Comparative Nutraceutical Analysis of *Capparis decidua* Edgew (Forssk.) Fruits at Different Maturation Stages

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy in Biotechnology

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بسم الله الرحمن الرحيم

Certificate of Approval

This is to certify that the Department of Biotechnology, Faculty of Biological Sciences, Quaid-I-Azam University Islamabad, Pakistan accepts the dissertation entitled "**Comparative Nutraceutical Analysis of *Capparis decidua* Edgew (Forssk.) Fruits at Different Maturation Stages"** submitted by in its present form as satisfying the dissertation requirement for the Degree of Master of Philosophy in Biotechnology.

Supervisor: _____

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Dedication

I dedicate my dissertation work to my parents without whom none of my success would be possible. A special feeling of gratitude to my loving parents, whose words of encouragement continually provide their moral, spiritual, emotional, and financial support. I also dedicate this thesis to my honorable supervisor and teachers who have supported me in developing my personality as a competent professional.

Masoud Ur Rasheed

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Declaration

I Masoud Ur Rasheed, hereby solemnly declare that the work presented in this thesis entitled "**Comparative Nutraceutical Analysis of *Capparis decidua* Edgew (Forssk.) Fruits at Different Maturation Stages**." is original. Further, I declare that this work has not been submitted for any degree or diploma to any other university or institution. I am aware of the terms copyright, and plagiarism and I will be responsible for any copyright violation found in this work.

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Acknowledgment

In the name of ALLAH who is the most merciful and Almighty without the help of whom I was unable to complete my work. All respect to Holly Prophet (S-A-W-W) the most exalted among human beings ever born on the surface of the earth, who is forever guidance and knowledge for humanity.

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Masoud Ur Rasheed

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List of Abbreviations

SD	Standard deviation
DW	Dry weight
FC	Folin-Ciocalteu
FRSA	Free radical scavenging activity
FW	Fresh weight
GAE	Gallic acid equivalent
MIC	Minimum inhibitory concentration
ROS	Reactive oxygen species
RNS	Reactive Nitrogen species
TFC	Total flavonoid content
TPC	Total phenolic content
TAC	Total antioxidant capacity
DPPH	2, 2-diphenyl-1-picrylhydrazyl
TRP	Total reducing potential
ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
MC	Metal chelation
AAE	Ascorbic acid equivalent
EDTA	Ethylene diamine tetra acetic acid
DMSO	Dimethyl sulfoxide
IAA	Indole-3-acetic acid
GA	Gallic acid
IS	Initial stage
PS	Premature stage
MS	Mature stage
dH_2O	Distilled water

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Abstract

Medicinal plants are the hub of active molecules and demand detailed scrutiny of biologically active compounds. The identification and purification of active components can be achieved by establishing a link between the extract processing procedure and bio-chemical activities. The purpose of this study was to explore *Capparis decidua* as a medicinal plant with diverse pharmacological properties. A Comparative Bioanalysis of the *Capparis decidua* plant with its different stages of Fruits Leaves' Flowers' and seeds was done in this study. Each sample was weighed out into an Eppendorf tube at a ratio of 100 mg to 1 mL DMSO. The extracts were centrifuged at 10,000 g for 15 minutes, and the resultant supernatant was applied for further biological assays. To assess phytochemical potential, the quantity of total phenolic content was lower in bark, leaves, and flower extract 28.71±1.44, 30.44±1.52, 62.94±3.15 µg GAE/mg DW extract, while higher in seed, and premature fruit extract 96.44±4.82, 86.96±4.30 µg GAE/mg DW, respectively. All the fractions of leaves, flowers, fruits initial, premature, mature, seed, and bark showed the total number of flavonoid contents 25.09±1.25, 22.72±0.91, 39.51±1.98, 39.63±1.59, 24.93±1.25, 42.39±2.12, 22.41±1.12 µg QE/mg DW respectively. Flowers and bark had a total antioxidant capacity of 266±13.3, 137.57±6.88 µg AAE/mg DW, which was below average in all fractions, while seeds had a high total antioxidant capacity of 389.90±19.49 µg AAE/mg DW. The highest reduction power was obtained from seed 258.01±10.32 µg AAE/mg DW, also the lowest in bark, leaves, and flowers extract i.e., 69.65±4.18, 146.06±7.30, and 225.92±11.30 µg AAE/mg DW respectively. Our current study results showed that *C. decidua* has supreme metal chelation capacity. The seed and premature fruit extracts had the greatest metal chelation activity, 63.203.17% & 60.853.1%, while the flowers had the lowest, 40.421.1% respectively. All the evidence indicates that the seed extract and premature fruits have the highest cytotoxic potential. Moderate antibacterial activity was observed by seed extract against different bacterial strains *E. coli, K. pneumonia,* and *P. aeruginosa* with a maximum inhibition zone of 12 mm. The significant enzyme inhibitory activity was also shown by all fractions. Maximum enzyme inhibition activity was demonstrated by fruit and seed extract as in amylase inhibition i.e., 69.29±3.41%, 96.79±4.83%, Urease inhibition i.e., 96.07%±4.83, and 95%.83±4.91%, and in Lipase inhibition i.e., 81.30±4.06%, and 75.75±3.78% respectively. Bioassay-guided isolation acknowledged its great potential for identifying active constituents. The plant and its parts have shown promising therapeutic effects, making them a strong option for drug development.

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Chapter 1

Introduction and Review of Literature

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1 Introduction

In the subcontinent, plant-based medicines have been utilized for treating illnesses since ancient times. It is based on their traditional practices and is typically taken in the form of powders, liquids, or combinations. In the past 20 to 25 years, there has been an increased intensity in the use of medicinal plants as a complementary and alternative therapy (Kaur & Arora, 2009). Historically, the natural pharmacopeia of human populations has included a variety of wild plant species. The therapeutic chemicals extracted from plants are increasingly appreciated as raw materials for modern medications and herbal remedies in addition to their usage in traditional therapies (Sarkhel, 2014). Sudden population development, habitat loss and change in climate, overuse, overgrazing, and deforestation have all contributed to an increase in demand for plant base medicines (Savikin *et al*. 2013). Since ancient times, people have employed medicinal plants as part of traditional medicine because of their curative abilities. Some bioactive chemicals found in these plants are what give them their pharmacological worth (Sathish Kumar *et al.,* 2022). Many of these phytochemicals are secondary metabolites that the plant consistently produces for its defense. depending on their origin in biosynthesis Phytochemicals are classified into phenolics, alkaloids, flavonoids, steroids, terpenes, saponins, etc.

The *Capparis* species have been used as food sources and medicinal plants. The *Capparis decidua* belongs to the Capparidaceae family. The most well-known species of this family are *C. spinosa, C. decidua, C. zeylanica,* and *C. ovata*, whereas *C. sepiaria, C. tomentosa*, and *C. shumilis* are less well-known. Alkaloids, flavonoids, steroids, terpenoids, and tocopherols are wide variety of bioactive compounds found in caper fruits (Nabvi *et al.,* 2016). All components of this plant have a lot of medical uses in addition to numerous socioeconomic and ecological advantages (Zia- ul- Haq *et al*., 2011). Traditional uses for the plant include treating toothaches, arthritis, asthma, coughs, inflammation, malaria, rheumatism, and swelling. Additionally, astringent, laxative, and vermifuge qualities are thought to exist (Singh *et al*., 2011). It is claimed that fruit powder and root bark alcohol extracts have anthelmintic effects. The fruits and seeds contain diuretic, and anti-diabetic qualities and are used to treat cholera, diarrhea, and urinary purulent discharges. Fruits with a spicy flavor are used as a bowel astringent, and a breath freshener, and are claimed to treat heart problems (Singh *et al*.,2011). It is useful in treating facial paralysis, enlarged spleen issues, and intestinal worm infestation. It is prescribed for scurvy, heart disease, and phthisis. The plant is used as a healthy camel feed in Rajputana. Fresh fruit juice destroys worms, also Liver

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problems are treated with a root powder. The root bark extract is administered twice daily for three days to treat hemorrhoids. The fruit is cooked and eaten like a vegetable. When fruits are green not fully mature and used as pickles. Processed seed oil is used to treat skin allergies and wounds (Sharma *et al*.,2011).

The plant is too drought resistant due to a lack of leaves. *C. decidua* plant flowers and fruits are a reliable source of electrolytic minerals such as calcium, zinc, and iron. By the usage of that plants, we can overcome the shortage of minerals in our diet (Gull *et al*., 2015). These plants are present in the tropical and sub-tropical arid regions of Thar deserts. They are small trees with a height of 5-6 m and have mini small green leafless branches. At the immature stage bark of plants appears green in color that changes into whitish grey with time. Young shoots have very tiny (2 mm long) and short-lived leaves, so the plant primarily is leafless. Plant leaves appear between January and February while the flowering season is between March to April. Flowers are red and purple that are present along the leafless branches. The shape of the fruit is round like a cherry. Fruits are initially green in appearance, changing to a reddish-gray color as they matured (Rathee *et al*., 2010)

According to published data aerial parts may be able to inhibit bacterial growth and be effective against resistant strains of bacteria. The reports highlight the urgent need to explore the antimicrobial properties of various *Capparis decidua* plant parts on distinct stages, due to limited data available regarding the full pharmacological spectrum of the particular plant. The purpose of that particular study was to evaluate the enzyme inhibition antimicrobial, cytotoxic, total phenolic, and flavonoids, reducing the potential of distinct parts of the *C. decidua* plant. Parts of plants that are involved in research are leaves flowers, fruits, and seeds which are collected at different initial, premature, and fully mature stages. The purpose of the present study to use multipolarity solvent extractions such as DMSO to scientifically gauge the antioxidant, antibacterial, cytotoxic, and reducing potential of *C. decidua* plant.

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Research Aims

1- A comparative bioanalysis of the *Capparis decidua* plant's leaves, flowers, fruits, and seeds at various premature and mature stages.

2- Exploration of *Capparis decidua* as a Source of Phytochemicals having potential for Natural Remedy.

3- Utilization of multiple biochemical assays to determine a plant's potential for antioxidative, antibacterial, and antidiabetic as well as total phenolic and flavonoid contents.

4-To look into the fractions of a designated plant employing a solvent system covering a broad polarity range to achieve optimal extraction efficiency concerning the extracted quantity of medicinally important secondary metabolites.

Objectives

The basic objectives involved were:

- To evaluate the phytochemical, and biological potential using many efficient assays i.e., total phenolic (TPC) and flavonoid content (TFC), antioxidant (DPPH, ABTS, CUPRAC, FRAP, metal chelating, total antioxidant capacity (TAC) and reducing power capability (TRP), enzyme inhibition assay (alpha-amylase, lipase, urease), antimicrobial (antibacterial).

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1.1 Review of Literature

Natural remedies that come from an ethnobotanical perspective are effective pharmacological warriors against a range of severe illnesses. The best options among these natural sources are medicinal plants because of their extensive range of secondary metabolites that have therapeutic potential. Many of the medications used today were originally derived from plants (Ishtiaq *et al*., 2015). Plant components, including seeds, fruits, leaves, branches, and flowers, are a plentiful supply of chemicals that can be used in the process of developing new medicines (Jamshidi *et al*., 2018). According to science, plants with therapeutic value are referred to as "medicinal plants." The discovery of biologically active plant chemicals opened a new way for therapeutic industries (Vaishnav *et al*., 2017).

For centuries, many plants have been used in conventional medicine. The study of medicinal plants and their traditional uses in various regions of the subcontinent has attracted more attention in recent decades. According to the World Health Organization (WHO), the population living in underdeveloped countries depends primarily on plants for their health care. Traditionally, plants served as the source of all pharmaceutical preparations, The benefits of adopting plant-derived medications include that they are generally safer than synthetic substitutes, and have significant therapeutic benefits (Chishty *et al.,* 2017). Approximately two hundred years ago, medicinal plants dominated our pharmacopeia, and nearly 24% of all prescribed worldwide pharmaceutics were derived from plants. Many plant substances used in traditional medicine are widely available in rural areas and significantly cheaper than modern pharmacopeia (Shad *et al*., 2014).

Modern medicines are based on substances found in nature. Microorganisms like superbugs become resistant to synthetic antibiotics due to their misuse. Director-General Margaret Chan from WHO has warned that the growing resistance of bacteria to basic antibiotics could mean "the end of contemporary medicine as we know it". Due to its greater social acceptance, well compatibility with the human body, and fewer side effects, herbal therapy continues to be the chief method of treatment for 70–80% of the world's population, primarily in poor nations (Dipti *et al*., 2016). With a sales volume of approximately $ 4 billion in 1996 and an expected increase to double by the turn of the century, herbal medications are presently sold in health food stores in the United States. India's herbal pharmaceutical sector is thought to be worth $1 billion, and the nation exports medications made from plants worth $80 million (Newman et al., 2016). In the USA and Europe, the current market for nutraceuticals which includes herbal remedies is expected to reach between $85 and $260 billion (Gull et al., 2015). The

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basic unique features recommended by WHO are: Evaluation of product quality: raw plant substantial; plant formulation; and final product. (ii) Safety evaluation, including toxicology investigations and credentials of safety based on experience. (iii) Stability: Shelf life of item (iv) Evaluation of effectiveness: Proven traditional use or activity determination (animals, human).

1.2 Categorization of phytochemicals produced from the plant.

Primary metabolites play a fundamental role in plant growth and development, although secondary metabolites are essential to the defensive systems of the plant (Newman and Cragg, 2016). Biosynthesis, bioassay profiling, isolation, and structure elucidation are all phases involved in the development of pharmaceutics. the basic concern is to identify the purposes of therapeutic plants and their applications. There are over four lake secondary plant metabolites, and nearly one lake of these has been chemically extracted (Aslam and Ahmad, 2016). They do not relate to usual physiological functions and are primarily focused on metabolic processes. They could belong to different genera or be connected to a certain species (Rathee *et al*., 2015).

The primary variables that influence the generation and number of secondary metabolites are Physiological states, developmental stage, tissue involvement, and environmental situations. High throughput screening has required highlighting the extraordinary bio synthetic characteristics of plants that are not fully available yet. Employing their genomic information, about twenty percent of plant genes are connected to a specialized metabolism that prepares the way for the synthesis of much more secondary metabolites (Gupta *et al.,* 2010). Some secondary metabolites are species-specific and may be used as a way of species confirmation.

1.3 Types of Secondary metabolites

Later, as the cell transitions from an active growth phase to a stationary state, significant amounts of secondary metabolites are produced. In addition, secondary metabolites are assumed to play a significant role in cellular metabolism and are dependent on primary metabolism for the provision of essential elements (enzymes, substrates, energy, and cellular machinery) (Tiwari *et al*., 2011).

Phytochemicals (secondary metabolites) produced by plants are essential oils, flavonoids, terpenoids, steroids, tannins, glycosides, and alkaloids. These secondary metabolites are the reason, medicinal plants are therapeutically effective. The antispasmodic, diuretic, analgesic,

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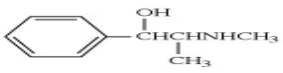
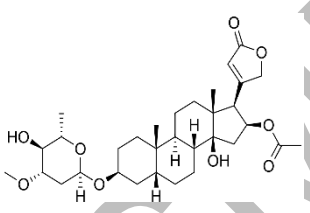
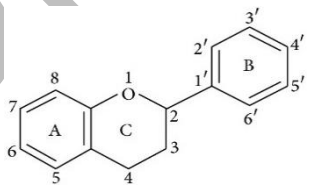
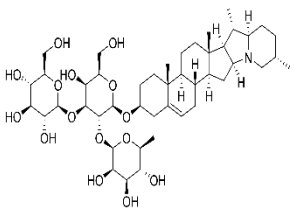
and antimalarial effects of alkaloids are well documented. (Mohammed et al., 2015) reported that 25k molecules were characterized as terpenoids, and they exhibit antiviral, antimicrobial, carcinogenic, antiparasitic, antimalarial, and anti-inflammatory properties. The antibacterial and antifungal properties of glycosides are addressed, and 21k have been identified so far. There are one million known phenols and flavonoids, which are well-known antioxidants with antibacterial and antiallergic characteristics. Saponins have antiviral, anti-inflammatory, and protection properties (Mishra *et al*., 2007).

Table 1.1: Secondary Metabolites are classified according to their chemical makeup.

Phytochemical Class	**General structure**	**Examples**	**Therapeutic Uses**
Alkaloid		Morphine, Quinine, strychnine nicotine, PVC, and ajmaline	Muscle relaxant, Antipyretic, anti-malarial, Analgesic
Glycosides		o-glucosides, s-glycosides, N-glycoside, c-glycoside, aglycon, cardiac-glycoside, aspirin, polygala, and sativoside	Cardiac diseases, poisons, Neisseria gonorrhea, viral infections, Antibacterial, anticancer,
Flavonoids		<u>Flavone;</u> Apigenin, luteolin, tangeritin, <u>Flavanol;</u> myricetin, fisetin, rhammazin <u>Flavanone;</u> rutin, naringenin,Hesperetin <u>Flavanonol;</u> taxifolin, erodictyl, luteolin.	Antiviral, antiplatelet, antioxidant, anticancer anti-inflammatory, Asthma, metabolic syndrome
Saponins		<u>Steroidic aglycon</u> spirostane, furostane, <u>Triterpene</u> aglycone oleanane, hopane,	Minimize cancer risk. Antidiabetic, Activate different cell cycle pathways.

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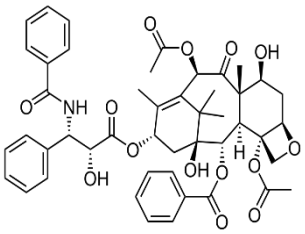
Terpenoids		Hemiterpenoids; prenol, isoprenol Monoterpenoid; citral, carvone Sesquiterpenoid; humulones, farnisol Diterpenoid; retinol, Triterpenoid; limonoids, sterols, carotenoids	Wound soothing, antimicrobial, malarial treatment, blood circulation, Antiinflammation, Antiviral, cancer drugs preparation.

Many researchers are studying various plant sources for the creation of natural medicines capable of countering the negative effects of synthetic drugs considering the factors and causes mentioned above. Because they have few side effects and are inexpensive, herbal medicines are becoming increasingly popular across the country. Particularly with the use of herbal medications, many severe types of disease-like issues have been resolved.

1.4 Importance of pharmaceutical plants

The ability of therapeutic plants to treat a variety of ailments makes them a role models for life. Drugs in general are risky in one way or another, but in this situation, plants are secure and economical (Shaw *et al*., 2013). Because plants are a powerful source for the discovery of new medicinal chemistry, most medications are plant-based. Folkloric uses for herbal medicines have been well-documented and serve as important linkages in the search for bioactive compounds. Several herbal remedies were not as significant until bioactive chemicals were discovered, for example, taxol from *T. breviolia* was known to be used as medicine in Asia before its isolation and identification as an anticancer agent (Rai et al., 1987). Just six percent of plant species have been investigated pharmacologically, while fifteen percent have been investigated phytochemically (Cragg and Newman, 2013). According to IUCN reports, there are over 1.5 million plant species that are not even well evaluated pharmaceutically. The ratio of threatened species is raising an urgent need for the creation of new medications utilizing plants due to global warming and environmental causes (Kunwar *et al*., 2022). There is a structural difference between synthetic and natural products due to higher chain lengths, fewer rings, less oxygen, more nitrogen, fewer chiral centers, low

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molecular weight, and more flexibility. These characteristics reduce their level of specificity. Natural products exhibit higher binding affinities, an incredible diversity of chemical compounds, and unique ADME/T (Absorption, distribution, metabolism, excretion, and toxicity) properties (Atanasov *et al*., 2013).

1.5 *Capparis decidua* plant

The *Capparaceae* family member *Capparis decidua* is a significant medicinal herb found in arid and dry areas of the subcontinent, Africa, and Saudi Arabia deserts and arid areas are where plants primarily grow. There are hundreds of common vernacular names for it; some of the most popular included *Kari, Delha, Caper, Kair, Karyal, Hanbag, Karil, and Kabra*. The significance of this plant in conventional medicine is acknowledged by all medical systems. (Chopra *et al*., 2006)

Distinct parts of *C. decidua* are well known for the antibacterial, antifungal, antihemolytic, antioxidant, antidiabetic, anthelmintic, anesthetic, antirheumatic, anti-arthritic, insecticidal, termiticidal, antiviral, antiplatelet, lipid-lowering, anti-aging, anti-atherosclerotic, anti-inflammatory, analgesic, and nociceptive diseases (Mann *et al*., 2013). Diets based on the plant are a rich source of secondary metabolites which have a good impact on human health and help to prevent diseases (Russel and Duthie., 2011)

In addition to its therapeutic value, *C. decidua* is considered a valuable supplier of essential minerals that enhance the nutritional value of the plant. Plants contain significant amounts of calcium and potassium, which are valuable for human and animal nutrition. A possible source of important electrolyte minerals is flowers and fruits. The plant contains substantial amounts of necessary minerals, especially Fe and Zn, supporting the potential for using it to treat mineral deficiencies in the human diet (Mann *et al*., 2013).

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Table 1.2: Profile of *Capparis decidua* plant

Vernacular Name		**Description of plant**	
English	Caper, caper bush, caper berry	Life form	They are small trees with a height of 5-6 m and have mini small green leafless branches
Hindi	Karira, kair , karo , kurrel, Delha.	Stems	Spreading green branches with crooked stipular spins that are thin smooth and spinous. Most branches are green in color but as they become older than pale grey barks emerge
Urdu	Kurry, karita , kair, karil	Flowers	Pink or red, infrequently yellow, pink, red-veined narrow oblong petals, 12mm length gynophore, androecium has eight stamens present near the base of the gynophore. Flowers mostly come on old branches
Arabic	Margh , Janbok , Tandip , sodad	Fruits	The shape of the fruit is round and globular like a cherry. Fruits are initially green in appearance, changing to a reddish-gray color as they mature and blackish on drying
Binomial Name	*Capparis decidua*	Flowering and fruit period	Plant leaves appear between January and February while the flowering season is between March to April and ripped in May and October.
Classification		**Topographical Distribution**	
Domain	*Eukaryota*	Pakistan	widely dispersed in Pakistan, from the arid region of Punjab to Sindh, Baluchistan, and north Khyber Pakhtunkhwa. Including Thal and Thar deserts Punjab and Sindh
Kingdom	*Plantae*	India	Plants are present in the western states of

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			Rajasthan, Gujarat, Rajputana, and Punjab.
Subkingdom	*Viridaeplantae*	South Africa	Tropical Africa, Tibesti, Sudan
Phylum	*Tracheophyta*	Western Asia	Arabia, Yemen, Egypt and Forsskhal
Subphylum.	*Euphyllophytina*		
Class.	*Magnoliposida*		
Subclass.	*Dilleniidae.*		
Orders	*Capperales.*		
Family	*Capparaceae*		
Genus	*Capparis*		
Species	*Decidua*		

1.6 Phyto chemistry

Biochemicals present in *C. decidua*, are alkaloids, phenols, sterols and glycosides, and fatty acids. According to the published data, the root bark of plants usually contains two sitosterols, codon carpine, stachydrine one diterpine alcohol, and one diterpine ester. It also has spermidine and spermines that play a significant role in the proliferation expansion and growth of mammalian cells. Due to their ability to promote cell proliferation, these polyamines also promote healthy hair growth (Rathee *et al.*, 2010). The arial part of *C.plant* has two acyclic terpenoids, two lupine terpenoids, two sterols, four fatty acids methyl isothiocyanate, glucapparinand and a shikimate derivative which prevent cancer cell proliferation (Fujisawa and Kadoma, 2005).

The flower of the *C. decidua* plant comprises nonacosane, triacontane, ascorbic acid (1195 mg kg-1), phytic acid (681 mg kg-1), oxalic acid (1.5 mg kg-1), Glucocapparin, Glucocappasalin, and phthalic acid. The phenolic components of leaves included 2-hydroxy-6-methoxy benzoic acid, sinapic acid, salicylic acid, caparison $C_{26}H_{31}N_3O_5$, protocatechuic acid, syringic acid, vanillic acid, hydroxybenzoic acid. All phytochemical compounds perform a vital role in the development of herbal medicines (Nazar *et al*., 2020)

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Table 1.3: Phytochemicals extracted from distinct parts of the *C. decidua* plant

Part of the plant selected	Bio chemicals	Fatty Acids	Concentration (%)
Shoot	Terpenoids	Hexa decanoic acid	20.5%
Root	Alkaloids	Octadecanoic acid	7.7%
Seed (berry)	Stachydrine, Capparisin, carotene	Tetra decanoic acid	0.9%
Flower	Hydrocarbons, glucocapparin	Oxalic Acid	11.6%
Fruit	Stachydrine	Oleic acid	58%

1.7 Medicinal and Nutritional value

Like many other plants, *C. decidua* has the potential to be both nutritive and therapeutic due to its phytochemicals such as glucosinolates, phenolics, polyamine alkaloids, and flavonoid constituents. Since the classical era, Capparis species have been utilized, in the Unani and Ayurvedic systems of medicine (Verma *et al*., 2010, Gupta *et al*., 2010). Stem and leaves are used to treat biliousness, while Fruits can treat rheumatism digestive problems, intestinal worms, and intermittent fever. Fruit and peel extracts have been used to treat asthma, coughs, stomachaches, ulcers, and other diseases. Additionally, the pulp of the fruit has demonstrated potential in the treatment of cardiac issues, phthisis, spleen enlargement, and scurvy. In ancient times, root paste was used to cure a scorpion bite and a broken bone (Meena and Yadav, 2010)

Most diseases affecting people in undeveloped and underdeveloped countries are due to malnutrition. According to literature in Pakistan, 39 percent of women and children suffer from malnutrition (Gull *et al*., 2015). Since ancient times, residents of dry and semi-arid areas have used caper berry pickles as a nutritious source of polypeptide, biological compound sugars, and vitamins. The branches are used as foodstuff for cattle due to their high nutritive value. A significant sign of an edible plant is the presence of minerals, fiber, carbohydrates, and proteins (Younus *et al*., 2016).

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Table 1.4: Nutritive components present in *C. decidua* plant.

Constituents	Nutritional value	Constituents	Nutritional value
Overall carbohydrates	74%	Iron	3.6mg/100g
Eatable carbohydrate	58%	Cellulose	8.99%
Fiber	31%	Fats	7.465
Protein content	15.87%	Carotene	4%
Oxalic acid	121mg/100g	Proline	12.76mg/100g
Na+	161mg/100g	Calcium	89mg/100g
Mg2+	50mg/100g	Cu2+	99.99mg/100g
Zn2+	1.8mg/100g	P3-	178mg/100g

1.8 Biofuel Production

Biodiesel is preferable to Petro diesel and can be used in its place. It offers several benefits and a crucial need for future. Biodiesel is an ecofriendly fuel that has roughly 10% built-in oxygen, no aromatics, and nearly no Sulphur (Foidl and Eder, 1997). Bio diesel is triglyceride fatty acid or methyl ethyl ester. An in-situ transesterification reaction can be used to produce biodiesel. This method makes it simple to transform triglycerides into long-chain fatty acid monoalkyl esters (Vivek and Gupta, 2004).

About 66% of the oil in the seeds of *Capparis decidua* is not edible. The *Capparis decidua* seed can release fatty acid methyl via in-situ transesterification. The biodiesel produced is inexpensive and has a low-slung viscosity. These plants' fuel can be utilized as a replacement in engines without any modifications (Sangamner, 2008)

Table 1.5. Comparison of biodiesel fatty acids content with American society for testing and material (ASTM) guidelines

Accomplishment Features	FAME) B.D. ASTM	*Capparis decidua*
KOH/gm	0.81	0.43-0.61
Sulfur	0.06	0.032
Phosphorus	0.001	0.002
Glycerin	0.02	0
Viscosity	1.8-5.9mm2/s	1.01

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1.9 Pharmacological activities of the phytochemical present in *Capparis decidua*

Antibacterial activity

Capparis decidua extract demonstrates distinct antibacterial properties by the presence of metabolites isoflavones, gamma thionin, and homo isoflavonoids carotenoids. Due to its high Sulphur content, it shows potential antibacterial activity. It has been noticed that plant portions like root leaves and flowers are efficient against distinct kinds of bacterial species (*Bacillus subtilis, helicobacter pylori, E. coli, and vibrio cholera*). The aglycon compound isothiocyanate found in plant seeds suppressed the activity of gram-negative bacteria such as *Vibrio cholera, Vibrio Inaba, Vibrioinaba,* and *Vibrio elector* (Rajshree Dahiya et al., 2017).

Antifungal activity

The wood, bark, and seeds of *C. decidua* showed strong antifungal action against *Aspergillus Niger, Aspergillus flavus, Candida albicans, and Fusarium moniliform*. The wood showed less activity while the green-whitish bark showed the most. Plant extract inhibitory zones were observed to range from 16.99 to 22.5 mm. A bear fruit extract has been claimed to have anti-tuberculosis properties (Jameel *et al*., 2018).

Antioxidant Activity

Exogenous antioxidants and phytoextracts of plant work as anti-aging mediators because they can be capturing ROS free radicals. In comparison to other plants, *Capper* is plentiful in biological active constituents like isothiocyanate glucoside, glucocapparin, stachydrine, and -sitosterol. isoginkgetin and ginkgetin, two antioxidants found in fruit extract, are responsible for scavenging activity. Fruit powder mixture condenses the negative effects of free radical's stress in alloxan-induced lipid peroxidation with abrupt changes in superoxide dismutase and catalase enzymes in erythrocytes, liver, and kidneys (Singh *et al*., 2011).

Anti-Cancer activity

Many cancer-fighting medicines are particularly costly, mutagenic, carcinogenic, and teratogenic. There is a need to develop substitute medications that are low-cost, safe, and highly effective. Literature reports that the pure chemical stachydrin may destroy tumor-forming cells and drastically lower chemokine receptor expression (Rahman *et al*., 2022). It predicts that stachydrine will be investigated as a possible anticancer drug, due to its anti-invasive and anti-anxiolytic properties. Leaves of the *Capparis decidua* plant are a rich

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source of stachydrine which play a potential role in cancer treatment. Lectin, especially blocks HIV-1 reverse transcriptase significantly, is found in the seeds of the plant and inhibits the growth of HepG2 and MCF-7 cells (Godara *et al.,* 2015).

Antidiabetic activity

C. decidua may be useful in reducing oxidative stress in diabetes and used as an anti-diabetic medication. Due to the hypoglycemic properties of leave extract, it reduces lipid peroxidation and changes free radical scavenging enzymes like catalase, amylase, and superoxide dismutase in the erythrocytes, liver, kidney, and heart. *C. decidua* has a sufficient concentration of -amylase and -glucosidase inhibitors like alloxan. According to the literature by using glucose tolerance tests in diabetic and healthy rats, the effects of methanol (295mg/kg) and pure (35mg/kg) stem extracts on blood glucose levels were investigated. The plant extract is helpful in the cure of diabetes (Shamim *et al*., 2022).

Antiplatelet activity

Platelets played a crucial role to maintain thrombotic processes in balance. Platelet abnormalities led to the development of vascular system diseases. The sesquiterpene lactones germacr-3-ol-7,9-dien-6,14-olide-15-oic acid and germacr-3-ol-12-ene-6,14-olide-15-oic acid, which was found in the methanolic extract of aerial portions of *C. decidua*, reduced arachidonic acid-induced platelet aggregation in a dose-dependent manner. These sesquiterpene lactones exhibit significant antiplatelet action, opening the door to the creation of other potent correspondents (Kunwar *et al*., 2022).

1.10 Justification for using plants in medical research?

Long before the ancient period, people employed plants for therapeutic purposes. There is proof that Indian vaids and Unani Hakeem have been using plants as medicine for more than 4,000 years. Traditional medicine is still widely used for many reasons, including the size of the population, an inadequate supply of medications, the cost and harmful effects of many chemically synthesized medications, and the emergence of drug resistance to presently prescribed medications for infectious illnesses. These factors have directed an increase in the demand for medicinal plants (Abayomi Sofowora *et al*., 2013). According to the WHO, 78% or 3 quarters of the biosphere depends mostly on plants and plant extracts for their medical requirements. Plant-based medications are thought to make up 25% of all drugs consumed in sophisticated nations such as the United States. The contribution can reach 87% in quickly developing nations like China and the subcontinent (Evan, 2008).

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The major benefit is that these drugs work in harmony with nature. The key factor is that herbal treatments can be used by people of all ages and genders. (Heinrich *et al*., 2010). According to old-fashioned scholars, a lot of health-related issues and diseases were solely treated by herbs. They performed research into the topic and conducted experiments to draw accurate conclusions regarding the efficacies of various herbs with medicinal worth. Many of these medications have no negative side effects or responses. This explains why herbal treatments are becoming more well-liked all around the world (Dahiya *et al*., 2019).

Even though our way of life becomes more technically advanced, we are turning away from nature. Although, since we are a part of nature, we cannot run away from it. However, as their delusional reliance on synthetic drugs has ended, people are coming back to natural products with the prospect of safety and security. It is now time to market internationally (Lordan *et al*.,2021)

1.11 Phytochemical Assays

To evaluate pharmaceutical tendency, the biochemical characterization of plants is crucial to identifying the various chemical constituent groups. These phytoconstituents are thought to offer antimicrobial and pest-preventative properties while not being considered nutrients (Doughari *et al*., 2009)

Total Phenolic Content

The most prevalent and diverse class of secondary metabolites present in plants are phenols. Phenolic acids, flavonoids, and polyphenols are among the important ones. According to Walton et al., phenols are made up of an aromatic hydrocarbon and a hydroxyl group (-OH) with a carboxylic acid attached. They are helpful to humans and essential for defense mechanisms (David *et al*., 2015). They are valuable to humans as well as being an essential part of defense mechanisms. Antioxidant qualities make them preventive agents against diseases brought on by free radicals. They fall under the classes of polyphenolics, flavonoids, and phenolic acids. Caffeic and ferulic acids are the two polyphenols that are most prevalent (Dai and Mumper, 2010). Phenolics are naturally occurring antioxidants and nutraceuticals that are contained in foods like apples, green tea, and red wine and are used to treat diseases like cancer. As phenolic acids, flavonoids, quinone benzene, and diols, they are also categorized according to the number of phenolic rings in their main structure. The Folin-Ciocalteu reagent is typically used to measure the polyphenol content (Anjum *et al*., 2020).

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Total Flavonoids contents

Low molecular weight phenolic compounds belong to the subgroup of flavonoids. Flavonoids can be found in apples, grapes, blackberries, chard, flowers, and leaflets of various plants. They have a broad range of biological properties that are widely known, including anti-inflammatory, anti-microbial, anti-allergic, and anti-cancerous (Harborne and Williams, 2000). These are a huge group of benzene-containing phenolic compounds that have more than one ring. Flavans are compounds that are the source of flavonoids. More than four thousand flavonoids are present in both higher and lower plants (Kumar and Pandy 2013). There are two diverse types of flavonoids, which depends upon the orientation of the benzenoid group: flavones (at the second position) and isoflavones (at the third position) (Pretorius, 2003). They can also be classified as iso flavonoids, flavones, flavanols, catechins, flavanones, and anthocyanins based on the degree of oxidation and ring replacement (Andersen and Markham., 2007). The location of the hydroxyl group is related to the antioxidant and free radical-scavenging properties of flavonoids (Doughari *et al*., 2012).

1.12 Antioxidant Assays

Free radicals are highly unstable molecules that frequently interact with other molecules to stabilize themselves. Reactive oxygen species including superoxide, peroxy, and hydroxyl radicals are produced as a result of metabolic activities in living organisms (Fernandes de Oliveria *et al*., 2012). Even though they are crucial for cell signaling, a rise in ROS and oxygen metabolism results in massive biomolecular oxidation, which converts normal cells into abnormal cells that promotes diseases including diabetes, lymphoma, and aging (Atanassova *et al*., 2011). Antioxidants play a crucial role in the process of defense against oxidative damage and are essential in inhibiting lipid peroxidation (Valko *et al*., 2004). Human bodies have their defense mechanisms that manage oxidative stress, but over time, these defense mechanisms become much less effective. To cure serious human diseases, there is a great demand for plant-based antioxidants (Mandal *et al*., 2010). According to an approximated study, human cells are exposed to about 1 million oxidative attacks (free radicals, reactive oxygen species) in a day. Antioxidants such as polyphenols, carotenoids, tocopherol, chlorophyll, and isoprenoids are naturally present in plant species which are safer for human consumption. The use of phytomedicines with strong antioxidant activity is a fantastic way to stop and treat diseases initiated by ROS (Sharma and Bhat, 2009).

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DPPH Assay

DPPH Having the molecular formula C18H12N5O6,2,2-diphenyl-1-picrylhydrazyl is a persistent cell permeable scavenger of free radicals. It is employed to calculate the antioxidant capacity of varied materials (Sharma and Bhat, 2009). The approach relies on the theory that the occurrence of antioxidant molecules in the test sample scavenges the DPPH reagent, which results from a shift in color from purple to yellow. The reagent is decreased by either absorbing or giving up an electron or hydrogen ion. The calorimetric analysis is used to detect color changes (Mishra *et al*., 2012).

In request to calculate the antioxidant capacity of samples, the redox potential of compounds is also employed. Due to the color transition from yellow to green, sample species reduce potassium ferricyanide to potassium ferrocyanide. This feature of the sample is due to the donation of a hydrogen atom to the free radical, which stops the free radical chain reaction or prevents the creation of peroxide. At a specific wavelength, a spectrophotometer is used to measure the changes (Javanmardi *et al*., 2003).

ABTS

The TEAC/ABTS (Trolox equivalent antioxidant capacity/2,2′-and-bis (3ethylbenzothiazoline-6-sulfonic acid) assay also serves to determine the antioxidant property of examined substances. The ability to inhibit ABTS formation by assessing early oxidation or by directly interacting with ABTS is used to determine the antioxidant capability (Schaich *et al*., 2015). Hydroxyl radicals are created when a sample is oxidized by ABTS to its vibrant free radical state. In essence, the green color would be lost when ABTS reacts with antioxidants (Mishra *et al*., 2012).

1.13 Enzyme Inhibition Assays

Alpha Amylase Assay

Hyperglycemia, a chronic metabolic disease linked to diabetes mellitus, is carried by either decreased insulin in the body or a sensitive cell reaction to already generated insulin. According to the data, fourteen crore people had diabetes in 2004, and that number is expected to rise to thirty crores by 2025 (Nair *et al*., 2013). Hyperglycemia can be cured by inhibiting two important enzymes (alpha-amylase and alpha-glucosidase), The primary function of alpha-amylase is the breakdown of carbohydrates. Its suppression is thought to be important for managing diabetes, delaying the release of glucose, and managing its rate so that patients with hyperglycemia can have stable serum glucose levels. Starch blocker is another name for the enzyme (Dinesh Kumar *et al*., 2010).

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Lipase

Identification of inhibitors of Lipase enzyme is necessary due to the prevalence of Pancreatitis diseases in patients of developed and emerging nations (Fats digesting enzymes) (Nickavar and Yousefian, 2011; Wickramaratne *et al*., 2016). The breakdown of fat and the absorption of fat-soluble vitamins are both carried out by lipases found in pancreatic secretions. For the pathophysiology of fat necrosis and acute and chronic pancreatitis, understanding the lipase function is essential. In some cholesterol-lowering drugs' mechanisms, lipases also play a crucial part (Simões *et al*., 2009). Non-enzymatic fat necrosis occurs during traumatic events, such as physical injury to breast tissue. Saponification of peripancreatic fat takes place in acute pancreatitis (Aloysius *et al.,* 2018). This results from injury to fat cells, which releases lipase, breaks down triglycerides, and releases fatty acids. (Chen *et al*., 2004). Orlistat is a weight loss medicine that works by inhibiting lipase. This medicine specifically inhibits pancreatic and gastric lipases. Lipase inhibition results in less digestion and absorption of dietary lipids. This can result in negative effects owing to decreased fat absorption, such as decreased absorption of fat-soluble vitamins. Abdominal pain, frequent bowel motions. Fibrates are generally used in clinical practice to decrease triglycerides. Fibrate side effects include cholesterol, gallstones, and rhabdomyolysis (Pamuk *et al*., 2018). There is a need to find natural inhibitors that do not have any negative effects.

1.14 Antimicrobial Assays

Pathogenic microorganisms are responsible for a variety of disorders (bacteria, viruses, a few algae, and fungi). They have always been initially treated with traditional therapies before being switched to antibiotics, antifungals, etc. It is necessary to isolate innovative therapeutic agents from medicinal plants due to the prevailing antibiotic and antifungal resistance generated by these bacteria' acclimation to the modern medication line (Hawkey, 2008). According to ethnos medicinal statistics, near about forty to eighty percent of antimicrobial agents are found in medicinal plants (Sivakumar *et al*., 2009).

Antibacterial Assay

Due to the prevalence of resistant strains of bacteria, and the increase in antibiotic resistance, the search for new therapies is necessary today. *E. coli* bacterium that occurs naturally, can also lead to gastroenteritis, stomachaches, and food poisoning. strains are a serious threat nowadays because their genetic makeup is very simple, alterable, and swiftly adapts to external stimuli (Chikere, 2008). Even Staphylococcus aureus has been linked to human

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subject dermatitis. A comprehensive examination of alternatives, such as extracts, decoctions, fractions, or even the discovery or isolation of active substances, is necessary due to the growing number of bacterial strains producing major health problems (Hon *et al*., 2005). Even though the area of medical sciences has made significant advancements, infectious microbes continue to pose a serious threat to the world's population.

Due to illogical prescriptions and patient noncompliance, the number of infectious diseases and mortality brought by microorganisms is rising every year. The experiments used to investigate new antimicrobials should be simple, reliable, and affordable (Valgas *et al*., 2007). The antibacterial susceptibility of plant extracts against diverse bacterial and fungal strains has been assessed in the present study using the agar disc diffusion method. In this technique, paper discs loaded with samples are placed over sterile plates filled with agar that have been swabbed with an inoculum of the desired strain. The minimum inhibitory concentration method is used to examine samples that have activity against a particular strain of microbes. The minimum inhibitory concentration of an antimicrobial agent is the lowest concentration at which the growth of visible microorganisms is inhibited (Nasir *et al*., 2015).

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Chapter 2

Materials and Methodology

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2 Materials and Methodology

2.1.1 Chemicals and Reagents

Solvents: n-Hexane, Chloroform, Acetone, Ethyl acetate, Methanol, Ethanol, and DMSO were applied in the research procedure and acquired from Sigma Aldrich Germany. Distilled water freshly prepared was also used; Acarbose was purchased from Sigma Aldrich USA. HCl, iodine, KI and surfactin, $AlCl_3$, ammonium molybdate, ascorbic acid, doxorubicin and $FeCl_3$, Gallic acid, monosodium dihydrogen phosphate, nutrient Agar, potassium acetate, potassium ferricyanide, Quercetin, sea salt, standard antibacterial drugs, Tetracycline, Sulfuric acid were purchased from Sigma (Sigma Aldrich Germany). Folin-ciocalteu reagent (FC) and Phosphate buffer saline were purchased from (Riedel de Haen Germany). Alpha amylase, Lipase, and Urease enzymes were purchased from (Unichem Laboratories India). Commercial. Bacterial strains involved both gram-positive and gram-negative strains. Gram positive bacteria strains *Staphylococcus aureus (ATCC-4617)* and *Bacillus subtilis (ATCC-6633)*. Gram negative bacterial strains, *Klebsiella pneumonia (ATCC-4617), E. coli (ATCC-25922)* and *Pseudomonas aeruginosa (ATCC- 15442).* Stock material culture was maintained at 4℃ while test bacterial strains are inoculated, cultured, and maintained on nutrient Agar slants and their incubation was done at 37-℃.

2.1.2 Apparatus and Equipment

Beakers, Flasks, Funnels, Petri plates, mortar, and pestle, sonicator, Tripod stand, Whatman filter paper, Bio compartment perforated tray, micropipette (Sartorius France), centrifuge, Freezer, Incubator, Microplate reader ELX800 (Biotech, USA), Rotary Evaporator, 96 well plates (SPL life science, Korea), weighing balance, Ph meter, Autoclave, Biosafety cabinet. Vortex tool and vernier caliper.

2.1.3 Collection of Plant material

Capparis decidua is also known as "Karir" or "Dela," is a member of the *Capparidaceae* family and was collected from the Desert of Muzaffargarh, Punjab. The fruits, flowers, and leaves were collected at various times and duration. the leaves were collected on 15th March 2022, and the fresh flowers and fruits at different mature and premature stages were collected in April and June 2022.

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2.1.4 Drying and Extraction

The collected plant parts (leaves, flowers, fruits, and seeds) were carefully washed with distilled water to remove dust and other impurities. The fresh weight (wet weight) of the flowers and fruits was 1 kg each. The plant sections were separated, chopped into small pieces, and dried for up to four weeks in a shady environment. After complete drying, the plant parts were then ground using mortar and pestle. The grounded material was then employed for the solution preparation. All the samples were crushed into a fine powder and put into Eppendorf with a quantity of 100mg and 200 mg respectively. The solvent system used for the extraction was Dimethyl Sulfoxide (DMSO). One milliliter of solvent (DMSO) was added to the Eppendorf and kept at room temperature for 24 hours. The extracts were centrifuged three times at 10000 g for 15 min. After that supernatant was collected and used for further process.

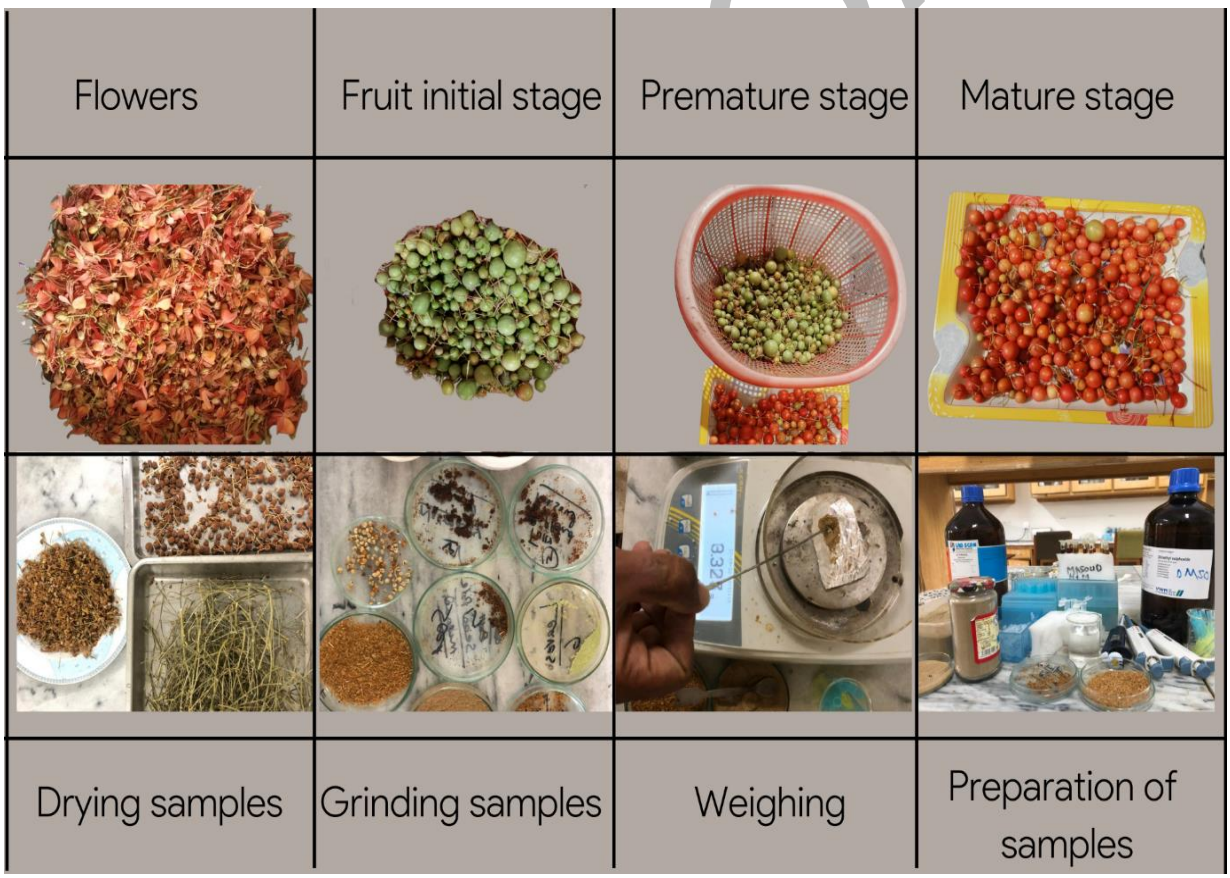

Figure 2.1 Overview of sample preparation stages

2.2 Phytochemical Analysis

2.2.1 Total Phenolic Content Assessment

Stock Solution

Distilled water was used to dilute the Folin-Ciocalteu reagent (FC) 10 times. 6 g of Na_2CO_3 were dissolved in 100 mL of distilled water to create a solution of sodium carbonate with a concentration of 6% (w/v). Gallic acid stock solution was made with a concentration of 1 mg/ml in DMSO.

Method

The total phenolic content was determined using the standard procedure outlined by Fatima *et al*.2015) using FC reagent (TPC). Each well of a 96-well plate was filled with an aliquot of 20 µL from the stock solution of 4 mg/ml DMSO for each sample, then 90 µL of the Folin-Ciocalteu reagent was added and followed 5 min incubations. After that 90 µL of sodium carbonate was incorporated into the reaction mixture. At 630 nm, absorbance was measured using a microplate reader (Biotech USA, microplate reader Elx 800). The results are presented as µg of gallic acid equivalent per milligram of the sample (µg GAE/mg DW) and the assay was carried out in triplicate. Gallic acid was used as the positive control.

2.2.2 Total Flavonoid Content Assessment

Standard solution

10g of $AlCl_3$ were dissolved in distilled water to produce an aluminum chloride solution with a 10% concentration having a total volume of 100mL. In distilled water, one molar (I98.125g/l) potassium acetate solution was made. A stock solution containing 1 mg/ml quercetin was formed in dimethyl sulfoxide. A 100 mg/ml DMSO stock solution of the crude extract was also formed.

Method

As earlier described method by Tabassum *et al*., was used. TFC was examined using the aluminum chloride spectrophotometric assay. A 96-well plate was filled with the specimen (20 µl of 100 mg/ml DW). Then, 10 µL of 10% $AlCl_3$ and 1 molar potassium acetate were added, followed by 160 mL of purified water. For 30 min, the mixture was allowed to settle at room temperature. The optical density was recorded at 415 nm with a spectrophotometer. As a regular positive control, quercetin was applied. The assay was conducted in triplicate,

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and the resulting TFC was expressed in milligram equivalents of quercetin (µg QE/mg DW sample).

2.3 Biological Evaluations

2.3.1 DPPH Assessment

Standard Solution

For the experiment, three separate standard solutions including DPPH, ascorbic acid, and sample extracts were made. DPPH stock reagent was made by dissolving 9.2 mg of DPPH in 100 mL of methanol, whereas ascorbic acid standard solution (1 mg/ml) was formed in DMSO. Furthermore, DMSO was also used to prepare a standard solution of 100 mg/ml crude extracts.

Method

Using the stable 2, 2-diphenyl-1, 1-picrylhydrazyl, or DPPH free radical, the samples' ability to scavenge free radicals was assessed spectrophotometrically reported by (Sajjad *et al.*, 2021). The extract was assessed to be active due to the presence of an antioxidant component, which reduced the DPPH radical, as seen by the change in the color of the DPPH from purple to yellow. In 96-well plates, 10 µL of the test extract was added. 190 µL of the DPPH solution was then added. The plates were incubated for 30 min. at 37 °C in the absence of light. The absorbance was measured at 515 nm using a microplate reader. A positive control was ascorbic acid, and a negative control was DMSO. The assay was performed three times. The calculation formula for percent radical scavenging activity (% RSA) is.

%RSA =(1- Sample Absorbance / Absorbance of negative control) * 100

2.3.2 Total Antioxidant Assessment

Standard Solution

0.2470 g of ammonium molybdate, 1.671 g of sodium phosphate, and 1.631 mL of sulfuric acid were dissolved in 50 mL of water to produce the TAC reagent stock solution. A stock solution of ascorbic acid was made with a final concentration of 1 mg/ml in DMSO. stock solutions of crude extracts with a concentration of 100mg/mL prepared in DMSO.

Method

The phosphor molybdenum method was used to calculate TAC which is reported by (Bibi et al., 2011). To prepare the reagent (0.6 M sulfuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate), 100 µL of each sample extract was combined with 100 µL of the

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reagent. The Regent was then added. Incubation of all the tubes took place for 90 min in a water bath at 95 ℃. The experiment was conducted in triplicates and a positive control, ascorbic acid, was used. Using a PDA spectrophotometer and a blank sample, the absorbance of each sample solution was measured at 695 nm. Micrograms of ascorbic acid equivalents per milligram of dry plant weight were used to measure the antioxidant potential.

2.3.3 Total Reducing Potential (TRP)

Standard Solution

To prepare a 0.2 M phosphate buffer solution, diluting agent 1.42 g of Na_2 $HPO_4.2H_2O$ along with one gram of NaH_2PO_4, were dissolved in 50 mL of purified water having a pH of 6.6. 0.1 g of ferric chloride was dissolved in 100 mL of distilled water to create the 0.1% ferric chloride solution. By mixing 1 g of potassium ferric cyanide in 100 mL of distilled water, a 1% potassium ferric cyanide solution was made. 10% trichloroacetic acid was made by mixing 10 g of trichloroacetic acid in 100 mL of distilled water. 1 mg of ascorbic acid per mL of DMSO was used to prepare it. A stock solution of crude extracts with a final concentration of 100 mg/ml was formed in DMSO.

Method

The procedure described by (Jafri *et al*.,2013) was applied to evaluate the test extract's reducing potential. sample 200 µL of test extract, 400 µL of phosphate buffer, and 1% potassium ferric cyanide was added to every well of a 96-well plate. The mixture was then incubated for 20 to 30 min at 50 ℃. before 10 min at 3000 rpm of centrifugation. A 400 µL solution of trichloroacetic acid was added to the solution following centrifugation. In the well plate, 150 µL of the supernatant and 50 µL of $FeCl_3$ were added. At 700 nm, absorbance was measured, and a rise in absorbance of the reaction mixture denotes a rise in reducing power. A positive control was ascorbic acid, and a negative control was DMSO. The essay was run in triplets, and each sample's reducing power was calculated as microgram ascorbic acid equivalent per milligram of plant dry weight.

2.3.4 Metal Chelating Assay

(Wang *et al*.,2009) reported the metal-chelating ability of SNPs in their study. 100 µl of a sample was mixed with 50 µl of $FeCI_2$ (2 mM) in 96 well plates after that 100 µl of ferrozine (5 mM), was added. Then the mixture was incubated at 37 °C for 10 min. in the dark. The absorbance was determined at 560 nm The subsequent formula was used to determine whether SNPs may chelate ferrous ions:

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%Metal chelating capacity calculated as "(Abs control - Abs sample) / (Abs control) ×100."

Ferrozine solution was replaced with EDTA-Na_2 as the standard, and 100 µL of distilled water served as the negative control.

2.4 Antibacterial Assay

2.4.1 Inoculum preparation

The affectivity of test samples against various bacterial strains was analyzed by the Disc diffusion method as explained by (Bibi *et al*., 2011) with slight alterations. The bacterial strains used in the biological assays included two Gram-positive "(*Staphylococcus aureus* (ATCC# 6538)", "SE" and three Gram-negative "*Escherichia coli* (ATCC 15224)", "*Pseudomonas aeruginosa* (ATCC# 9721)", "*Klebsiella pneumoniae* (ATCC# 4619)". The 100 mg/ml stock solution of the test samples was used for this procedure. tetracycline was formulated by dissolving 4 mg of standard drug/ml DMSO with a final concentration of 20 µg per disc. The given strains were re-cultured by inoculating them from stored slants onto nutrient broth media, for further use and turbidity was tested using McFarland 0.5 $BaSO_4$ turbidity Standard. The nutrient agar medium was crafted by dissolving 37 g of nutrient agar in 1000 ml of purified water. Its pH was maintained at 6.5 and autoclaved.

Method

As previously reported, the in vitro antibacterial capacity of the test extracts was evaluated using the agar disc diffusion method. To create lawns on Nutrient Agar plates, a new bacterial culture was employed with pre-adjusted seeding density. A 5 µL concentration of test extract was added to a disc of sterile filter paper before being directly applied to seeded plates. Tetracycline disc was used as a positive control. As a negative control, a disc containing DMSO was used. After that, the plates were incubated for 24 hours at 37 ℃, and respectively, a zoon of inhibition round the sample and positive negative control was calculated by vernier caliper and reported.

Statistical Analysis:

All of the assays were performed in triplicate, and the findings are presented as mean with standard deviation. The means were further evaluated using analysis of variance (ANOVA) and least significant difference (LSD) at the probability level $p<0.05$.

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Chapter 3

Results

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3 Results

The objective of the present study was to assess the biological effects and antioxidant capacity of the extract of the *Capparis decidua* plant. To better understand the cytotoxic and biological potential of a particular plant, several biological activities were carried out. The outcomes of each assay and activity are listed below.

The DMSO extract of leaves, flowers, seeds, and fruits that were collected at various early, premature, and mature stages of *Capparis decidua* were analyzed for their phytochemical (total phenolics and flavonoids) analysis, free-radical-scavenging activity, total reducing power, total antioxidant, antimicrobial and enzyme inhibition capacity.

3.1 Phytochemical Analysis of Extracts

3.1.1 Total Number of phenolic Contents

The results demonstrate that phenolics and flavonoid contents were present in significant concentration in the extracts of *Capparis decidua*. Relative to leaves, flowers, and fruit extracts, the quantity of total phenolic content was lower in bark, leaves, and flower extract (30.44, 28.71, 62.94 µg GAE/mg DW extract) and higher in seed extract (96.43 µg GAE/mg DW). The quantity of total phenolic content varied depending on the stage of the fruit. At premature, and completely mature phases of development, fruits had total phenolic contents of (85.18, 86.96, and 69.75 µg GAE/mg DW) respectively. From the findings, it is evident that seed and fruit at a premature stage have higher phenolic contents than fruits and flowers.

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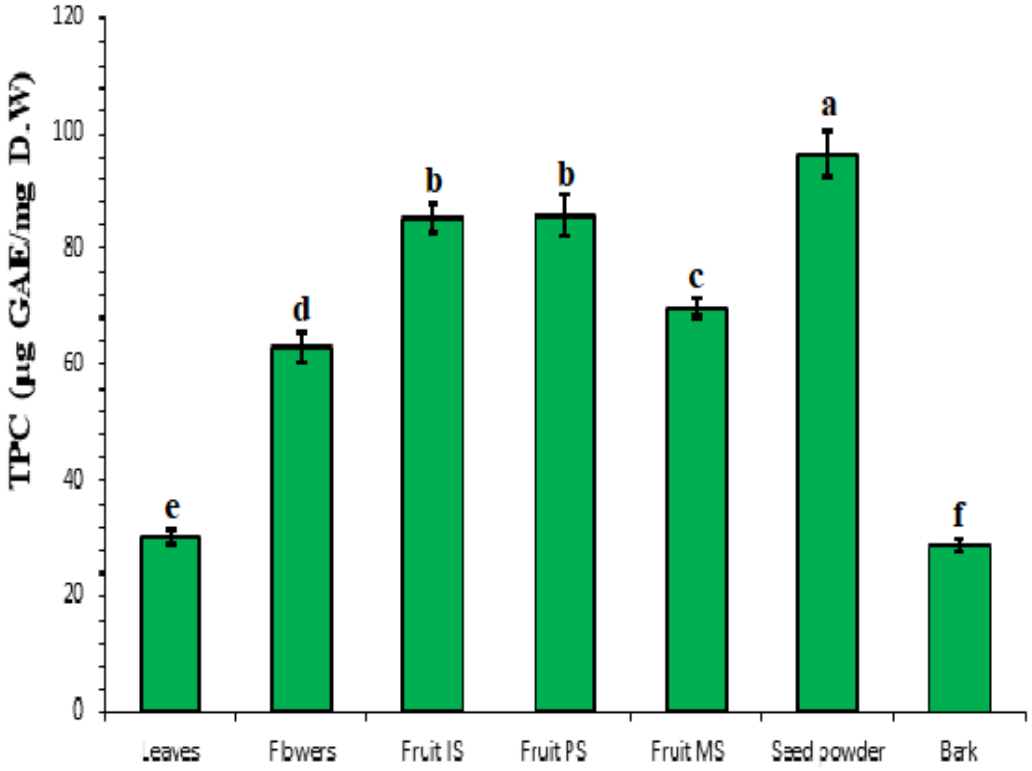

Figure 3.1. Phytochemical analysis of different *C. decidua* (100 mg/ml DMSO) fractions. The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.1.2 Total Number of Flavonoid Contents

When comparing the extracts from bark, leaves, flowers, fruits, and seeds, it was found that the bark, leaves, and floral extract had a lower concentration of flavonoids (25.09, 22.41, 22.71 µg QE/mg DW, respectively), whereas the seed extract had a greater concentration (42.38 µg QE/mg DW. The amount of flavonoid content in fruits at initial, premature, and fully mature developmental stages was i.e., 39.51, 40.63, and 24.93 µg QE/mg DW, respectively. Compared to leaves, flowers, and fruits that are harvested at completely mature phases, the results reveal that seeds and fruits that were taken at premature phases contain a higher number of flavonoids.

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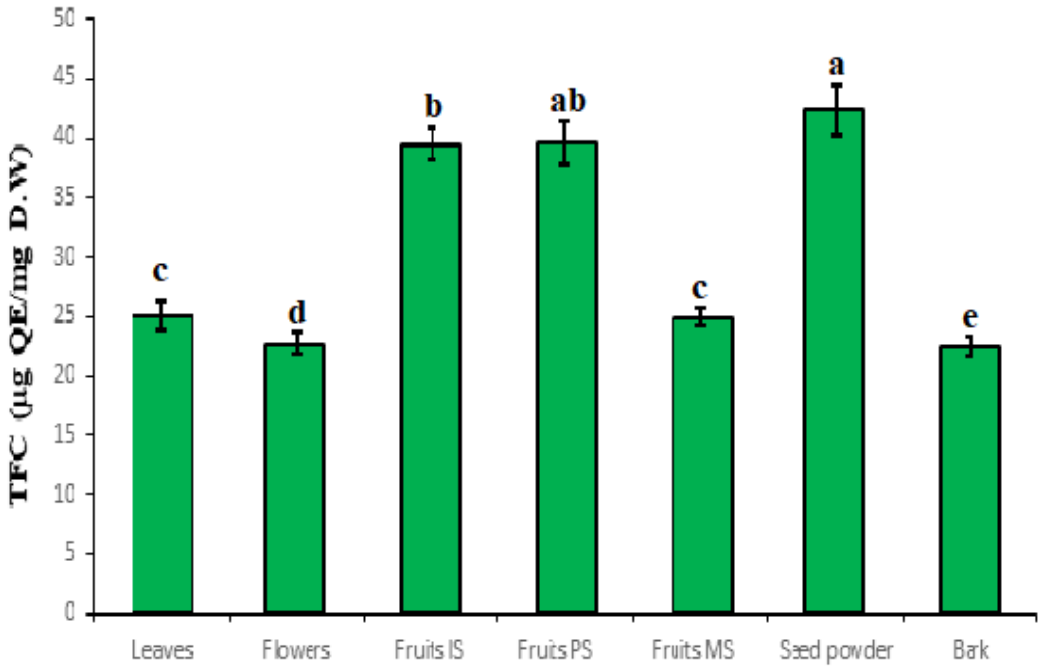

Figure 3.2. Graphical representation of total flavonoid contents at different *C. decidua* fraction (100 mg/ml DMSO). The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.2 Antioxidant Assays

3.2.1 DPPH

The potential for scavenging free radicals by using crude extracts was also examined. Both seeds and fruits that were prematurely harvested displayed a variety of radical scavenging activity patterns. The DPPH solution's discoloration was used to gauge the crude extracts' percent free radical scavenging activity (%FRSA). This process relies on reducing the purple DPPH to a stable, yellow-colored diphenyl picrylhydrazine molecule by receiving an electron or hydrogen radical from a donor antioxidant (Haq et al.,2005).

The aqueous extract of seeds revealed the highest antioxidant activity in the DPPH assay was 73.36%, whereas the scavenging activity of flowers was the lowest 45.36, and 54.34%, respectively. Among all the fruit extracts the most potent radical scavenging potential was exhibited by the premature fruit with a value of 68.14%. The activity of the extract of leaves, and fruits at their early and fully mature developmental phases is 66.71, 59.76, and 60.1% respectively. Among all the extracts, the highest level of radical scavenging activity was demonstrated by seed extract and fruit at its premature phases.

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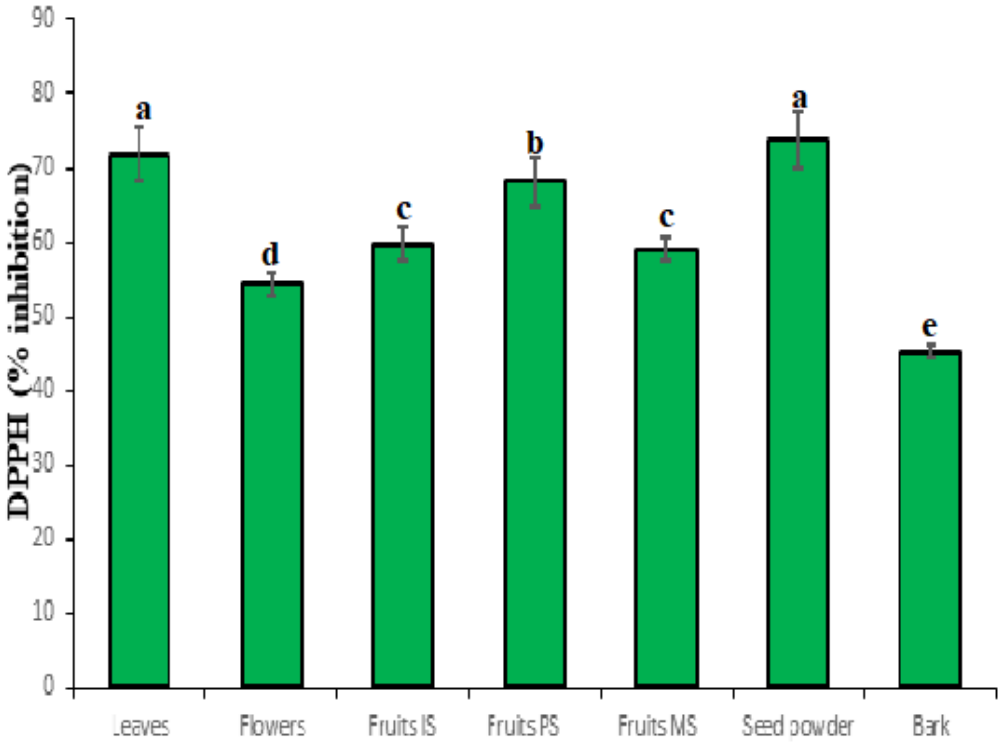

Figure 3.3. DPPH free radical scavenging activity of different *C. decidua* (100 mg/ml DMSO) fractions. The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.2.2 Total Antioxidant capacity (TAC)

The technique depends on the antioxidant mediators reducing Mo (VI) to Mo (V), which leads to the development of a green phosphate/Mo (V) complex with a maximum absorbance at 630 nm (Zia-Ul-Haq et al., 2011). To assess the extract's antioxidant potential, a total antioxidant analysis was conducted. Results were presented, and they show that both fruit and seed extract had an extremely prominent level of overall total antioxidant capacity.

Flowers had a total antioxidant capacity of 266 µg AAE/mg DW, which was below average in all fractions, while seeds had a high total antioxidant capacity of 389.90 µg AAE/mg DW. All flower extracts at initial, premature, and mature phases shown antioxidant activity 290.44, 350.55, and 270.50 µg AAE/mg DW, respectively. The premature extract had the highest level of antioxidant activity 350.55 µg AAE/mg DW, of all of them.

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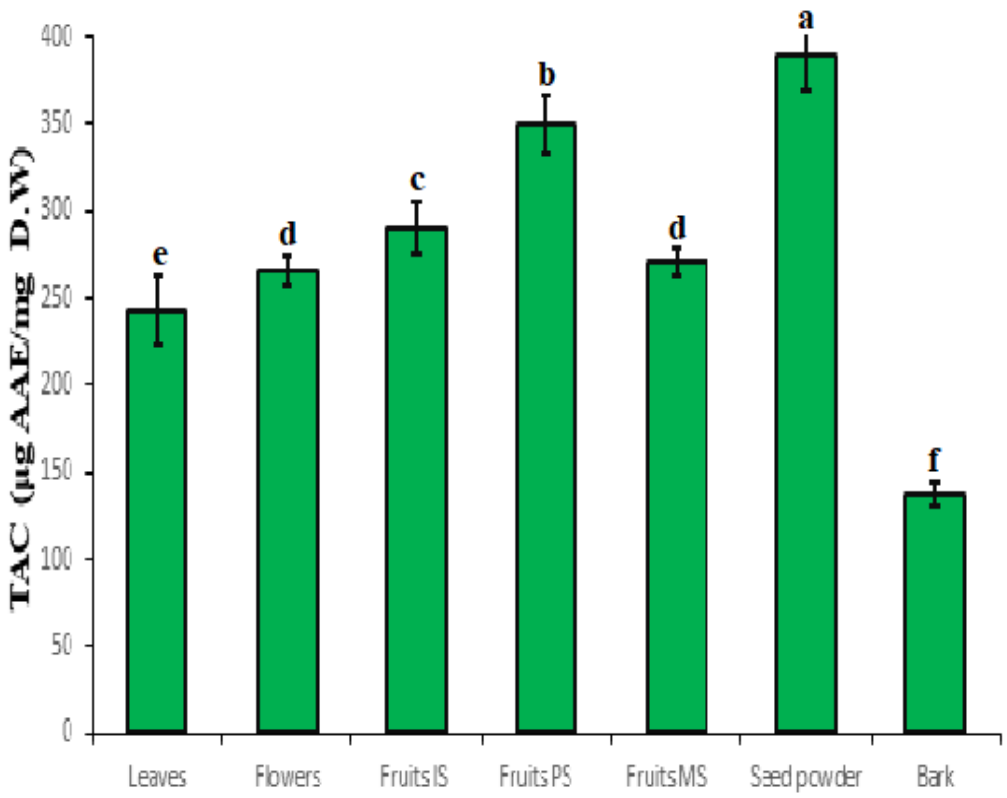

Figure 3.4. Graphical representation of Total antioxidants capacity (TAC) of *C. decidua* fractions (100 mg/ml DMSO). The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

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3.2.3 Total Reducing Potential (TRP)

Capparis decidua crude extracts' total reduction power (TRP) was determined as µg AAE/mg DW. The presence of reductones is connected to the antioxidant action and breaking of the chain of free radicals by donating a hydrogen atom, which demonstrates how the reducing characteristics are typically associated. Consequently, a link has been found between plant extracts' reducing power and antioxidant capacity (Jafri *et al*., 2014).

The highest reduction power in the current investigation was obtained from the DMSO extract of seed 258.01µg AAE/mg DW, also the lowest from the extract of bark, leaves, and flowers i.e., 69.65, 146.06, 225.92µg AAE/mg DW, respectively. Fruit extract at various initial, premature, and mature stages 245.71, 257.04, and 244.87 µg AAE/mg DW, have reducing power respectively. All the findings indicated that the seed extract and fruits that were harvested at premature stage had the greatest reduction potential.

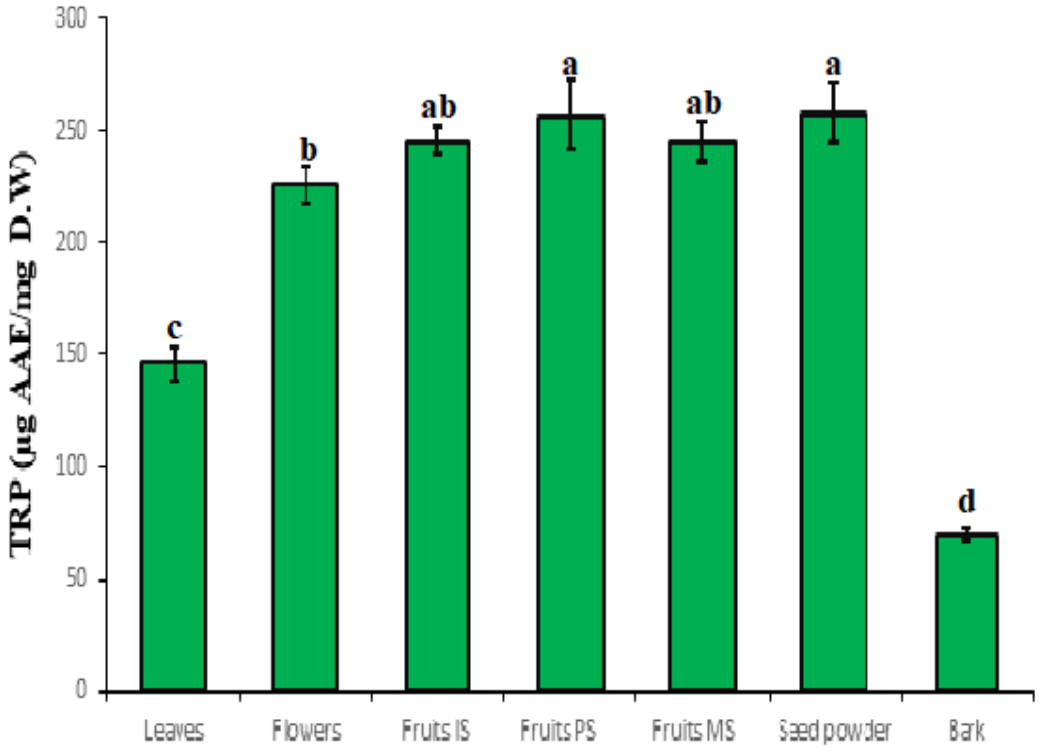

Figure 3.5. Graphical representation of total reducing power capacity (TRP) of *C. decidua* (100 mg/ml DMSO) fractions. The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.2.4 ABTS

The 2, 2'-azino-bis-ethylbenzthiazoline-6-sulfonic acid (ABTS+) radical cation was used to study the radical scavenging activity. The DMSO extract of seeds in the current study showed the highest capacity to scavenge free radicals 79.81%, while the extract of bark, leaves flowers had the lowest 23.40, 25.66, and 31.98% Scavenging, respectively. The fruit extract demonstrated free radical scavenging potential at various initial, premature, and mature phases 39.21, 73.84, 37.19% separately. All the findings indicated that the maximal ABTS capability was present in seed extract and fruits that were harvested at a premature stage.

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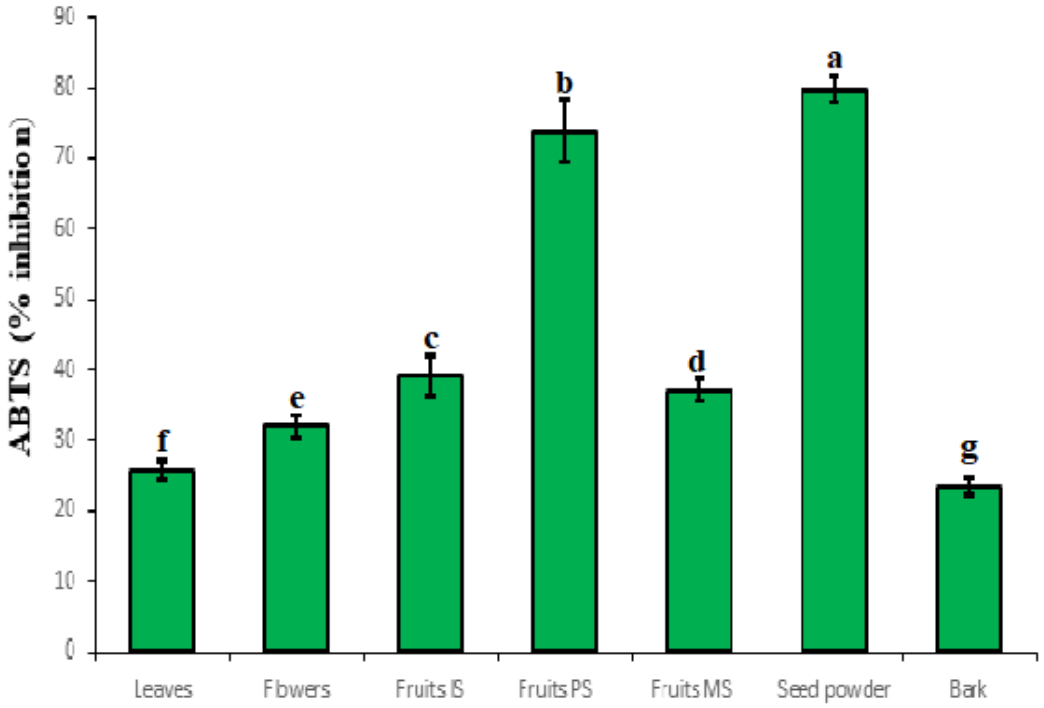

Figure 3.6. Graphical representation of ABTS inhibition capacity of *C. decidua* (100 mg/ml DMSO) fractions. The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level.

3.2.5 Metal Chelation

Extract ability to chelate transition metals can also be used to estimate antioxidant activity. Several transition metals, including Fe_2+ and Cu_2+, are dangerous for human health (Loef and Walach, 2012). Copper and iron ions must be chelated because they are involved in the production of ROS species (Fenton and Haber-Weiss reactions). In a slightly acidic media pH 6, phenolic compounds bind some Fe_2+, while the remaining Fe_2+ interacts with ferrozine to generate a blue-colored complex that can be measured spectrophotometrically. However, the development of the metallic complex is disrupted in the presence of phenolic compounds due to the binding of Fe2+ in the phenolic structure, which results in a decrease in the absorbance at 562 nm.

The current study results showed that *Capparis decidua* has maximum metal chelation capacity. Maximum metal chelation capacity was displayed by the aqueous extract of the seed 63.20%, fruit at the premature stage 60.85%, and minimum chelation capacity was noted in flowers 40.42%. Fruits at initial, premature, and mature stages were shown 46.88%, 60.85%, and 44.41% respectively. All stages have metal chelation ability but seed and fruit at the premature stage showed maximum capacity.

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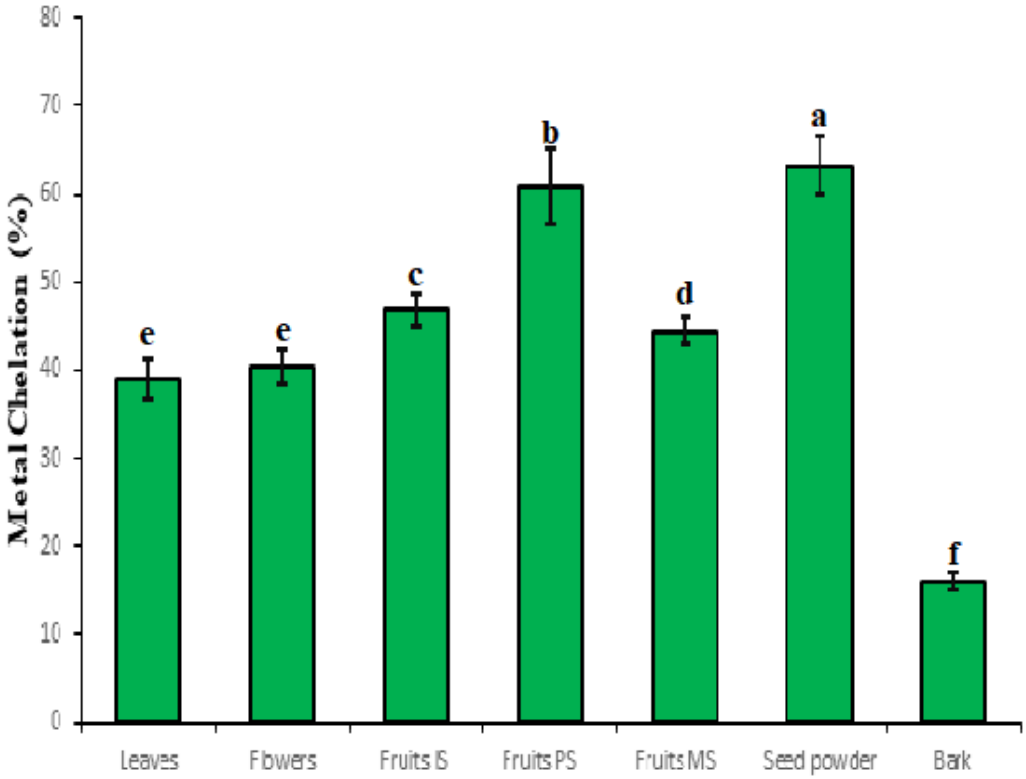

Figure 3.7. Graphical representation of Metal chelation capacity (MC) of *C. decidua* (100 mg/ml DMSO) fractions. The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.3 Antibacterial Assay

3.3.1 Antibacterial activity

The disc diffusion method was applied to evaluate the antibacterial sensitivity of all strains against the extracts of *Capparis decidua*. According to the results, it was found that the seeds extract showed a high zoon of inhibition (10mm, 11mm, 10mm, 12mm, 9mm, 11mm) against *k. pneumoniae, SE, MRSA, E.coli, S. aureus, P. aeruginosa*, respectively. At the second position fruits at the premature stage showed maximum zone of inhibition (9mm, 12mm, 9mm, 10mm, 9mm,11mm) against the following strains correspondingly. Flowers and leaves also showed antibacterial activity against strain which is presented in the table.

Samples were deemed to be antibacterial if they produced an inhibition zone more than or equal to 8, 9 mm. Comparing the activity of plant extracts, the activity of seeds was dominant as compared to other Fractions. Tetracycline was used as a positive control which showed a zone of inhibition of 18mm.

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Table 3.1: Antibacterial activity of *Capparis decidua* DW Extract fractions against different bacterial Strains

S. No	Antibacterial Assay						
	Diameter of zone of inhibition zone measured in (mm) Mean ± SD						
	Samples	***K. Pneumoniae***	***S. Enterococcus***	***MRSA***	***E. coli***	***S. aureus***	***P. aeruginosa***
1	**Leaves**	8±0 .4	7 ± 0.35	7 ± 0.35	8 ± 0.4	7± 0.3	8 ± 0.4
2	**Flowers**	7±0.5	8 ± 0.4	8 ± 0.4	7± 0.35	8± 0.4	7 ± 0.35
3	**Fruit IS**	8±0.4	8 ± 0.41	7 ± 0.35	9± 0.45	7± 0.3	8 ± 0.4
4	**Fruit PS**	10±0.6	11 ± 0.55	9 ± 0.45	11± 0.5	12± 0.6	9 ± 0.45
5	**Fruit MS**	8± 0.4	9 ± 0.45	8 ± 0.4	7± 0.35	9± 0.45	8 ± 0.4
6	**Seeds**	11±0.5	10± 0.5	12 ± 0.6	10± 0.7	12± 0.6	11 ± 0.55
Controls							
8	**Tetracycline**	18 ± 0.9	20 ± 1	18 ± 0.9	18± 0.9	20± 1	21 ± 1
9	**DMSO**	0	0	0	0	0	0

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3.4 Enzyme Inhibition Assays

3.4.1 Alpha Amylase Inhibition Assay

Alpha-amylase is an important carbohydrate hydrolyzing enzyme that can be inhibited to keep blood sugar levels within the acceptable range (Nair *et al*., 2013). Alpha amylase inhibition assay was carried out to screen the plant for its anti-diabetic property. The purpose of this research was to assess the inhibitory effects of *Capparis decidua* plant extracts in enzymes that take part in diabetes, obesity, and many other diseases. The results reveal that maximum and minimum enzyme inhibition 96.79%, 60.22% was achieved by the seeds and flower extract. Fruits that were collected at different initial, premature, and mature stages showed enzyme inhibition 72.94%, 91.52%, 69.29% respectively. In all fractions of fruits, Activity was high in premature fruit extract.

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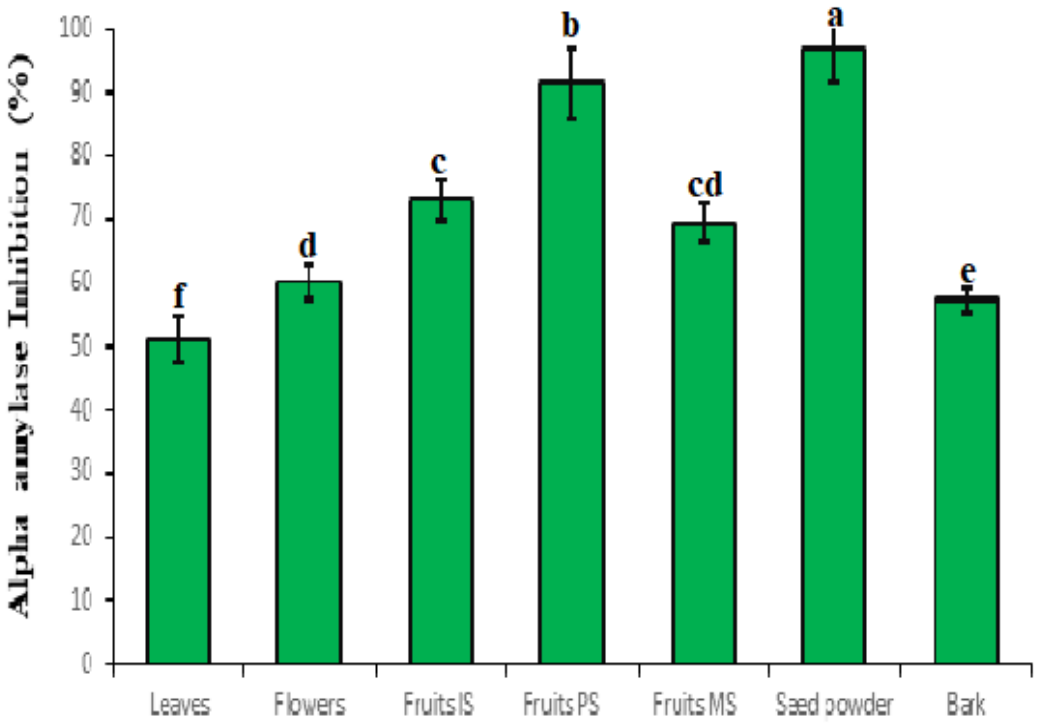

Figure 3.8. Graphical representation of Alpha-amylase inhibition assay of *C. decidua* fractions (100 mg/ml DMSO). The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.4.2 Lipase Assay

The inhibition of key fat-splitting enzyme lipase is a real approach to keeping glycerol levels within the acceptable range (Nair *et al*., 2013). It is found in blood, pancreatic secretions, and intestinal juices which are involved in the hydrolysis of lipids into fatty acids and glycerol molecules. A lipase inhibition assay was conducted to screen the plant extract for any inhibition properties.

Our results revealed that maximum and minimum enzyme inhibition (81.30%, 58.42%) was achieved by the seeds and flower extract. Fruits that were collected at different initial, premature, and mature stages showed enzyme inhibition (72.01%, 75.75%, and 60.21%) respectively. In all fractions of fruits, Activity was high in premature fruit extract.

Orlistat was used as a positive control which showed enzyme inhibition activity at 91.40% respectively. DMSO was used as a negative control.

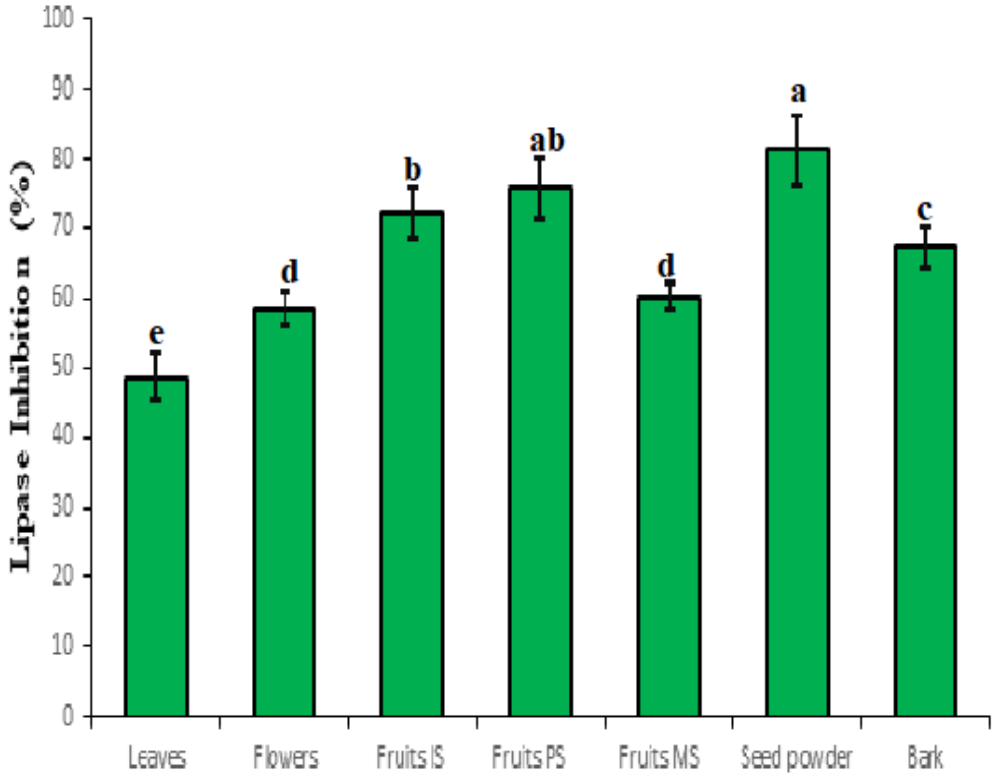

Figure 3.9. Graphical representation of lipase inhibition assay of *C. decidua* fractions (100 mg/ml DMSO). The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

3.4.3 Urease Inhibition Assay

The functional group and phytochemical constituents are linked with aromatic rings which display interaction in enzyme active site with Ni ions, resulting in urease inhibition. The results reveal that maximum and minimum enzyme inhibition (95.17%, 75.66%) was achieved by the seeds and flower extract. Fruits that were collected at different initial, premature, and mature stages showed enzyme inhibition (95.66%, 96.07%, 95.93%) respectively. In all fractions of fruits, Activity was significantly high in premature fruit extract.

Thiourea was used as a positive control which showed enzyme inhibition activity at 90.30% respectively. DMSO was used as a negative control.

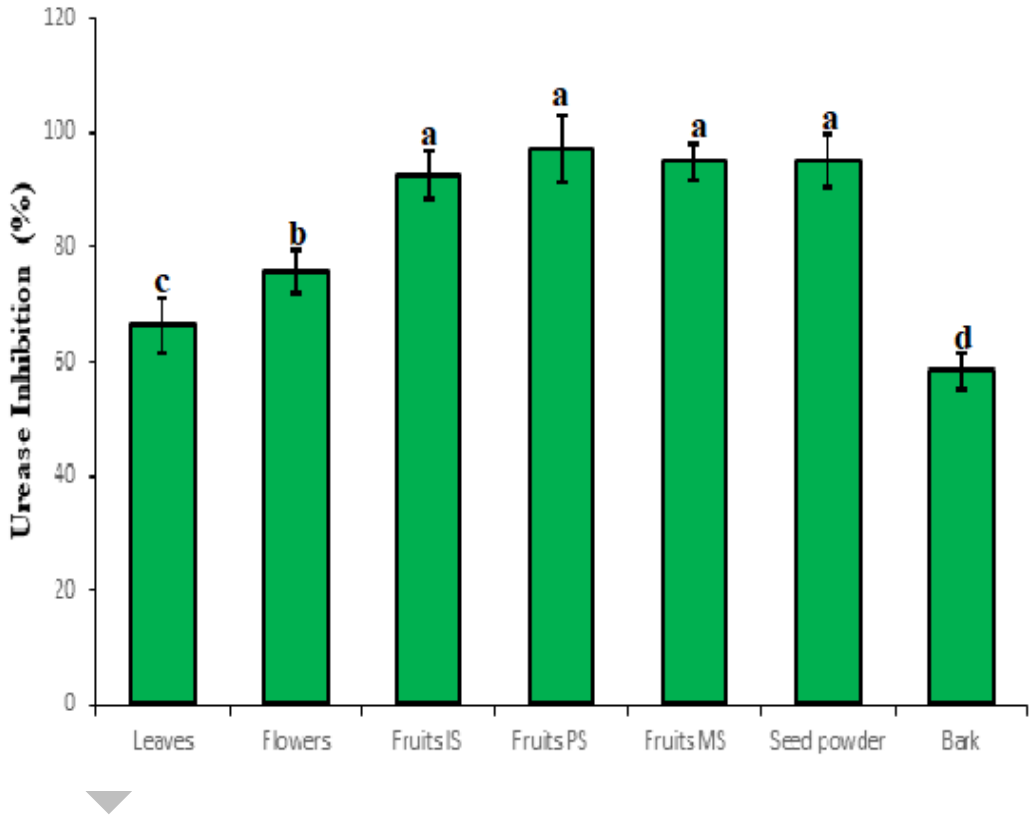

Figure 3.10. Graphical representation of Urease inhibition assay of *C. decidua* fractions (100 mg/ml DMSO). The results are reported as Mean± S.D. Small letters mentioned on each value show significant differences among mean values at ($p \leq 0.05$) probability level using LSD.

Chapter 4

Discussion

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4 Discussion

Natural remedies that come from an ethnobotanical perspective are effective pharmacological warriors against a range of illnesses. The best options among these natural sources are medicinal plants because of their extensive range of secondary metabolites that have therapeutic potential. Many of the medications used today are originally derived from plants (Ishtiaq *et al*., 2015). Plant components, including seeds, fruits, leaves, branches, and flowers, are a plentiful supply of chemicals that can be used in the process of developing new medicines (Jamshidi *et al*., 2018). The discovery of biologically active plant chemicals that open a new way for therapeutic industries (Vaishnav et al., 2017). Many plant substances used in traditional medicine are widely available in rural areas and significantly cheaper than modern pharmacopeia. The roots, leaves, shoots, and bark of plants, among other parts, contain plant-derived chemicals that are used to prepare medicines (Shad *et al*., 2014).

The *Capparis* species have been used as food sources and for medicinal purposes. Alkaloids, flavonoids, steroids, terpenoids, and tocopherols are a wide variety of bioactive compounds found in caper fruits (Nabavi *et al*., 2016). All components of this plant have a lot of medical uses in addition to numerous socioeconomic and ecological advantages (Zia Ul Haq *et al*., 2011). Traditional uses for the plant include treating toothaches, arthritis, asthma, coughs, inflammation, malaria, rheumatism, and swelling. Additionally, astringent, laxative, and vermifuge qualities are thought to exist (Singh, Mishra, Srivastava, Jha, & Khosa, 2011). It is asserted that the alcoholic extract of fruit pulp and root bark has anthelmintic properties. The fruits and seeds contain diuretic, and anti-diabetic qualities and are used to treat cholera, diarrhea, and urinary purulent discharges. Fruits with a spicy flavor are used as a bowel astringent, a breath freshener, and are claimed to treat heart problems (Singh *et al*., 2011). When fruits are green not fully mature and used as pickles. Processed seed oil is used to treat skin allergies and wounds (Sharma, Kumar, & Joshi, 2011). According to published data plant, aerial parts may be able to inhibit bacterial growth and be effective against resistant strains of bacteria (Gull, Sultana, Bhatti, & Jamil, 2015. The reports highlight the urgent need to explore the antimicrobial properties of various *Capparis decidua* plant parts on different stages, due to limited data available regarding the full pharmacological spectrum of the *Capparis* plant.

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The current study examines the phytochemical investigation of kair leaves, and flower fruits at different stages for the qualitative evaluation of total phenolic, flavonoid, and Antioxidant contents using different spectrophotometric approaches.

Different biological assay techniques were used for the quantitative measurement of flavonoids, antioxidants, and enzyme inhibition. The findings provide insight into the phytochemical makeup of these wild nutritious plant species. It may be more helpful to explore additional bioactive principles that are responsible for their therapeutic efficacy in depth to gain knowledge that will be useful for the pharmaceutical business.

Phytochemicals

Total Number of phenolic Contents

The results demonstrate that phenolics and flavonoid contents are present in significant amounts in the extracts of *Capparis decidua*. Relative to leaves, flowers, and fruit extracts, the quantity of total phenolic content was lower in bark, leaves, and flower extract 30.44, 28.71, 62.94 µg GAE/mg DW and higher in seed extract 96.43 µg GAE/mg DW. The quantity of total phenolic content varied depending on the stage of the fruit. At the initial, premature, and completely mature phases of development, fruits had total phenolic contents of 85.18, 86.96, and 69.75 µg GAE/mg DW respectively. From the findings, it is evident that seeds and fruit at a premature stage have higher phenolic contents than fruits and flowers. The presence of diverse phytochemicals agrees with a previous study that reported several secondary metabolites from this plant (Kheta Ram *et al*., 2022).

The same research was carried out by utilizing the accepted techniques, qualitative analysis was performed for phytochemical screening. The previous study investigated that the methanolic extract of *C. decidua* fruits was calculated to have a Total Phenol Content of 37.265 mg GAE/g of the extract on a dry weight (DW) basis (Sonali Bhagat et al.,2021). The Folin-Ciocalteu colorimetric method was used to determine the TPC of various C. Magna extract components. The examined crude extracts of flowers young leaves had TPCs that ranged from 0.81 to 5.73 mg GAE/g DW (Surayot panitan and Pattawat Seekhaw., 2018). According to multiple studies that have established a close connection between these chemicals and antioxidant activity in fruits, plants, and vegetables, it can be assumed that phenolics and flavonoids are responsible for antioxidant activity (Adebiyi *et al*., 2017). Several food and medicinal plants have been processed for primary and secondary metabolite

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determination (Schmeda Hirschmann et al., 2020) to establish a possible link between their Phyto components and beneficial bioactivities. The presence of polyphenols (both phenolic and tannin) in plant extracts indicates that there may be a variety of functions that can be used to treat various disorders and diseases.

Total Number of Flavonoid Contents

Flavonoids are long-chain phenol derivatives that give plants their flavor and color. They are the most abundant polyphenol in human diets. Dietary polyphenols may activate endogenous defense systems and regulate cellular-signaling processes (Molina *et al.,* 2003; Shen *et al*., 2007). They can spread fruit, promote spore germination, and grow seedlings (Griesbach., 2010). When comparing the extracts from bark, leaves, flowers, fruits, and seeds, it was found that the bark, leaves, and floral extract had a lower concentration of flavonoids (25.09, 22.41, 22.71 µg QE/mg DW, whereas the seed extract had a greater concentration 42.38 µg QE/mg DW, respectively. The amount of flavonoid content in fruits at initial, premature, and fully mature developmental stages were (39.51 mg, 40.63, 24.93 µg QE/mg DW correspondingly. Compared to leaves, flowers, and fruits that are harvested at completely mature phases, the experimental result revealed that seeds and fruits that were taken at premature phases contain a higher number of flavonoids.

The previous study investigated the total flavonoid content in the methanolic extract of *C. decidua* fruit was 18.58 mg QE/g DW (Sonail Bhagat *et al*.,2021). In the current study, the amount of phenolic and flavonoid compounds in the DMSO extract of *Capparis decidua* fruits was calculated. Previous results showed that the phenolics and flavonoids, which are principally responsible for the antioxidant, antibacterial, and antidiabetic potential, which are present plentiful in the leaves of *C. decidua* (Zia-ul-Haq *et al*., 2011).

Antioxidants

TAC

Antioxidants play a crucial role in the process of defense against oxidative damage and are essential in inhibiting lipid peroxidation (Valko *et al*., 2004). Hydrogen atoms are donated by reductant molecules, and free radical chains are broken up (Abdel-Hameed et al., 2009). Good electron donors include phenols and reductants (Shon *et al*., 2003). A deficiency in free radicals can cause illnesses and cancer by damaging DNA, protein, and lipid molecules as well as by disrupting membrane phospholipids (Mocan *et al*., 2016). Human bodies have

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their defense mechanisms that manage oxidative stress, but over time, these defense mechanisms become much less effective. To cure serious human diseases, there is a great demand for plant-based antioxidants (Mandal *et al.*, 2010). Plant extracts have a lot of medicinal potentials including antioxidant, anti-inflammatory, and anti-cancer activities (Das *et al*., 2021). To assess the extract's entire antioxidant potential, a total antioxidant analysis was carried out. Results were presented, and they show that both fruit and seed extract had extremely high levels of overall total antioxidant capacity.

Leaves, bark, and Flowers had a total antioxidant capacity of (242.81,137.57, 266 µgAAE/mg DW) which was below average in all fractions, while seeds had a high total antioxidant capacity of 389.90 µgAAE/mg DW. All fruit extracts at initial, premature, and mature phases shown antioxidant activity (290.44, 350.55, and 270.50 µgAAE/mg DW) respectively. the premature extract had the highest level of antioxidant activity 350.55 µgAAE/mg DW, of all of them. If we were to proceed stage by stage, activity has increased progressively. Previous research indicated that the plant's leaflets, and flowers, had a strong antioxidant activity that can neutralize a variety of radical types as well as ferric oxidation (Zia-Ul-Haq *et al*., 2011). The same study was conducted by (Kinnera Padi *et al*., 2018) who measured the absorbance of ethanolic extracts of the aerial and underground parts of *Capparis decidua* at 695 nm. The total reported antioxidant capacity was 105 ± 0.235 ± 43.2 ± 0.567 g/ml. The secondary metabolites flavonoids, alkaloids, terpenoids, tannins, and saponins are among those found in *C. decidua*, which has recently been shown by (Rehman *et al*., 2013) to have excellent antioxidant activity. the same research was conducted by (Zia-Ul-Haq *et al*., 2011), *C. decidua* extracts demonstrated strong antioxidant activity, which reduced several radical types. The investigated extracts had various degrees of success in lowering the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, with IC50 values ranging from 68.1 g/mL for fruit extract to 103.17 g/mL for leaves extract.

DPPH

In the current research, we also examined the potential for scavenging free radicals by using crude extracts. Both seeds and fruits that were prematurely harvested displayed a variety of radical scavenging activity patterns. The DPPH solution's discoloration was used to gauge the crude extracts' percent free radical scavenging activity (%RSA). This process relies on reducing the purple DPPH to a stable, yellow-colored diphenyl picrylhydrazine molecule by receiving an electron or hydrogen radical from a donor antioxidant (Haq *et al*., 2005).

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The aqueous extract of seeds revealed the highest antioxidant activity in the DPPH assay 73.36%, whereas the scavenging of flowers was the lowest (45.36%, and 54.34%) respectively. Among all the fruit extracts the most potent radical scavenging potential was exhibited by the premature fruit with a value of 68.14%. The activity of the extract of leaves, and fruits at their early and fully mature developmental phases is (66.71%, 59.76%, and 60.1%) respectively. The antioxidant capacity of extracts was determined by their ability to scavenge the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical and decrease ferrous ions (FRAP). (Ayat et al., 2016) conducted the same study and concluded that the DPPH scavenging activity of petroleum ether was 48±0.02 and n-butanol extracts were moderate at 43±0.10, but chloroform extracts were low at 23±0.05. The same work was carried out by Kinnera Padi et al. (2018), who assessed the Hydroxyl radical scavenging activity of ethanolic extracts of the aerial and underground sections of *Capparis decidua* at 695 nm. The overall recorded scavenging of hydroxyl radicals' activity was 53.49±1.50 43.95±1.17 g/ml extract.

TRP

Capparis decidua crude extracts' total reduction power (TRP) was determined as µg AAE/mg DMSO. The presence of reductones has been connected to the antioxidant action and breaking of the chain of free radicals by donating a hydrogen atom, which demonstrated how the reducing characteristics are typically associated.

The highest reduction power in our current investigation was obtained from the DMSO extract of seed 258.01 µg AAE/mg DW, also the lowest from the extract of bark, leaves, and flowers (69.65, 146.06, 225.92 µg AAE/mg DW). Fruit extract at various initial, premature, and mature stages (245.71, 257.04, and 244.87 µg AAE/mg DW, have reducing power respectively). All the findings indicated that the seed extract and fruits that were harvested at a premature stage had the greatest reduction potential. Kinnera Padi et al. 2018, investigated the overall reduction capacity of ethanolic extracts of the aerial and underground portions of *Capparis decidua* at 695 nm. The overall reducing activity was 68.43 µg/ml, 60.86 µg/ml respectively. Previous research on *C. decidua* found that the leaves and roots, exhibit significant antioxidant activity, lowering several types of radicals as well as ferric reducing antioxidant capacity (Zia-Ul-Haq et al., 2011). As a result of this, we may conclude that the existence of carbohydrates, steroids, tannins, phenolics, and flavonoids is confirmed in the *Capparis decidua* extract. By comparing current and previous data,

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we concluded that the extracts of *Capparis decidua* of leaves, and flowers both have some antioxidant activity, while the DMSO extract of seed and premature fruit has more antioxidant activity.

ABTS

The 2, 2'-azino-bis-ethylbenz thiazoline-6-sulfonic acid (ABTS+) radical cation was used to study the radical scavenging activity. The DMSO extract of seeds in the current study showed the highest capacity to scavenge free radicals (79.81%), while the extract of bark, leaves flowers had the lowest (23.40%, 25.66%, 31.98%) respectively. The fruit extract demonstrated free radical scavenging potential at various initial, premature, and mature phases (39.21%, 73.84%, and 37.19%) separately. All the findings indicated that the maximal ABTS capability was present in seed extract and fruits that were harvested at a premature stage.

Prior research on *C. decidua* was performed by (Surayot panitan and Pattawat Seekhaw., 2018), and they reported that the leaves and roots have remarkable antioxidant activity. The IC50 values of ABTS were represented in ranges from 0.310 ± 0.02 to 3.861 ± 0.14 and 0.92 ± 0.04 to 3.02 ± 0.16 mg/ml, respectively.

Metal Chelation

A sample's or extract's ability to chelate transition metals can also be used to estimate antioxidant activity. Several transition metals, including Fe_2+ and Cu_2+, are dangerous for human health (Loef and Walach, 2012). Copper and iron ions must be chelated because they play important role in the production of ROS species (Fenton and Haber-Weiss reactions). In a slightly acidic media (pH 6), phenolic compounds bind some Fe_2+, while the remaining Fe_2+ interacts with ferrozine to generate a blue-colored complex that can be measured spectrophotometrically. However, the establishment of the metallic complex is disrupted in the existence of phenolic compounds due to the binding of Fe_2+ in the phenolic structure, which results in a decrease in the absorbance at 562 nm. metal chelation is a crucial property, to estimate the impact of the tested sample.

In the present study, results showed that *Capparis decidua* has supreme metal chelation capacity. Maximum metal chelation capacity was displayed by the aqueous extract of the seed (63.20 %) and minimum chelation capacity was reported by bark, leaves, and flowers

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extract which was (40.42%, 32.05%, and 37.54%). Fruit extract at different initial, premature, and mature stages showed metal chelation capacity (46.88%, 60.85%, 44.41%). All stages have metal chelation ability but seed and fruit extract at the premature stage showed maximum metal chelation capability. In a Comparison Ayat *et al.* (2016), reported that *Capparis decidua* seeds have strong 36 ± 0.04 metal chelation activity. The literature has very few, if any, previous publications on the antioxidant activity of *C. decidua*, making it incredibly challenging to compare our results to those of other investigations. However, the results of the present study are consistent with numerous other studies on the antioxidant properties of other Grewia species, such as G. Asiatica, which is endemic to Asia and has a wide range of pharmacological activity (Zia-Ul-Haq *et al*., 2013). These findings are also in line with research demonstrating that the antioxidant activity is correlated to the phenolic content, which depends upon the solvent system used (Najjaa *et al*., 2011; Sousa *et al*., 2015). The current research extracts demonstrated antioxidant activity by the iron-chelation and radical-scavenging assays, it indicates the presence of both primary and secondary antioxidants. While secondary antioxidants restrict radical generation and guard against oxidative damage, primary antioxidants combat free radicals to halt the start and spread of oxidative chain reactions (Liorent - Martnez et al., 2017) .

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Antibacterial Assay

Antibacterial activity against multiple strains of bacteria.

The disc diffusion method was used to evaluate the antibacterial susceptibility of all strains against the test extract of *Capparis decidua*. According to the results, it was found that the seeds extract showed a very high zoon of inhibition (10mm, 11mm, 10mm, 12mm, 9mm, 11mm) against *k. pneumoniae, SE, MRSA, E. coli, S. aureus, P. aeruginosa* respectively. At the second position fruits at the premature stage showed maximum zone of inhibition (9mm, 12mm, 9mm, 10mm, 9mm,11mm) against the following strains correspondingly. Flowers and leaves also showed antibacterial activity against the following strain which are presented in a table. These results supported the findings of (Elhadi and El Imam., 2013) on the methanol extract of stems as well as those of (Eldeen and Van Staden's.,2007) declaration of the antibacterial action of dichloromethane, ethyl acetic acid, and ethanol extract of *C. decidua* twigs against *B. subtilis, S. aureus, E. coli, and P. pneumoniae*. According to the result of (Ahmad Shahzad *et al*., 2020) methanol extract of *C. decidua* leaves showed a variety of antibacterial activities. Zone of inhibition was reported against *B. Cereus, E.coli, S. aureus,* and *Sp* (21.1, 19.44, 10.52 mm) respectively. It has been discovered that various plant portions are efficient against various bacterial types *(Bacillus subtilis, Pasteurella multocida, Escherichia coli,* and *Staphylococcus aureus* (Gul *et al*., 2015). Bark, fruits, and seed displayed the most activity, whilst wood displayed the least activity. It was discovered that the plant extracts' inhibitory zones ranged from 12 to 18 mm (Abdal Rahman *et al*., 2016)

Samples were deemed to be antibacterial if they produced an inhibition zone more than or equal to 8-9 mm. Comparing the activity of plant extracts, the activity of seeds was dominant as compared to other Fractions. Tetracycline was used as a positive control which showed a zone of inhibition of 18mm.

Enzyme inhibition Assay

Alpha Amylase Inhibition Assay

Alpha amylase is an important carbohydrate hydrolyzing enzyme that can be inhibited to keep blood sugar levels within the acceptable range (Nair *et al*., 2013). Alpha amylase inhibition assay was carried out to screen the plant for any antidiabetic properties. The goal of this study was to evaluate the inhibitory effects of *Capparis decidua* plant extracts in

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enzymes that take part in diabetes, obesity, and many other diseases. Our results revealed that maximum and minimum enzyme inhibition (96.79%, 60.22%) was achieved by the seeds and flower extract. Fruits that were collected at different initial, premature, and mature stages showed enzyme inhibition (72.94%, 91.52%, 69.29%) respectively. In all fractions of fruits, Activity was high in premature fruit extract. According to (Zia ul Haq *et al*., 2011) plant fruit and flower extract significantly inhibited both enzymes. In a different study, it was examined methanol (300 mg/kg) and pure (30 mg/kg) stem extracts decreased blood glucose levels in diabetic and healthy rats when measured by the glucose tolerance test (Dangi and Mishra *et al*.,2011). The enzyme inhibition activities of this plant have not previously been documented, like other parameters. The use of this plant as a dietary supplement for the treatment of diabetes is supported by the findings of the current investigation. To determine the mechanism by which these plant extracts inhibit -amylase and -glucosidase, more research is needed.

Lipase Assay

The inhibition of key fat-splitting enzyme lipase is an effective strategy to keep glycerol levels within the permissible range (Nair et al., 2013). It is found in blood, pancreatic secretions, and intestinal juices and is involved in the hydrolysis of lipids into fatty acids and glycerol molecules. A lipase inhibition assay was carried out to screen the plant for any inhibition properties. Our results revealed that maximum and minimum enzyme inhibition (81.30%, 58.42%) was achieved by the seeds and flower extract. Fruits that were collected at different initial, premature, and mature stages showed enzyme inhibition (72.01%, 75.75%, and 60.21%) respectively. In the same pattern of research (Zia ul Haq *et al*., 2011) reported that *Capparis decidua* methanol extract 100 µg/mL of leaves and flowers have good lipase inhibition activity at 46% and 50% respectively. In all fractions of fruits, Activity was high in premature fruit extract. Many inhibitors include acarbose, miglitol, and voglibose which are used in clinical practice to control enzyme activities. However, these medications cause several digestive tract infections in many patients. Finding and researching amylase inhibitors from natural sources that have fewer negative effects is therefore urgently needed. In the current study, every extract showed remarkably potent inhibitory effects. Similarly, significant urease and lipase inhibitory action was seen.

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Urease Inhibition Assay

The functional group and phytochemical constituents are linked with aromatic rings which display interaction in enzyme active site with Ni ions, resulting in urease inhibition. Our results revealed that maximum and minimum enzyme inhibition (95.17%, 75.66%) was achieved by the seeds and flower extract. Fruits that were collected at different initial, premature, and mature stages showed enzyme inhibition (95.66%, 96.07%, 95.93%) respectively. In all fractions of fruits, Activity was significantly high in premature fruit extract. Zia ul Haq et al. (2011) reported *Capparis decidua* methanol extract 100 g/mL of leaves and flowers exhibit good urease inhibition activity by 53% and 61% respectively (Zia ul Haq et al., 2011).

Additionally, this research shows that enzyme inhibition like- amylase and lipase may contribute, at least in part, to the plant's ability to lower blood glucose levels. In conclusion, the results of our investigation support the appropriate use of *C. decidua* in conventional medicine to treat diabetes by preventing the activity of -amylase. To determine the active principles and clarify additional potential mechanisms of action, more research is necessary.

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Conclusion

From this study, it is concluded that *Capparis decidua* is a very important medicinal plant with diverse biological actions. we examined leaves, flowers, fruits, and seeds in their different stages by doing multiple biological phytochemicals evaluating assays including antioxidants, total phenolic, flavonoid contents, antibacterial, and enzyme inhibition respectively. These findings provide insight into the phytochemical makeup of these wild nutritious plant species. It may be more helpful to explore additional bioactive principles that are responsible for their therapeutic efficacy in depth to gain knowledge that will be useful for the pharmaceutical business. From the result of different assays. All the fractions showed excellent biological actions and antioxidant effects. Among all the assays Relative to leaves, flowers, fruit, and seed extracts, the amount of total phenolic content was lower in bark, leaves, and flower extract and higher in seed correspondingly. The amount of total phenolic content varied depending on the stage of the fruit. Compared to bark leaves, flowers, fruits, and seed fractions, the experimental result revealed that seeds and fruits that were collected at premature phases contain a significant amount of bioactive metabolites, and these fractions could be used for the isolation of bioactive compounds that need to be further studied for their anticancer anti diabatic activities. The plant can be used as a nutraceutical, as evidenced by its antioxidant capabilities. The significant enzyme-inhibitory activity of amylase lipase and urease shows plant potential against several diseases. Amylase and lipase inhibition activity support its preliminary use against digestive disorders. While commencing this research there is necessary to coordinate with industry so that the exploration leads to modern plant-based therapeutics and patents. Bioassay-guided isolation acknowledged its great potential for identifying active constituents. The plant and its constituent parts have shown promising therapeutic effects, making them a strong option for drug development. Compounds that were isolated showed a lot of promise as an agent to reduce oxidative stress.

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Chapter 5

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