.0 ± 6.0 µg EAA/mg, and IC50 (DPPH) & EC50 (FRAP) proved to 30 µg/mL as well as 120 µg/mL, correspondingly. An outstanding inhibition zone was observed against most bacterial strains, notably *K. pneumoniae* showing the highest inhibition zones at 14.27 ± 0.42 a respectively, indicating a substantial antibacterial activity of EOCA. The *K. pneumoniae* had MICs of 21.35 ± 0.76 mm showed. Significantly, EOCA demonstrated significant antifungal efficacy against every strain of fungal, with inhibition zone widths against every fungal species varying to 19.44 ± 1.10 mm. Notably, the MICs of EOCA against *C. albicans* and the *F. oxysporum* was 19.38 ± 0.58 g/mL while 18.65 ± 0.94 g/mL, correspondingly. The current study's findings demonstrated that the EOs from *C. arvensis* might be utilized to conceptualize medications for the management of infections along with radical-related disorders (38).

Asaad Abdulwahed (2014) investigated the cytotoxicity of methanolic and aqueous extract of stem, roots and leaves *C. arvensis* against the Rhabdomyosarcoma (RD) human tumor cell via in-vitro. Additionally, the impact of the glycoside on the RD cellular line's mitotic index was examined. Tetrazolium bromide (MTT) is utilized via Elisa Reader to quantify the optical density (OD) for the proliferation of cells at the wavelength range of 492 nm. After 24 hours, glycoside FI and leaf extracts in aqueous and methanolic forms showed increased cytotoxic effects around 10 mg/ml. When compared to other doses, proteoglycan along with glycoside FI over 10 mg/ml showed extremely significant cytotoxic activity following 48 hours. When compared to other extracts, glycoside 10 mg/ml exhibited greater cytotoxic inhibition during 72 hours. After 24 hours, 48 hours, and 72 hours, respectively, glycoside cytotoxicity content 50% (CC 50%) was 1.775, 0.870, as well as 0.706 mg/ml. Compared to other extracts, the root extracted with water showed a lower cytotoxic impact after 72 hours (30).

Serap Sunar (2021) investigate that the genotoxic and inhibitory effects of *C. arvensis* methanolic extracts upon the overall dissolved protein content and the viability of the genome templates against maize *Zea mays* investigated in this study. Corn seed was treated with methanol extracts from *C. arvensis* branches, leaves, and root that had been diluted into 50, 75, as well as 100 micro-Litres concentrations. The results of

genomic template integrity and total soluble-protein concentration were contrasted with control. [14].

Uzma Saleem et al., (2020) design to assess the extracted form *C. arvensis* anti-arthritic properties and acute oral toxicity. The acute-oral toxicity research was used to evaluate safety. In vitro (inhibition of denaturation of protein) and the in-vivo induced arthritis with carrageenan-induced inflammatory) techniques were used to investigate the anti-arthritic effect.

By measuring ferric reducing power, DPPH restriction, while H_2O_2 scavenging tests, antioxidant potential was ascertained. Moreover, molecular docking has been employed to examine the interactions between the COX-1 and the COX-2 cyclooxygenases with plant components. HPLC was used to quantify quercetin, gallic acid, caffeine, syringes acid, and vanillic acids. GC-MS analysis was used to identify eight different chemicals. In the toxicology trial, no abnormalities in biochemical markers or death were noted. Important tissue histological analyses corroborated these biochemical findings. The alcohol-based extract of *C. arvensis* leaf shown dependent on dose anti-arthritic as well as antioxidant activity, according to in-vitro and in-vivo investigations. When compared to regular ibuprofen, docking of molecules revealed that plant chemicals interacted with cyclooxygenases more effectively. Strong anti-arthritic properties of the *C. arvensis* support the traditional usage of herbal remedy (15).

Nurul et al., (2015) used several methods in this work to assess the antioxidant effectiveness of the ethanolic extract of *C. arvensis Linn (CA*). The (ABTS) radical and the ferric reducing-power for antioxidants as well as the oxygen radical absorption capacity (ORAC) measured indicate the antioxidant activity. The results were 1.62 mmol 1.71 mmol TE/g DW, and 2.11 mmol TE/g DW, respectively. By measuring electron-magnetized resonance (EPR), the carbonaceous ethanol extract demonstrated scavenging efficacy from methoxy radical that was generated by the Fe reaction. In burgers containing 0.1% and 0.3% (w/w) CA kept in modified atmospheric packaging (80% O_2 and 20% CO_2), the antioxidant effects of lyophilized CA were assessed. An initial investigation of a gelatin-based films containing CA revealed higher antioxidant capability in preventing the lipid in muscle meals from breaking down. Accordingly, the current findings suggest that CA extract has potential use as an all-natural antioxidant (41).

Ali Esmail and Al-Snafi (2016) stated that the phytochemical analyses revealed that scammonia included scammonin substance, dihydroxy nicotine, beta-methylesculetin, i-puranol, reducing sugar, and starch, while convolvulus arvensis contained alkaloids, phenolic substances, flavonoids, dietary carbohydrates, sugars, mucilage that sterols, resin, tannins, and unsaturated sterols/triterpenes. Prior pharmacological research demonstrated that *Convolvulus scammonia* displayed purgative, vaso relaxat, anti-platelet aggregation, anticancer, and cellular protective activities; in contrast, *C. arvensis* had cell-damaging, antioxidant that vaso-relaxat, immune stimulant, hepatoprotective, antibacterial, antidiarrheal, and diuretic actions. The components and pharmacological consequences of *C. scammonia* and *C. arvensis* will be highlighted in this study (5).

Shakir Ullah et al,. (2018) discovered the Arrange Sari Ghar War-affected mountain in Bajaur Agency, Pakistan yielded species of medicinal plants: Rumex dentatus L., Rumex hastatus L., *C. arvensis* L. After washing, air drying, and powdering, the leaves of the chosen medicinal plants were used. Leaf sample extracts in methanolic, ethanolic, and chloroform were utilized for the phytochemical analysis (qualitative and quantitative) and antibacterial activity. Verifying the effectiveness of antibacterial agents and the existence or absence of phytochemical elements in all the chosen medicinal plants was the main goal of the current study. The phytochemical evaluation of these therapeutic plants revealed the presence of alkaloids, proteins, glycosides, phenols, tannins, flavonoids, carbohydrates, and phenols. When it was discovered that all of the chosen therapeutic plants contained terpenoids. Rumex hastatus had the highest quantity of phenol (0.81±0.10 mg/g) in the methanolic extract, next to *Canabis indica* (0.68±0.11 milligrams per), *C. arvensis* were (0.58±0.20 mg/g), and *Solanum nigrum* (0.10±0.11 mg/g) with the lowest amount of flavonoids. Rumex hastatus was able to identify the greatest level of inhibition towards *Staphylococcus aureus* as well as *Salmonella typhi*, measuring 24.17 and 21.00 mm, respectively. Regarding *Escherichia coli, Rumex hastatus* displayed a 17.00 mm a zone of inhibition. The secondary metabolite found in plants is highly valuable in the pharmaceutical industry and is used extensively in the development of novel medications intended to treat a variety of ailments (42).

Soukaina Hrichi et al., (2020) extracted the leaves of *Convolvulus L by*

Using spray ionization mass spectrometry, 22 polyphenols, 11 chlorophyll derivatives, and 10 carotenoid compounds were identified; polyphenolic compounds were more prevalent in extracts from leaves collected in the winter, while pigments were more prevalent in the spring collections. The extracts' antioxidant activities were assessed using the DPPH radical scavenging method, which revealed an IC50 ranging from 0.1369 ± 0.0272 mg g~1 to 0.432 ± 0.00 18 mg g~1; there was no correlation between seasonal fluctuations. In terms of antifungal assays, ethyl acetate and ethanol extracts were particularly active against skin diseases such as (*T. rubrum*, *T. menthargrophytes*, and *M. canis*), with restricting percentages accomplishing 100% with 50 mg mL~1. Additionally, ethyl acetate and 6.25 mg mL~1 in cultures of Candida spp. At an amount of 3.1 mg mL1, the winter collection of these extracts had a 90% inhibitory impact on the development of *Candida albicans* germ tubes. The seasonality appears to have an impact on the natural chemicals found in *C. althaeoides L*. leaves, which are primarily responsible for their antioxidant and antifungal properties (13).

Utilizing GC/MS along with LC/MS/MS chromatographic techniques, the natural components in extracts made with methanol of *C. arvensis* parts of plants were identified. Both fresh and dried samples were used in the investigation. The examination shows that the extracts include a wide variety of chemicals. Some of them, together with their biological activity, were previously described and reported in a number of other plants. In every phase, every portion of the plant received the same treatment. Major peaks are visible in the GC/MS chromatograms (peak area/total peaks area>0.2%). Seventeen distinct, crisp, well-resolved, and easily quantifiable chemicals were found in the leaf extract. Neophytadiene, hexadecanamide, 9-octadecanamide, and 1, 2-benzendicarboxylic acid are the four primary peaks. Four main peaks out of the thirteen chemicals found in the stem extract are as follows: hexadecanamide, 1, 2-benzendicarboxylic acid, 9-octadecanamide, and the streptant-5-en-3-ol. Twelve chemicals were found in the root extract, with the three main peaks being 1, 2-benzendicarboxylic acid, 9-octadecanamide, and hexadecanamide. Neophytadiene, the acid stearic, hexadecanamide, benzendicarboxylic acid, the antioxidant vitamin E, streptent-5-en-3-ol, and 5-beta-pregn-7-en-3, 20-dione are the eight primary chemicals found in the dry extract. Following the completion of the

LC/MS/MS measurements, a correlation was discovered between the outcomes of the two separate chromatographic procedures, indicating consistency.

The two chromatographic techniques identify the main components. Historically, the fresh and dry plant was investigated for its potential to cure many illnesses. Convolvulus arvensis, both fresh and dry, is used to treat skin cuts and wounds in order to halt bleeding and encourage rapid damage healing. This is why the study was conducted (43).

Balah (2015) described that the root exudation is one of the primary mechanisms used by weeds to produce allelochemicals, and it plays a significant role as a regulatory agent in nearby plants and microorganisms. When compared to non-rhizosphere soil, the rhizosphere microbial communities were unquestionably richer in field bindweed (*C. arvensis L.*) regions with both high and low densities. In high density areas, bindweed root exudates suppressed rhizosphere soil bacteria and fungus more than in low density ones. For seedling biomass fresh weights, the EC50 of the bindweed ethyl acetate root exudates extract was 1625 µg/g (*Lactuca sativa* L.) and 1360 µg/g (*Phalaris paradoxa* L.). Total phenolics varied from 0.3 to 0.57 mg/g in non-rhizosphere soil and from 0.7 to 0.9 mg/g in rhizosphere soil, respectively. The amount of total phenolics was greater in rhizosphere soil. Six have been revealed by the exudates profile analysis. Six substances were identified by LC-MS analysis of the exudates profile: umbelliferone (C9H6O3), propranolol (C15H10O7), gentisic acid glycoside, cambriatic acid the glycoside and ferulic glycoside (C16H20O9) (44).

Sepideh Hamedi et all., (2017) studied a quick and environmentally friendly way to synthesize nanoparticles of silver using Convolvulus arvensis leaf extract. The SPR band, which was visible in UV–visible spectra at about 430 nm, demonstrated the AgNP production. Within 150 minutes, silver ions were reduced to nanoparticles. The production of AgNPs with an average size of 28 nm and a PDI of 0.2 was confirmed by particle size analysis. The crystalline character of the generated AgNPs was confirmed by XRD patterns. The existence of spherical nanoparticles without any signs of aggregations was confirmed by SEM images. The role of the phenolic ingredients in AgNP production was confirmed by FTIR analysis. The antibacterial activity of biosynthesized AgNPs against the human pathogen E. coli was discovered. Additionally, nanoparticles have the ability to break down biofilms against both bacterial and fungal species (45).

CHAPTER 3

METHODOLOGY

3.1 APPARATUS NECESSARY

3.1.1 GLASS WARES

- Beakers (500mL, 300 mL, 250 mL and 100 mL),
- Micropipette,
- Test tubes,
- Conical Flask (100mL),
- Pipette (10mL),
- Measuring cylinder (50 and 100mL),
- Viols (20mL),
- Petri dishes,
- Centrifuge tube,
- Funnel etc.

3.1.2 OTHERS

- Filter papers,
- Aluminum foil,
- Burner, Tripod stand, Spatula , Matchbox,
- Magnetic stirrer, Pistol /Mortar.

3.2 REQUIRED CHEMICALS AND SOLVENT

De-ionized H_2O /distilled water, Fresh *C. arvensis* herbs, Methanol /Ethanol, Agar/Peptone, Nutrient broth, Ciprofloxacin (1 mg), Quercetin, Gallic acid, egg white, DPPH, ABTS.

3.3 MICROORGANISM USED

- *Escherichia coli* (Gram-negative); MTCC 443
- *Pseudomonas aeruginosa*,
- *Staphylococcus aureus* (Gram-positive); MTCC 7443
- *Bacillus cereus* (Gram-positive) : MTCC(9817)

3.4 INSTRUMENTATIONS

- Weighing balance (SHIMADU LIBROR, CAPACITY OF 220.0g)
- Incubator/furnace
- Sonicate
- UV-Visible spectrophotometer(shamzudu 1700)
- Centrifuge (GenFuge 24D)
- FTIR Spectrophotometer (IRAffinity-1S)

3.5 EXPERIMENTAL WORK

The compounds as well as reagent were used have analytically grade, hence no any necessity to purify as well as autoclave afore using them excepting antimicrobial activity.

3.5.1 COLLECTION OF PLANT

The fresh herb of *C. arvensis* was collected from Nasir bagh and oval ground of Government College University Lahore.



Figure 3.1 Air-dry leaves and stem sample of *C. arvensis*

3.5.2 IDENTIFICATION OF PLANT

Dr. Zaheer-ud-din Khan Professor Government College University, Lahore identified the selected plant sample and voucher specimen submitted to the herbarium of the Botany Department for the purpose of future reference.

3.5.3 SAMPLE PREPARATION

After the identification, the herb was wash away with the distilled water then keep in the shade to dry for about 35 to 40-days, subsequently its stem and flowers are separated through the handpicking using small pliers. Weighted about 100 g of each stem and leaf in digital weight machine then prepared its fine powders via pistol/mortar then stored for the experimental work.



Figure 3.2 Handpicking separation of leaf and stem of *C. arvensis*

3.5.4 PREPARATION OF LEAVES AND STEM AQUEOUS EXTRACT

- Weighted about 10 leaves and stem fine powder
- Added the weighted powder into the separated conical-flask and added about 100 ml of the distilled water, stirred, prepared an homogenized mixture
- Keep underneath shade for about 72-h for the extraction
- After 78-h of period filtered by using Whatmann-42-filter paper, labeled the residue and filtrate and stored at 4℃.

- Evaporated the whole solvent with the help of rotatory evaporator to obtained the crude extract, that was stored at 4℃ for furthers usage.
- All the procedure was performed in the absence of sunlight.

3.5.5 PREPARATION OF LEAVES AND STEM METHANOLIC EXTRACT

- Weighted about 10 g fine powder of leaves and stem via digital weight machine.
- Added weighted sample in the separated conical-flask followed by added 100 ml of (90%) methanol, stir and prepared a homogenized mixture
- Keep in shade for about 72-h for the extraction
- After 72-h filtered it via Whatmann -42 filter-paper, and labeled filtrate and stored at a temperature of 4℃.



Figure 3.3: Filter the aqueous and the methanolic extract of plants sample

3.5.6 PHYTOCHEMICAL SCREENING

The phytochemical screening carried through both qualitative and quantitative method.

Qualitative Test for tannins, saponin, terpenoids, carbohydrate, protein, lipids, steroid and other chemical was performed by using both aqueous and methanolic extract.

Quantitative analysis for total phenolic, flavonoids, alkaloids, terpenoids and other quantification will perform by through UV- visible spectrophotometric method [18].

3.5.6.1 QUALITATIVE PHYTOCHEMICAL SCREENING

The various biochemical test of the *C. arvensis* extract was performed for the qualitative analysis of different bioactive compounds through standard methodology.

3.5.6.1.1 ALKALOIDS

Took 2mL crude extract in test-tube and added 3 to 4 drops of Mayer's reagent (Mercuric chloride 1.36 g and potassium iodide 5 g in 100mL DI water). Observed the formation of white precipitation, which indicated the presence of alkaloids in *C. arvensis* extract.

3.5.6.1.2 FLAVONOIDS TEST

2mL crude extract solution was taken in clean test-tube and added 2.5 mL of NaOH (2%) solution. Observed the intense yellow-color, which was, change into colorless by addition of 3-4 drops of dilute HCl that has indicated flavonoids presence in extract.

Ammonia solution about 5 mL was taken in test-tube and added the aqueous extract of sample followed by few drops of sulphuric acid (H_2SO_4). Yellowish precipitate was formed that confirm the flavonoid in sample.

The Liberman-Nitroso reaction and Phthalein dye test were performed for the qualitative determination of polyphenol in sample.

3.5.6.2.3 BRAYMER'S TEST

1 mL aqueous extract and 3 mL distilled water was taken in test tube and added 2-3 drops of 10% ferric chloride. The dark green/blue black color were observed which indicate the presentence of tannins in sample

3.5.6.2.4 GELATIN TEST

The crude extract was dissolved in 4 mL distilled water in a test tube and added 1mL gelatin (1%) solution followed 10% sodium chloride. The white precipitated formed which indicated the presence of phenol and Tannins in extract.

The crude extract of plant mixed with distilled water (5 mL) in a test tube after that shaken test-tube vigorously. The foam formation taken as conformation of the saponin present in extract.

Molish test and Benedict test performed for the identification of presence of carbohydrate.

The protein were identified by performing the Biuret test and Ninhydrin test.

Other phytochemical were also qualitatively analyses by using various approaches.

3.5.6.2 TOTAL PHENOLIC CONTENT (TPC) DETERMINATION

The total phenolic content present in various part of *C. arvensis* plant were determine through Folin-Ciocalteu (FC) reagent by UV-visible spectrophotometry as determined by with little modification. Gallic acid, Folin-Ciocalteu (FC) reagent and sodium carbonate (20%).

About 10 mg of each plant sample were dissolve in 5 mL of solvent and taken diluted 0.5 mL each plant sample in test tubes and added 0.5 mL dilute FC reagent and stand at room temperature for 3-minutes. Then, 1 mL of 20% Na_2CO_3 solution (20 g sodium carbonate in 100 mL water) was added shake well and incubated for 1-hour at 25 ℃. Finally, at 750 nm the absorbance was measured through UV-visible spectrometer (UV-1700 Shimadzu).The Gallic acid standard curve used for the determination of TPC in all plant sample. For preparing, the standard stock solution measured 1 gram of Gallic acid that dissolved in methanol (10 mL) and transfer into 100 mL volumetric flask diluted to volume with distilled water. Prepare series of dilution from stock, which contain 0, 50, 100, 150, 250 and 500 µg/mL and same procedure carried to find the absorbance. The total phenolic content in all sample was represent in mg of Gallic acid equivalent (GAE) per gram of dried plant sample.



Figure 3.4: Complete Protocol of TPC determination

3.5.6.3 TOTAL FLAVONOIDS CONTENT (TFC) DETERMINATION

The total flavonoid content of different part of plant determined through Aluminum chloride with some modification from Woisky and Salatino procedure that were reported in literature. The Quercetin used as standard for make calibration curve.

Aluminum chloride 10%, Quercetin, 1M potassium acetate, ethanol and distilled water.

About 1 mg of standard quercetin dissolved in ethanol (80 %) and make series of diluted 30 µg/mL, 25µg/mL, 20 µg/mL, 15 µg/mL, 10 µg/mL, 5 µg/mL and blank solution. The all diluted solution mixed separately with 95% ethanol (1.5 mL), 10% aluminum chloride (0.1 mL), 1M potassium acetate (0.1 mL) and distilled water (2.5 mL). Incubated all the solution at room temperature for half-hour and find absorbance at about 512 nm by UV-visible spectrometer (UV-1700 Shimadzu). Take 10 mg of the plant sample and dissolved in 5 mL of solvent and diluted about to sample solution in test tube. The diluted sample of 0.1 mL, ethanol (0.3 mL), 10% aluminum chloride (0.3 mL) and distilled water (2.5 mL) added in test tube. The mixture containing test tube incubated for 30 minutes at room temperature in incubator. Measure the absorbance at 412 nm. The total flavonoid content in all sample was represent in mg of Quercetin equivalent (QE) per gram of dried plant sample.

3.6 ANTIBACTERIAL ACTIVITY OF STEM AND LEAF EXTRACT

Select gram-positive and gram-negative bacterial strains to be use against plant methanolic and aqueous extract, Preparation of the bacterial cultures, Agar well-diffusion methodology will be use evaluation of antibacterial activity and find out the zone of inhibition [19]. The herbal extract's antibacterial activity was evaluated using four distinct bacterial strains:

Gram-*negative Escherichia coli*, and *Pseudomonas aeruginosa*, and Gram-positive *Bacillus cereus, Staphylococcus aureus*.

Using well-diffusion approach, the plant extract's antibacterial capacity was investigated. The procedure was carried out in a laminar flow, and twentieth milliliters of nutrient-agar mixture were made, autoclaved, and then evenly put onto the culture plates that had been labeled along with sterilized. With caution, the autoclave's equipment and agar material were removed. The equipment and agar medium have been permitted to cool down sufficiently to handle. If the agar in the flask is allowed to cool for an extended length of time, it may harden. The agar medium was moved, in a sterile setting, like a laminar flow, to the petri plates. The agar was evenly spread and approximately half an inch deep when it was added to the petri dish.

Use a permanent marker to mark the bottom of the petri plates and split them into four parts. Since the petri plate cover can be changed while it is in use, do not mark it. Label the samples and positive control in the designated section as well.

The inoculation process was first heated on a Bunsen burner until it became brilliant orange in color. Next, allow the loop to cool before taking an agar media-filled petri dish. The stock cultivation of bacteria had been submerged into the sterilized inoculating loop. To begin the streaking procedure, a loop holding the culture of bacterium was put on the agar surface. To ensure uniform distribution of the bacterial culture, zigzag-streak the petri plates. Sterilize the loop used for inoculating after streaking one strain of bacteria, then carry out the same procedure with the remaining strains. Additionally, petri plates were grown in an incubator set to 30–37 °C for a whole day.

The colonies of bacterial cells from the culture dishes where bacteria were allowed to grow were taken out and placed in a sterile environment to create the bacterial suspension. Using a sterilized inoculating loop, brush the bacterial colonies and place

them in the Eppendorf that has the nutritional liquid in it. Shake well and then put the Eppendorf in the freezer. The Bunsen burner flame, which produces an impure atmosphere in laminar flow, was used to work on bacterial colonies in close proximity. Carefully immerse the sterile cotton swab in the properly mixed bacterial mixture by opening the container. The bacterial suspension's lid was replaced to prevent contamination.

Keeping your distance from the Bunsen burner, you placed the agar plate so that the surface faced you. Now, slowly begin wiping the cotton swab from the center over the agar's surface. To distribute the bacteria evenly, the cotton tip was first swabbed upward and then downward. Then, the petri dish was rotated, and the process was repeated, making sure to swab the brush swab on the plate's edges as well. As soon as the culture plate was ready, the petri dish was covered with a lid. To reduce the possibility of contamination, close the petri dish as soon as you can. The antibacterial activity was carried out using the well diffusion technique. Using blue micropipette tips, wells were created on the agar. Using yellow micropipette tips, sample solutions have been introduced into the appropriate wells following the creation of wells. Ofloxacin solution (1 mg/mL) was used as a positive control. To prevent contamination, every petri dish was placed within a zipper bag. Put a title and number of rolls on the bag with zippers to prevent any accidents. Petri dishes were in the incubator at 30-37 °C for a whole day.

3.6.1 MEASUREMENT OF INHIBITION ZONE

The inhibition zone could be seen after a whole day. Samples and the positive control both show antibacterial activity. The petri plate was first placed on a non-reflective surface, and the inhibition zone was measured from the rear of the plate. To get the radius, take a centimeters ruler and set its zero value in the center of the antibiotic or sample disk. Because the zone of the inhibition was calculated in diameter, double the radius by two. To translate the reading from centimeters to millimeters, the numbers were then multiplied by ten.

3.7 ANTIOXIDANT ACTIVITY OF STEM AND LEAF EXTRACT

Conducting the assay such as DPPH to be evaluated the antioxidant capability of plant. UV-visible spectrophotometer was used for absorbance measurements [20].

3.8 ANTI-INFLAMMATORY ACTIVITY

3.8.1 BOVINE SERUM ALBUMIN ASSAY (BSA)

The anti-inflammatory activities of the plant extracts were determined by using the modified version of the BSA assay. Employing the BSA (bovine serum albumin) test, this in-vitro study evaluated the anti-inflammatory effect in a laboratory setting. Denatured proteins were produced by BSA at an alkaline pH of 6.8. By introducing to BSA, data anti-inflammatory capabilities of the standard (Diclofenac) and sample (stem and leaves) extracts from sample plant were evaluated. The method utilized to compute the percentage of inhibition of breakdown was based on the observed absorbance. The data that was gathered was analyzed using descriptive statistics.

3.9 DATA ANALYSIS

Statistical approach was used for the analyzing phytochemical, antibacterial, and antioxidant activity data.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PHYTOCHEMICAL ANALYSIS

All the qualitative phytochemical Analyses was carried at laboratory and the quantitative analysis carry out by using the UV-vis spectrometer from Department of the Chemistry Government College University Lahore.

4.1.1. QUALITATIVE ANALYSIS

The plant stem and leaves aqueous extract and methanolic extract were analysis for identification of the presence of different phytochemical constituent namely flavonoids, alkaloids, sterol, polyphenol, carbohydrate, protein, terpenoids etc.

Table 4.1 Different Phytochemicals present in *C. arvensis* methanolic and aqueous extract of leaves and stem

	LEAVES		**STEM**	
Phytochemical	**Aqueous extract**	**Methanolic extract**	**Aqueous extract**	**Methanolic extract**
Alkaloids	+	-	+	+
Flavonoids	+	+	+	+
Phenol and Tannins	+	+	+	+
Saponin	+	-	-	-
Reducing sugars	-	+	+	-
Protein	+	+	-	+
Glycosides	+	-	+	-
Terpenoids	+	-	-	-
Steroids	-	+	-	+
Polysterol	-	+	-	+
Coumarins	+	-	+	+

+ = Present, - = Absent

4.1.2 QUANTITATIVE ANALYSIS

4.1.2.1 TOTAL PHENOLIC CONTENT

The total phenolic content present in various part of *C. arvensis* plant were determine through Folin-Ciocalteu (FC) reagent by UV-visible spectrophotometry as determined by with little modification.

Prepare series of dilution from stock, which contain 0, 50, 100, 150, 250 and 500 µg/mL and same procedure carried to find the absorbance. The total phenolic

content in all sample was represent in mg of Gallic acid equivalent (GAE) per gram of dried plant sample

Table 4.2 Standard Gallic acid solution absorbance for the determination of TPC in sample

Sr. No.	Concentration	Absorbance (nm)
1	0	0
2	50	0.12
3	100	0.29
4	150	0.41
5	200	0.55
6	250	0.69
7	300	0.80

Table 4.3 *C. arvensis* leaves and stem extract absorbance for TPC

Test no.	Plant sample	Absorbance
1	Aqueous extract leaves	0.101
2	Methanolic extract of leaves	0.09
3	Aqueous extract of stem	0.032
4	Methanolic extract of stem	0.042

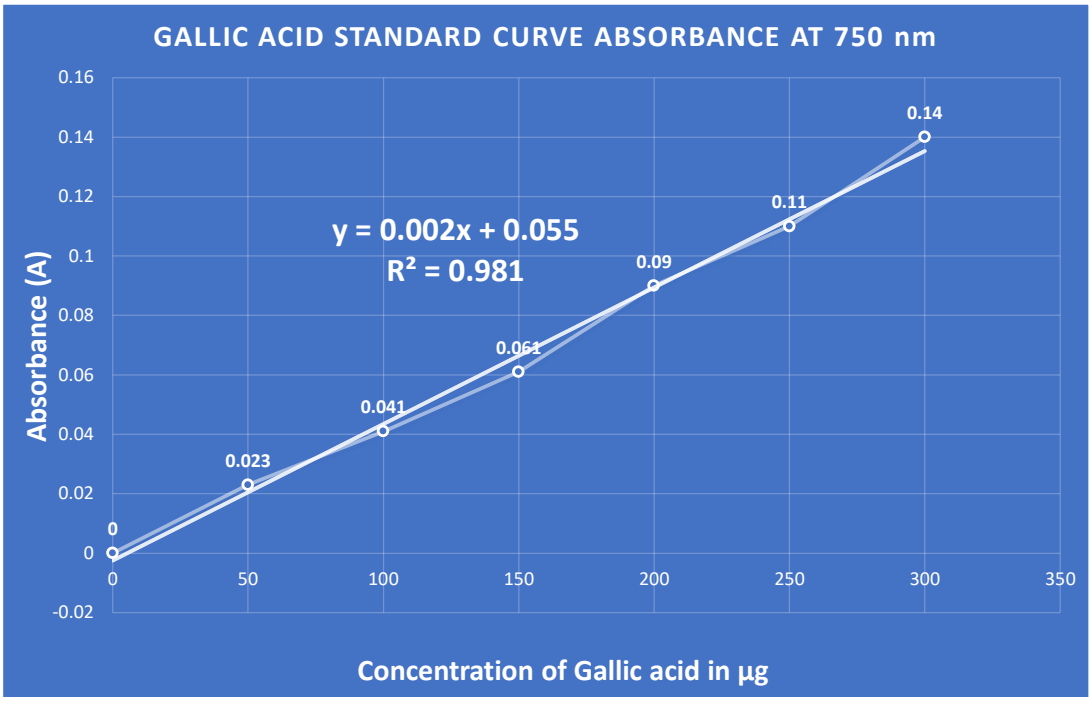


Figure 4.1 Calibration curve of gallic acid each point represents the mean (750)

Calculation of total phenolic content

$$Y = mx + c$$

$$Y= 0.002x + 0.055 \text{ and } R^2 = 0.9811$$

The absorbance showed by the unknown sample determine through UV-visible spectroscopy.

$$X=(y-c) /m$$

Initially 10 mg of extract was dissolve in 5mL of distilled water. So 2 mg of extract in each 1 mL of aqueous exact solution.

The polyphenol amount in mg/g of sample = $x/2 \times 1000$

$$= X \text{ mg/gram}$$

The total phenolic content of the different extract expressed as mg Gallic acid equivalent (GAE) per gram of dry weight sample (mg/gram).

The TPC calculated by using following formula:

$$TPC= c (v/m)$$

C= concentration of Gallic acid obtained from calibration curve in mg/mL

M= mass of plant extract in gram

V=volume used in mL

Table 4.4 Total phenolic contents of aqueous and methanolic extract of *C. arvensis*

Plant sample	Polyphenol (mg/g) (mg Gallic acid equivalent)
Aqueous extract leaves	180.4 ± 0.11
Methanolic extract of leaves	191± 0.10
Aqueous extract of stem	32± 0.09
Methanolic extract of stem	42± 0.09

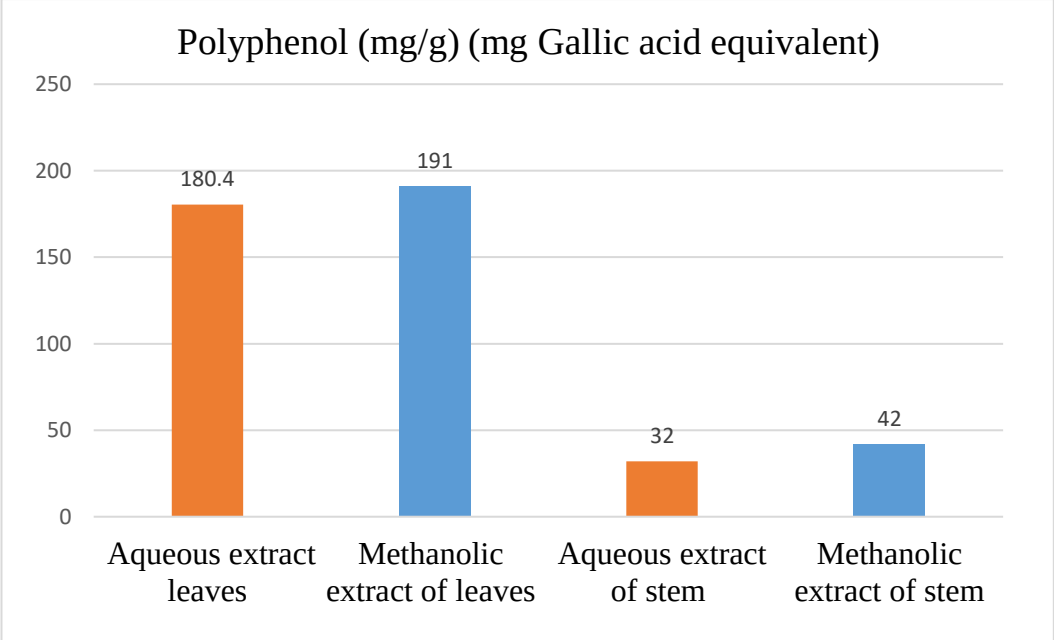


Figure 4.2 Graph showing Total phenolic contents of aqueous and methanolic extract of *C. arvensis*

The TPC calculation showed that in the methanolic extract of leaves contain higher amount of phenolic compound as compare to aqueous and other extract of plant which demonstrated that the leaves are good source of phenolic constituent that plays significant role in biological activities and exhibit higher antimicrobial proficiency (46).

Total phenolic were estimated by gallic acid (Figure 4.1) and expressed by way of mg gallic acid equivalent (GAE)/g of extract. Table 4 represents the analytical data for phenolic content of the methanolic and aqueous extract of *C. arvensis*. Data clearly showed a considerable quantity of total phenolic content present in the leaves methanolic extract.

4.1.2.2 TOTAL FLAVONOIDS CONTENT

The total flavonoid content in all sample was represent in mg of Quercetin equivalent (QE) per gram of dried plant sample

Table 4.5 Standard Quercetin solution absorbance for the determination of TFC in sample

Quercetin standard		
Test no.	Concentration	Absorbance (750nm)
1	0	0
2	50	0.023
3	100	0.041
4	150	0.061

5	200	0.09
6	250	0.11
7	300	0.14

Table 4.6 *C. arvensis* leaves and stem extract absorbance

Test no.	Plant sample	Absorbance
1	Aqueous extract leaves	0.111
2	Methanolic extract of leaves	0.189
3	Aqueous extract of stem	0.034
4	Methanolic extract of stem	0.054

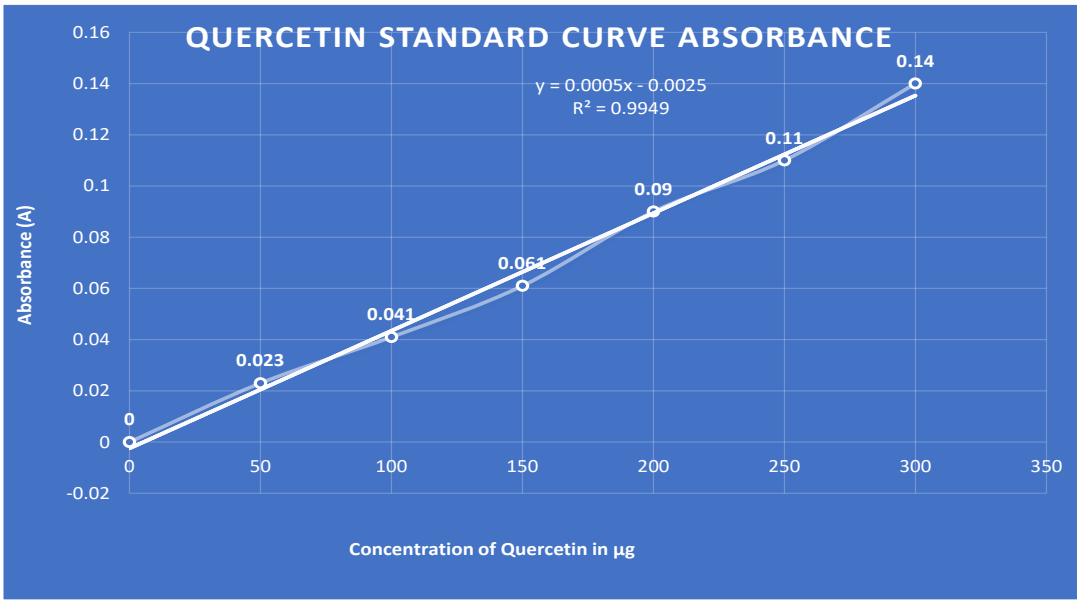


Figure 4.3 Calibration curve of Quercetin each point represents the mean

Calculation of total flavonoids content:

$$Y = mx + c$$

$$Y = 0.0005x + 0.0025 \text{ and } R^2 = 0.9949$$

The absorbance showed by the plant extract determine through UV-visible spectroscopy

$$X=(y-c)/m$$

The total Flavonoid content of the different extract expressed as mg Quercetin equivalent per gram of dry weight sample (mg/gram).

The TFC calculated by using following formula:

TFC= c (v/m)

Table 4.7 Total Flavonoid contents of aqueous and methanolic extract of *C. arvensis*

Plant sample	Flavonoid (mg/g) (mg Quercetin acid equivalent)
Aqueous extract leaves	168 ± 0.09
Methanolic extract of leaves	187± 0.01
Aqueous extract of stem	32± 0.08
Methanolic extract of stem	45± 0.01

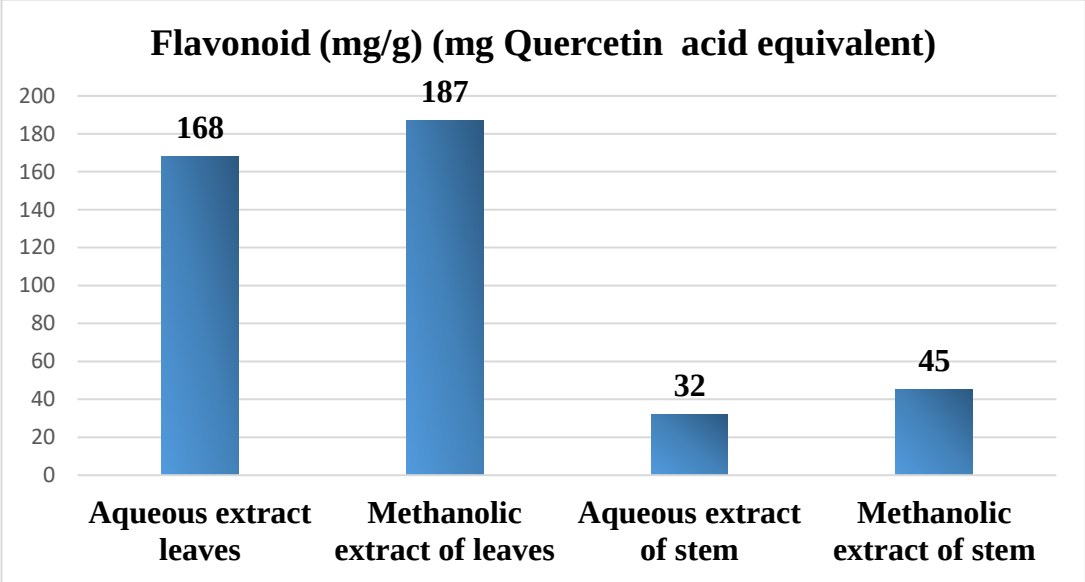


Figure 4.4 Graph showing Total Flavonoid contents of aqueous and methanolic extract of *C. arvensis*

The TFC calculation showed that in the methanolic extract of leaves contain higher amount of flavonoid compound as compare to aqueous and other extract of plant, which demonstrated that the leaves are good source of flavonoid constituent that plays significant role in pharmacological activities and exhibit higher antimicrobial proficiency (47).

4.2 ANTIOXIDANT ACTIVITY

With a temperature control set at 30 °C, the ABTS radical cation was mixed with 50% methanol to get an initial absorbance of around 0.71±0.01 at 745 nm. In a micro cuvette, 350 µL of the test sample and 3.0 ml of the ABTS working standard were combined to measure the free radical scavenging activity. After mixing the solution, the absorbance decreased by exactly one minute and then by up to six minutes.

The percentage inhibition was calculated according to the formula:

Scavenging effect (%) = [(Control absorbance – sample absorbance)] × 100

Table 4.8 Antioxidant Activity of C. *arvensis* Samples by DPPH and ABTS Assay Radical Scavenging Assay

Plant extracts/chemical	DPPH radical	ABTS radical (0-6 min)
Aqueous extract leaves	179±0.4	159±4
Methanolic extract of leaves	295±0.3	187.0±3
Aqueous extract of stem	42.0±1	19.0±0.3
Methanolic extract of stem	27.0±0.9	35.0±0.2

4.2.1 DPPH ASSAY

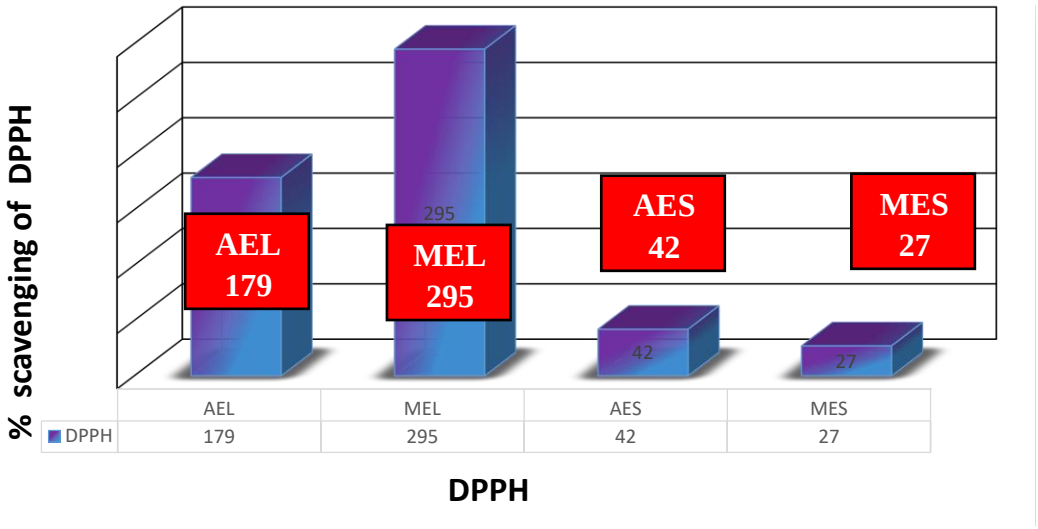


Figure 4.5 Graph showing the DPPH assay of different extract of plant sample

AEL= Aqueous extract of leaves

MEL= Methanolic extract of leaves

AES= aqueous extract of Stem

MES= methanolic extract of stem

Antioxidant activities evaluated by the DPPH radical capacity showed by methanol extracts of leaves is 295±0.3, which higher as compare to all other plant sample presented in Table 8.

4.2.2 ABTS ASSAY

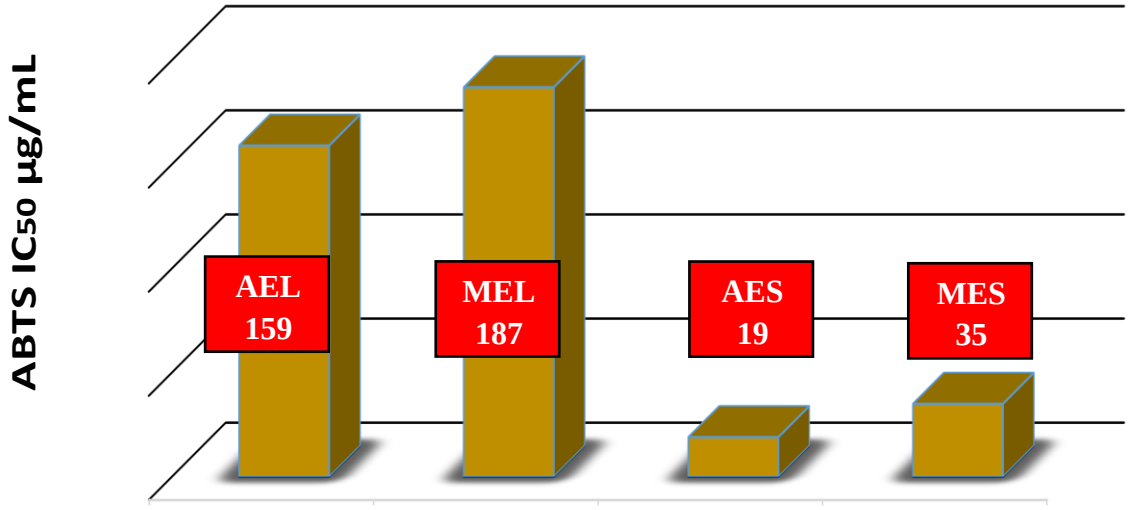


Figure 4.6 Graph showing the ABTS assay of different extract of plant sample

Antioxidant activities evaluated by the ABTS radical capacity showed by methanol extracts of leaves is 187 ±0.3, which higher as compare to all other plant sample presented in Table 8.

4.3 ANTI-INFLAMMATORY ACTIVITY

4.3.1 BOVINE SERUM ALBUMIN ASSAY (BSA)

The anti-inflammatory activities of the plant extracts were determined by using the modified version of the BSA assay.

The percentage inhibition of precipitation (protein denaturation) was determined on a percentage basis, relative to the negative control using the following equation:

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times ([Vt/Vc] - 1)$$

Where

Vt = absorbance of test sample,

Vc = absorbance of control.

Table 4.9 showing the anti-inflammatory activity of *C. arvensis* with egg albumin denaturation assay

Concentration (µg/mL)	Rate of inhibition (%)	
	Inhibition Leaves	Inhibition Stem
Control	-	-
20	74±0.01	65±0.41
40	76±0.41	77±0.39
60	79±0.39	69±0.41
80	82±0.41	89±0.38
100	98.2±0.39	94±0.42

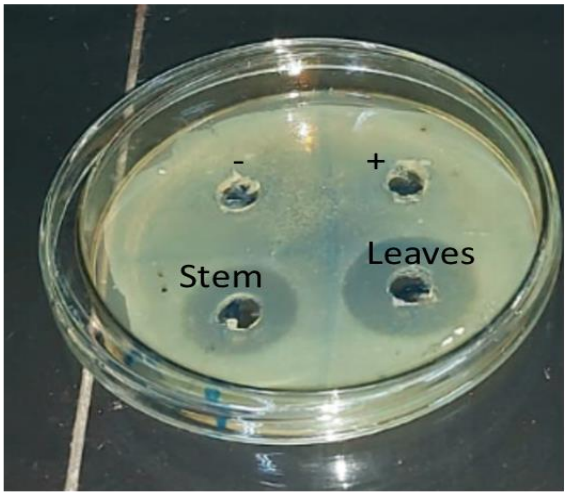
The plant extract induced significant ($P < 0.01$) anti-inflammatory effect. The anti-inflammatory effect of positive control (diclofenac) anti-inflammatory effect was 14.32%. Influence of standard drugs against protein denaturation.

Table 4.10 showing the anti-inflammatory activity of Rate of inhibition (%) diclofenac sodium

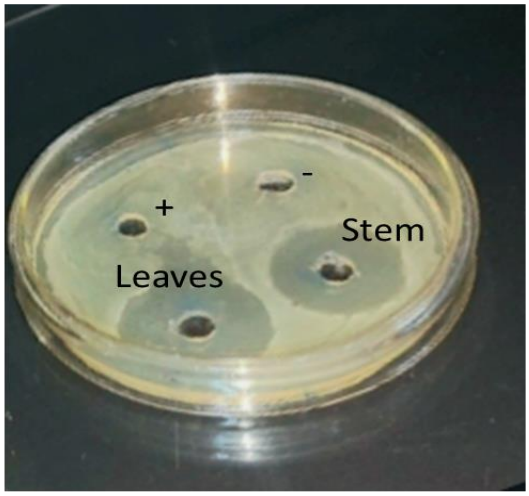
Concentration (µg/mL)	Rate of inhibition (%) diclofenac sodium
Control	-
20	11±0.22
40	20±0.41
60	29±0.39
80	36±0.22
100	42±0.38

4.4 ANTI-BACTERIAL ACTIVITY

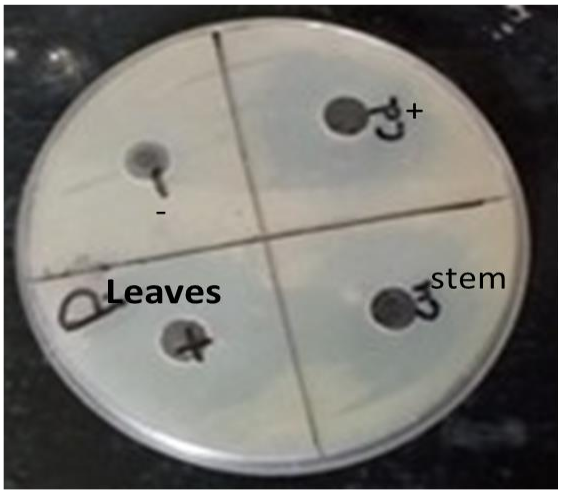
Using a well-diffusion approach, the inhibition zone was checked. Following a 24-hour incubation period, each bacterial strain's zone of inhibition was determined using a plant extract, and positive control. Efficacy zone of inhibition of plant extract against Gram-negative and Gram-positive is being investigated in this study.



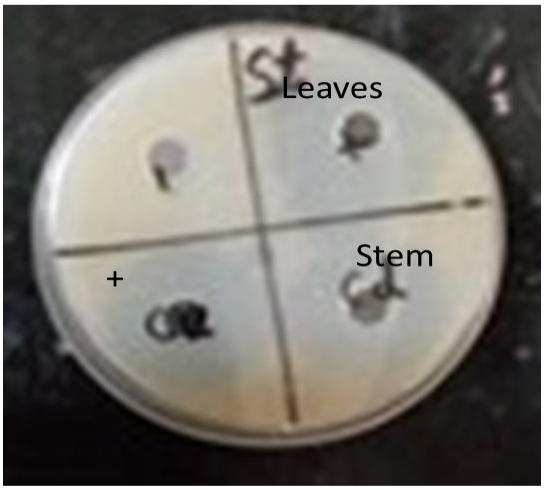
Bacillus cereus



Escherichia coli



Pseudomonas aeruginosa



Staphylococcus aureus

Figure 4.7 Zone of inhibition showed by the plant sample and standard against gram-negative and gram –positive bacterial strain

As showed in Figure (4.5) the zone of inhibition exhibited by leaves extract is higher than the other positive control which existed given in the Table.

Table 4.11 Zone of inhibition value showed by sample against bacterial strain

Name of Bacterium	Zone of Inhibition (ZOI) in (mm)		
	Leaves (5µL/mL)	Stem (5µL/mL)	Positive (1mg/mL)
Escherichia coli	23.5	18.2	11.1
Pseudomonas aeruginosa	20.2	17.4	5.2
Bacillus cereus	14.9	13.8	3.2
Staphylococcus aureus	17.2	15.1	3.1

The ZOI values shows, the leaves extract of plant showed to be remarkable bactericidal activity against various bacterial strains.

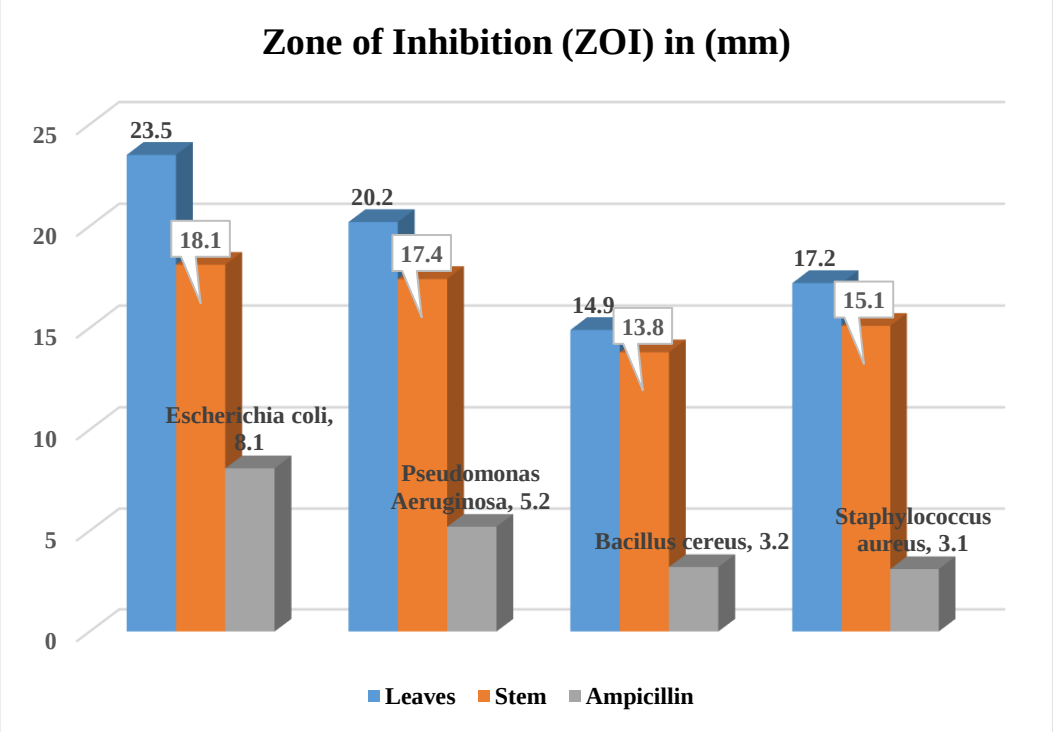


Figure 4.8 Zone of inhibition value showed by sample against bacterial strain

At same period, the plant leaves extract exhibited ZOI of 23.5, 20.2, 14.9 and 17.2 mm and the stem extract showed 18.2, 17.4, 13.8 and 15.1 mm against gram-negative *E.coli* and *P. aeruginosa* (gram-negative) and *B. cereus* besides *S. aureus* respectively. The maximum zone of inhibition exhibited by leaves extract against *E. coil* as paralleled to gram-positive bacterium. Thus, the leaves of *C. arvensis* might be the potential to treat of microbial infections.

CONCLUSIONS

The plant is rich in Phytochemicals like flavonoids, phenolic. Tannins, saponin, terpenoids, carbohydrate, protein, lipids, steroid.

The plant has high TPC and TFC. The leaves have highest TPC and TFC than stem.

The plant shows significant antioxidant, anti-inflammatory and antibacterial potentials

Furthermore, it revealed strong hope to development of additional novel chemotherapeutic mediators from plants, that in the future might be serve in production of the synthetically reworded therapeutic agents. Supplementary studies are suggested to explore different parts of plants significance which frequently occurring in nature.

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APPENDICES

APPENDIX- 1

1. L-BROTH (Kahn *et al.*, 1979)

Sr. No.	Ingredients	gL-1
1.	Tryptone	12
3.	NaCl	5

pH adjusted to 7.0.

APPENDIX- 2

2. NUTRIENT-AGAR (Gerhardt *et al.*, 1994)

Sr. No.	Ingredients	gL-1
1.	Peptone	5
2.	plant extract	3
3.	NaCl	3

pH adjusted to 7.0.

APPENDIX- 3

3. SCHAEFFER'S MEDIUM (Schaeffer *et al.*, 1965)

Sr. No.	Ingredients	gL-1
1.	plant extract	3
2.	Peptone	5
3.	NaCl	8
4.	Agar	12

pH adjusted to 7-7.2.

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