Solvent Based Extraction and Phytochemical and Biological activities of *Teucrium Stocksianum*

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy (M.Phil.) in Biotechnology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

DECLARATION

I hereby declare that the work **"Solvent Based Extraction and Phytochemical and Biological Activities *T. Stocksianum*"** accomplished in this thesis is the result of my research carried out in the Nanobiotechnology Laboratory, Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan. This thesis has not been published previously nor does it contain any material from the published resources that can be considered a violation of international copyright law. Furthermore, I also declare that I am aware of the term "copyright" and plagiarism. If any copyright violation is found in this research work. I will be responsible for the consequence of any such violation**.**

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Certificate of Approval

This is to certify that the research work presented in this thesis, entitled **"Solvent Based Extraction and Phytochemical and Biological Activities *T. Stocksianum*"** was conducted by Ms. Sumbal Komal under the supervision of **Dr. Muhammad Zia.**

No part of this thesis has been submitted anywhere else for any degree. This thesis is submitted to the **Department of Biotechnology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan** in partial fulfillment of the requirements for the degree of Master in Philosophy in the field of Biotechnology from the **Department of Biotechnology, Faculty of biological sciences, Quaid-I-Azam University, Islamabad, Pakistan.**

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Examination Committee

1. Examiner Signature: ____________________

2. Supervisor Signature: ____________________

3. Chairperson Signature: ____________________

Dated: ____________________

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DEDICATION

I dedicated this dissertation, with all my heart, to my beloved Aba & Amma, Khala, Mamu, Friends and my respected supervisor. Without their support, a bunch of sincere prayers, and sacrifices it would not have been possible for me to accomplish my work.

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May Allah bless you all with eternal happiness and success! Ameen

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LIST OF ABBREVIATIONS

DPPH	1, 1-diphenyl-2-picrylhydrazyl
DW	Distilled water
µg/mg DW	Microgram/milligram Dry Weight
GAE	Gallic acid equivalents
QE	Quercetin equivalents
TFC	Total flavonoid content
TRP	Total reducing potential
TAC	Total antioxidant capacity
TPC	Total phenolic content
MRSA	Methicillin-resistant Staphylococcus aureus
MC	Metal Chelating
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

DRSML QAU

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ..... **V**
TABLE OF CONTENTS ..... **VII**
LIST OF FIGURES ..... **IX**
LIST OF TABLES ..... **X**
Abstract ..... **XI**
1. INTRODUCTION **1**
1.1. Aims and Objectives 3
2. REVIEW OF LITERATURE **4**
2.1. Geographical Distribution 4
2.2. Scientific Classification 4
2.3. Others name 5
2.4. Botanical description 5
2.5. Medicinal uses of *Teucrium stocksianum* 5
2.6. Economic importance 5
2.7. Phytochemistry 6
2.8. Biological Assays 6
2.8.1. Types of bioassays 6
2.8.2. Antioxidant assays 6
2.8.3. Total antioxidant capacity 7
2.8.4. Total Reducing Power Assay 8
2.8.5. Metal Chelating Assay 8
2.8.6. ABTS Radical Scavenging Assay 8
2.8.7. Antimicrobial assays 9
2.8.8. Antibacterial assay 9
2.8.9. Enzyme Inhibition Assay 11
3. MATERIALS AND METHODS **12**
3.1. Plant collection and extraction 12
3.2. Solvents and reagents 12
3.3. Preparation of Crude Extract 12

DRSML QAU

3.4. Biochemical and Biological screening of plant extracts 12
3.4.1. Phytochemical Analysis 13
3.4.2. Antioxidant Assays 13
3.4.3. Antibacterial Assay 15
3.4.4. Enzyme Inhibition Assays 16
3.5. Statistical analysis 17
4. RESULTS 18
4.1.1. Total Phenolic & Flavonoid Content 18
4.1.1.1. Phenolic contents 18
4.1.1.2. Flavonoid contents 19
4.1.2. Antioxidant assay 21
4.1.2.1. DPPH free radical scavenging activity 21
4.1.2.2. Total antioxidant capacity 22
4.1.2.3. Reducing power assay 23
4.1.2.4. ABTS 25
4.1.2.5. Metal Chelating assay 26
4.1.3. Antibacterial activity of *T. stocksianum* 27
4.1.4. Enzyme inhibition assay 29
4.1.4.1. Alpha- amylase % inhibition assay 29
4.1.4.2. Urease % inhibition 30
4.1.4.3. Lipase % inhibition 31
5. DISCUSSION 33
4.1.5. Phytochemical analysis 33
4.1.6. Antioxidant activities 34
4.1.7. Antimicrobial activity 36
4.1.8. Enzyme inhibition assay 37
6. CONCLUSION 38
7. FUTURE PROSPECTIVE 38
8. REFERENCES 39

DRSML QAU

LIST OF FIGURES

Fig 1.1: ***Teucrium stocksianum*** plant……………………………………………….…………4

Figure 4.1 Total phenolic contents of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD.………………..……………… ……………………………………………………...….21

Figure 4.2 Total flavonoid contents of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD……………………………………………………………………………………………22

Figure 4.3 DPPH free radical scavenging activity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD ……………………………………………………………………………………….…...24

Figure 4.4 Total antioxidant capacity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD……………………………………………………………….…………………….....25

Figure 4.5 Total reducing power of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD…………………………………………………………….…………………………...26

Figure 4.6 ABTS Scavenging activity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD…………………………………………………………………………..……………..27

Figure 4.7 MC % of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at (p≤0.05) probability using LSD ……………………………………………………………………………..……………….28

DRSML-QAU

LIST OF TABLES

Table 4.1 Antibacterial activity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane) against 6 bacterial strains…………………………………………………………………...29

Table 4.2 Alpha- amylase % inhibition *of T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane)……………………………………………………………………………….……..31

Table 4.3 Urease % inhibition of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane)……………………………………………………………………………………...32

Table 4.4 Lipase % inhibition of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane)……………………………………………………………………………………...34

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Abstract

Throughout human civilization, plants have been a provider of inspiration for innovative medication, as plant-based medicines have significantly improved human health and wellbeing. Around 10,000 to 53,000 plant species are utilized as traditional medicines around the world, but very few of these treatments have undergone biological screening, which is a necessary step before validating and examining the full pharmacological potential of these plants. *Teucrium stocksianum* is one of the economically significant medicinal plants. To determine the most effectual plant sample and solvent system for bioactivity, a range of solvent extracts (distilled water, methanol, ethanol, acetone, ethyl acetate, n-hexane, chloroform) were put through phytochemical and biological tests. The methanolic extract of leaves had the highest. TPC, TFC, DPPH, TAC, and TRP values of all the extracts (38.92 ± 1.94 µg/GAE/mg, 40.39 ± 2.01 µg/QE/mg, 87.29 ± 4.36%, 298.59 ± 14.92 µg/ AAE/mg, and 218.20 ± 10.91 µg/ AAE/mg, respectively). Whereas methanolic extract of stem and root displayed the highest ABTS value (91. 92 ± 4.59%, and 91.31 ± 4.56 % respectively). The highest values of MC %, alpha-amylase, urease, and lipase inhibitory activities were unveiled by the aqueous extract of leaves (64.42 ± 3.32%, 88.57 ± 4.42%, 78.71 ± 3.93%, and 72.89 ± 3.64% respectively). Methanolic extract of root displayed anti-microbial potential against six gram-positive and gram-negative bacterial strains. The results showed that *T. stocksianum* can be thought of as an alternative source of antioxidants, anticancer, and antimicrobial chemicals. Additionally, it underlines the necessity of further screening, isolation, and characterization of its biologically active components and supports the traditional therapeutic usage of *T. stocksianum*.

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1. INTRODUCTION

For millennia, medicinal plants have served as the foundation of traditional medicine and are considered a possible source of biologically active compounds with elevated medicinal potential. 90% of the over 1300 medicinal plants used in Europe are found in the wild; approximately 118 of the top 150 prescription drugs in the United States are derived from natural sources. Furthermore, around 25% of prescribed pharmaceuticals in rich countries come from wild plant species, whereas up to 80% of people in developing countries rely on herbal medicines for basic healthcare (Khan and Shinwari, 2016).

Pharmaceutical companies are focusing on specific chemicals from medicinal plants with different metabolic activities using cutting-edge research approaches. Some well-known examples of drugs created from medicinal plants include aspirin, artemisinin, atropine, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, reserpine, Taxol, tubocurarine, vincristine, and vinblastine. The traditional uses of diverse plants and conventional treatments are the main foundations for the discovery of natural product medicines (Gilani et al., 2005).

Around the world, between 10,000 and 53,000 plant species are used as traditional remedies, but very few of these cures have been biologically tested, necessitating this step to authenticate and thoroughly examine the pharmacological potential of these plants (Sarwat and Ahmad, 2012).

Teucrium stocksianum Boiss. commonly known as speer botay, is a perennial plant that is commonly found in the United Arab Emirates and Pakistan's highlands (Ahmad *et al*., 2002), Iran, and northern Oman (Mojab et al., 2003). The height of *T. stocksianum* ranges from 10 to 25 cm, and its stem is densely packed with grey-green leaves. According to a previous phytochemical study, *T. stocksianum* contains potent physiologically active substances such as alkaloids, tannins, flavonoids, certain essential oils, and phenolic compounds.

Traditional medicines found in *T. stocksianum*'s leaves and young shoots are used to treat a variety of conditions, including diabetes, digestive problems, and inflammatory illnesses. (Radhakrishnan et al., 2001). Additionally, the decoction of *T. stocksianum* offers digestive-protective qualities. *T.*

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stocksianum has been used to treat both diabetes and the condition known as "burning feet syndrome." This herb is also utilised as a blood purifier, a hypertension medication, and an epileptic treatment (Ibrar and Hussain, 2009).

The far more widely used method for separating plant antioxidant components is solvent extraction. Due to the presence of various antioxidant compounds with varying chemical properties and polarities that may or may not be soluble in a particular solvent, the extract yields and subsequent antioxidant activities of the plant materials are, however, highly dependent on the type of extracting solvent. Polyphenols are frequently extracted from a plant matrix using polar solvents. Aqueous combinations of ethanol, methanol, acetone, and ethyl acetate are the best solvents. These solvents can be hot or cold (Peschel et al., 2006).

Plants include a wide range of components with various physicochemical characteristics that are diverse in both structure and chemical composition (Shaheen and Shinwari, 2012). Since they have a wide range of affinities and solubilities, the extraction solvents used frequently may not completely cover potentially active components (Sarwat and Ahmad, 2012). As a result, different plant screening processes produce variable results. A survey of the literature revealed that both the use of a sequence of bioassays to ascertain the subject plant's underlying pharmacological potential and to conduct a pharmacological spectrum investigation of *T. stocksianum* utilizing different solvent systems covering a wider polarity range were absent. Our study's objectives were to apply a variety of polarity-based solvents (acetone, chloroform, ethanol, methanol, ethyl acetate, and n-hexane) for the extraction, to detect, identify, and quantify the extracted phytoconstituents, and to screen all the extracts for potential antioxidant, antimicrobial, and antidiabetic properties of *T. stocksianum* by using a variety of in vitro bioassay.

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1.1. Aims and Objectives

- To evaluate in vitro activities of different parts of *T. stocksianum* plant crude extracts made in various solvents for screening different biological activities.
- To compare the antioxidant capacity of diverse plant extracts prepared from the same plant in various solutions.
- Evaluation of antibacterial and enzyme inhibitory properties of *Teucrium stocksianum* components prepared in various solvents.

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2. REVIEW OF LITERATURE

Teucrium stocksianum is a perennial aromatic herb with grayish-white leaves and sessile blooms, growing 10 to 30 cm tall. *Teucrium* species are known to have medicinal benefits, including antioxidant, antibacterial, and antifungal qualities (Yildirim et al., 2004, Ahmad et al., 2007). In traditional medicine, this plant is used to cure abdominal pain, diarrhea, coughing, and jaundice. Numerous scientific research has been done to justify and establish its therapeutic potential because of its uses in the conventional medicine system. *Teucrium* has hepatoprotective and cytoprotective effects on the stomach (Rasheed et al., 1995). Its crude saponins have cytotoxic and anthelminthic properties after being isolated (Gul, 2012), while antispasmodic activity has also recently been discovered. These research investigations suggest that *T. stocksianum* has significant therapeutic potential. Researchers determined the chemical composition and therapeutic potential based on its natural growth and the presence of beneficial phytochemicals in other members of this genus.

2.1. Geographical Distribution

The perennial, woody, and aromatic herb *Teucrium stocksianum* Boiss. (Lamiaceae), often referred to as Speer botay, is a native of the highland United Arab Emirates (UAE), northern Oman, and other places (Nadaf et al., 2003), Iran, and also found in the Northwest of Pakistan (Dir, Swat, Malakand, and Hazara) (Akram et al., 2002).

2.2. Scientific Classification

Kingdom	Plantae
Phylum	Tracheophyta
Class	Equisetopsida
Order	Lamiales

Figure 1.1: *Teucrium stocksianum* plant

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Family Lamiaceae

Genus Teucrium

Specie stocksianum

2.3. Others name

Local name/ vernacular name: Speer botay

Scientific name *Teucrium stocksianum* Boiss

2.4. Botanical description

Teucrium stocksianum Boiss is a member of the Lamiaceae family. It is a perennial aromatic herb with sessile blooms and grayish-white leaves that can reach heights of 10 to 30 cm. In Pakistan, only four of the family's 340 species have been documented.

2.5. Medicinal uses of *Teucrium stocksianum*

The plant's leaves are a common local remedy for jaundice, cough, diarrhea, abdominal pain, and blood purification. Researchers looked at the extracts' potential to treat diabetes mellitus, epilepsy, and hypertension as well as their antiulcerogenic, analgesic, and anti-inflammatory activities (Radhakrishnan et al., 2001).

2.6. Economic importance

Teucrium stocksianum is an exceptional source of free radical-fighting natural antioxidants. In the food and pharmaceutical industries, *T. stocksianum* may therefore be valuable (Irum et al., 2019). The essential oils from this plant contain several advantageous components with noteworthy medicinal use, including thymol and methanol. They frequently appear in plenty of cosmetic and drug preparations.

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2.7. Phytochemistry

Among the potent, physiologically active ingredients found in *T. stocksianum* include alkaloids, tannins, flavonoids, phenolic compounds, and certain essential oils. In the genus *Teucrium*, alpha-pinene, linalool, caryophyllene oxide, Germacrene D, beta-caryophyllene, and delta cadinene have all been shown to be the major constituents of essential oils. These phytochemicals are good beginning points for the development of novel drugs due to their antibacterial, cytotoxic, and phospholipase and esterase inhibiting activities (Radhakrishnan et al., 2001).

2.8. Biological Assays

A biological test is an experiment that evaluates the effectiveness of a chemical, material, preparation, or process when employed on living organisms. Utilizing an intermediate tissue or cell model in vivo or in vitro under controlled conditions is a native way for evaluating the efficacy of a biologically active chemical. In the realm of photochemistry research, biological tests are widely used to determine the phytochemical, secondary metabolite, or bioactive plant extract (Rehman et al., 2013).

2.8.1. Types of bioassays

It is feasible to conduct both quantitative and qualitative bioassays. (A) Biostatistics, which deals with measuring the concentration or potency of drugs by examining the biological effects they have, performs quantitative bioassays or quantitative analyses. (b) Qualitative bioassays assess a substance's quality and are used to assess any unquantifiable physical consequences, like abnormal development.

2.8.2. Antioxidant assays

Antioxidants are a subset of highly reactive chemicals. Living beings produce various kinds of reactive oxygen species (ROS), such as hydroxyl radicals (HO), superoxide anion radicals (O2), and hydrogen peroxide (H2O2) (Halliwell, 2008). In most living things, the antioxidant system controls the production of reactive oxygen species. Numerous cellular processes depend on encouraging the phagocytosis process and redox control of the signal transmission. However, there

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are some circumstances here a living thing produces too many reactive oxygen species, which may make them attract different macro and micro molecules or cellular components like cell membrane, protein, and DNA, and ultimately cause degradation, destruction, and toxicity of those components, which may lead to various diseases (Heo et al., 2005).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is based on a method that allows the radical scavenging activity of substrates, foods, and beverages to be examined. This approach provides the speed, simplicity, and affordability to accurately assess the samples' capacity to scavenge free radicals. For DPPH, methanol and ethanol (Shikanga et al., 2010) can be used as the solvent. Scientists claim that this experiment can be performed using a variety of wavelengths, including 492 nm, 517 nm, 540 nm, and 516 nm. To compare radical scavenging action, a variety of standards are utilized, with ascorbic acid (vitamin C) being the most frequently used as a standard medication (Kwon et al., 2003). The sample reduces as a result of the reaction with DPPH. The absorbance decreases and the color of the DPPH solution change from purple to yellow when it is lowered to DPPHH. More color changes will take place when the antioxidant sample's scavenging potential (power of hydrogen donation) increases (Oktay et al., 2003).

2.8.3. Total antioxidant capacity

Several human disease illnesses can be brought on by reactive oxygen species. Inequality in these species' synthesis and scavenging processes causes cellular damage that impairs the body's ability to operate normally. Consuming plant-based natural products and medicinal plants with significant antioxidant activity can help prevent illnesses brought on by reactive oxidative species. There are various methods for carrying out this type of experiment, but the ones that involve producing free radical species that are then neutralized by antioxidant chemicals are those that are most frequently employed for antioxidant assays (Arnao et al., 2001). Different in vitro tests are frequently used in the present scientific period to evaluate an antioxidant's ability to combat reactive oxygen species (ROS) and free radicals. These assays may be carried out very quickly and expertly. To achieve consistent results, the majority of these assays needed spectrophotometric measurement and a specific amount of reaction time (Aruoma, 2003).

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2.8.4. Total Reducing Power Assay

Redox characteristics of the sample can also be used to estimate antioxidant capability; these characteristics are crucial for determining whether a substance will absorb and neutralize free radicals, burn off singlet and triplet oxygen, or produce rancid peroxides. Potassium ferricyanide ($Fe3+$) can be reduced to potassium ferrocyanide ($Fe2+$) by samples with a high antioxidant potential, which ultimately transforms into ferric ferrous complexes. The real reducing power assay is based on watching the reaction mixture's produced ferric ferrous complex absorbs light at 700 nm. Following are some explanations of reactions:

Ferric chloride +Potassium ferricyanide →Ferrous chloride + Potassium ferrocyanide (Benzie and Szeto, 1999).

2.8.5. Metal Chelating Assay

In a combination with free ferrous ions (Fe(II)), ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazinep,p′-disulfonic acid) can produce a chromophore with strong absorbance at 562 nm. The concentration of the Ferrozine-Fe (II) complex decreases due to the ability of certain compounds to chelate Fe(II), which causes a drop in absorbance at 562 nm (Sudan et al., 2014).

Fe(II) + Ferrozine ———→ Ferrozine * Fe(II) (highly colored)

Fe(II) + Chelator + Ferrozine ———→ Chelator * Fe(II) + Ferrozine (less color)

[Fe(II) chelators reduce Ferrozine-Fe(II) formation which generates less color]

2.8.6. ABTS Radical Scavenging Assay

The ABTS assay compares antioxidants' relative capacity to scavenge ABTS produced in the aqueous phase to a Trolox (a water-soluble vitamin E analog) reference. The pre-generated ABTS•+ radical cation and an antioxidant interact to form the basis of the ABTS/PP test. The bleaching of the absorption spectra characteristic maxima at 414, 417, 645, 734, and 815 nm makes it simple to quantitatively detect ABTS•+ scavenging (Miller and Rice-Evans, 1997).

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2.8.7. Antimicrobial assays

Microbes of different sorts, such as bacteria, algae, and fungi, are responsible for a multitude of diseases in humans, animals, and plants. This increase in mortality rates in higher creatures is brought on by these contagious microorganisms (bacteria and fungi). Over 2000 years ago, several techniques were employed in ancient cultures to cure infections with mixtures that have antibacterial characteristics. A. Fleming is credited with discovering penicillin, which is now acknowledged as one of the pioneering breakthroughs in the history of medicine. Different novel antibiotics were extracted from plants and soil microbes after this one was discovered. The rise of resistant microprobes has been attributed to inappropriate treatment and excessive use of such medicines. Antimicrobial agent development, synthesis, and discovery are thus ongoing processes. The development of new antimicrobial agents has been significantly aided by novel strategies, contemporary techniques, and the growing diversification of chemicals and plant-based chemical extracts (Khan et al., 2008).

2.8.8. Antibacterial assay

The primary issue is resistant bacteria, which have emerged as a result of improper antibiotic use. These antibiotics were once thought to be vital for preserving life, but since the 1950s, drug-resistant microorganisms have emerged (Hawkey, 2008). The introduction of new medications or drug compounds can address this issue. Using well diffusion techniques, the antibacterial activity of the plant extracts was investigated in this work against many kinds of pathogenic bacteria, including:

2.8.8.1. Staphylococcus aureus

The gram-positive, facultative anaerobe *Staphylococcus aureus* causes atopic dermatitis in people and is a part of the natural flora. This type of bacterium can contaminate food and cause human food poisoning since it is found on the skin and the inner surface of the nose (Dinges et al., 2000).

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2.8.8.2. *Escherichia coli*

Gram-negative bacteria include *E. coli*. The majority of *E. coli* strains are not dangerous and are typically found in the intestine. However, some of them lead to various issues for humanity, including gastroenteritis, newborn meningitis, and other dangerous stomach diseases, in addition to food poisoning. They contribute to urinary tract infections as well. The virulence factor is expressed by all pathogenic strains that are disease-causing. Their genetic makeup is rather straightforward and easily manipulable (Nataro and Kaper, 1998).

2.8.8.3. *MRSA*

Staphylococci are opportunism bacteria that frequently infect hospital patients and invade their epithelia. Methicillin-resistant *MRSA*, a particularly virulent strain of *Staphylococcus aureus*, can lead to life-threatening diseases. They commonly exhibit various forms of antibiotic resistance and can create a wide range of toxins. The majority of these characteristics are present on the mobile genetic components of the genome (MGEs). Methicillin resistance is mostly caused by the mecA gene, which is not native to this species and codes for a modified penicillin-binding protein (PBP2a) with low affinity for -lactams (Stefani et al., 2012).

2.8.8.4. *Pseudomonas aeruginosa*

Pseudomonas bacilli, which are gram-negative bacteria, are frequent occupants of the soil, freshwater, and marine environments. Since *Pseudomonas aeruginosa* also causes human diseases and is an opportunist infection, it attracts more attention. The majority of strains produce bacteriocins. The fact that the entire genome of this bacterium was sequenced in 2000 demonstrates the interest in it (Wu et al., 2015).

2.8.8.5. *Salmonella enterica*

Globally, both human and animal health are seriously threatened by the infectious pathogen *Salmonella enterica*. It has a significant impact on mortality and morbidity for people all over the world. *S. enterica* can successfully colonize people, animals, and plants in addition to existing in the environment. While other serovars are host-adapted, *S. typhimurium* and *S. enteritis* are two serovars that have a broad host range (*S. typhi* and *S. paratyphi* A and C). After consumption, *S.*

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enterica colon and ileum, infiltrating the intestinal epithelium to either cause sepsis or to travel throughout the body and result in neutrophilic gastroenteritis. It thrives in the intracellular niche, allowing for extended colonization and innate antibiotic resistance in a few very uncommon cases (Knodler and Elfenbein, 2019).

2.8.8.6. Klebsiella pneumoniae

Klebsiella pneumonia is an infectious agent that often affects persons with weakened immune systems and is known to cause nosocomial infections. A subpopulation of highly pathogenic *K. pneumonia* serotypes with high production of capsule polysaccharide can cause life-threatening community-acquired infections in previously healthy persons, including meningitis, endophthalmitis, necrotizing fasciitis, and severe pneumonia. Several virulence factors, including fimbriae, outer membrane proteins, capsule polysaccharides, lipopolysaccharides, and determinants for iron acquisition and nitrogen source usage, are used by *K. pneumonia* during infection (Li et al., 2014).

2.8.9. Enzyme Inhibition Assay

The assay aims to ascertain whether the injection of a second molecule affects the activity of a medication, such as an enzyme. In common words, this indicates that a DDI, or drug-drug interaction assay, can be used to evaluate the impact of one drug on another (drug-drug interaction). Three different kinds of enzyme inhibition assays exist (Gubareva et al., 2002).

- Alpha-amylase inhibition assay
- Lipase Inhibition Assay
- Urease Inhibition Assay

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3. MATERIALS AND METHODS

3.1. Plant collection and extraction

In May 2022, fresh *Teucrium stocksianum* plant components were taken from the deer's Swat, Khyber Pakhtunkhwa, Pakistan habitat. The plant was dried and divided into leaves, roots, and shoots for additional screening. Using a professional blender, these were each processed into powder and kept in a different bottle.

3.2. Solvents and reagents

Analytical-grade reagents and solvents were used in the current study. Methanol, ethanol, ethyl acetate, chloroform, n-hexane, acetone, and dimethyl sulfoxide were obtained from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent and DPPH reagent was purchased from Sigma-Aldrich (Germany). Sodium hydroxide, ferrous chloride, aluminium chloride, quercetin. Gallic acid, ascorbic acid, caffeic acid, rutin, kaempferol, myricetin, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and (+)-catechin.

3.3. Preparation of Crude Extract

Seven common solvents were used to extract the root, shoot, and leaf of *Teucrium stocksianum*: methanol, n-hexane, acetone, chloroform, ethyl acetate, ethanol, and distilled water. By placing 200 mg of every sample into 5 ml of each above-mentioned solvents, with the exception of distilled water, which is 1 ml. Then own wards, mixture was stored at room temperature for three days. After three days, centrifugation at 1400 rpm was performed for 20 min. Pellet was discarded after collection of supernatants. After drying, supernatant was then dissolved in 1 ml of DMSO. The extracts were then used in a number of assays.

3.4. Biochemical and Biological screening of plant extracts

Plant extracts of *Teucrium stocksianum* roots, shoots, and leaves in seven different solvents were obtained and subjected to different biological assays. These assays include:

- Phytochemical analysis

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- Antioxidant assays
- Antibacterial assays
- Enzyme inhibition assays

3.4.1. Phytochemical Analysis

3.4.1.1. Total phenolic content (TPC) Analysis

96-well plate was utilized to perform this assay. A 20 µL sample was put to each well of a 96-well plate. Thenceforth, 90 µL of Folin-Ciocalteu reagent was added. At 37 °C, after an incubation of 5 minutes, sodium bicarbonate (90 µL) was added to each well of the plate. By employing microplate reader at 630 nm, optical density of all sample extracts was measured. For negative control, DMSO was utilized. In two-fold serial dilutions (2.5, 5, 10, 20, 40 µg/ml), gallic acid was employed as positive control. The experiment's findings are represented as µg of gallic acid equivalent (GAE) per milligram of the extract (Ali et al., 2017). The assay was conducted in triplicate.

3.4.1.2. Total flavonoid content (TFC) Analysis

The findings were reported as µg quercetin equivalent (QE)/mg of extract (Oktay et al., 2003). The 96-well plate was used to conduct the assay. Each well consisted of 20 µL aliquot from test extracts, 160 µL of distilled water, 10 µL of 1 molar potassium acetate, and 10 µL of 10% aluminum chloride. By employing microplate reader at 415 nm, optical density of all sample extracts was measured after 30 min settlement. For negative control, DMSO was utilized. Quercetin served as a positive control in two-fold serial dilutions (2.5, 5, 10, 20, 40 g/ml). The experiment's findings were represented as mg equivalents of quercetin (QE)/mg. The assay was conducted in triplicate.

3.4.2. Antioxidant Assays

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3.4.2.1. Radical scavenging activity-DPPH assay (DPPH)

Stable free radical (DPPH) was used to test antioxidant capacity of compound. A combination of 190 µL of DPPH solution and 10 µL of sample (4mg/ml DMSO) was used to obtain reaction mixture's final concentrations of 200 µg/ml.

After 30 minutes of incubation at 37°C in dark, the absorbance at 515 nm was measured using a microplate reader. The research applies the following formula to determine the proportion of free radical scavenging activity.

FREE RADICAL SCAVENGING ACTIVITY = 1 – As/Ac * 100

Where As and Ac represent the absorbance of the sample and the negative control, respectively. Ascorbic acid was used as a positive control and DMSO as negative control in the test, which was repeated three times (Sajjad et al., 2021).

3.4.2.2. Total antioxidant capacity (TAC) estimation by phosphomolybdenum-based assay

Phosphomolybdenum-based assay was utilized to determine total antioxidant capacity of each test sample. 100 µl of test extract and 900 µL of TAC reagent (0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM ammonium molybdate) were used as a mixture. Ascorbic acid was employed as positive control while for negative control methanol was used. Incubation of mixture was done for 90 mins at 95 °C in water bath. Absorbance of standard and test solution was then measured at 630 nm following cooling. As g AAE/mg of DW, the antioxidant potential was expressed. The assay was conducted in triplicate.

3.4.2.3. Total reducing power (TRP) estimation by potassium ferricyanide colorimetric assay

Extract's reducing power was computed using a potassium ferricyanide colorimetric test. Potassium ferricyanide 250 µL was mixed with 200 µL of phosphate buffer (0.2 M, pH 6.6) and 100 mL of each test extract (4mg/ml DMSO). After that incubation was performed for 20 mins at 50 °C in water bath. Each sample was combined with trichloroacetic acid 200 µL, mixture was centrifuged at 3000 rpm for 10 mins at room temperature. The 50 µL of 0.1 percent w/v FeCl3 in distilled water on a 96-well plate were then added to the 150 µL of supernatant. 700nm wavelength was used to measure absorbance. DMSO was employed as negative control and ascorbic acid

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served as the positive control. Ascorbic acid equivalent (AAE) was used to measure each sample's reducing power, which is given as µg AAE/mg (i.e. µg ascorbic acid equivalent per mg).

3.4.2.4. Metal Chelating Assay

The fact that SNPs can chelate metal was described by (Wang et al., 2009). 100 aliquots of each substance were added to a 96-well plate together with 50 aliquots of FeCI2 (2 mM) and 100 aliquots of ferrozine (5 mM), and the mixture was then incubated at 37 °C in the dark for 10 minutes. At 562 nm, the absorbance was determined. The ability of SNPs to chelate ferrous ions was evaluated using the following formula: (Abs control - Abs sample) / (Abs control) 100 gives the percentage of metal chelating ability.

In place of the ferrozine solution, EDTA-Na2 was used as a standard, and 100 l of deionized water was used as a negative control.

3.4.2.5. ABTS Radical Scavenging Assay

Scavenging the radical cation of 2, 2'-azino-bis-ethylbenzthiazoline-6-sulfonic acid (ABTS. +) was used to test the radical scavenging activity. In a 1:1 ratio, 2.45mM potassium persulfate solution and 7mM ABTS were combined to make the ABTS reagent. After incubating the solution at room temperature for 12-14 hours in the dark, it acquired an absorbance of 0.700 0.01 at 734 nm. An aliquot of 10 l of each SNP stock solution was put on a microtiter plate with 190 l of ABTS reagent and incubated for 10 mins at 37 °C. At 734 nm, the absorbance was measured. Following formula was employed to calculate percent scavenging activity of SNPs.

ABTS $^{+\%}$ Scavenging = ((AB-AA)/AB)100),

Positive control was ascorbic acid, and negative control was DMSO, where AB denotes ABTS radical absorbance + methanol and AA denotes ABTS radical absorbance + sample (Sajjad et al., 2021).

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3.4.3. Antibacterial Assay

The disc diffusion method was used to conduct an antibacterial experiment (Akintelu et al., 2020). Six bacterial strains were used: Gram-negative *Salmonella enterica*, MRSA, Gram-negative *E. coli,* Gram-negative *Klebsiella pneumonia*, Gram-positive Staphylococcus aureus, and Gram-negative *Pseudomonas aeruginosa.* New bacterial strains with altered

seeding densities were produced on nutrient agar plates. Discs made of sterile filter paper were covered with 5 µl of samples. Cefixime and roxithromycin were the employed positive controls. Following inoculation, the average diameter of clear ZOI was measured at 37°C for 24 hours. ZOI of less than 12mm was considered crucial.

3.4.4. Enzyme Inhibition Assays

3.4.4.1. Alpha-amylase inhibition assay

Ahmed et al., (2017) highlighted a method which was employed to determine the test samples' ability to inhibit alpha-amylase. Phosphate buffer (15µl) was added to a 96-well plate (pH 6.8). Then, in successive phases, 40 µl of a starch solution (2 mg/ml in potassium phosphate buffer), 10 µl of a test sample (4 mg/ml in DMSO), and 25 µl of alpha-amylase enzyme (0.14 U/ml) were added. After 30 minutes of heating at 50 °C, 20 µl of 1M HCL and 90 µl of iodine reagent were added (5 mM iodine, 5 mM potassium iodide). As a positive control, acarbose was taken in doses ranging from 5 to 200 g/ml. The amylase enzyme and test sample were not used to create the blank. The data were read using a microplate reader, and measurements at 540 nm were taken.

3.4.4.2. Lipase Inhibition Assay

The lipase inhibition activity was investigated using the approach published by (McDougall et al., 2009) with minor modifications.. After centrifuging the supernatant at 16000 rpm for 5 minutes, the lipase solution (10 mg/ml) was utilized. As an assay buffer, 100 mM of Tris-Buffer with a pH of 8.2 was used. The substrate utilized was olive oil, it was 0.08% by volume diluted in 5 mM sodium acetate (pH of 5) containing 1% Triton-X-100 and boiled in water for one minute before cooling to room temperature. 350 µl of buffer, 50 µl of the test sample (4 mg/ml in DMSO), and 150 µl of lipase were placed in each Eppendorf dish. The substrate was then added to begin the reaction in 450 µl. An Eppendorf without any material was used as a blank, and orlistat was used as an inhibitor. Before 200 µl was added to the wells of the microtiter plate, each sample was centrifuged for one minute at 16000 rpm after being incubated at 37ºC for two hours. After readings at a wavelength of 400 nm in a UV

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spectrophotometer, the outcomes were compared with orlistat, a common inhibitor. The following formula was used to determine the percentage of enzyme inhibition in the reaction:

Enzyme Inhibition Percentage =OD(b)-OD(s) OD(b)*100

Where OD (s) = the abs. value of the test sample

OD (b) = the absorbance of the blank.

3.4.4.3. Urease Inhibition Assay

The assay combination for urease inhibition consisted of 25 µl of urease, 50 µl of phosphate buffer (3 mM, pH 4.5, 100 mM urea), and 10 µl of test samples (4 mg/ml in DMSO). The assay mixture was then incubated in 96-well plates at 30ºC for 15 minutes. Each well was then treated with the phenol reagent (1% (w/v) phenol and 0.005% (w/v) sodium nitroprusside) and the alkali reagent (0.5 (w/v) NaOH and 0.1%) NaOC1. The inhibiting action was evaluated using a clear, strongly scented ammonia product (Biglar et al., 2012). A UV spectrophotometer was used to record the measurement at 630 nm following a 50-minute incubation period at 30 °C. Thiourea was used as a urea inhibitor control. However, for the blank, 60 µl rather than 50 µl of the buffer were used, and neither the test sample nor the control were used. The following formula was used to determine the percentage of enzyme inhibition in the reaction:

Enzyme Inhibition as a Percentage = OD (b)-OD(s) OD (b)*100 Where OD (b) is the absorbance of the blank and OD (s) is the absorption value of the test sample.

3.5. Statistical analysis

Each of the aforementioned trials was carried out in triplicate, synchronized and thrice. To formalize statistical analysis, the mean value was determine in each experiment and the standard error was calculated using Microsoft Excel Program. All figures were created using Origin program (8.5). A mean standard deviation was used to present the outcome data.

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4. RESULTS

T. stocksianum leaves, roots, and stems were subjected to extraction using seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). To identify the ideal solvent for extraction and isolation of *T. stocksianum* bioactive component. Four in vitro experiments were conducted sequentially to determine the biological activity of *T. stocksianum* extracts.

i. Phytochemical analysis was done by the evaluation of phenolic and flavonoid contents.
ii. The antioxidant assay was implemented using DPPH procedure, total antioxidant capacity, ABTS, reducing power assay, and metal chelating.
iii. Using the disc diffusion method, an antimicrobial experiment was performed on five bacterial strains.
iv. Alpha-amylase, Urease, and Lipid inhibition assays were performed on all seven extracts of the plant's roots, shoots, and leaves.

4.1.1. Total Phenolic & Flavonoid Content

Figures 4.1 and 4.2 show the phenolic and flavonoid content of T. stocksianum's leaves, roots, and stems in different solvents.

4.1.1.1. Phenolic contents

The total phenolic content of the leaves and roots was maximum in the methanolic extract (38.92 ± 1.94 and 33.55 ± 1.67 µg GAE/mg, respectively), but the aqueous extract has the highest phenolic content of the stem (33.92 ± 1.69 µg GAE/mg). However, n-hexane extract of the stem was found to have the lowest amount of phenolic content, leaves, and roots i.e. (3.52 ± 0.17, 3.73 ± 0.18, 5.74 ± 0.28 µg GAE/mg respectively) followed by chloroform extracts of leaves, stem, and root (8.18 ±0.40, 8.37 ± 0.41, 9.96 ± 0.49 µg GAE/mg respectively). Acetone and ethyl acetate extracts of root, stem, and leaves displayed appreciable results as shown in figure 4.1.

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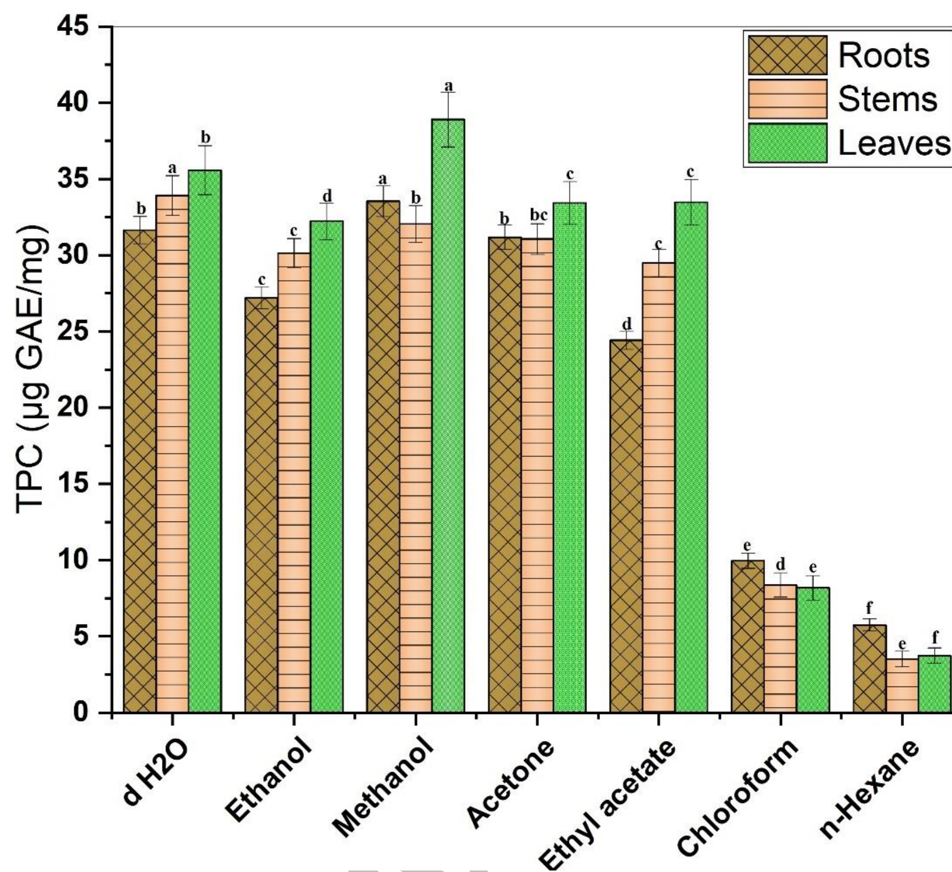

Figure 4.1 Total phenolic contents of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability using LSD.

4.1.1.2. Flavonoid contents

The methanolic extract had the highest flavonoid concentration of all the leaf extracts (40.39 ± 2.01µg QE/mg). Similarly, in the case of root and stem highest flavonoids were quantified in methanolic extract (25.31 ± 1.26 and 27.32 ± 1.36 µg QE/mg respectively). Aqueous leaves and stem extracts also unveiled the highest flavonoid content (30.12 ± 1.50, 26.74 ± 1.33 µg QE/mg). While the flavonoid concentration of the n-hexane extract of the stem, leaves, and roots was found to be the lowest i.e. (2.13 ± 0.10 µg QE/mg, 2.32 ± 0.11 µg QE/mg, 4.18 ± 0.20 µg QE/mg)

followed by chloroform extracts of leaves, stem, and root (4.02 ± 0.20 µg QE/mg, 4.60 ± 0.23 µg QE/mg, 5.26 ± 0.26 µg QE/mg). As can be seen in figure 4.2, the root, stem, and leaf extracts in acetone and ethyl acetate produced noticeable results.

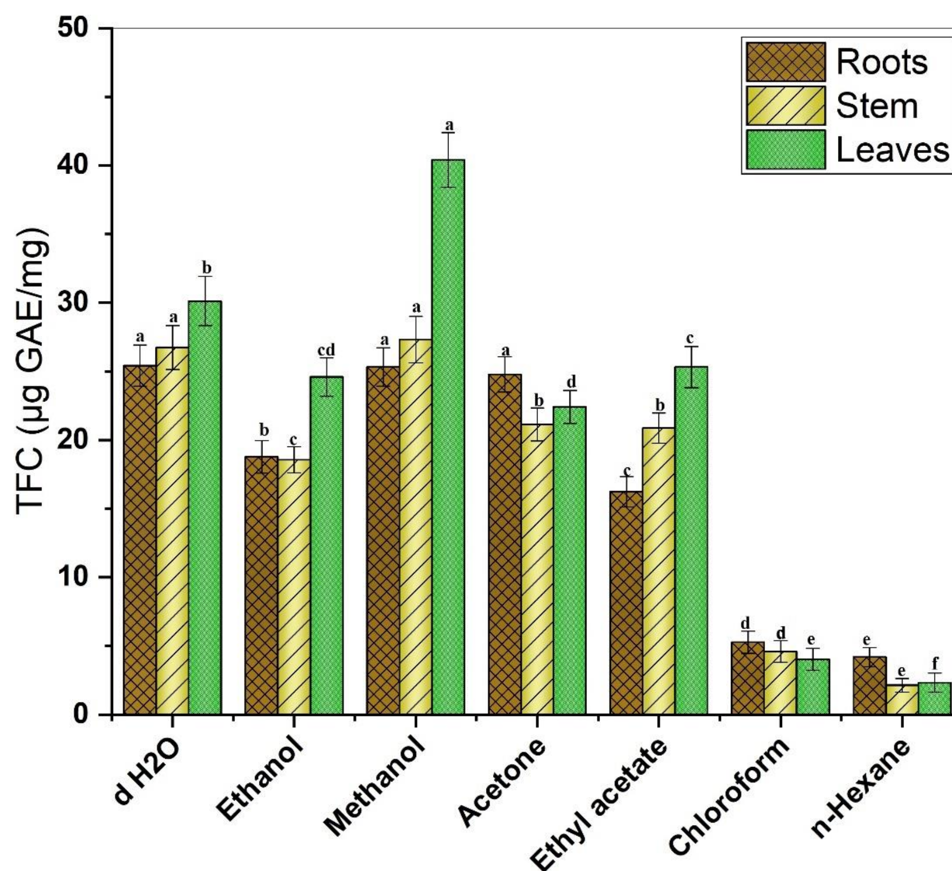

Figure 4.2 Total flavonoid contents of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability using LSD.

4.1.2. Antioxidant assay

The antioxidant assay on *T. stocksianum* leaves, root, and stem extracts in different solvents was carried out using DPPH, TAC, metal chelating, ABTS, and TRP.

4.1.2.1. DPPH free radical scavenging activity

By evaluating the reducing ability of the tested compounds, DPPH assay is regarded as straightforward, suitable, and efficient approach for screening plant extracts or compounds for their antioxidant potential. Each extract's ability to neutralize DPPH free radicals in various solvents was assessed. A positive control was used, which was ascorbic acid, a renowned antioxidant standard. By providing superior DPPH values, each extract demonstrated strong antioxidant activity. Methanolic extract of leaves, stems, and roots showed maximum value (87.29 ± 4.36% and 86.73 ±4.33%, and 86.10 ± 4.30% respectively) followed by aqueous extract of leaves, stem, and root by giving a value of 80.89 ± 4.04%, 78.18 ± 3.90%, and 75.11 ± 3.7% respectively. Detailed results are displayed in figure 4.3.

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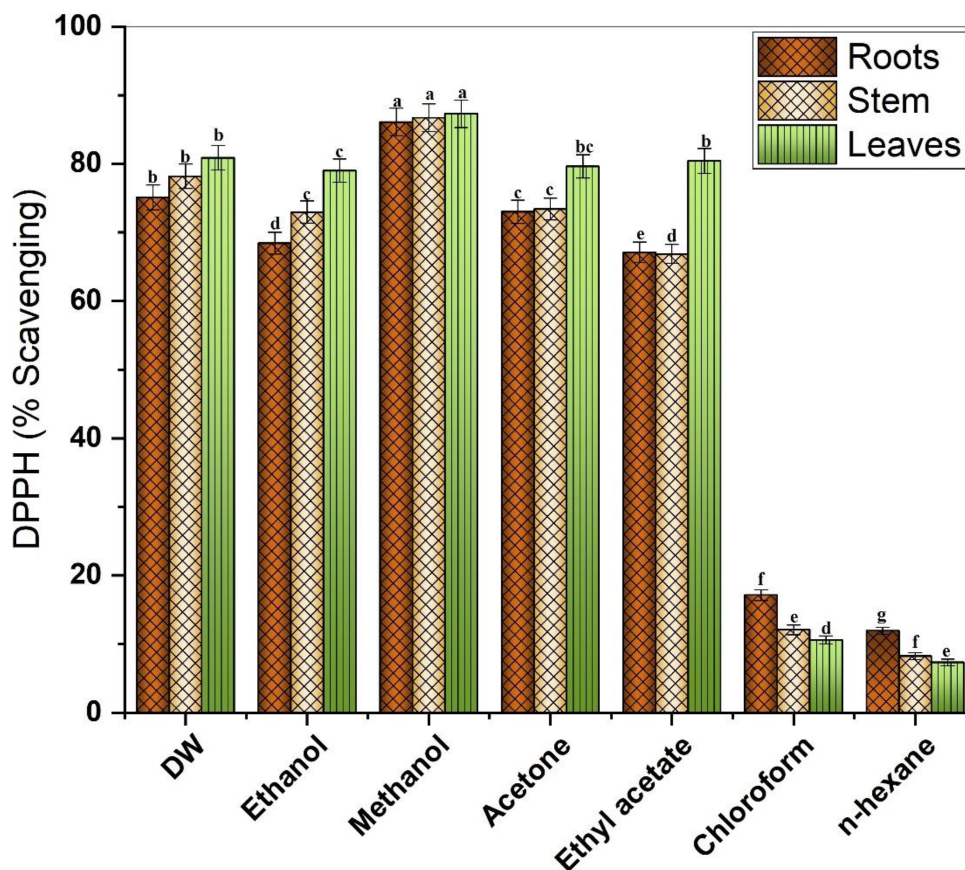

Figure 4.3 DPPH free radical scavenging activity of *T.stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability using LSD.

4.1.2.2. Total antioxidant capacity

The antioxidant activity of the extracts was determined using the phosphomolybdenum technique. The methanolic extract of leaves, stems, and roots was shown to have the maximum reducing property (298.59 ± 14,9 µg AAE/mg, 293.58 ± 14.6 µg AAE/mg, and 289.16 ± 14.4 µg AAE/mg respectively). The significant reducing potential was detected in the leaves' aqueous, ethanolic, methanolic, and acetone extracts (260.35 ± 13.0 µg AAE/mg, 246.97 ± 12.34 µg AAE/mg, 298.59

± 14.9 µg AAE/mg, and 248.77 ± 12.4 µg AAE/mg, respectively). The n-hexane extract of leaves and stems was found to have the lowest reducing property (15.16 ± 0.75 µg AAE/mg and 15.57 ± 0.77 µg AAE/mg respectively). Figure 4.4 displays the complete findings.

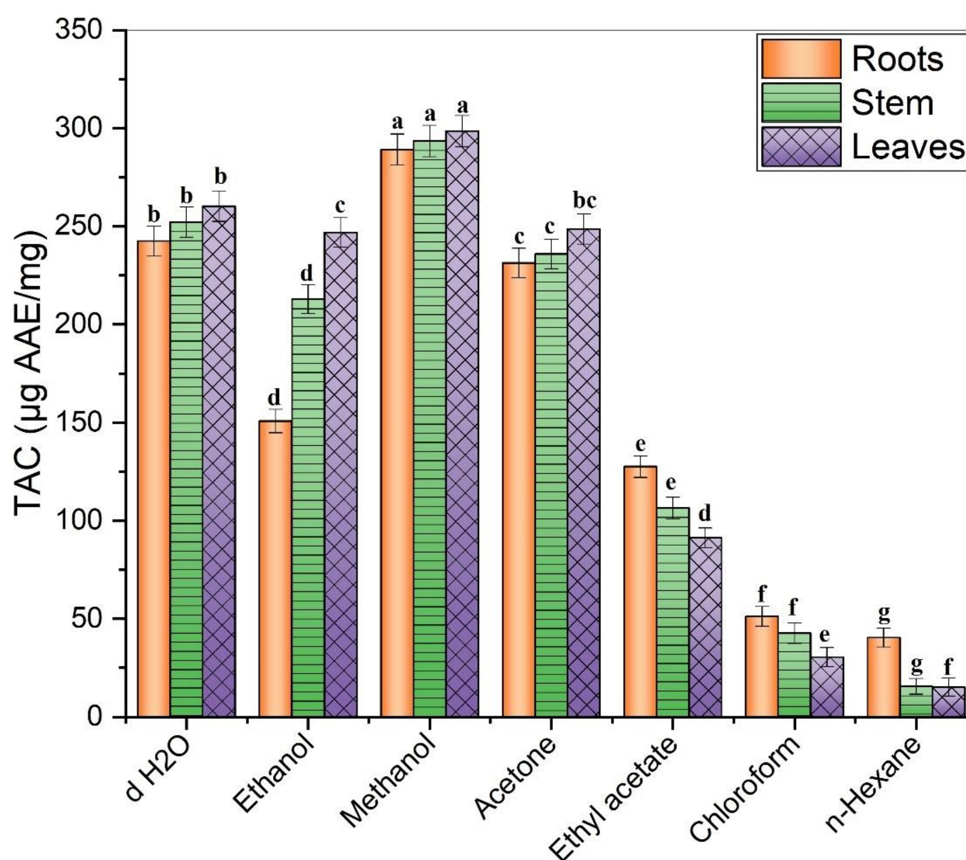

Figure 4.4 Total antioxidant capacity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability using LSD.

4.1.2.3. Reducing power assay

All the extracts showed a potent reducing property. The highest reducing property was reported in the methanolic extract of leaves, stems, and roots (218.20 ± 10.9 µg AAE/mg, 214.52 ± 10.7 µg

AAE/mg, and 206.36 ± 10.3 µg AAE/mg respectively). Aqueous, ethanolic, methanolic, and acetone extract of the leaves displayed significant reducing potential (213.20 ± 10.6 µg AAE/mg, 200.93 ± 10.0 µg AAE/mg, 218.20 ± 10.9 µg AAE/mg, and 202.59 ± 10.1 µg AAE/mg respectively). The lowest reducing property was reported in the case of n-hexane extract of leaves and stems (11.86 ± 0.59 µg AAE/mg, 13.78 ± 0.68 µg AAE/mg). Complete results are given in figure 4.5.

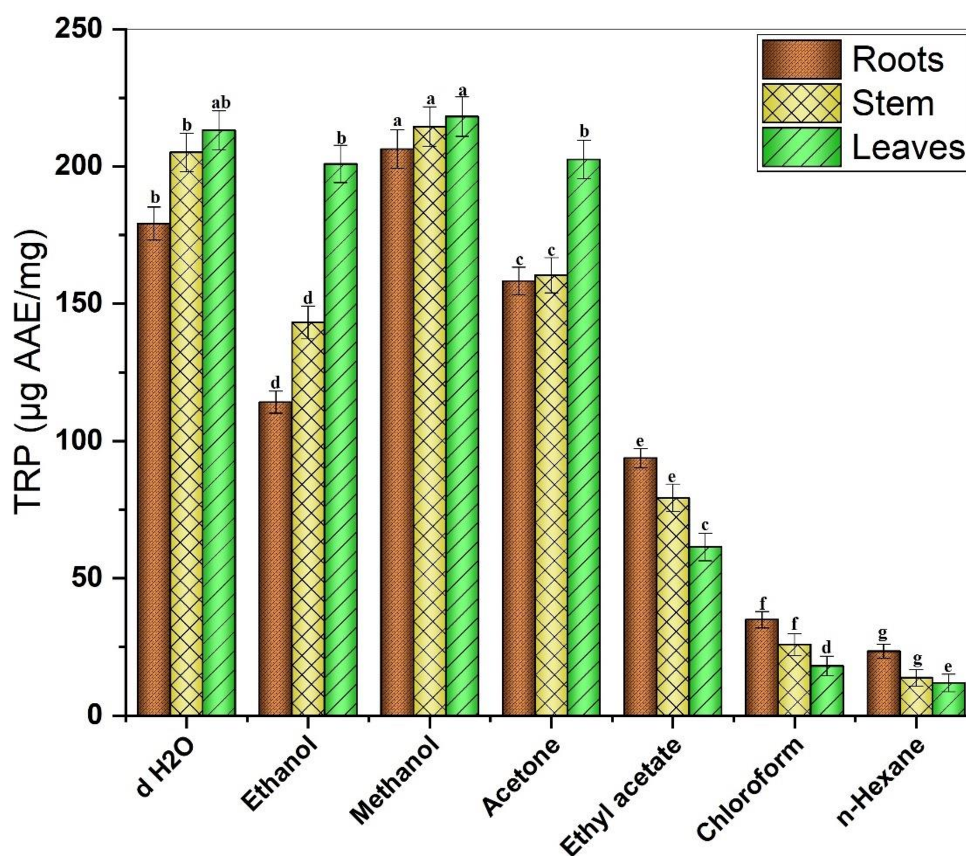

Figure 4.5 Total reducing power of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability using LSD.

4.1.2.4. ABTS

In the case of the stem, root, and leaves highest ABTS activity was reported in methanolic extract (91.92 ± 4.59%, 91.31 ± 4.56%, and 86.54 ± 4.32%). Acetone root and stem extract also unveiled the highest ABTS activity (89.23 ± 4.46%, 89.11 ± 4.45%). Whereas the minimum ABTS activity was reported in n-hexane extract of leaves, roots, and stem i.e. (25.24 ± 1.26%, 29.16 ± 1.45%, and 43.33 ± 2.16%). As can be seen in figure 2, the root, stem, and leaf extracts in ethyl acetate also produced noticeable results. Detailed results are shown in figure 4.6.

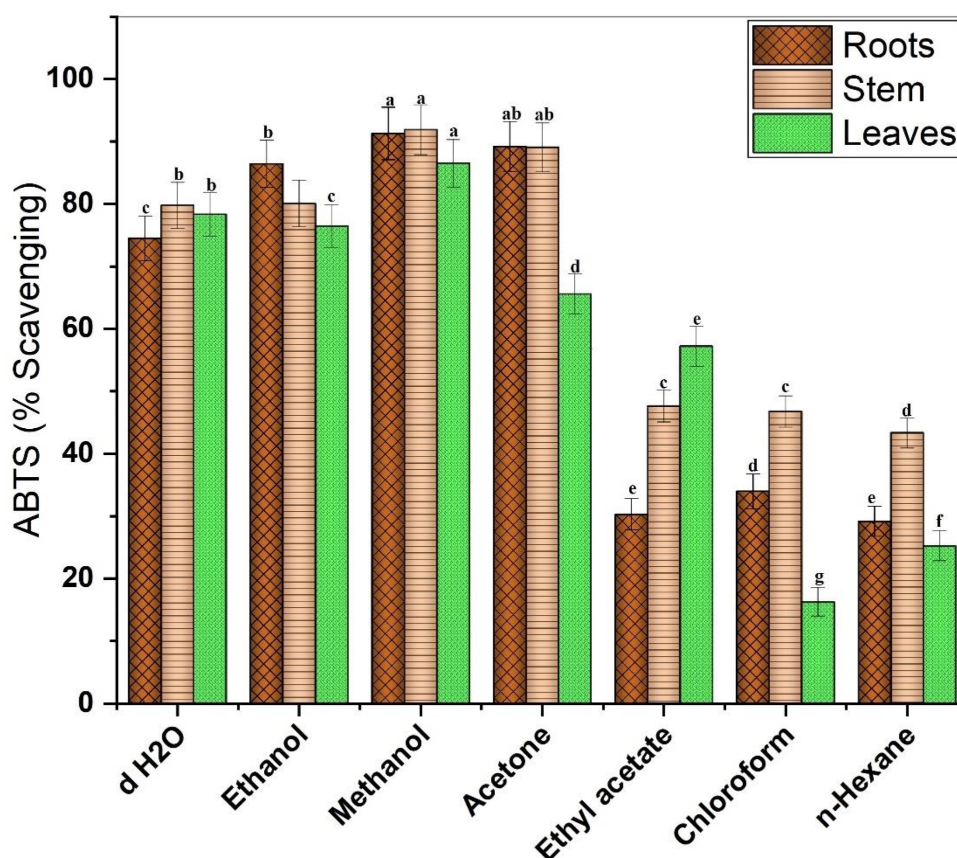

Figure 4.6 ABTS Scavenging activity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability using LSD.

4.1.2.5. Metal Chelating assay

In the present case, the highest MC % was reported in aqueous leaves, root, and stem extract (64.42 ± 3.22 %, 61.89 ± 2.18%, and 43.68 ± 3.09 %,). Ethyl acetate stem and root extract displayed the lowest value (11.47 ± 0.57 %, and 13.32 ± 0.66 %,) respectively. Ethanolic extract of leaves, stems, and roots also showed significant results (45.03 ± 2.25 %, 39.80 ± 1.98 %, and 37.10 ± 1.85 %). Figure 4.7 depicts the detailed results.

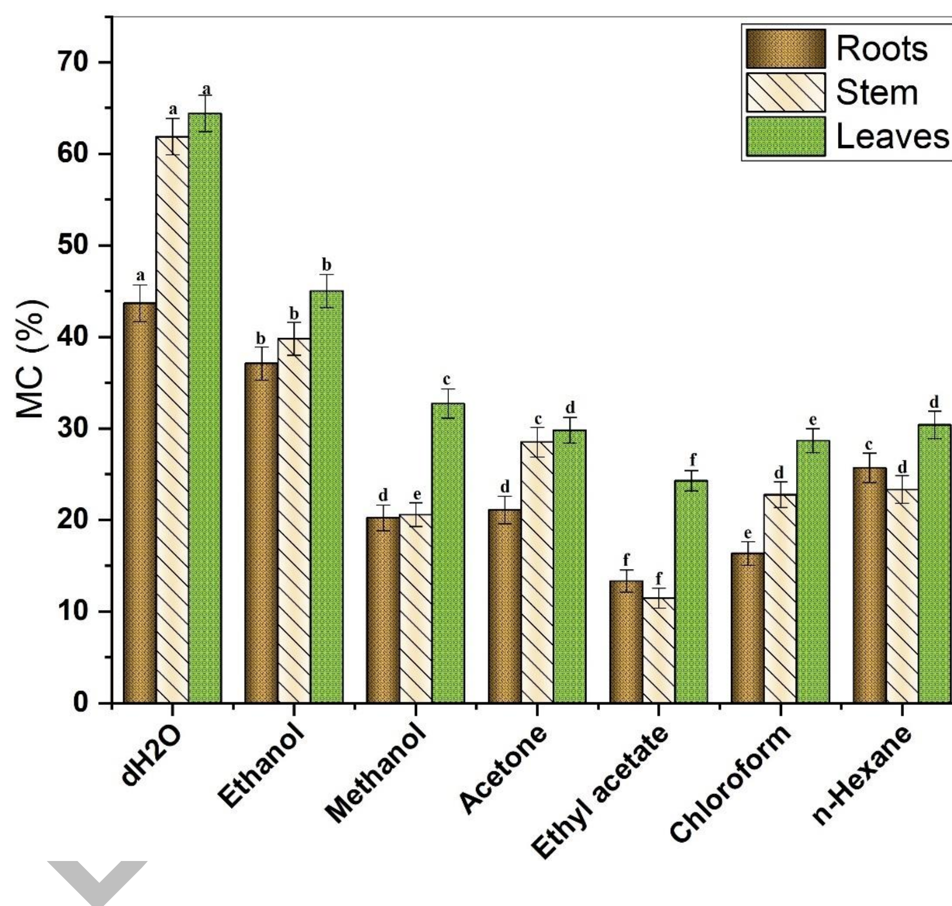

Figure 4.7 MC % of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane). Test results were presented as mean ± S.D. Small letters mentioned on each value show significant differences among mean value at ($p \leq 0.05$) probability

4.1.3. Antibacterial activity of *T. stocksianum*

Antibacterial activity of *T. stocksianum* leaves, roots and stem extracts in seven different solvents (distilled water, methanol, ethanol, acetone, chloroform, n-hexane, ethyl acetate) was assessed against 6 bacterial strains.

Staphylococcus aureus and *MRSA* constituted two gram-positive bacterial strains. Four other strains were gram-negative, whereas the other two (*Klebsiella pneumonia, Salmonella enterica, Pseudomonas aeruginosa, and Escherichia coli*). In Table 4.1, the outcomes of each extract are displayed. The antimicrobial activity of various leaf extracts demonstrated the larger zone of inhibition, for example, the aqueous extract of leaves presented the maximal inhibition zone against *Salmonella enterica, Escherichia coli, and Staphylococcus aureus* (7 mm ± 0.35 each).

With the exception of *Pseudomonas aeruginosa*, leaf methanolic extract was effective against all bacterial strains. The methanolic extract of leaves could only demonstrate a maximum value of 9mm ± 0.45 of inhibitory zone against *Klebsiella pneumonia*. In the case of the stem, n-hexane extract (8mm ± 0.4 zone of inhibition against *Pseudomonas aeruginosa*) demonstrated the highest antibacterial efficacy. Root extracts in acetone and methanol were effective against all bacterial strains.

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Table 4.1 Antibacterial potential of *T. stocksianum* roots, stem, and leaves extracts in seven different solvents (distilled water, methanol, ethanol, acetone, chloroform, n-hexane, and ethyl acetate) against 6 bacterial strains.

Part	Solvent used	*MRSA*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*E. coli*	*Salmonella enterica*	*Klebsiella pneumoniae*
Root	DW	-	6.5±0.32	-	-	-	-
Root	Ethanol	-	-	-	-	-	-
Root	Methanol	7±0.35	7±0.35	8±0,4	7±0.35	8±0.4	6.5±0.32

Root	Acetone	7±0.35	7±0.35	7±0.35	8±0.4	7±0.35	7±0.35
Root	Ethyl acetate	-	7±0,35	7±0.35	7±0.35	-	7±0.35
Root	Chloroform	7±0.35	-	-	7±0.35	-	-
Root	n-hexane	-	-	7±0.35	-	-	-
Stem	DW	-	6.5±0.32	6.5±0.32	7±0.35	-	-
Stem	Ethanol	7±0.35	6.5±0.32	6.5±0.32	-	-	-
Stem	Methanol	7±0.35	8±0.4	-	-	-	-
Stem	Acetone	8±0.4	7±0.35	7±0.35	-	-	-
Stem	Ethyl acetate	-	6±0.3	-	6.5±0.32	-	-
Stem	Chloroform	7±0.35	7±0.35	7±0.35	-	-	-
Stem	n-hexane	-	-	8±0.4	-	7±0.35	-
Leaves	DW	-	7±0.35	-	7±0.35	7±0.35	-
Leaves	Ethanol	7±0.35	-	8±0.4	-	-	8±0.4
Leaves	Methanol	7±0.35	7±0.35	-	8±0.4	8±0.4	9±0.45
Leaves	Acetone	-	7±0.35	-	7.5±0.37	-	7±0.35
Leaves	Ethyl acetate	-	8±0.4	7±0.35	6.5±0.32	8±0.4	-
Leaves	Chloroform	-	7±0.35	7±0.35	7±0.35	7±0.35	-
Leaves	n-hexane	7±0.35	7±0.35	-	-	8±0.4	-

Standard	Tetracycline hydrate	21±1.05	15±0.75	14±0.7	22±1.1	25±1.25	21±1.05
Standard	DMSO	-	-	-	-	-	-

4.1.4. Enzyme inhibition assay

4.1.4.1. Alpha- amylase % inhibition assay

Aqueous and ethanolic extracts of leaves displayed the highest % inhibition (88.57 ± 4.42 **%** and 87.62 ± 4.38 **%).** n-hexane extract of stem showed the lowest inhibitory activity (26.38 ± 1.31 %). In the case of roots, the aqueous extract showed the maximum % inhibition, while the extract of n-hexane unveiled the minimum inhibitory value. Table 4.2 details the findings.

Table 4.2 Alpha- amylase % inhibition of *T. stocksianum* leaves, roots, and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane).

Part	Solvent used	Alpha-amylase % inhibition
Root	DW	75.91 ± 3/79
Root	Ethanol	42.25 ± 2.11
Root	Methanol	48.89 ± 2.44
Root	Acetone	71.57 ± 3.57
Root	Ethyl acetate	59.26 ± 2.96
Root	Chloroform	46.00 ± 2.29
Root	n-hexane	28.18 ± 1.40
Stem	DW	83.40 ± 4.42
Stem	Ethanol	73.60 ± 3.68
Stem	Methanol	62.98 ± 3.14
Stem	Acetone	69.26 ± 3.46
Stem	Ethyl acetate	47.83 ± 2.39
Stem	Chloroform	37.12 ± 1.85

Stem	n-hexane	26.38 ± 1.31
Leaves	DW	88.57 ± 4.42
Leaves	Ethanol	87.62 ± 4.38
Leaves	Methanol	70.33 ± 3.51
Leaves	Acetone	74.76 ± 3.73
Leaves	Ethyl acetate	84.23 ± 4.21
Leaves	Chloroform	49.28 ± 2.46
Leaves	n-hexane	28.62 ± 1.43
Standard	Acarbose	95.80 ± 4.79

4.1.4.2. Urease % inhibition

In the case of roots, acetone and ethyl acetate extract showed the highest urease % inhibition i.e. 75.66 ± 3.78% and 75.66 ± 3.78%. Ethanolic extract of roots also displayed appreciable results i.e. 75.12 ± 3.75%. Ethyl acetate extract of leaves showed maximum inhibitory activity of 79.79 ± 3.78%. Overall, all the leaf extracts showed excellent results. Table 4.3 presents the results in detail.

Table 4.3 Urease % inhibition of *T. stocksianum* leaves, roots, and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane).

Part	Solvent used	Urease % inhibition
Root	DW	74.98 ± 3.74
Root	Ethanol	75.12 ± 3.75
Root	Methanol	46.23 ± 2,31
Root	Acetone	75.66 ± 3.78
Root	Ethyl acetate	75.66 ± 3.78
Root	Chloroform	66.20 ± 3.31
Root	n-hexane	62.7 ± 3.13

Stem	DW	75.25 ± 3.76
Stem	Ethanol	75.79 ± 3.78
Stem	Methanol	48.53 ± 2.42
Stem	Acetone	74.30 ± 3.71
Stem	Ethyl acetate	75.66 ± 3,78
Stem	Chloroform	65.39 ± 3.26
Stem	n-hexane	63.18 ± 3.15
Leaves	DW	78.71 ± 3.93
Leaves	Ethanol	76.07 ± 3.80
Leaves	Methanol	75.93 ± 3.79
Leaves	Acetone	75.52 ± 3.77
Leaves	Ethyl acetate	79.79 ± 3.98
Leaves	Chloroform	65.66 ± 3.28
Leaves	n-hexane	65.93 ± 3.29
Standard	Thiourea	90.30 ± 4.51

4.1.4.3. Lipase % inhibition

Ethyl acetate extract of root showed a maximum inhibitory value of 76.94 ± 3.84%. In the case of stem and leaves, ethyl acetate displayed the highest lipase % inhibition 80.46 ± 4.02% and 81.34 ± 4.06% respectively. n-hexane extract of root, stem, and leaves showed the lowest inhibitory value. Table 4.4 presents the results in great detail.

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Table 4.4 Lipas**e %** inhibition of *T. stocksianum* leaves, roots, and stem extracts in seven different solvents (water, absolute methanol, ethanol, acetone, chloroform, ethyl acetate, and n-hexane).

Part	Solvent	Lipase % inhibition
Root	DW	72.18 ± 3.60
Root	Ethanol	61.44 ± 3.07
Root	Methanol	67.25 ± 3.36
Root	Acetone	71.65 ± 3.58
Root	Ethyl acetate	76.94 ± 3.84
Root	Chloroform	56.02 ±2.80
Root	n-hexane	52.32 ± 2.61
Stem	DW	70.25 ± 3.51
Stem	Ethanol	43.13 ± 2.15
Stem	Methanol	65.14 ± 3.25
Stem	Acetone	68.84 ± 3.44
Stem	Ethyl acetate	80.46 ± 4.02
Stem	Chloroform	60.89 ± 3.04
Stem	n-hexane	47.22 ± 2.36
Leaves	DW	72.89 ± 3.64
Leaves	Ethanol	68.66 ± 3.43
Leaves	Methanol	67.67 ± 3.38
Leaves	Acetone	73.42 ± 3.67
Leaves	Ethyl acetate	81.34 ± 4,06
Leaves	Chloroform	62.30 ± 3.11
Leaves	n-hexane	57.61 ± 2.88
Standard	Orlistat	91.40 ± 4.57

5. DISCUSSION

Due to their less detrimental effects on humans than synthetic medications, plant-based natural remedies are utilized to treat various diseases. The utilization of medicinal plants to cure various illnesses, including infections, diabetes, cancer, swelling, heart conditions, and others, has many benefits in the world. (Awal et al., 2004). Native people frequently use herbal remedies in developed and developing nations alike, and these remedies have been studied to determine their active ingredients. Various plant species are being employed in herbal treatments in traditional medical systems, where the entire plant, a part of it, or an extract is taken. Recently, people have become more aware of the negative consequences of synthetically created chemicals, which has led to 80% of people using plant-derived medicines to treat illnesses and understanding their advantages (Hamayun et al., 2006).

The present study deals with in vitro assays of various extracts of the medicinal plant, *T. stocksianum*. In vitro testing involved four different assays: antioxidant, antibacterial, phytochemical analysis, and enzyme inhibition assays.

4.1.5. Phytochemical analysis

To measure the TPC and TFC, various *T. stocksianum* extracts studied in various solvents. The class of organic aromatic compounds known as phenolics includes those in which an aromatic hydrocarbon is connected to a -OH group. One of the indicators used to assess oxidative stress is total phenolics (Inderjit and Nilsen, 2003). Polyphenols and other oxygenated phytoconstituents are considered to be highly antioxidant and capable of scavenging free radicals (Devasagayam et al., 2004). Polyphenols in medicinal plants have received increased attention as a result of some interesting discoveries about their biological functions. The most significant pharmacological effect of polyphenolic compounds is their antioxidant capacity, particularly their ability to scavenge free radicals and prevent lipid peroxidation. In the current study, phenolic contents ranged from 38.92 ± 1.94 to 3.52 ± 0.17 µg GAE/mg. The highest quantities of phenolics were found in the methanolic extract of the leaves and roots, which had phenolic concentrations of 38.92 ± 1.94 µg GAE/mg and 33.55 ± 1.67 µg GAE/mg, respectively. Phenolic levels in n-hexane were significantly lower (3.52 ± 0.17 µg GAE/mg), which is consistent with other comparable results.

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(Sahreen et al., 2010). The chemical composition of the extraction solvent affects the detection of phenolic components in plant extract (Kumar et al., 2013).

Because phenols have antioxidant, antimutagenic, and anticancer activities, the amount of phenolic components in the compounds is closely related to their antioxidant activities (Ahmad and Mukhtar, 1999). Flavonoids, like phenolic substances, are secondary plant metabolites that are subjected to a variety of adverse situations such as low nutrition levels, oxidative stress, low temperatures, damage, or infection. Natural substances called flavonoids, or polyphenols, can be found in foods including fruits, vegetables, nuts, and alcoholic beverages such as red wine, coffee, and tea (Nabavi et al., 2008). In the present study, flavonoid contents were seen in the ranges from 40.39 ± 2.01 to 2.13 ± 0.10 µg QE/mg. The highest amount was observed in the methanolic extract of leaves (40.39 ± 2.01 µg QE/mg), whereas the lowest was reported for the n-hexane extract of the stem (2.13 ± 0.10 µg QE/mg). According to earlier research, plants possess a high concentration of flavonoids. The results suggest that flavonoids and phenolics are the main sources of the antioxidative properties and inhibitory effects against oxidative processes in vitro and in vivo.

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4.1.6. Antioxidant activities

Because antioxidants function in a variety of ways, the mode of action of all antioxidants in a sophisticated structure cannot be replicated in a single assay. Two methods describe the mechanism of antioxidants i.e. free radical scavenging by hydrogen donation to form a stable compound (Prior et al., 2005) and reduction of free radicals by donating an electron. With a nitrogen core and the ability to take an electron or a hydrogen radical to form a stable diamagnetic molecule, DPPH is a stable free radical. When DPPH radicals interact with the proper reducing agents, the amount of electrons consumed determines the color loss spectrophotometrically at various wavelengths (Singleton et al., 1999). The DPPH test, a non-enzymatic method, is being employed to deliver fundamental knowledge on free radical scavenging. Commercially, DPPH is offered in a rich purplish color. All extracts showed an increase in DPPH activity. The fundamental knowledge of the capacity of various substances to scavenge free radicals is provided by this assay. The substance reduces DPPH to diphenyl picryl hydrazine, a pale-yellow substance. This

characteristic was used to assess the scavenging capacity of leaves, fruits, and their various fractions. All fractions in the current investigation demonstrated strong antioxidant activity by providing outstanding DPPH values. Methanolic extract of leaves, stem, and root show maximum value (87.29 ± 4.36, 86.73 ± 4.33, and 86.10 ± 4.30% inhibition) followed by aqueous extract of leaves, stem, and root by giving values of 80.89 ± 4.04, 78.18 ± 3.90, and 75.11 ± 3.75% inhibition, respectively.

Total antioxidant capacities of different fractions were checked using the phosphomolybdenum method and capacities are expressed in ascorbic acid equivalent. This test relies on the reduction of phosphomolybdate ion in the presence of an antioxidant, which results in the formation of a green phosphate complex, which is detected spectrophotometrically (Prieto et al., 1999). The result of the extracts of *T. stocksianum* in different solvents has great antioxidant potential. All extracts showed a good antioxidant capacity especially the methanolic extract of leaves, stem, and root showed maximum antioxidant capacity (298.59 ± 14.92, 293.58 ± 14.67, and 289.16 ± 14.45µg AAE/mg, respectively). n-hexane and chloroform extract of leaves, stems, and roots showed comparatively low antioxidant activity. In this test, the extract converts Mo (VI) to Mo (V) under acidic conditions, resulting in the formation of a phosphate Mo (V) complex that is green in color (Prieto et al., 1999). The reducing power capabilities of *T. stocksianum* extracts, given as ascorbic acid equivalents, were also assessed. By detecting the change of potassium ferri-cyanide ($Fe3+$) to potassium ferrocyanide ($Fe2+$) in the presence of plant extract, the reductive capability was examined (Oyaizu, 1988). The presence of reductants, which use antioxidant action by donating a hydrogen atom and breaking the free radical chain, is typically linked to the reducing property of the extracts. The maximum reductive ability was found in the methanolic extract of the leaves, stems, and roots in our investigation. *T. stocksianum* extracts' lowering ability suggests that they will probably contribute significantly to the overall antioxidant impact. It has been proven that plant extracts' antioxidant activity, however, works through a number of different mechanisms, including the binding of heavy metal ions to ion catalysts, the dissolution of peroxides, the prevention of chain initiation, the reductive capacity on metals, and radical scavenging. Numerous researchers that explored the connection between total polyphenol content and the ability to scavenge free radicals found a linear link between the two. Therefore, it can be concluded that the

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numerous polyphenols included in *T. stocksianum*. The regulation of oxidative stress, metal reductive capacity, and radical scavenging are all significantly influenced by extracts. Another radical cation decolorization technique to assess the antioxidant capacity of samples is the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay. It works on the principle of hydrogen atom transfer (Sajjad et al.) mechanism. When ABTS is incubated with $Na_2S_2O_8$ it forms ABTS cation (ABTS•+) that is deep blue and is highly reactive towards antioxidants. When mixed with hydrogen-donating antioxidants, it rapidly decolorized by accepting an electron pair and become nonradical (Kouris-Blazos and Belski, 2016). In the case of the stem, root, and leaves highest ABTS activity was reported in methanolic extract (91.92 ± 4.59%, 91.31 ± 4.56%, and 86.54 ± 4.32%). The capacity to chelate metals is crucial because it lowers the metal concentration, which catalyzes the oxidation of lipids. In addition, because they lower the redox potential and hence stabilize the oxidized metal ions, metal chelating. All the extracts displayed appreciable MC% in the current study. The highest MC % was shown by aqueous root, stem, and leaves extract.

Moreover, the present study displayed a positive correlation between TFC and ABTS which implies that maximum flavonoids present in callus culture were responsible for enhanced ABTS activity.

4.1.7. Antimicrobial activity

The plant part, extraction solvent, and organism being tested all have a major impact on predictions of the antibacterial activity of herbal compounds isolated from plant parts. The present perception of antibiotic resistance as a severe problem necessitates the development of novel strategies and the identification of novel antimicrobial compounds with therapeutic promise (Latha et al., 2007). For the investigation of powerful and effective antibacterial drugs, numerous studies had been approved. In the current investigation, root, stem, and leaf extracts of *T. stocksianum* were subjected to an antibacterial assay against six bacterial strains in various solvents. By measuring the inhibitory zone surrounding the disc, the activity was estimated. All of the chosen strains were inhibited by the root's acetone extract's antibacterial activity. Methanolic extract of the root also showed results against all the strains. Ethanolic extract of root did not display activity against any

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strain. This antibacterial activity might be helpful to isolate compounds of biological significance and for rational drug designing. The antibacterial efficiency of *T. stocksianum* oil was previously evaluated using the disc diffusion method against a panel of seventeen bacterial and six fungal species. All test organisms and the growth of the yeasts, yeast species, *Staphylococcus* and *Streptococcus* species were significantly and variably suppressed by the oil (Hisham et al., 2006).

One of the proposed mechanisms for phenolic toxicity to microbes is enzyme inhibition by the oxidized compounds, perhaps through reactivity with sulfhydryl groups or through more general interactions with the proteins (Cowan, 1999).

4.1.8. Enzyme inhibition assay

The conversion of oligo- and/or disaccharides to monosaccharides are carried out by the -glucosidase enzymes, such as -amylase. Inhibitors of these enzymes cause a notable decrease in the rate of glucose uptake, which delays the subsequent rise in plasma glucose and extends the period of time needed for starch digestion overall. Acarbose, miglitol, and voglibose are a few examples of these inhibitors that are utilized in medical practice to manage diabetes. However, these medications are known to cause several gastrointestinal adverse effects in patients, (Bhutkar and Bhise, 2012). 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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6. CONCLUSION

Research on *T. stocksianum* extracts shows that this plant exhibits significant antioxidant and free radical scavenging properties. This study demonstrates extracts of leaves, stems, and roots of *T. stocksianum* in various solvents have significant in vitro activities. Methanolic extract of leaves, stem, and root part showed excellent antioxidant, phenolic, and flavonoid contents, and enzyme inhibition. The presence of phenolics and flavonoids in the root's methanolic extract may have contributed to its superior antimicrobial performance when compared to other extracts during antimicrobial screening of different extracts.

7. FUTURE PROSPECTIVE

The findings of this study suggested that this plant species is advantageous from a therapeutic perspective. However, more research is needed to isolate and purify the chemicals of biological relevance found in this plant as well as to understand its potential mode of action in the management of oxidative stress and other bioassays. To emphasize the importance of in vitro screening, further biological activity comparisons may be useful. In vivo research should be conducted shortly, according to the pharmacological perspective.

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