PHYTOCHEMICAL SCREENING AND ANALYSIS OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES IN *OXYSTELMA ESCULENTUM* R.BR.

A
Thesis

Submitted to the
UNIVERSITY OF KOTA

in the Partial Fulfillment of the Requirements for the
Award of the Degree of
DOCTOR OF PHILOSOPHY

in
Botany

under the
Faculty of Science

Submitted by
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Under the Supervision of
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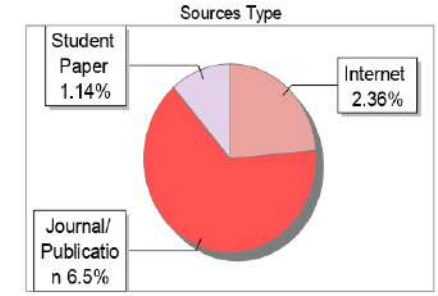

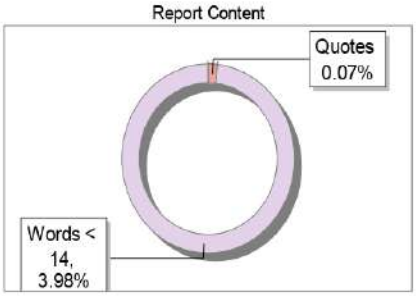

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Regards

(**Rajendra Meena**)

Research Scholar

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ABSTRACT

Oxystelma esculentum R.Br. is an important medicinal plant belongs to Asclepiadaceae family and commonly known as 'Jaldudhi'. It is a perennial twiner found throughout the plains of the Indian sub-continent near water-logged areas. The plant is used as laxative, antiseptic, depurative, anthelmintic, antiulcer, aphrodisiac, hepato protective and useful in leucoderma and bronchitis. Decoction of plant is used in ulcer, sore-throat and itches. Milky juice is used as galactogogue, antiperiodic, antiulcer and as a vulnerary. Leaves are used as antiperiodic. Its root is prescribed in jaundice. Fruit is bitter, tonic, expectorant, anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent.

Major objectives of the research work are preliminary phytochemical investigation, to assess the antibacterial and antifungal activities of the methanol crude extract of flowers and fruits, to check the antioxidant potential of the plant parts (flowers and fruits) and to study their free radical scavenging effects and to separate and identify the compounds from methanol extract using GC-MS, HPLC and FT-IR.

In the present study, the preliminary phytochemical screening of methanol extract of fruits and flowers of the plant was carried out and the results revealed the presence of alkaloids, terpenoids, glycosides, steroids, flavonoids, phenolics, tannins and saponins etc. In qualitative phytochemical screening, the methanol extracts of fruits contained strong phytochemicals such as alkaloids, terpenoids, phenolics, saponins and flavonoids followed by flowers. In quantitative phytochemical screening, the methanol extracts of fruits showed better results than flower extracts. The radical scavenging effects of crude extract of fruit and flower were evaluated against DPPH, ABTS·+, hydrogen peroxide, superoxide radical, nitric oxide radical, hydroxyl radicals scavenging assay, ferric reducing antioxidant power, total phenol, total flavonoids content and total antioxidant activity. Fruit extracts in methanol demonstrated higher levels of tested antioxidants than flower extracts. *O. esculentum* R.Br. was further tested for antibacterial and antifungal activity. The methanol extract was tested for antibacterial activity against gram positive *Staphylococcus aureus* and gram

negative *Escherichia coli*. Antifungal activity was tested against *Candida albicans* and *Aspergillus flavus*. When compared to other tested strains, the methanol extract of fruits of the plant had the highest mean zone of inhibition against *Staphylococcus aureus* (22.5±0.35 mm at 1000 µg/disc). Methanol extracts had the lowest minimum inhibitory concentration (21.50 µg/mL) and minimum bactericidal concentration (38.25 µg/mL). The lowest zone of inhibition (9.5±0.43 mm at 250 µg/disc) was observed in a methanol extract of the plant flowers against *E. coli*. In fungi, the highest mean zone of inhibition (12.5±0.50 mm at 1000 µg/disc) was recorded with methanol extract of flowers of *O. esculentum* R.Br. against *Candida albicans*. Methanol extracts of fruits and flowers had the lowest MIC (500 µg/mL) and MFC (1000 µg/mL) values against *Aspergillus flavus* and *Candida albicans*. As a positive control for bacteria, ciprofloxacin (10 µg/disc) was used and the mean zone of inhibition ranged between 24.0±0.65 mm and 29.80±0.56 mm. Positive controls for *Candida albicans* and *Aspergillus flavus* were amphotericin-B (100 units/disc) and ketaconazole (5 µg/disc), respectively and the mean zone of inhibition ranged from 14.50±0.50 to 21.0±0.76 mm. The methanol extracts of fruits of *O. esculentum* R.Br. was further subjected to carry out GC-MS characterization due the highest activity against tested microbes. The compounds were identified in the crude extract such as 2-Chloroethyl methyl sulfoxide, Acetic acid, methyl ester, Trichloromethane, n-Hexadecanoic acid,9(E), 11(E)-Conjugated linoleic acid, 6-Octadecenoic acid, (Z)- etc. The methanol extracts of flowers of *O. esculentum* R.Br. showed presence of 1- Heptacosanol, .beta.-Sitosterol acetate, 9-Octadecen-1-ol, acetate,(Z), Phenol, 2,4- bis(1,1-dimethylethyl)-, phosphite,dl-.alpha.-Tocopherol compounds in GC-MS characterization.

In conclusion, the study proved the efficiency of antimicrobial and antioxidants properties of *Oxystelma esculentum* R.Br. and advocates the potentiality of the plant as a source of alternative medicine. Presence of antioxidants in the crude extracts leads to delay or prevent oxidative damage to compounds. Therefore, antioxidants may serve to control the level of free radicals. So, use of natural products, especially fruits of *Oxystelma esculentum* R.Br. may be considered as a new source of natural antioxidant and antimicrobial agents.

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LIST OF ABBREVIATIONS

°C	-	Degree Celsius
µg	-	Microgram
µL	-	Microlitre
ABTS	-	2,2'-Azinobis-(3-thylbenzothiazoline-6-sulfonic acid)
BHT	-	Butylated hydroxyl toluene
DMSO	-	Dimethyl sulphoxide
FT-IR	-	Fourier transform infrared spectra
g	-	Gram
GAE	-	Gallic acid
GC-MS spectrometry	-	Gas chromatography mass
H_2O_2	-	Hydrogen peroxide
HPLC chromatography	–	High performance liquid
IC_{50}	-	Half maximal inhibitory concentration
L	-	Litre
MBC	-	Minimum bactericidal concentration
MFC	-	Minimum fungicidal concentration
mg	-	Milligram
MIC	-	Minimum inhibitory concentration
mL	-	Millilitre
mm	-	Millimeter
R_f	-	Retention factor
TLC	-	Thin layer chromatography

CHAPTER – I

INTRODUCTION

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INTRODUCTION

The Vedic period (or Vedic years) (2500 BC to 600 BC) was the period in Indian history when the Vedas, the oldest Indian texts, were named. In Rigveda, three groups of plants were recognized, viz. trees (Vriksha), herbs (Osadhi) and creepers (Virudh). In the Atharvaveda genus, the structure and growth of plants are also described. In Yajurveda four groups of medicinal plants are described.

The most famous plant often mentioned in Rigveda and later Samhitas is the Soma plant. Vedic Indians worship Soma as King of the jungle (Vanaraja). Ownership of Soma plants, however, has not been determined to this day. Potential candidates are Ephedra (Gymnosperm); Sarcostemma (flowering plant) and Mushrooms (a fungus commonly called Agaricus). The second most mentioned plant was Peepal or Asvattha (*Ficus religiosa* L.) during the Vedic period. Rigveda refers to vessels and utensils made of wood from the Asvattha tree. Some of the other trees mentioned in the Vedas are Shalmali (*Salmalia malabaricum*), Khadira (*Acacia catechu*), Simsupa (*Dalbergia sissoo*), Vibhitaka (*Terminalia bellerica*), Sami (*Prosopis sp.*), Plaksa (*Ficus infectoria*), lksu (*Saccharum offcinarum*) is mentioned as a cultivated plant in Atharvaveda, Maitaryani Samhita, and other sources.

Vedic Indians were familiar with many flowering and fruit-bearing plants, such as Palas (*Butea monosperma*), two varieties of Kamala (Lotus)- white (Pundarika) and blue (Puskara), Kumuda (White lily), Urvaruka (Cucumber), Badara (*Zizypus jujuba*), Dumbara (*Ficus glomerata*), Kharjura (*Phoenix dactylifera*) and Bilva (*Aegle marmelos*), etc. Various ancient written documents, in the form of manuscripts, are available in Sanskrit and a few other Indian languages. Sanskrit books include the Vedas, Upanisada, and epics such as Ramayana and Mahabharata. Professional literature on medicinal plants with details of morphology and pharmacology is described in works such as Charaka Samhita and Sushruta Samhita, dictionaries such as Medinikosa and Amarakosa, and encyclopedic works such as Arthashastra and Brihat Samhita are also available.

Atharvaveda also contains descriptions of many medicinal plants. Common references to many regions within the plant are found scattered throughout Rigveda and almost complete details of the plants are found in Atharvaveda. Here we can say that Atharvaveda is perhaps the first recorded authority on plant life.

Charaka Samhita written by Maharishi Charaka is the first text in Ayurveda. Along with Sushruta Samhita, It is among the two most important surviving texts in ancient India. The earliest books were written in the period 900 BC-600 BC while the later editions of Charaka Samhita were written centuries later.

Sushruta Samhita is an important document of classical Sanskrit in medicine. It was written by Maharishi Sushruta, usually written about 600 BC. It is among the earliest Ayurvedic inscriptions near Charaka Samhita. Sushruta Samhita, in its present form, in 184 chapters contains descriptions of diseases, medicinal plants, preparations from mineral sources and animal sources.

For over five millennia, the Siddha system of medicine, also recognized as the Tamil system of medicine, has served humanity. Siddha is said to have come from Lord Shiva, according to legend. It was then handed on to Parvathi, Nandhi, and lastly Sitars, his consort. It has a holistic approach that values the body, mind, and spirit equally. Its goal is to harmonize the mind and body. The therapeutic materials were divided into three categories: i) herbal goods, ii) inorganic substances, and iii) animal products (Thaavaram). Siddha treatments are widespread in rural areas of our country and are simple and inexpensive.

Unani medicine is an Indian subcontinent-based traditional medicine system. The Unani medical system started in Greece. Unani medicine evolved over four centuries in four separate geographical belts: 1. The Greek period, 2. The Arab-Persian period, 3. The Spanish period, and 4. The Indian period. The human body, according to this traditional philosophy, consists of four basic elements: earth, air, water, and fire, which have cold, hot, wet, and dry temperaments, respectively. Blood, phlegm, yellow bile, and black bile are the four senses of human that make up the body fluids. Pulse, stool, and urine examinations, as well as standard physical examinations, are used in traditional

Unani diagnosis. In the Unani system, there are three types of treatment: Regimental therapy (Ilajbil tadbeer), Pharmacotherapy (Ilajbil dava), Surgery (Ilajbil Yad).

According to the Indian Medicines Plant Board (AYUSH Department, Government of India), India is one of the 17 mega countries featuring an extensive array of natural resources. India provides about seven percent of the global biodiversity. From the Himalayas to the sea, from the desert to the natural rain forest ecosystem, medicinal plants are found. More than 7000 plant species are referred to as the medicinal plants of 17,000-18,000 flowering plants in India. The Ayurveda, Siddha and Unani herbal systems contain more than 90% plant compounds.

In 2014-15, There is anticipated domestic demand for medicinal herbs to be 1,95,000 MT. In 2014-15, the overall use of raw herbal medicines in the country was predicted to be 5,12,000 MT. About 22% of the product is dependent on investments in the 1,178 kinds employed in 242 types that trade over 100 MT each year. Between 2014 and 2015, the export the market for therapeutic plants is anticipated to reach 1,34,500 MT, with an export value of rupees 3,211 crore.

The study of phytochemicals, which notably the secondary metabolites that plants make and store, is referred to as phytochemistry. It considers the structural makeup, metabolic routes, and functions of living organisms, such as plants. Microbiology, environmental sciences and restoration, botany, dietary intake, biochemistry, medicine, pharmacy, and biotechnology and other fields all use phytochemistry. It might be classified as a botany or chemical subfield. This field of study is critical for determining the active components in medicinal plants, quantifying them, and analyzing the positive and negative impacts on human health. It also discusses how to source these active substances, how to classify them based on the functional organic chemical groups to which they belong, and how to ensure quality using analytical techniques. This is the chemical-based evaluation of plant primary and secondary metabolites.

Herbal remedies are a valuable resource for new medicines worldwide. Consumption of therapeutic plant material is growing rapidly around the world, due to the rising need for complementary therapies, natural health products, and

secondary metabolites of therapeutic plant material. Worldwide, between 50,000 and 80,000 species of flowering plants are employed in medical purposes, as per the International Union for Conservation of Nature, the World Wildlife Fund. About 15,000 certain species face extinction due to over harvest and habitat loss, and 20% of their wildlife resources have already been reduced due to population growth and crop use. Plants used for medicinal purposes are at risk of extinction because of the quick extinction of species and habitat damage worldwide, particularly in China, India, Kenya, Nepal, Tanzania and Uganda.

Several sets of conservation recommendations have been compiled, including the need for integrated conservation measures based on both *In-situ* as well as *Ex-situ* strategies, in addition to the development of animal species selection and status monitoring systems. Continued use of wild resources can be an effective conservation strategy for therapeutic plants with restricted growing availability. Because of the high requirement for the population, the situation in India, China and South Africa is much worse.

The availability of species is hampered by extreme exploitation, indiscriminate property collection, uncontrolled deforestation, and habitat destruction. Specificity of habitat, distribution range, population size, species diversity, growth rate, and reproductive system are all biological factors linked to endangered species.

Recent studies on herbal remedies attribute their therapeutic capacity to their complex structure, that is composed of primarily of highly bioactive compounds, minerals, vitamins, and furthermore. Medicines, in general, contain only one active substance that is synthesized, whereas herbal remedies are essentially a combination of dozens or even hundreds of chemicals that act synergistically. Furthermore, medicinal plants contain a significant number of vitamins and minerals that are easily engulfed in the human body.

Many recent research articles indicates that vitamins and minerals derived from chemical synthesis do not possess the similar beneficial effect as comparable natural products. It might be because vitamins, minerals, and enzymes have a synergistic and complementary action in natural products, whereas synthetic compounds (vitamins or minerals) are secluded and even obtained in a different

enantiomeric form. Drugs, conversely, have significant disadvantages when compared to medicinal plants, such as various negative consequences, restrictions, and interactions with other substances, drug resistance, and expensive and time-consuming research. Natural compounds, conversely hand, have a greater structural diversity, a more intricate structure, and multiple stereocenters. These are just a few of the arguments in favour of herbal medicines. Furthermore, the World Health Organization (WHO) seeks to increase the application of traditional medicine to enhance the health-care system.

Several studies have been conducted to investigate this plant derived compounds. Natural substances are classified into the following major categories based on their function in living organisms:

- Primary metabolites, molecules that are found universally in the plant kingdom and are found in all biological systems because they are components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle. Primary metabolites are essential due to the importance of these and other primary pathways in allowing a plant to synthesise, assimilate, and degrade organic compounds. Proteins, fats, sugars, nucleic acid, and other substances are main examples of primary metabolite.
- Seondary metabolites, are compounds that may be unique to different species due to the evolution of a particular phylogenetic group. Many thousands of secondary metabolites have been isolated from plants, and plenty of these individuals have the powerful biochemical consequences in humans and are applied as pharmaceuticals. Secondary metabolites have only been clearly recognized as having important functions in plants since the late twentieth century. Terpenoids, Phenolic acids, Alkaloids, Flavonoids, Saponins, and Tannins are some examples of metabolites that are secondary. Its only role in plants for the sake of safety, food storage, energy, and pathogen resistance.

The alkaloids are a kind of broad range of metabolites that are secondary, found in plants that heterocyclic compound having nitrogen atoms. "Naturally occurring basic, complex, nitrogenous organic, heterocyclic ring compound of plant origin having physiological and pharmacological qualities," Ladenburg characterized alkaloids. The first alkaloid, Morphine was discovered by the

German chemist Friedrich Serturner from *Papaver somniferum* in 1805, sparked their research in the nineteenth century. Till date, about twelve thousand alkaloids are known to exist.

The majority of the alkaloids are bitter, crystalline, solid, and very toxic. Conine and nicotine are two examples of liquids among them. The residual alkaloids readily dissolve in a variety of solvents, including benzene, chloroform, and ether. These biochemicals react with inorganic acids to generate salts due to their alkaline nature. Alkaloids shield plants from animals and insects. As insecticides and pesticides, nicotine sulphate and azadirachtin, which come from tobacco and neem, respectively, are applied. As a N reservoir, they could take part in protein synthesis, according to some experts. Certain alkaloids are used as medications, including morphine, ergotine, quinine, and emetine.

Terpenes are a class of secondary metabolites that have variety of functions. Many terpenes are essential components of vital oils and play important roles in primary metabolism, such as electron carriers, hormones, terpene–derived compounds, and photosynthetic pigments. There are several types of terpenoids, including monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids (carotenoids), and polyterpenoids (Raaman, 2006). Lycopene, a tomato red pigment, is a tetraterpenoid. Triterpenoids are found in both angiosperms and gymnosperms. Fruits contain a lot of tetraterpenoids and carotenoids. Terpenoids, they perform as growth hormones, antioxidants, pollinator attractants, and reserve sources of food. They are primarily found in leaf glandular trichomes, bud exudates, and bark resins. likewise, it stimulates the healing of plant wounds.

Phenolic substances consist of those that include one or more benzene rings additionally hydroxyl groups. Phenolics have antioxidant effects and may help to prevent oxidative damage disorders. These chemicals are necessary for the reproduction and growth of plants. They also develop as an immune mechanism against infections. All other phenolic group members are phenol derivatives. phenolic chemicals include flavonoids, lignin, anthocyanin, flavonols, tannins, and quinine etc.

Flavonoids are phenolic compounds derived from the C-15 body of flavones. Their central pyran ring's degree of oxidation distinguished them from other phenolic compounds. Their biological properties are altered by the varying degree of oxidation. These metabolites are soluble in water and made up of carbohydrates combined with either hydroxyl compounds or carbohydrates combined with other non-carbohydrate molecules like glucose & phenolic compounds. Flavonoids are biomolecules that have extraordinary physiological effects in plants.

Saponins are glycosides that have foaming properties. They are chemically composed of sugars linked to polycyclic aglycones known as sapogenins. Sapogenins are either triterpenes or steroids.

These primary and secondary metabolites are utilized in medications, laxatives, anticancer, hormones, contraceptives, anaesthetics, anti-inflammatory, and antioxidant agents, among other things. Plants produce metabolites for both essential tasks like growth and development (primary metabolites) and specific functions like pollinator attraction or herbivory defence (secondary metabolites).

The medicinal value of plants is found in certain chemical substances that have a specific physiological action on the human body; these chemical substances are known as phytochemicals. In herbal and homoeopathic medicines, these phytochemicals were used to treat the disease (Chitravadivu *et al*., 2009). Several active principles of many medicinal plants have been isolated and introduced as valuable drugs in modern medical systems thanks to advances in phytochemical techniques. Most plants include antimicrobial chemicals that defend them from invading organisms, particularly bacteria (Silva and Junior, 2010).

Plants have been used to treat various diseases since ancient times. India is well-known for its traditional plant uses; however, in order to promote the proper use of herbal medicine and determine its potential as a source of new drugs, it is necessary to conduct more systematic and scientific research on it (Asolkar *et al*., 1992; Cordell, 2000).

In recent years, there has been an increase in the occurrence of antibiotic resistance to many common bacterial pathogens such as *Staphylococcus aureus*,

Streptococcus pyogenes, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella flexneri*, and *Proteus mirabilis*, as well as fungal pathogens such as *Aspergillus flavus* (Russell, 2002). Drug resistance in human pathogens to commonly used antibiotics has necessitated the search for new antimicrobial substances from other sources, including plants. Making antibacterial therapy effective, safe, and affordable has been a major focus in recent years (Sharma *et al*., 2002).

Many developing countries have shown a keen interest in researching herbal repositories as a source of new antibacterial agents over the last twenty-five years. Another reason for such research is the emergence of drug resistance and the occurrence of unfavourable side effects from certain antibiotics (Farnsworth, 1988; Jain, 1991; Khan *et al*., 2011). As a result, steps must be taken to address this issue, such as limiting the use of antibiotics, conducting research to better understand the genetic mechanisms of resistance, and continuing investigations aimed at developing drugs from natural sources (Dash *et al*., 2001).

The majority of synthetic drugs have side effects, and the majority of microbes have developed resistance to the synthetic drugs (Chanda and Rakholiya, 2011). Antimicrobial compounds derived from potential plants should be investigated to address this issue. These plant-based drugs are less toxic, have fewer side effects, and are also less expensive. They are effective in the treatment of infectious diseases while also reducing many of the side effects associated with synthetic antimicrobials (Harishchandra *et al*., 2012).

Antioxidant rich foods found in medicinal plants aid the human body in reducing oxidative damage caused by free radicals and active oxygen. Flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans, and lignins are common phenolic compounds found in medicinal plant parts (Cai *et al*., 2004; Kahkonen *et al*., 1999; Larson, 1988). Because of their redox properties, ability to chelate metals, and quenching of singlet oxygen, phenolic acids and flavonoids have antioxidant properties (Rice-Evans *et al*., 1996). Flavonoids can be devided as flavanols, flavones, flavanones, and anthocyanins on the basis of saturation of the flavan ring and the extent of hydroxylation (Youdim *et al*., 2002).

Antioxidants are defined as any substance that, when present very little amounts relative to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1999). Oxidation is a chemical reaction in which electrons are transferred from a substance to an oxidizing agent. Free radicals can be produced during oxidation reactions, which can set off chain responses that harm cells. By eliminating free radical intermediates, antioxidants halt these chain reactions. Additionally, by oxidizing themselves, antioxidants prevent other oxidation events. Consequently, reducing agents are often times antioxidants such thiols, ascorbic acid, or polyphenols (Hyoung, 1992).

The role of antioxidants and antioxidant enzymes in the cure and preservation of many diseases is currently the aim of research. Antioxidants can protect against reactive oxygen species (ROS) toxicity by preserving ROS construction, disrupting ROS attack, scavenging reactive metabolites and converting them to loss reactive molecules, or increasing the resistance of sensitive biological targets to ROS attack.

Antioxidants are well-known for their ability to act as chemo-preventive agents against oxidative damage. Natural antioxidants have been shown to have a variety of biological effects, including antibacterial, antiviral, hepatoprotective, anti-inflammatory, antiallergic, antithrombic, vasodilatory, anticarcinogenic, anti-immunogenic, and anti-aging activity. As a result, compounds capable of protecting against ROS-mediated damage, particularly those derived from natural sources, may have potential applications in disease prevention and/or treatment. Traditional medicine is a rich source of potentially beneficial new compounds for the development of chemotherapeutic agents.

Oxystelma esculentum R.Br. is an important medicinal plant belongs to Asclepiadaceae family and commonly known as ‘Jaldudhi’. It is a perennial twiner found throughout the plains of the Indian sub-continent near water-logged areas. This plant is native to China, South Asia, Southeast Asia, Northeastern Africa, and South-west Asia. Plant genus derives its name from two words: Oxys (sharp) and Stelma (crown), which describe the acute lobes of the corolla.

Characters such as a milky latex, climber, opposite leaves, pointed leaf tip, white (outside) and pink (inside) coloured flowers, pointed petal tips with hairy edges, presence of pollinia, and smooth follicular fruit with many hairy tiny seeds can be used to identify it in the field. The plant serves as laxative, antiseptic, depurative, anthelmintic, antiulcer, aphrodisiac, hepato protective and effective for leucoderma and bronchitis. Decoction of plant is given in ulcer, sore-throat and itches. Milky juice is employed as galactogogue, antiperiodic, antiulcer and as a vulnerary. Leaves are used as antiperiodic. Its root is prescribed in jaundice. Fruit is bitter, tonic, expectorant, anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent.

The plant collection was done in Baran district of Rajasthan (India). Baran is located at 24°25′ N to 25°25′ N latitude and 76°12′ E to 77°26′ E Longitude. It is surrounded by three Rivers Kalisindh, Parvati and Parban. The city is situated on the border of Rajasthan and Madhya Pradesh. The average annual rainfall in the district is 895.2 mm. It has an average elevation of 262 metres (859 ft). January is the coldest month with the average daily maximum temperature of 24.3°C and the average daily minimum temperature of 10.6°C.

Oxystelma esculentum R.Br. was tested for antibacterial activity, antioxidant potential, phytochemical analysis, and phytocompound identification in this study.

The current investigation was conducted with the following goals in mind:

1. Selection and authentication of the medicinal plant from the natural habitat.
2. To make crude extracts from the flowers and fruits within the plant using different solvents.
3. Preliminary phytochemical investigation of the plant.
4. To evaluate the antifungal and antibacterial properties of the crude extracts of flowers and fruits of the plant.
5. To ascertain each extract's minimum fungicidal concentration (MFC), minimum bactericidal concentration (MBC), inhibitory zone, and minimum inhibitory concentration (MIC).

6. To assess the antioxidant capacity of the plant's fruits and flowers and investigate how they scavenge free radicals.
7. To distinguish and recognize the compounds from methanol extract using GC-MS, HPLC and FT-IR.

Importance of proposed research work in brief:

1. The proposed study will provide actual data of the phytochemicals (flavonoids, Alkaloids, glycosides, steroids, tannin, terpenoids etc.) present in the studied plant. The anti-diuretic, anti-inflammatory, anti-analgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites.
2. The phytochemical analysis of the selected plant is also important and has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.
3. Proposed study will provide more attention towards conservation, cultivation for effective utilization of the selected plant with balancing threat to the biodiversity.
4. The given research proposal is try to discover hidden pharmacological activities present in selected medicinal plant which is used as traditional/ethno medicine by tribes.
5. This research work again tries to isolate the active compound(s) from the plant which is responsible for such pharmacological activities. It will also makes the addition of one more herbal drug for effectively commercialize and to export.

Plate 1. Whole plant of *Oxystelma esculentum* R.Br.

Plate 2. Flower part of *Oxystelma esculentum* R.Br.

Plate 3. Leaves of *Oxystelma esculentum* R.Br.

Plate 4.Matured leaves of *Oxystelma esculentum* R.Br.

Plate 5. Collection of *Oxystelma esculentum* R.Br.

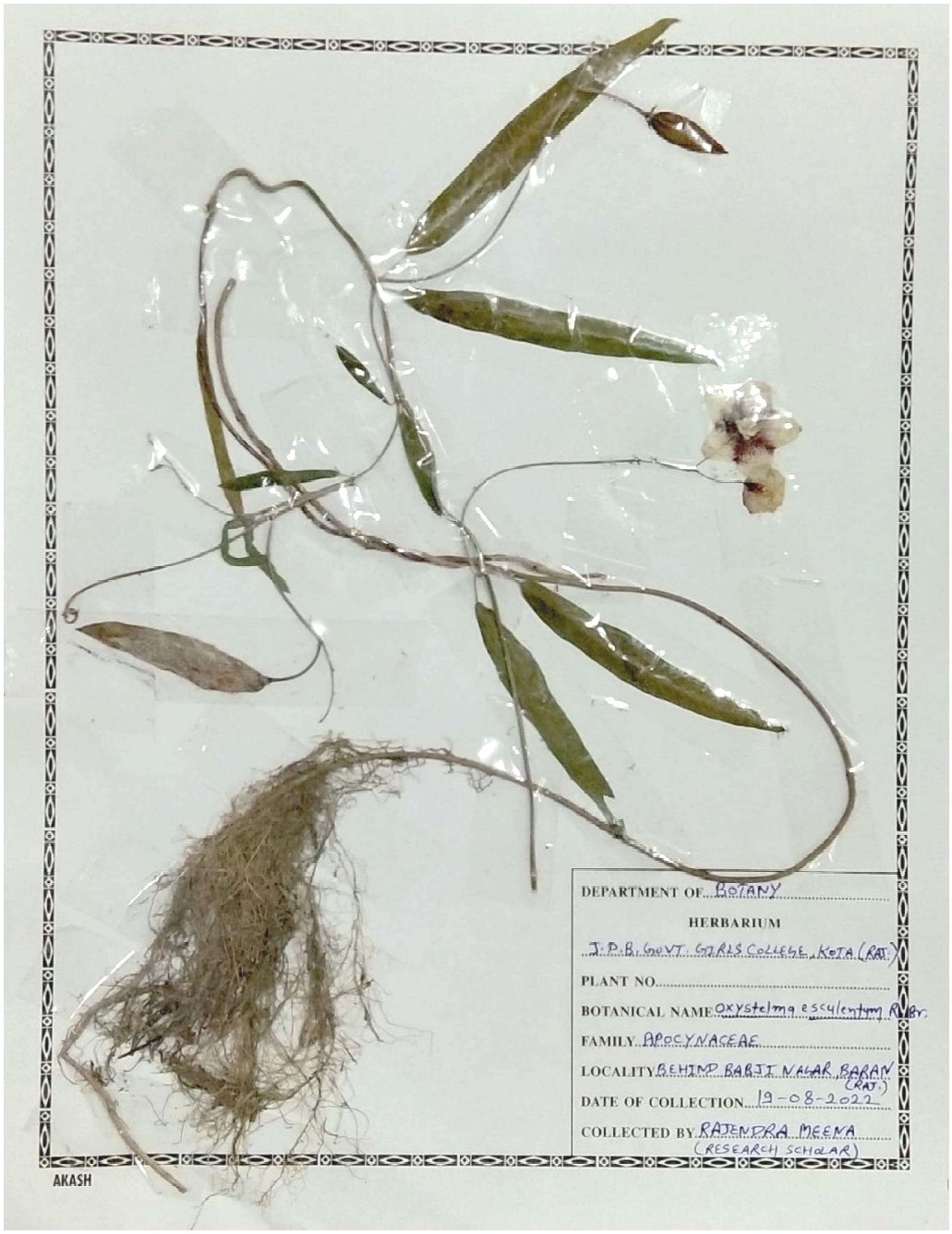

Plate 6. Specimen of *Oxystelma esculentum* R.Br.

Plate 7. Collection site of *Oxystelma esculentum* R.Br.

Near NH-27, Baran,Rajasthan, India

(Coordinates 25°05'41.2"N 76°29'41.2"E)

CHAPTER - II
REVIEW OF LITERATURE

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Since thousands of years, traditional healthcare in many regions of the planet has relied heavily on plant-based medicines. At the moment, there is increased interest in employing plants as sources of agents against microbial infections (Chariandy *et al*., 1999). Herbal remedies largely contribute to the basic healthcare of individuals and communities in many underdeveloped nations (Ghosh, 2003). Traditional plant-based knowledge is now recognized as a valuable tool in the search for new drugs and nutraceuticals (Sharma and Mujumdar, 2003). The isolation and characterization of Pharmacologically potent substances derived from therapeutic plants are still be a focus of research. The isolated natural products from these plants and from its derivatives have led to medicines used for various clinical purpose (Balunas and Kinghorn, 2005). The crude extract form of plant exhibits a fascinating combination of activities, highlighting the vast capabilities of therapeutic plants not only as sources of novel medications but also for use as botanicals in both developing and industrialized countries (Gilani and Rahman, 2005). Medicinal plants have served as a crucial source of drugs for millennia and remain fundamental to traditional medical practices worldwide (Pan *et al*., 2009). These plants provide significant therapeutic benefits for various ailments, with an estimated 20,000 species from various families used for medicinal purposes. Additionally, approximately 80 percent of the global population relies, wholly or partially, on plant-based medicines (Kuete *et al*., 2008).

India, one of the world's most medico-culturally diverse countries, maintains a respected tradition of using medicinal plants (Bopana and Saxena, 2007). Of the estimated 300,000 species of higher plants, about 1% (roughly 3,000 species) are used for food, while around 10,000 species have medicinal uses. This suggests a vast potential for further discoveries within the plant kingdom for pharmaceutical applications (McChesney *et al*., 2007).

Natural products, such as plant extracts in pure or standardized forms, offer limitless opportunities for microbial control due to their chemical diversity. Beyond their antimicrobial properties, many plants are used in various health-

related areas, including old medicine, foods, dietary supplements, and recombinant protein synthesis. Phytochemicals, particularly polyphenols, flavonoids, anthocyanins, and carotenoids, dominate the market (Patwardhan *et al*., 2004).

Plant-produced secondary metabolites are a significant source of biologically active compounds. There is now more scientific interest raised in these metabolites because ongoing search for sources of novel plant-based medicinal compounds, driven by the rising resistance of microorganisms to many current antimicrobial drugs. According to the World Health Report on infectious diseases from 2000, Combating resistance to antibiotics is a major priority for the WHO in the current era. Consequently, the past decade has seen a surge in research into plants as sources for managing human diseases (Prashanth *et al*., 2001). Additionally, it is analyzed that two-thirds of the world population relies on traditional remedies, primarily due to the limited availability and high cost of many pharmaceutical products (Tayboto and Townson, 2001).

Plant-derived antibacterial chemicals fall into various important categories, including phenolics and polyphenols (such as normal phenolic acids and phenols, flavones, quinones, flavanols, flavonoids, coumarins and tannins), vital oils, terpenoids, lectins, polypeptides, and alkaloids.

Plant products have been used since ancient times for flavoring food and beverages and for medicinal purposes, with varying success in curing and preventing diseases (Borris, 1996). In recent years, numerous studies across different countries have aimed to prove the efficacy of plant products (Zeng *et al*., 2012). Thousands of compounds isolated from plants are claimed to possess antimicrobial or medicinal properties. The use of plant extracts with known antimicrobial properties can significantly enhance food preservation. The value of these plants lies in the chemical substances they contain, which produce specific effects on the microbiological, chemical, and sensory qualities of food. These phytochemicals are categorized into groups such as polyphenols, flavonoids, tannins, alkaloids, terpenoids, isothiocyanates, lectins, polypeptides, or their oxygen-substituted derivatives (Edeoga *et al*., 2005).

Medicinal plants that have been utilized for centuries yield a wide range of chemicals with established medical benefits. Materials that are either able to stop the spread of infections or eliminate them completely without endangering host cells are thought to be good candidates for use in the creation of novel antimicrobial medications. More and more reports about the antibacterial qualities of medicinal plants from around the globe have surfaced in recent years. Plant extracts that target other locations than traditional antibiotics are expected to be beneficial against drug-resistant microbial infections (Ahmad and Beg, 2001).

The presence of several phytochemicals in extracts of P. *granatum* peel, whole fruit, and seeds was studied by Bhandary *et al.* (2012). It was discovered that triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrates, and vitamin C were present in the three distinct peel extracts. Triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrates, and vitamin C were discovered to be present in the three distinct fruit extracts.

Yadav and Agarwal in 2011, examined phytochemical presence to ascertain the overall flavonoid and phenolic and constitutes of the different medicinal plants.

Four medicinal herbs of India were examined for phytochemical investigation by Zahin *et al.* (2009). Phytocompounds like glycoside, alkaloids, phenolics, and saponins were found, according to analysis.

The phytochemical performance of *Synclisia scabrida* leaf extract in *n*-hexane was evaluated by Fredrick *et al*. (2013). They pointed out that the analyzed extract included various phytochemicals, including alkaloids, flavonoids, saponins, and glycosides.

Savithramma *et al*. (2011) used qualitative phytochemical analysis to screen fresh leaves of twenty various medicinal plants for secondary metabolites. They found that the leaves contained a variety of secondary metabolites, like triterpenoids, flavonoids, saponins, steroids, phenols, tannins, and others.

Savitha *et a*l. (2014) demonstrated that the existence of tannins, terpenoids, saponins, alkaloids, and glycosides in *P. daemia* and assessed the phytochemicals' effectiveness against specific infections.

Five plant species in the Cucurbitaceae family had their phytochemical activity examined by Sood *et al*. (2012). The examination revealed the presence of phytochemicals such as tannins, phytosteroids, polysaccharides, resins, saponins, and cardiac glycosides.

Coccinia cordifolia's phytochemical analysis was evaluated by Rahman *et al*. (2015). Flavonoids, vitamins, phenol, proteins, carbs, and lipids were some tested phytochemicals from the plant.

In a 2019 study, Behera *et al*. tested phytochemicals from 19 families of medicinal plants. It was observed that the substance contained terpenoids, alkaloids, tannins, steroids, leucoanthocyanin, coumarin, phenol, etc.

In 2022, Rajendran *et al*. identified the phytochemical characteristics of the *S. alata* leaf extract. The phytochemical examination showed the existence of secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, anthroquinone, phenols, saponin, tannin, and carbohydrates. By quantitative phytochemical examination, the amounts of alkaloid (49.2%), flavonoid (69.72%), and phenolic (69.72%) content were found.

The preliminary phytochemical content of hydroalcoholic extracts from *H. arifolia* leaves were evaluated by Priya *et al*. in 2021. In the separation, it was discovered that the phenol concentration was 31.78%, the flavonoid content was 1.02%, and the alkaloid content was 30.40%. The study suggests that leaf extracts could be a natural source of antioxidants and supports their application in ethnomedicine.

Cassia alata has been employed in conventional medicine to treat a range of ailments, including skin disorders (Sri Fatmawati *et al*., 2020). In addition, plants may possess antifungal, anticancer, antioxidant, anti-inflammatory, and anti-allergic qualities. Among the metabolites that was determined by the plant are alatinon, alanonal, flavones, flavonols, flavonoids, and glycosides.

Herin *et al*.'s 2019 study examined the phytochemical content of methanol extracts from five Pteridaceae family ferns by employing both quantitative and qualitative screening approaches. Five fern extracts underwent a qualitative examination and their phytochemical content was determined using standard procedures. Steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic

compounds, flavonoids, saponins, tannins, and amino acids were among these constituents. All of the fern extracts underwent a quantitative analysis to see whether any notable secondary metabolites, such as alkaloids, phenolic compounds, flavonoids, saponins, and tannins, were present.

Alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins, and reducing sugars were discovered in the initial phytochemical investigation of *Centella asiatica* leaf and callus extracts (Arumugham *et al*. 2011).

The screening of phytochemicals over eighteen significant medicinal plants, which are utilized to treat a variety of illnesses across the nation, by Savithramma *et al*. (2011) investigated. These plants include a variety of phytochemicals, like saponins, terpenoids, steroids, anthocyanins, coumarins, fatty acids, tannins, leucoanthocyanins, and emodins, according to a qualitative phytochemical examination of the plants.

Plants have been related with human health from time immemorial. Sickness was once thought to hold the position of punishment from the gods; thus, it was handled with prayers and ceremonies, maybe including 'magic potion' made from local herbs. Since the birth of human civilization, medicinal plants have played an important part in man's health and healing. Despite considerable advancements in the area of allopathic medicine over the twentieth century, plants continue to be a key source of pharmaceuticals in both modern and traditional medical systems (Farnsworth and Soejarto, 1991).

According to Tenover (2006) and Senhaji *et al*. (2020), the continued use of traditional medicine is due to cultural and economic factors as well as the ineffectiveness of many medications currently on the market, a lack of efficient treatments, the resistance of current antibiotic-resistant pathogens, and oxidative stress to novel therapeutic agents derived from plants (Aruoma,1998; Michael *et al*., 2006; Berrino *et al*., 2009).

Antimicrobial substances are abundant in medicinal plants (Mosihuzzanman and Chowder, 2008). The World Health Organization claims that medicinal plants are the main source of new medications (Santos *et al*., 1995). Plants used for medicinal purposes are utilized as herbal medications to treat common bacterial infections because they contain a variety of phytoconstituents.

The possibility for creating antibacterial chemicals from natural products seems to be satisfactory because it prevents the evolution of bacterial strains that are resistant to several drugs (Kushwah and Pratab, 2012). Natural herbal remedies have been shown to be successful in the treatment of infectious bacterial infections, and they appear to be more affordable and to have less side effects than manufactured antibiotics (Moses *et al*., 2013).

Anand *et al.* (2007) examined the effects of medicinal plant extracts from *Curcuma longa*, *Acalypha indica*, and *Annona squamosa* against isolates with dermatophytes. C. *longa*'s antifungal abilities were proven against *Microsporum gypsum* and *Trichophyton rubrum*. It has been demonstrated that *Acalypha indica* and *Annona squamosa* are resistant to these two organisms. *Cocculus hirsutus* leaf extract was exposed to a phytochemical analysis, which demonstrated the existence of carbohydrates and glycosides and demonstrated the extract's potent antibacterial action against microorganisms that are both gram(+) and gram(-) (Panda *et al.*, 2007).

Staphylococcus aureus, *Bacillus subtilis*, *Micrococcus luteus*, *E. coli*, *Pseudomonas aeruginosa*, *Serratia macrescens, Mycobactrium smegmatis, Menilia sitophila, and Candida albicans* were the bacteria against which, extracts from *Lippia alba's* roots and leaves were assessed for their ability to fight microorganisms. The findings demonstrated that methanol extracts of leaves inhibited *Mycobacterium smegmatis, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus*, and *Monila sitophila* (Aguiar *et al.*, 2008). Saranraj *et al.* (2010) tested the ethanol and ethyl acetate extracts of *Acalypha indica* leaves for their capacity to prevent the expansion of human pathogenic bacteria and discovered that the ethanol extract possessed higher level of inhibitory effect.

Cardiospermum halicacabum leaf and stem crude extracts were exposed to phyto chemical and antibacterial screening against a number of microbiological infections in various solvents. Acetone and chloroform leaf extracts demonstrated the most inhibitory effects against *Salmonella typhi* and *Streptococcus subtilis*, respectively, among the various solvent extracts. Stem extracts in acetone and benzene both demonstrated a significant amount of

inhibitory action against S. *typhi* and E. *coli*, respectively (Viji and Murugesan, 2010).

The ability of an ethanol extract of *Bauhinia variegata* leaves to inhibit microbial infections was examined. All of the investigated bacterial species were inhibited to varied degrees by ethanol extracts. *Salmonella typhi* had the most inhibitory activity, followed by *Vibrio cholerae*, *Klebsiella pneumoniae*, E. *coli* and *Staphylococcus aureus* (Gunalan *et al*., 2011). Alcoholic (methanol and ethanol) extracts from the *Solanum nigrum* stem, berries and entire plant were examined for their ability to combat against *Bacillus subtilis*, E. *coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. More antibacterial activity was displayed by the methanolic extracts than the ethanolic extracts (Parameswari *et al*., 2012).

Singh *et al*. (2012) used disc diffusion to test the antimicrobial properties of nine medicinal plants against B. *subtilis*, E. *coli*, S. *aureus*, E. *aerogenes*, K. *pneumoniae*, P. *vulgaris*, and P. *aeruginosa*. The results showed that leaf extracts of R. *communis*, B. *asiatica* and C. *opaca* had the greatest zone of inhibition against all of the pathogens examined, whereas E. *hirta*, Z. *armatum* and A. *vesica* had the smallest zone of inhibition. The antibacterial activity of *Passiflora subpeltata* fruits and leaves was examined (Lingaraju *et al*., 2015). E. *coli*, *Enterobacter aerogenes*, E. *faecalis*, *Klebsiella pneumonia* and *Staphylococcus aureus* were tested for antibacterial activity. They discovered that ethyl acetate and methanol extracts of both leaves and fruits outperformed petroleum ether and extracts in chloroform in terms of antibacterial activity. The ability to fight bacteria of P. *subpeltata* was greatest against E. *aerogenes*, E. *faecalis* and S. *aureus*.

Das and Mandal (2016) used disc diffusion to investigate the ethanolic extract's antimicrobial properties of *Syzygium cumini* and *Mangifera indica* seeds. E. *coli* and K. *pneumonia* were suppressed by ethanolic seed extracts of S. *cumini* and M. *indica* in the same way that standard antibiotics were. Both extracts had a synergistic impact against all of the tested microorganisms when combined with trimethoprim and vancomycin. According to research done by Elisha *et al*. (2017), several plants, including *Hypericum roeperianum*, *Cremaspora triflora*,

Heteromorpha arborescens, *Pittosporum viridiflorum*, *Bolusanthus speciosus*, *Calpurnia aurea*, *Maesa lanceolata*, *Elaeodendron croceum* and *Morus mesozygia* have antibacterial properties. When compared to other gram-positive (S. *aureus*, E. *faecalis*, and B. *cereus*) and gram-negative (S. *typhimurium* and P. *aeruginosa*) bacteria, acetone extract of leaves of nine plant species demonstrated excellent antimicrobial efficacy against E. *coli*.

Gomphrena globosa flower tests were conducted on extracts by Kusmiati *et al*. (2017) for their ability to combat *Staphylococcus aureus*, E. *coli*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*. The extract made in n-butanol was said to have the strongest inhibitory effect. Neem oil and three plant extracts in aqueous form were tested *in vitro* for their ability to prevent the expansion of three post-harvest fungal pathogens: *Rhizopus arrhizus*, *Sclerotium rolfsii* and *Fusarium solani*. Among the leaf extracts *Lasonia inermis*, Neem oil and *Cocculus hirsutus* shown the strongest antifungal effectiveness in opposition to the infections (Devi *et al*., 2017).

Making use of the disc diffusion method, Mostafa *et al*. investigated five plant extracts' antibacterial properties against B. *cereus*, S. *aureus*, E. *coli*, P. *aeruginosa* and S. *typhi* (2018). The studied bacterial strains were vulnerable to the extracts of ethanol from P. *granatum*, *Syzygium aromaticum*, *Zingiber officinale* and T. *vulgaris* and the extract of S. *aureus* from *Cuminum cyminum*. According to Manandhar *et al*. (2019), four Various plant extracts were examined to determine their ability to fight off bacteria such E. *coli*, S. *typhi*, K. *pneumoniae*, *Citrobacter koseri*, S. *aureus* and *Rhizopus* sp. The antibacterial properties of methanolic extracts of *Ageratina adenophora*, *Artemisia vulgaris*, *Oxalis corniculata*, and *Cinnamomum tamala* were assessed using the disc diffusion method. The findings showed that the vast majority of the extracts have antibacterial properties. O. *corniculata* extract shown the highest level of effectiveness against E. *coli*, S. *typhi*, K. *pneumoniae*, and C. *koseri*. A. *vulgaris*, C. *tamala* and A. *adenophora*'s methanolic extract all demonstrated effectiveness in opposition to S. *aureus*.

Alsudani *et al*. (2019) examined the ethanolic extract's antibacterial properties of *Daucus carota* (seeds), *Rheum ribes* (roots), *Rumex vesicarius* (leaves), and *Punica granatum* (peels). These extracts were tested against some gram-negative bacteria (*Proteus mirabilis*, *Helicobacter pylori* and K. *pneumoniae*), gram-positive bacteria. The findings revealed that P. *granatum* and D. *carota* extracts possess the highest levels of antibacterial action in opposition to every tested bacterial strains. The obtained results demonstrated that P. *granatum* extract and D. *carota* extract both exhibited the highest levels of inhibition against a fungal isolate. Two cardamom extracts (fruit and seeds), which are high in volatile chemicals, were tested for their antibacterial effectiveness against the main periodontal pathogens by Souiss *et al*. in 2020. The growth of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* was inhibited by the examined plant extract's antibacterial characteristics.

Canarium patentinervium leaf and bark extracts were subjected to a thorough antibacterial activity screening by Mogana *et al*. (2020), and it was discovered that the ethanolic extract from the leaves and bark had antimicrobial activity against all four ATCC and eight clinical isolates. The antibacterial and antibiotic-modulating efficacy of *Baccharis coridifolia* essential oil was studied by Freitas *et al*. (2020) against multidrug-resistant (MDR) microorganisms. When tested against strains the essential oil of S. *aureus* and P. *aeruginosa* demonstrated considerable antibacterial activity. The bactericidal and antioxidant properties of *Annona squamosa* leaf extracts were assessed by Al-Nemari *et al*. in 2020. Bacteria that are gram (+) and gram (-) were sensitive to the extracts' potent qualities that are both antibacterial and antioxidant.

The bactericidal and antioxidant properties of freeze-dried extracts rose-colored fruits were studied by Cendrowski *et al*. (2020). The maximum inhibitory activity was found in the rose fruits against the majority among the examined pathogens. The *Bacillus cereus* strain of Gram-positive bacteria, together with K. *pneumoniae* and E. *coli* of Gram-negative bacteria, turned out to be the most susceptible to the rose extract. *Achillea fragrantissima, Teucrium polium, Peganum harmala* and *Solenostemma argel* are four commonly used wild

medicinal plants whose phytochemicals, antioxidant capacity, and antibacterial potential were studied by El-Zayat et al. (2021). Against strains of B. *cereus*, B. *subtilis*, E. *coli*, K. *pneumoniae*, *Listeria innocua*, L. *monocytogenes*, P. *aeruginosa*, S. *enterica* and S. *typhimurium*, the studied extracts demonstrated wider range antimicrobial effectiveness. The extracts MIC varied between 0.049 and 1.56 mg/ml.

Namukobe *et al*. in 2021 proved the antimicrobial and antioxidative properties of extracts from *Erlangea tomentosa, Spermacoce princeae, Plectranthus caespitosus* and *Psorospermum febrifugum*. The microorganisms utilized in this investigation were *Pseudomonas aeruginosa*, S. *aureus*, E. *coli*, K. *pneumoniae*, S. *pyogenes* and S. *typhi*. The studied plant extracts were effective against the bacteria and exhibited MIC values that ranged from 3.12 to 12.5 mg/ml. *Measa lanceolata* leaf extracts were evaluated for their antibacterial properties by Ismael *et al*. in 2021. They had examined the ability of three gram negative and two-gram positive bacterial strains to inhibit bacteria *in vitro*. The bacterial strains responded well to both methanol and water extracts, but S. *aureus*, E. *coli*, P. *aeruginosa* and P. *vulgaris* were unaffected by extracts ofchloroform and ethyl acetate.

The antibacterial effectiveness of five medicinal plants *e.g. Momordica charantia*, *Alchornea cordifolia*, *Myrianthus arboreus*, *Psidium guajava* and *Justicia flava* was investigated by Owusu *et al*. in 2021 with MDR bacterial pathogens that were extracted from postoperative wounds. Of all the microorganisms used, they claimed that the A. *cordifolia* displayed the largest growth inhibition. The antibacterial effectiveness against eight pathogens, comprising Gram-positive and Gram-negative bacteria, were tested using methanolic extracts was examined by Jebali *et al*. in 2022. The highest widths of the inhibitory zones were found in gram-positive bacteria, which ranged *Clostridium tetani* measures 22 mm, while S. *aureus* measures 33 mm.

Barakadze *et al*. (2023) examined the antimicrobial characteristics of two biopolymers, poly[2-methoxycarbonyl-3-(3,4-dihydroxyphenyl) oxirane] (PMDHPO) and poly[3-(3,4-dihydroxyphenyl) glyceric acid] (PDHPGA), derived from six plants in the Boraginaceae family: *Symphytum asperum*, S.

caucasicum, S. *grandiflorum*, *Cynoglosum officinale*, *Anchusa italica* and *Borago officinalis*. The research found that the antibacterial activities were only mild, and that only three plants biopolymers exhibited antibacterial activity against all examined microorganisms. Contrarily, the antifungal activity outperformed the antibacterial action. The highest activity with MIC/MFC were seen with biopolymers from the stems of *Borago officinalis* at concentrations of 0.37-1.00 mg/mL and 0.75-1.5 mg/L, respectively.

In their study published in 2023, Dubale *et al*. investigated the antimicrobial effects of extracts from plants on K. *pneumoniae*, P. *aeruginosa*, S. *aureus*, E. *coli* and C. *albicans*. All fractions of the passages taken from the root of T. *rhynchocarpum*, one of the chosen plant species, demonstrated the greatest amount of action against all strains that were tested. Specifically, the crude extract from T. *rhynchocarpum* demonstrated MIC values of 0.98 µg/mL in opposition to K. *pneumoniae* and P. *aeruginosa* 0.48 µg/mL in opposition to S. *aureus* and E. *coli*, which are even higher compared to those of the control medicines, gentamicin and clotrimazole.

Dembetembe *et al*. (2023) assessed the antibacterial activity of several extracts against *Neisseria gonorrhoeae, Candida albicans* and *Gardnerella vaginalis*, three STD pathogens. The minimum inhibitory concentrations (MICs) of 0.78 and 0.39 mg/mL, respectively, for the root extract of *Rhoicissus tridentata* against C. *albicans* and N. *gonorrhoeae*, demonstrated the strongest antibacterial activity. Additionally, with a 20.3±4.7 mm region/zone of inhibition situated at 2 mg/mL, the R. *tridentata* extract demonstrated the most notable antifungal efficacy against M. *furfur*.

An antioxidant is a substance that, when present in relatively low concentrations compared to an oxidizable substrate, effectively retards or prevents the oxidation of that substrate. In other words, antioxidants help protect substances from damage caused by oxidation by inhibiting or slowing down the oxidative processes that can lead to degradation or deterioration. They are commonly used in various industries and are also essential for maintaining the health of living organisms by neutralizing harmful free radicals.

In a study by Chew *et al*. (2009), the *in vitro* antioxidant capacity and polyphenolic composition of leaves and flowers from three different plant species, namely *Bauhinia kockiana*, *Caesalpinia pulcherrima*, and *Cassia surattensis*, were examined. Among these plants, the flowers of *Bauhinia kockiana* were discovered to have the highest levels of total phenols, scavenging free radical activity, and reducing ability. This suggests that *Bauhinia kockiana* flowers are particularly rich in antioxidants and might be promising health benefits because of its ability to combat oxidative stress.

In analysis done by Garzon and Wrolstad in 2009, an investigation was carried out on the petals *Tropaeolum majus* to assess their anthocyanin content, ascorbic acid content, total phenolic content, and their ability to scavenge radicals such as ABTS and DPPH. The results of the study revealed that these flower petals exhibited excellent free radical scavenging activities. Additionally, they were found to contain high levels of phenolic compounds and ascorbic acid. These findings indicate that the *Tropaeolum majus* flowers possess strong antioxidant properties.

In analysis done by Zahin *et al.* in 2009, the effectiveness of antioxidants in several medicinal plants was evaluated. The results indicated that *Plumbago zeylanica* exhibited the highest antioxidant potential, followed by *Holarrhena antidysenterica*, *Acorus calamus*, and *Hemidesmus indicus*. These medicinal plants demonstrated antioxidant activity comparable to well-known commercial antioxidants like butylated hydroxytoluene (BHT), L-ascorbic acid, and tocopherol. This suggests that these natural plant sources could serve as effective alternatives or supplements to synthetic antioxidants in various applications, owing to their strong antioxidant properties.

In a study done by Tyagi *et al*. in 2010, the researchers examined the *in vitro* antioxidant activity of water and methanolic extracts derived from *Flacourtia indica Merr*. They measured the extracts' overall antioxidant capacity and found it to be 260 µg/ml for the extract of methanol and 180 µg/ml for ascorbic acid for the aqueous extract. These results suggest that both of the methanolic and extracts in water exhibited strong antioxidant effects. Additionally, the researchers conducted a preliminary phytochemical screening of

the freshly prepared extracts to identify various phytoconstituents present in them. This screening likely helped in identifying the potential compounds responsible for the antioxidant properties observed in the extracts.

In research done by Panda *et al.* in 2011, the researchers evaluated the antioxidant characters of extracts collected from the aerial parts of a specific plant species using alcohol as the solvent. They utilized various assays involving 1,1-diphenyl-2-picryl hydrazyl, nitric oxide, superoxide, and hydroxyl radicals to assess antioxidant activity. The study found that the extract exhibited antioxidant properties in a concentration-dependent manner. This effect was due to the existence of phenolic compounds and antioxidant vitamins in the extract. The researchers emphasized that since free radicals are implicated in numerous diseases, the antioxidant properties of naturally occurring phytochemicals have gained significant importance in healthcare and research.

In research conducted by Daniels *et al*. in 2011, It was discovered that the fruits and flowers of *Gethyllis multifolia* and *Gethyllis villosa* contained higher levels of total polyphenols, oxygen radical absorbance capacity, ferric reducing antioxidant power, radical scavenging ability, flavonols, and flavanones in contrast to the leaves, bulbs, and roots of these plants. This implies that the flowers and fruits among these kinds are rich in antioxidants. Similarly, in another study conducted by Badaturuge *et al.* in 2011, the alcoholic extract of the aerial parts of *Cassia auriculata* exhibited potent antioxidant activities. These activities were assessed using various methods, including DPPH scavenging, lipid peroxidation inhibition, and reducing power analysis. The researchers also fractionated the crude extract using solvents of increasing polarity and discovered that the fraction containing ethyl acetate had the strongest antioxidant activity, followed by the chloroform fraction. The petroleum ether, n-butanol, and water fractions exhibited less antioxidant activity compared to the crude extract. These findings highlight the potential of *Cassia auriculata* as a source of antioxidants, particularly in its ethyl acetate and chloroform fractions.

In a research project that by Meena *et al*. in 2012, the antioxidant qualities of two well-known medicinal plants known for their memory-enhancing properties, *Bacopa monnieri* and *Centella asiatica*, were determined. The

researchers also evaluated the levels of antioxidant compounds, including ascorbic acid, total phenols, and tannins, in these plants. The study revealed significant differences ($P<0.05$) in the antioxidant values between *Bacopa monnieri* and *Centella asiatica*. Specifically, the methanolic extract of the whole leaf powder of *Bacopa monnier*i exhibited significantly increased action of antioxidants compared to *Centella asiatica*. Furthermore, the antioxidant components, such as ascorbic acid, total phenols, and tannins, were found to be present in higher concentrations in *Bacopa monnieri* as compared to *Centella asiatica*. These findings suggest that *Bacopa monnieri* may have stronger antioxidant properties and higher levels of antioxidant compounds compared to *Centella asiatica.*

In investigation done by Bhadauria *et al.* in 2012, the antioxidative capacity of the hydro methanolic root extract of *Coccinia grandis* was examined. The researchers found that the antioxidant properties of the fractions obtained from the extract varied depending on their concentration. They attributed this antioxidant activity due to flavonoid presence and phenolic compounds within the hydro methanolic extract. These findings highlight the potential health benefits of *Coccinia grandis* due to its natural antioxidant properties.

In a research project headed by Jain *et al.* in 2012, they investigated the antioxidant and antibacterial properties of *Pergularia daemia*. The researchers evaluated extracts from the entire plant, prepared using various solvents including acetone, methanol, chloroform, hexane, petroleum ether, and dichloromethane. They also identified specific compounds in the plant, including lupeol, β-sitosterol, α- and β-amyrin, quercetin, acetate, oleanolic acid, and kaempferol, with the lowest IC50 (half-maximal inhibitory concentration) values. Among the different extracts, higher levels of antioxidant activity were seen in the methanol extract compared to the others. This suggests that *Pergularia daemia* may contain valuable antioxidant compounds, particularly in its methanol extract.

In research done by Karthishwaran and Mirunalini in 2012, they investigated the antioxidant capacity of the methanolic extract from *Pergularia daemia* through *in vivo* as well as *in vitro* experiments. They focused on 7,12-dimethylbenz(a)anthracene(DMBA)-induced hamster buccal pouch carcinogenesis as their model. The researchers observed that pre-treatment of the

DMBA-exposed test animals with the methanolic extract (two hundred milligrams per kilogram) led to significant increases ($P<0.05$) in the levels of erythrocyte lysate and plasma antioxidants, including vitamin C, vitamin E, and glutathione (GSH). Additionally, in the buccal tissue, the levels of vitamin E and GSH also increased when compared to the hamsters induced with DMBA alone. These findings suggest that the extract of methanol from *Pergularia daemia* possesses antioxidant properties that might be advantageous in protecting against the stress caused by oxidation associated with DMBA-induced carcinogenesis in hamster buccal pouches.

In research done by Jaberian *et al.* in 2013, various parts of three different plants, namely *Primula auriculata*, *Fumaria vaillantii*, and *Falcaria vulgaris*, were extracted using three different solvents. The researchers investigated the bactericidal and antioxidant properties of these extracts against nine different Gram (+) and Gram (-) bacteria. The DPPH radical test for scavenging showed that all three plants demonstrated strong antioxidant activities, with more than 80% inhibition at a level of 50 µg. Among the plants, *Falcaria vulgaris* showed a high content of carvacrol, which accounted for 29.8% of its composition. Additionally, the methanolic-water extracts of these plants contained significant amounts of carvacrol and fumaric acid, with concentrations of 1119 mg/L and 1966 mg/L, respectively. These findings highlight the chemical makeup and antioxidant capacity of these plants, particularly *Falcaria vulgaris*, which contains carvacrol as a primary component.

Aegle marmelos, a plant belonging to the Rutaceae family, is extensively available in India and holds significance in both medicinal and religious contexts. Varughese and Tripathi (2013) conducted a study on *Aegle marmelos* fruits at various stages of ripening. They identified a suitable solvent extract through the phytochemical evaluation of different solvent extracts. It was established to exhibit free radical and scavenging hydroxyl radical activity, along with *in vitro* antioxidant activity, as reported by Gupta *et al*. in 2012. Their research suggested that the methanolic hydro extract of *Aegle marmelos* contains several phytochemical constituents in charge of the plant's antioxidant properties.

In research done by Upadhyay *et al.* in 2013, the researchers investigated the overall phenolic content and protective qualities of *Tinospora cordifolia* stem extracts, specifically using ethanol and methanol as solvents. The study found that the ethanolic stem extract had the highest phenolic content, measuring at 66.28±0.82 mg/g. Interestingly, the same ethanolic stem extract also exhibited greater comparative antioxidant activity between the antioxidant activity of the methanolic stem extract. This suggests that *Tinospora cordifolia*, particularly its ethanolic stem extract, contains a substantial number between phenolic substances and possesses strong antioxidant properties.

Anjum *et al.* (2013) carried out research on *Callicarpa macrophylla* leaves, evaluating their physiochemical traits and phytochemical composition. Various extracts were utilized to analyze the existence of substances that are bioactive in these leaves. Additionally, Ashwagandharistha, which was prepared using both traditional and modern methods, exhibited high levels of total phenols and flavonoids. Furthermore, it demonstrated dose-dependent antioxidant activity, as reported by Tiwari and Patel in 2013. The antioxidant potential of both types of Ashwagandharistha was investigated using two different *in vitro* models: superoxide radical scavenging activity and lipid peroxidation assay. These studies shed light on the antioxidant properties of *Callicarpa macrophylla* leaves as well as their uses in traditional and modern formulations like Ashwagandharistha.

In research done by Meena *et al*. in 2014, the *in vitro* antioxidant capacity of the methanolic extract from *Cocculus hirsutus* was evaluated. The researchers found that the extract of methanol from the stem showed the maximum DPPH (2,2-diphenyl-1-picrylhydrazyl) action that scavenges radicals, followed by the extract of methanol from the leaf, and then the callus. However, it's worth noting that the activity of these extracts was lower than that of the standard antioxidant, ascorbic acid. This suggests that *Cocculus hirsutus* may have some antioxidant properties, However, they might not have the same strength as the well-known antioxidant ascorbic acid.

In research done by Umamaheshwari and Sangeetha in 2014, they investigated the anti-inflammatory and anti-oxidant properties of the ethanolic leaf extract of *Cocculus hirsutus*. To assess the antioxidant properties, they used

the DPPH assay, nitric oxide radical generation assay, and the reducing power assay. For evaluating anti-inflammatory activity, they employed a human erythrocyte assay. The study stated that the extract's anti-inflammatory properties were due to the presence of flavonoids. This suggests that *Cocculus hirsutus* may have potential therapeutic applications due to its antioxidant and anti-inflammatory properties, possibly mediated by its flavonoid content.

In research done by Shalini *et al*. in 2014, The antioxidative capacity of ripe fruits of *Coccinia grandis* was investigated. The study indicated the existence of both metabolites, both primary and secondary in these fruits. They prepared five different extracts using various solvents and discovered that the extracts of ethyl acetate and chloroform exhibited higher scavenging free radical activity. Additionally, these extracts were rich in flavonoid and phenolic contents, which are known for their antioxidant properties. Another study by Alamagir *et al*. in 2014 explored the same plant species, *Coccinia grandis*, for its antimitotic, cytotoxic, and qualitative properties. They found that the leaf extract exhibited a suppressive wheat seed germination and the growth of seedling roots. Furthermore, the extract displayed an impediment to tumors when tested on potato discs, without causing harm to the tumor-inducing agent, *Agrobacterium tumefaciens*. These findings suggest potential applications of *Coccinia grandis* in both anti-oxidant and anti-tumor contexts.

In a research conducted by Kaur and Mondal in 2014, the antioxidant activity and total phenolic content of alcoholic extracts from seven medicinal plants were evaluated. The plants included *Asparagus racemosus*, *Ocimum sanctum*, *Cassia fistula*, *Piper betel*, *Citrus aurantifolia*, *Catharanthus roseus*, and *Polyalthia longifolia*. The results showed that the extracted material's total phenolic content varied between 212 to 366 mg per 100 grams on a fresh weight basis. Among all the medicinal plants tested, *Citrus aurantifolia* showed the greatest level of antioxidant activity, with a percentage of 87.05%, followed by *Ocimum sanctum* at 81.80%, and *Catharanthus roseus* at 71.4%. Additionally, the highest tannin content was found in *Catharanthus roseus* at 7.14%, and the highest anthocyanin content was observed in *Polyalthia longifolia* at 0.65 mg/L.

These findings provide insights into the capacity for antioxidants and phytochemical composition of these medicinal plants.

In research done by Pawar *et al*. in 2015, the capacity of antioxidants in ethanol and extracts of chloroform from four Indian medicinal plants—*Grewia asiatica*, *Caesalpinia bonducella*, *Syzygium samarangense*, and *Asteracantha longifolia*—was analyzed. The researchers employed various assays to assess antioxidant activity, including the reducing power assay and scavenging effects on several radicals, including DPPH, hydroxyl, hydrogen peroxide, and nitric oxide radicals. The results of the study indicated that among the four medicinal plants, the seeds of *Asteracantha longifolia* exhibited good antioxidant activity. This suggests that *Asteracantha longifolia* seeds contain compounds with strong antioxidant properties.

In research done by Ashraf *et al.* in 2015, the antioxidative capacity of different extracts (methanol, hexane and aqueous) from fresh *Euphorbia royleana* was investigated. The researchers used the ferric reducing antioxidant power (FRAP) assay to assess the antioxidant potential of these extracts. The results showed that the hexane extract exhibited the highest ferric reducing power, measuring at 12.70±0.49 mg gallic acid equivalents per gram of plant extract. Additionally, the hexane extract contained the highest levels of phenolic compounds, with a concentration of 47.47±0.71 mg gallic acid equivalents per milligram of plant extract, and flavonoids, with a concentration of 63.68±0.43 mg quercetin equivalents per milligram of plant extract. These findings suggest that the hexane extract of *Euphorbia royleana* is rich in antioxidant compounds and exhibits strong antioxidant activity.

In research done by Stellus and Nair in 2015, *Trichosanthes cucumerina* was investigated for its antioxidant activity and phytochemistry using extracts prepared from the plant in five different solvents: petroleum ether, chloroform, methanol, benzene, and distilled water. It was discovered that *Trichosanthes cucumerina*'s methanolic extract included phenols, flavonoids, tannins, and alkaloids. Additionally, this extract exhibited DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging properties, and the scavenging activity increased with the concentration level of the extract. *Tichosanthes cucumerina* is

well-known for its numerous therapeutic properties, including being anthelmintic, antifertility, anti-HIV, and cardioprotective. The existence of antioxidants and phytochemicals within this plant could contribute to these therapeutic qualities and make it an important asset in traditional and alternative medicine.

An investigation into the historical usage of S. *alata* in the management of different illnesses was completed in 2017 by Ita and Ndukwe. In particular, they looked at the root extracts of S. *alata*'s antioxidant capacity in acetone, ethanol, and aqueous solutions. They evaluated the extract's ferric reducing power, metal chelating activity, polyphenol content, and capacity to scavenge DPPH and ABTS radicals. In the DPPH and ABTS tests, the ethanol extract showed the strongest antioxidant activity, with IC50 values of 45.18 and 39.14 µg/ml, respectively. Additionally, there were notable amounts of flavonoids and total phenolics in this extract. In addition, the aqueous extract outperformed the other extracts in terms of metal-chelating and metal-reducing abilities. The findings show that the root of S. *alata* may be a useful antioxidant source that might help lower oxidative stress.

Rajeshwari Prabha Lahare *et al*., in their 2020 research, examined *S. alata*, a member of the Leguminaceae family, which is an appealing flowering plant commonly referred to as ringworm bush and candle bush. Plant was primarily recognised for its antifungal characteristics, this plant also possesses noteworthy pharmacological value, as it is utilised for its antiviral, antifungal, antibacterial, antioxidant, anticancer, and anti-mutagenic qualities. Its leaves, which have anti-parasitic qualities, are used to cure ailments like eczema, ringworm, asthma, bronchitis, and toxic bug bites. Its bark is used to heal skin disorders. They found that the plant displayed notable antioxidant activity in extracts obtained from its leaves, stems, and roots using both methanol and aqueous solvents. This function of antioxidants is likely linked due to the plant's flavonoid and phenolic component contents. Given its robust antioxidant properties, investigating, and characterizing the bioactive components of this plant could be valuable for the development of medicinal formulations.

Priya *et al*., in their 2021 study, conducted an assessment of the antioxidant capacity of hydro alcoholic leaf extracts from *H. arifolia*. They evaluated the ability of these extracts to counteract free radicals through various

tests, including the DPPH assay, ABTS assay, reducing power assay, and nitric oxide test. The hydro alcoholic extract, when concentrated in the solvent, exhibited significant antioxidative capacity. The ABTS, DPPH, reducing power, and nitric oxide tests revealed that have inhibitory concentrations of 667.75 µg/ml, 734.25 µg/ml, 791.58 µg/ml, and 899.67 µg/ml, respectively. This research findings support the potential utilization of *H. arifolia* hydro alcoholic leaf extracts in conventional medical practice, suggesting that they could serve as an organic source of antioxidants.

In Mohammed *et al.*'s 2022 study, it was discussed that various phytochemicals can be found in different parts of Senna species, along with other constituents. Research experiments demonstrated that extracts from Senna plants possess strong antioxidant characteristics. The efficiency of Senna plant extracts regarding them biological activity is closely linked to the existence of bioactive compounds.

Rajendran Raja Priya *et al*., in their 2022 research, highlighted that oxidative stress, caused by free radicals in the body, is responsible for numerous health issues. Study was conducted in order to examine the phytochemical makeup, characteristics, as well as an antioxidant's hydroalcoholic leaf extract from S. *alata*. The study aimed to evaluate the in vitro antioxidant capabilities and perform a phytochemical screening of S. *alata*. To gauge the hydroalcoholic extract's ability to neutralize free radicals, various tests including the DPPH assay (715.26 µg/ml), reducing power assay (715.26 µg/ml), ABTS test (643.95 µg/ml), and nitric oxide test (856.79 µg/ml) were employed.

Iqbal Ahamad *et al*. (2006) used infrared spectroscopy to identify significant categories of chemicals as the most active fraction of extracts from four plants.The bioactive group of compounds in the dry leaf powder of *Calotropis gigantea* were tested by FTIR analysis by Ramamoorthi and Kannan in 2007. Using FTIR spectroscopy, Kareru *et al*. (2008) found saponins in the crude dry powder of 11 different plants. *Eclipta alba* and *Eclipta prostrata* leaf, stem, and root powder samples were subjected to FTIR spectroscopic investigation by Muruganantham *et al*.

Gaurav kumar *et al*. performed an FTIR analysis on the aqueous methanolic leaf extracts of *Bauhinia racemosa* to look for phytochemical components (2010). Using a spectroscopic approach, Ragavendran *et al*. (2011) identified the functional groups in several *Aerva lanata* samples. Using FTIR spectroscopic technique, Thangarajan Starlin *et al*. (2012) identified the elements and functional groups in the extract of the ethanol from the entire *Ichnocarpus frutescens* plant. The ethanol extract of Ampelocissus latifolia leaves contained functional groups subjected to an FTIR spectroscopic examination by Paraj A.

Ashok kumar *et al.* (2014) done Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. FTIR analysis of methanol fraction of leaves of *Oxystelma esculentum* R.Br. was completed by Savitha *et al.* in 2015.

CHAPTER - III

MATERIALS AND METHODS

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MATERIALS AND METHODS

Oxystelma esculentum R.Br. plant was collected from near NH-27 (Coordinates 25°05'41.2"N 76°29'41.2"E) Baran district, Rajasthan, India during the month of August, 2022. Herbarium was deposited in Department of Botany, Janki Devi Bajaj Government Girls College, Kota. The collected specimens were washed with tap water, then surface-sterilized with a 10 percent sodium hypochlorite solution, rinsed with sterile distilled water, and allowed to shade dry at room temperature. The samples were then ground into a fine powder using an electric blender.

One hundred grams of each powdered plant material (fruits, and flowers) was successively extracted with different solvents using a Soxhlet apparatus for 8 hours (Vogel, 1978). The extracts were filtered, combined, and the solvents were evaporated using a rotary evaporator (Heidolph, Germany) under reduced pressure at 40°C. The crude extracts were then stored at 4°C in a refrigerator for further action.

After being stored for four hours in a chromic acid cleaning solution (10 g of potassium dichromate in 1000 mL of 36 N sulfuric acid), the glassware was thoroughly cleaned with a detergent solution using tap water and then rinsed with distilled water. It was then dried in a dust-proof cabinet and oven dried at 160°C for one hour before being stored for future use.

The media were autoclaved for 30 minutes at 15 psi (pounds per square inch) to sterilize them. A hot air oven set at 160 degrees Celsius was used to disinfect the glassware for one hour. All glass syringes and test tubes, which needed to be plugged with cotton wool, were sterilized using a hot air oven. Inoculation loops and forceps tips were sterilized by heating them in a Bunsen flame until they became red hot. The mouths of culture tubes, as well as scalpels, needles, and other objects, were occasionally flamed and then submerged in methylated spirit before being briefly exposed to the Bunsen flame.

3.1 PHYTOCHEMICAL ANALYSIS

3.1.1 Qualitative Phytochemical Screening

3.1.1.1 Alkaloids

One milliliter sample will be diluted in 5 millilitres of 10% ammonia solution, then extracted using 15 millilitres of chloroform. After the chloroform component has evaporated completely, the residue left behind will be examined for the existence of alkaloids.

3.1.1.1.1 Mayer's reagent (Bertrand's reagent)

A few droplets of Mayer's reagent will be introduced to an acidic solution in a test tube. This portion will be watched for any opalescence or precipitate that is yellowish that may indicate the alkaloids' existence.

3.1.1.1.2 Dragendorff's reagent

10% ammonia solution will be added to a test tube holding two milliliters of acidic solution in order to neutralize it. To the above neutralize mixture, after adding Dragendorff's reagent, turbidity or a precipitate will be seen, this suggests that alkaloids are there.

3.1.1.2 Saponins (Frothing Test)

Ten milliliters of distilled water and three milliliters of the extract solution will be mixed together in a test tube. After stopping the test tube and giving it a good shake for roughly five minutes, after allowing it to remain upright for thirty minutes, the presence of saponins will be determined by looking for the production of honeycomb foam.

3.1.1.3 Flavonoids

3.1.1.3.1 Shibita's reaction test

Five to six drops of strong HCl and metal magnesium will be added after one milliliter of the extract has been boiled to 50% in one or two milliliters of methanol. Flavonols are present in the solution when it goes red, while flavones are present when it turns orange.

3.1.1.3.2 Pew's test

Five milliliters of the extract solution will be mixed with eight milliliters of concentrated sulfuric acid and 0.1 g of metallic zinc. The mixture should have a reddish tint, which indicates the presence of flavonols.

3.1.1.4 Tannins (Ferric chloride test)

Some drops of a pale yellow, 10% ferric chloride solution will be mixed with 2 millilitres of the extract solution. Gallic tannins were suggested by the existence of a blackish blue hue, while catechol tannins were indicated by the existence of a greenish-blackish tint.

3.1.1.5 Steroids

3.1.1.5.1 Liebermann-Burchard's test

Ten millilitres of anhydrous chloroform will become accustomed to dissolve one millilitre of the extract before it is filtered. For the next tests, the solution will be split divided into two equal parts. One millilitre of acetic anhydride will be combined with the initial portion of the fix above, and then one millilitre of strong sulphuric acid will be added down the test tube's side to create a layer underneath. We'll look for green colouring in the test tube, this suggests the use of steroids.

3.1.1.5.2 Salkowski's test

The second part of the solution above will be carefully mixed with a lower layer of strong sulfuric acid, and the interface will be examined for the presence of a steroid ring by looking for a reddish-brown color.

3.1.1.6 Phenols

To One ml of extract solution add a neutral solution of ferric chloride slowly dropwise. Observe the change in colour. A red, blue, green or purple colouration indicates the presence of phenol.

3.1.1.7 Carbohydrates

3.1.1.7.1 Molisch's test

A small amount of concentrated sulfuric acid will be allowed to drip down the side of the test tube to form a layer without shaking after two milliliters of the extract solution and a few drops of Molisch's solution have been combined. The presence of carbohydrates will be indicated by a purple color on the interface.

3.1.1.7.2 Barfoed's test

After adding one ml of the extract's aqueous solution and one ml of Barfoed's reagent to using a test tube and heating it in a water bath for roughly

two minutes; the presence of monosaccharides was indicated by the red precipitate.

3.1.1.8 Ketones

To 2 mL of the extract's aqueous solution, a little amount of resorcinol crystals and an equivalent volume of strong HCl were added. After heating the combination over a spirit lamp flame, the mixture was examined for the presence of ketones by looking for a rose coloration.

3.1.1.9 Pentoses

2 mL water-soluble extract filtrate was introduced to a quantity of strong hydrochloric acid that was comparable that contained a small amount of phloroglucinol. This is heated over a spirit lamp flame, and the presence of pentoses is detected by looking for red coloration.

3.1.1.10 Phlobatannins (HCl test)

After adding 2 milliliters of the extract's aqueous solution to diluted HCl, researchers looked for a crimson precipitate, this demonstrated that phlobatannins were found.

3.1.1.11 Cardiac glycosides

Three droplets of a strong lead acetate solution were mixed with two milliliters of the extract's aqueous solution. This was well combined and then filtered. In a separating funnel, 5 ml of chloroform was agitated with the filtrate. In a little evaporating dish, the layer of chloroform vanished until it was completely dry. The residue was placed on top of two milliliters of sulfuric acid concentration in a testing tube after being dissolved in glacial acetic acid with a hint of ferric chloride. The presence of cardiac glycosides was seen in the bluish-green coloration of the topmost layer and the reddish-brown coloration of the interface between the two layers.

3.1.1.12 Anthraquinones (Borntrager's reaction for free anthraquinones)

One milliliter of the extract and twenty milliliters of chloroform were placed into a dry test tube. This was steamed for five minutes in a steam bath. After being heated, the extract was cooled after being filtered. The addition of a 10% ammonia solution in an equal proportion to the filtrate. After shaking it, the brilliant pink colour of the top portion is watery layer—which is suggestive of the

existence of anthraquinones. In a test tube, for the control test, 10 milliliters of a 10% ammonia solution were mixed with 5 milliliters of chloroform.

3.1.1.13 Standard test for combined reducing sugars

A hydrolyzed extract was used in 1 mL by heating it in five millilitres of dil. HCl. Solution of sodium hydroxide was used to neutralize this. The Fehling's test was performed again as previously mentioned, and the existence of combination reducing sugars was determined by looking for a reddish-brick precipitate in the tube.

3.1.1.14 Standard test for free reducing Sugar (Fehling's test)

Fehling's solutions I and II were combined in a test tube with 2 milliliters of the extract. The mixture was then warm in a bath of water for approximately two minutes. Reducing sugars were present because of the brick-red precipitate.

3.1.2 Quantitative Phytochemical Screening

3.1.2.1 Alkaloids

One milliliter of extract was taken. To it 5.0 ml of phosphate buffer and 5.0 ml of BCG was added followed by 4.0 ml of chloroform and shaken vigorously. The layer of chloroform was collected in a 10.0 ml volumetric flask and made up the volume with Chloroform. The absorbance was measured at470 nm. Atropine (100 mg/ml) was taken as standard.

3.1.2.2 Saponins

The method used was that of Obadoni and Ochuko (2001). One milliliter (1 ml) extract was put into a conical flask and 100 ml of 20% ethanol is included in the sample. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a soak in the water at about 90ºC. The concentrate was then transferred into a 250 ml separating funnel and diethyl ether (20 ml) was added to the extract and vigorously shaken. The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process was repeated. 60 ml of *n*-butanol was included and the combined *n*-butanol extracts were washed twice with 10 ml of 5% sodium chloride. The remaining solution

was then heated in a water bath and after evaporation; the samples was dried in the oven to a constant weight and values was expressed as mg/g of extract.

3.1.2.3 Flavonoids

Using Chang's approach, the sample's total flavonoid content was estimated. A slightly modified colorimetry approach was employed to ascertain the flavonoid concentration. After combining a 0.5 ml aliquot of the specimen solution using two milliliters of distilled water, 0.15 ml of 5% $NaNO_2$ solution was added. Subsequently, 2 millilitres of 4% NaOH solution were added to the mixture, and after 6 minutes, 0.15 millilitres of 10% AlCl3 solution was included, and left to stand for 6 minutes. Water was included right away to make the final volume 5 ml. After that, the mixture was well mixed and let to stand for an additional 15 minutes. At 510 nm, the mixture's absorbance was measured in relation to a water blank. As standard, 100 mg/ml of Quercetin was used.

3.1.2.4 Tannins

Tannins were determined by the method of Peri and Pompei. One milliliter (1 ml) of the sample extracts was taken in a test tube. The volume was made up to 1ml with distilled water and one milliliter of water serves as the blank. To this 0.5 ml of Folin’s phenol reagent (1:2) followed by 5 ml of 35% sodium carbonate was added and the temperature was maintained at room 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 10mg/ml) was plotted, from which the tannin content of the passage was determined.

3.1.2.5 Steroids

One milliliter (1 ml) of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulfuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), was added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20ºC for 30 minutes with occasional shaking and diluted to the mark using purified water. In comparison to the reagent blank, absorption was determined at 780 nm.

3.1.2.6 Total Phenolic Content (TPC)

With minor adjustments, the Folin-Ciocalteau technique developed by Slinkard and Singleton was used to calculate the extract's total phenolic content.

Briefly, 0.1 ml of extract, 1.9 ml distilled water and 1 ml of Folin-Ciocalteau's reagent was included in a tube, and then 1 ml of sodium carbonate was included. The combination used in the reaction was incubated at 25°C for 2 h and the ability to absorb of the mixture was read at 765 nm. A standard graph (gallic acid- 10 mg/ml) was plotted, from which the phenolic content of the extract was determined.

3.1.2.7 Carbohydrates

For three hours, in a boiling water bath, 10 milliliters of the extract and five milliliters of 2.5N HCl underwent hydrolysis. After bringing it to room temperature, solid sodium carbonate was added and stirred until the effervescence subsided. After centrifuging the contents, Distilled water was introduced to the supernatant to make 100 millilitres. This allowed for the pipetting of 0.2 ml of sample to make 1 ml of volume using distilled water. Next, 1.0 millilitre of phenol reagent and 5.0 millilitres of sulphuric acid will be added. The tubes were maintained for 20 minutes at 25–30°C. After measuring the absorbance at 490 nm and using glucose (10 mg/ml) as a reference, the concentration was computed.

3.1.2.8 Proteins

After extracting 0.2 millilitre, 1.0 millilitre was created using distilled water. After adding 5.0 ml of alkaline copper reagent to each tube, they were let to stand for ten minutes. After adding 0.5 ml of Folin's Ciocalteau reagent, the mixture was left to incubate for 30 minutes in the dark. At 660 nm, the color's developed intensity was measured and BSA (1mg/ml) was taken as standard and from which the concentration of proteins was calculated.

3.1.2.9 Determination of Percentage Lipid

Petroleum ether was combined with 10ml of the extract (100 ml) to cause a reflux and the lipid was extracted for 3 hours by heating on an electric hot plate at 50°C. The extractant (petroleum ether) was distilled off and the lipid recovered by cooling the flask in a dessicatorand its value calculated by reweighed flask and content.

3.1.2.10 Quantification of Terpenoids

10 ml of each extract was soaked in alcohol for a day. Later on it was

filtered and petroleum ether was use for purpose of extraction. The extracted material was calculated and considered as terpenoids.

3.1.2.11 Estimation of total glucosinolate content by spectrophotometric method

100 µl of extract was used for estimation. 0.3 ml double distilled water and 3 ml of 2 mM sodium tetrachloro palladate (58.8 mg Sodium tetrachloropalladate +170 µl concentrated HCl +100 ml double distilled water) were incorporated into it. After a one-hour incubation period at room temperature, absorbance was measured at 425 nm using a spectrophotometer. A blank was set following the same procedure without the extract. Total glucosinolates was calculated by putting the OD of each sample taken at 425 nm in formula.

3.1.2.12 Quantitative Analysis of Total Anthraquinones

The extract (1ml) was accurately measuredand distilled water (29 ml) was included. The concoction was mixed and refluxed on a water bath for 15 minutes. The flask was permitted to cool, and the concoction was centrifuged at 4000 rpm for 10 minutes. 20 ml of the supernatant liquid was transferred to a separatory funnel and acidified with 2 M hydrochloric acid. Fifteen milliliters of chloroform was added, the mixture was extracted and the chloroform layer was discarded. The aqueous layer was separated and 0.10 g of sodium bicarbonate was included. The concoction was then shaken for 3 minutes and centrifuged at 4000 rpm for another 10 minutes. Ten milliliters of the supernatant liquid was transferred to a 100 ml flask. The solution of 10.5% w/v ferric chloride hexahydrate (20 ml) was included and mixed the concoction was refluxed on a boiling water bath for 20 minutes. Concentrated hydrochloric acid (1 ml) was included and the concoction was heated for 20 minutes, with frequently shaking to dissolve the precipitate. The mixture was cooled, transferred to a separatory funnel and shaken with 167-172 ml diethyl ether. The diethyl ether extracts were combined and washed with 15 ml distilled water twice. The combined diethyl ether was then transferred to a 100 ml volumetric flask and adjusted to volume. Twenty-five milliliters of the resolution was evaporated to dryness. The residue was dissolved with 10 ml of 0.5% w/v magnesium acetate in methanol yielding a red solution. The UV absorbance was measured at 515 nm.

Statistical Analysis

The experiments were conducted in triplicate, and the recorded data was analyzed for mean and standard deviation using MS Office Excel 2007. Linear correlation coefficient and correlation analysis were performed. The linear regression equation for a straight line, Y=mx+c, was applied, where Y represents the absorbance of the extract, m is the slope of the calibration curve, x denotes the concentration of the extract, and c is the intercept. Quantitative analysis was conducted using the regression equation based on the calculated concentrations of each extract.

Standard Deviation (SD)

It was computed using the subsequent formula:

$$\mathrm{SD} = \sqrt{\frac{\sum |x - \bar{x}|^2}{n}}$$

Whaere,

SD = Sample standard deviation

Σ = Sum of

$\bar{x}$ = Sample mean

n = Number of scores in sample

3.2 ANTIMICROBIAL ANALYSIS

3.2.1 Used Media for Screening

3.2.1.1 Bacterial Medium and It's Preparation
(i) Muller - Hinton Agar

Ingredients	Gram/litre
Beef infusion	300.0
Casein acid hydrolysate	17.5
Agar	17.0
Starch	1.5

After bringing the pH to 7.3±0.2 at 25°C, 38 g of Mueller Hinton Agar was suspended in 1000 mL of distill water. The agar was then heated to completely dissolve the medium. The medium was well combined and autoclaved for 15 minutes at 15 lbf/sq. inch pressure (121°C) prior to pouring.

3.2.1.2 Fungal Medium and It's Preparation
(i) Sabouraud Dextrose Agar (MO63)

Ingredients	Gram/litre
Mycological peptone	10.00
Dextrose	40.00
Agar	15.00

The medium was completely dissolved by boiling the agar after the pH was adjusted to 5.6±0.2 at 25°C and 65 g of powdered Sabouraud Dextrose Agar was suspended in 1000 milliliters of distilled water. The agar was sterilized by autoclaving it for 15 minutes at 121°C and 15 lbf/sq inch of pressure.

3.2.2 Assessing Antimicrobial Efficiency *in Vitro*

3.2.2.1 **Microorganisms Used**

Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and the two fungus species *Aspergillus flavu*s and *Candida albicans* were all isolated clinically. On Sabouraud dextrose agar for fungi and Muller Hinton agar for bacteria, the stock cultures were kept at 4ºC. Muller Hinton Agar (MHA) was used to measure *in vitro* antibacterial activity and Sabouraud Dextrose Agar was used to measure in vitro antifungal activity (SDA).

3.2.2.2 Preparation of Inocula

Physiological saline was added to a 24-hour-old culture of particular bacteria and fungus to achieve 0.5 (CFU) per milliliter, the Mac Farland turbidity standard. The next step involved injecting physiological saline in sterility to correct the turbidity. The mould fungus was subcultured and incubated on SDA for 24-48 hours at 28°C for Candida sp. and 72-96 hours at 30°C for Aspergillus sp. To come to a spectrophotometric standard for the fungal solution at 530 nm absorbance of 0.600, the expansion was thoroughly scraped, ground, and thoroughly macerated in deionized, sterilized water. (Espinel-Ingroff and Kerkering, 1991).

3.2.2.3 Making the Assessment Solution and Disc

The solution for testing consisted of 100 mg of unrefined extracts in 5% DMSO. Twenty micro liters of the extract (corresponding to fifty, twenty-five, and twelve-five mg/mL of crude extracts) was deposited onto test discs for sterile susceptibility, and they were then left under nitrogen at RT.

3.2.2.4 Sterility Check

To verify their purity, all of on MHA and SDA, the extracts were streaked plates. The plates underwent scrutiny following the incubation time (24 hours for bacteria and 24-48 hours for fungi).

3.2.3 Antimicrobial Assay

3.2.3.1 Disc Diffusion Method

Using the disc diffusion technique, Bauer *et al*. (1966) evaluated the antibacterial qualities of crude extracts of *Oxystelma esculentum* R.Br. In petridishes, 20 mL of MHA/SDA was poured and allowed to settle. Plates were dried before inoculating the entire agar surface with 0.1 mL of standardised inoculum suspension (bacteria/fungi). The discs with various crude extract concentrations were made and aseptically put to using sterile forceps to gently press against the petri plate surface in order to make contact with the infected agar surface. Positive controls included ciprofloxacin (5µg/disc) for bacteria,

amphotericin-B (discs containing 100 units) for *Candida* and ketoconazole (10 µg/disc) for *Aspergillus*. In these tests, 10% DMSO was utilised as negative control. Finally, the inoculation plates underwent incubation for 24 hours at 37°C for bacteria, 24 hours at 28°C for *Candida* sps., and 72-96 hours at 30°C for *Aspergillus* sp. The inhibitory zones were examined and recorded in millimeters. In these trials, each test was performed three times.

3.2.3.2 Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration was measured in MHB for bacteria and SDA for fungi using the broth macro dilution technique (Ericsson and Sherris, 1971). The plant extracts were diluted in 10% DMSO to yield 2 mg/mL. To obtain a concentration of 1000 to 15.62 µg/mL, 0.5 mL of MHB and 0.5 mL of the standard solution were combined. Each tube received 50 µL of standardised test organism suspension. The control tube contained only organisms and no plant extracts. The culture tubes were cultured for 24 hours at 37°C for bacteria and 24–48 hours at 28°C for Candida species and 30°C for 72-96 hours for *Aspergillus* spp. After macroscopic examination, the lowest concentrations that didn't indicate any expansion of the tested organism were identified as MIC.

3.2.3.3 Minimal Fungicidal Concentration (MFC) and Minimal Bactericidal Concentration (MBC)

By plating 100 µL of growth-inhibited material in every MIC test tube into freshly made MHB and SDA and incubating the plates at 37°C for 24 hours (for bacteria) and 28°C for 48–72 hours (for SDA) (fungi), Kartnig et al. (1991) evaluated the MBC and MFC of the extracts. Using the extracts at the lowest concentrations that prevented any discernible bacterial or fungal colony development on the agar plate during the incubation period, the amounts of MBC and MFC were calculated.

3.3 ANTIOXIDANT ANALYSIS

Different portions of *Oxystelma esculentum* R.Br. tests were carried out on crude extracts to their ability to scavenge radicals utilizing the subsequent methods: hydrogen peroxide, superoxide radical, nitric oxide radical, hydroxyl radical scavenging assay, ferric reducing antioxidant power, and overall antioxidant activity.

3.3.1 Free Radical Scavenging Activity of DPPH

The action that scavenges radicals of DPPH of various extracts isolated from flowers and fruits of *Oxystelma esculentum* R.Br. was assessed following the methodology outlined by Hatano *et al.* in 1988. The radical DPPH scavenging potential of various plant-based extracts in contrast to the stable DPPH radical was determined through spectrophotometry. In this procedure, one milliliters of each extract having various concentration was combined with three milliliters of a 0.004 percent free radical solution in methanol. After a 30-minute incubation period, the absorbency of the resulting solutions was 517 nm in measurement by a UV spectrophotometer. This measurement was then by contrast with the absorption of a standard solution containing ascorbic acid.

To establish a baseline, methanol was employed to zero the spectrophotometer absorbance of the radical in the absence of antioxidants. The actual reduction in absorbance caused by the tested sample, indicated by a change in color from deep violet to light yellow, was compared to that of the positive control, which contained ascorbic acid. The inhibition of the free radical DPPH was estimated by a specific formula, and the extract's concentration required to achieve 50% inhibition (IC50) was determined.

Effect of scavenging (%) = (Absorbance of control- Absorbance of sample) /Absorbance of control ×100

3.3.2 ABTS Scavenging Effects

The ABTS.+ in contrast to the scavenging activity was assessed utilizing the procedure introduced by Re *et al.* in 1999, with a few adjustments. To evaluate the effects of the ABTS. + radical, a 5 mL aqueous solution of ABTS. + (7 mM) combined with 88 µL of a potassium persulfate solution 140 mM

(resulting in a final concentration of 2.45 mM). This mixture was maintained in the dark at 29°C for 14 hours before use and was subsequently diluted with ethanol to achieve an absorbance reading of 0.7000±0.02 units at 734 nm using a UV-Vis spectrophotometer (specifically, a U-200 Hitachi model. Reference substances, such as ascorbic acid, were allowed to react with 3 mL of the resulting blue-green ABTS. + radical solution under dark conditions. The scavenging behavior of solvent methanol was evaluated at different concentrations. Three duplicates of each experiment were carried out.

Effect of scavenging (%) = (Absorbance of control- Absorbance of sample) /Absorbance of control ×100

3.3.3 Superoxide Radical Scavenging Assay

The riboflavin-light-NBT system proposed by Hyland *et al*. (1983) supported Superoxide anion radical scavenging evaluate activity. In summary, 1 ml of sample was mixed with 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT (0.5 mM) at different concentrations (25 to 500µg/ml). The reaction mixture in was illuminated by a fluorescent bulb in order to initiate the reaction. At 560 nm, Measurements were made of absorbance following a 20-minute incubation period. The standard was ascorbic acid. The following formula accustomed to calculate the plant extract's scavenging capacity.

Effect of scavenging (%) = (Absorbance of control- Absorbance of sample) /Absorbance of control ×100

3.3.4 Nitric Oxide Radical Scavenging Assay

The activity of nitric oxide scavenging was assessed using spectroscopic techniques, following the method outlined by Govindarajan *et al*. in 2003. Various extracts were introduced into different test tubes at different concentrations (100, 150, 200, 250, and 300 µg/mL). To each test tube, Sodium nitroprusside at 5 mM in a phosphate buffer was added to reach a total volume of 1.5 mL. After that, these solutions were incubated for 30 minutes at 25ºC. Afterward, 1.5 mL of Griess reagent (comprising 1% sulphanilamide, 0.1% naphthylethylenediamine

dichloride, and 3% phosphoric acid) was added to each test tube. The absorbance was promptly measured at 546 nm, and the percentage of scavenging activity was determined, using ascorbic acid as the standard for reference.

Effect of scavenging (%) = (Absorbance of control- Absorbance of sample) /Absorbance of control ×100

3.3.5 Hydrogen Peroxide (H_2O_2) Scavenging Assay

H_2O_2 scavenging activity was assessed following the procedure described by Ruch *et al*. in 1989. A phosphate buffer (pH 7.4) was used to prepare a solution of H_2O_2 with a concentration of 40 mM. The concentration of H_2O_2 was determined using spectrophotometry at 230 nm. Various concentrations (100, 150, 200, 250, and 300 µg/mL) of different extracts obtained from *Oxystelma esculentum* R.Br. were subsequently included in 0.6 mL of the 40 mM H_2O_2 solution. After 10 minutes of incubation, the absorbance of the resultant mixture was determined at 230 nm. This measurement was compared to a blank solution containing residual oil within phosphate buffer but lacking H_2O_2. The percentage of H_2O_2 scavenged was calculated using the following formula.

Effect of scavenging (%) = (Absorbance of control- Absorbance of sample) /Absorbance of control ×100

3.3.6 Hydroxyl radical scavenging assay

Efficient scavenging of hydroxyl radicals by various extracts was assessed following the protocol developed by Klein and colleagues in 1991. Different concentrations (100, 150, 200, 250, and 300 µg/mL) of these extracts were combined with a solution composed of 1.0 milliliters of iron-EDTA solution (containing 0.13% Ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of 0.85% dimethylsulphoxide (DMSO) in a 0.1 M phosphate buffer with a pH of 7.4. The combination used in the reaction was quenched by adding 0.5 mL of ascorbic acid (0.22%), as well as the mixture's incubation at a temperature between 80-90°C for 15 min. in a water bath. After incubation, the response was quenched with 0.1 milliliters of ice-cold TCA (17.5% w/v). Following this, three mL of Nash reagent (a mixture of 75.0 g of

ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone, diluted to 1 L with distilled water) was included and given permission to sit at room temperature for 15 min. A control sample, which did not have extract was prepared, using vitamin C. Using a spectrophotometer, the resultant color's intensity was determined at 412 nm with a blank reagent. The proportion of OH radical scavenging efficiency was estimated by the below formula.

$$\text{Effect of scavenging (\%)} = (\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control} \times 100$$

3.3.7 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was conducted using a modified version of the method described by Chu *et al.* in 2000. To perform this assay, 2.5 mL of 0.1 M potassium phosphate buffer with a pH of 6.6 was combined with 2.5 mL of a 1% potassium ferricyanide solution. Subsequently, 1 mL of extracts at various concentrations was included in this mixture. After that, the resulting mixture was left to stand for 20 minutes at 50°C. After the incubation period, 2.5 mL of 10% trichloroacetic acid was introduced into the mixture. Following this, 2.5 mL of water and 0.5 mL of a 0.1% $FeCl_3$ solution to the mixture of the reaction, were added. The solution was further 30 minutes of room temperature incubation was employed to facilitate the development of color.The amount of Ferric reducing antioxidant power was determined in terms given as milligrams of Gallic acid equivalents (GAE) per gram of dry weight.

3.3.8 Total Antioxidant Activity

The overall antioxidant efficiency of different crude extracts from *O. esculentum* was assessed following the method developed by Prieto *et al*. in 1999. In a nutshell, three milliliters of a solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with 0.3 milliliters of the material. This reaction mixture was then incubated at 95°C for 90 minutes in a water bath. After the incubation period, the absorption ability all the sample mixtures were calculated at 695 nm. The overall antioxidant efficiency was calculated and expressed regarding equivalents of ascorbic acid.

3.4 FTIR ANALYSIS

Perhaps the most efficient tool for determining the kinds of chemical bonds (functional groups) present in compounds is the Fourier Transform Infrared Spectrophotometer (FTIR). As can be seen in the annotated spectrum, the wavelength of light absorbed is indicative of the chemical bond. The chemical bonds in a molecule can be identified by analysing the infrared absorption spectra. FTIR analysis was conducted using dried powdered methanol solvent extracts of each plant material. To create translucent sample discs, 100 mg of KBr pellet and 10 mg of the dried extract powder were combined. Each plant specimen's powdered sample was placed into a Shimadzu IR Affinity 1 FTIR spectroscope, which has a scan range of 400 to 4000 cm^{-1} and a resolution of $cm.^{-1}$

3.5 GC-MS ANALYSIS

Gas chromatography (GC) analysis was conducted using a Varian 3800 gas chromatograph equipped with a mass selective detector connected to a type 1079 front injector. The chromatograph utilized a DB 5 MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The injector temperature was set at 280°C. The oven temperature was initially set to 45°C, then increased to 300°C at a rate of 10°C per minute, and finally held at 200°C for 5 minutes. The carrier gas was helium, flowing at a rate of 1.0 mL/min. One microliter of the sample, diluted with acetone at a ratio of 1:10, was injected in split mode at a ratio of 1:100. The compound's percentage composition was ascertained by the GC peak areas.

The compound's GC–MS analysis was carried out using the Varian 3800 gas chromatograph paired with a Varian 1200 L single quadrupole mass spectrometer. The GC conditions and column were the same as those used in the GC analysis. The mass spectrometer operated in electron impact mode at 70 eV, with the ion source and transfer line temperatures maintained at 250°C. Mass spectra were obtained using a centroid scan over the mass range of 40 to 1000 amu. Compounds were identified by comparing their retention indices (RI), retention times (RT), and mass spectra using information from the WILEY and NIST libraries and literature data (Adams, 2009).

3.6 HPLC ANALYSIS

Two Shimadzu LC-10 ATVP reciprocating pumps, a Shimadzu SPD-10 AVP UV-VIS detector, and a Rheodyne Model 7725 injector with a twenty-micro milliliter loop size were all part of the HPLC apparatus (Shimadzu Corporation, Kyoto, Japan). The sample was extracted using a mixture of alcohol, water, and hydrochloric acid (50:20:8). The mobile phase was prepared from a mixture of methanol, water, and phosphoric acid (100:100:1). Standard solutions were prepared by accurately weighing quantities of USP Quercetin RS, kaempferol, and isorhamnetin, which were then dissolved in methanol and diluted to achieve a standard solution of 1 µg/mL.

For the test solution, 10 g of the sample was finely powdered, accurately weighed, and transferred to a 250 mL flask fitted with a reflux condenser. After adding 78 mL of extraction solvent, the mixture was refluxed on a hot water bath for 135 minutes. The mixture was then transferred to a volumetric flask. Subsequently, 20 mL of methanol was added to the 250 mL flask and sonicated for 30 minutes. The resulting filtrate was collected in a 100 mL volumetric flask, and the residue was washed with methanol. This methanol wash was also filtered and collected in the same volumetric flask, then diluted to volume and mixed.

The liquid chromatograph had a 4.6 mm × 25 cm column filled with L1 and a 270 nm detector. The flow rate was approximately 1.5 mL per minute. Standard solution 1 was chromatographed, and the peak responses were recorded. Equal volumes (about 20 µL) of each standard solution were separately injected into the chromatograph. The chromatograms were then recorded, and the major peak areas were measured. The percentage of the compound in the sample was subsequently calculated.

CHAPTER - IV

RESULTS

CHAPTER - IV

RESULTS

1. Qualitative Phytochemical Screening

Table 1

Preliminary phytochemical analysis of medicinal plant using methanol extracts

S. No.	Parameters	Sample Fruit	Sample Flower
1.	Alkaloid- Dragendorff reagent	++	+
2.	Alkaloid- Mayers reagent	+	-
3.	Tannins- Gallic	++	-
4.	Saponin	+	+
5.	Reducing sugar	+	-
6.	Free reducing sugar-Fehling solution	-	-
7.	Ketones	+	-
8.	Pentoses	+	-
9.	Phlobatannins	+	-
10.	Cardia Glycosides	+	-
11.	Steroids- Libermann test	-	+
12.	Steroids- Salkowsk's test	+	-
13.	Flavanoids- Shibita test	++	+
14.	Flavanoids- Pew's test	+	-
15.	Anthraquinones	-	-
16.	Carbohydrates- Molish test	+	-
17.	Carbohydrates- Barfoed test	+	+

(+) = Present; (-) = Absent; ++ = Strong

The crude methanol fruit extract of the plant was tested for qualitative assay for phytochemicals, of which alkaloids, flavonoids and tannins were strongly positive. Whereas other phytochemicals like saponins, reducing sugars, ketones, pentoses, phlobatannins, cardia carbs, glycosides, and steroids also showed their presence.

Similarly, flower plant extract showed presence of alkaloids, saponins, Steroids, flavanoids and carbohydrates.

Table 2.

Preliminary phytochemical analysis of medicinal plant using acetone extracts

S. No.	Parameters	Sample Fruit	Sample Flower
1.	Alkaloid- Dragendorff reagent	++	+
2.	Alkaloid- Mayers reagent	++	-
3.	Tannins- Gallic	+	+
4.	Saponin	+	+
5.	Reducing sugar	+	-
6.	Free reducing sugar- Fehling solution	+	-
7.	Ketones	+	-
8.	Pentoses	+	+
9.	Phlobatannins	+	-
10.	Cardia Glycosides	++	+
11.	Steroids- Libermann test	-	+
12.	Steroids- Salkowsk's test	+	-
13.	Flavanoids- Shibita test	++	+
14.	Flavanoids- Pew's test	++	+
15.	Anthraquinones	-	+
16.	Carbohydrates- Molish test	+	-
17.	Carbohydrates- Barfoed test	++	+

(+) = Present; (-) = Absent; ++ = Strong

The crude acetone fruit extract of the plant was tested for qualitative assay for phytochemicals, of which alkaloids, flavonoids, cardia glycosides and carbohydrates were strongly positive. Whereas other phytochemicals such as tannins, saponins, reducing sugars, free reducing sugars, ketones, pentoses, phlobatannins and steroids, also showed their presence.

Similarly, flower extract of the plant showed presence of alkaloids, tannins, Saponins, pentoses, cardia glycosides, steroids, flavanoids, anthraquinones and carbohydrates.

Table 3.

Preliminary phytochemical analysis of medicinal plant using chloroform extracts

S. No.	Parameters	Sample Fruit	Sample Flower
1.	Alkaloid- Dragendorff reagent	+	+
2.	Alkaloid- Mayers reagent	++	-
3.	Tannins- Gallic	+	++
4.	Saponin	+	+
5.	Reducing sugar	+	+
6.	Free reducing sugar- Fehling solution	-	-
7.	Ketones	++	-
8.	Pentoses	+	+
9.	Phlobatannins	+	-
10.	Cardia Glycosides	++	++
11.	Steroids- Libermann test	-	+
12.	Steroids- Salkowsk's test	+	-
13.	Flavanoids- Shibita test	+	+
14.	Flavanoids- Pew's test	++	+
15.	Anthraquinones	+	-
16.	Carbohydrates- Molish test	+	+
17.	Carbohydrates- Barfoed test	++	+

(+) = Present; (-) = Absent; ++ = Strong

The crude chloroform fruit extract of the plant was tested for qualitative assay for phytochemicals, of which alkaloids, ketones, cardia glycoside, flavonoids and carbohydrates were strongly positive. Whereas other phytochemicals such as tannins, saponins, reducing sugars, pentoses, phlobatannins, steroids, anthraquinones also showed their presence.

Similarly, flower plant extract showed strong the existence of tannins and cardia glycosides. alkaloids, saponins, reducing sugars, pentoses, steroids, flavanoids and carbohydrates were also present in the extract.

2. Quantitative Phytochemical Screening

Table 4.

Summary of preliminary quantitative phytochemical analysis of medicinal plant

S. No.	Parameters	Sample CF-Fruit (Mean ± S.D) *	Sample MF-Flower (Mean ± S.D) *
1.	Alkaloids	94.1±0.81 mg/ml	183.5±0.32 mg/ml
2.	Saponins	0.065±0.0057 mg/g	0.0133±0.06 mg/g
3.	Flavonoids	11.7±0.66 mg/ml	0.781±.008 mg/ml
4.	Tannins	11.9±0.58 mg/ml	3.2±0.24 mg/ml
5.	Steroids	916±8.17 µg/ml	1286±12.8 µg/ml
6.	Total Phenolic Content	6.2±0.54 mg/ml	3.3±0.34 mg/ml
7.	Carbohydrates	10.5±0.81 mg/ml	12.4±0.54 mg/ml
8.	Proteins	1.69±0.13 mg/ml	1.03±0.10 mg/ml
9.	Lipids (%)	1.00%	0.50%
10.	Terpenoids	1.86±0.16 gm/10ml	1.3±0.20 gm/10 ml
11.	Total glucosinolate	28.37±0.51 µmol/gm	75.88±0.86 µmol/gm
12.	Total Anthraquinones	33.60±0.71 µg/ml	19.90±0.31 µg/ml

*Values are expressed as mean±SD of three replicates.

Summary of phytochemicals found in quantitative phytochemical analysis of fruits and flowers is given in **Table. 4**. Experiments are performed in three replicates and results are shown in Mean ± S.D.

4.1 Alkaloids

Table 5

Absorbance of standard compound (Atropine) and extracts for estimation of alkaloids

S.No.	Standard/extract	Concentration (mg/ml)	Absorbance at 470 nm (O.D.)
1.	Atropine	20	0.12
		40	0.25
		60	0.4
		80	0.52
		100	0.65
2.	MF-Flower	183.5±0.32*	1.09
3.	CF-Fruit	94.1±0.81*	0.554

*Values are expressed as mean±SD of three replicates

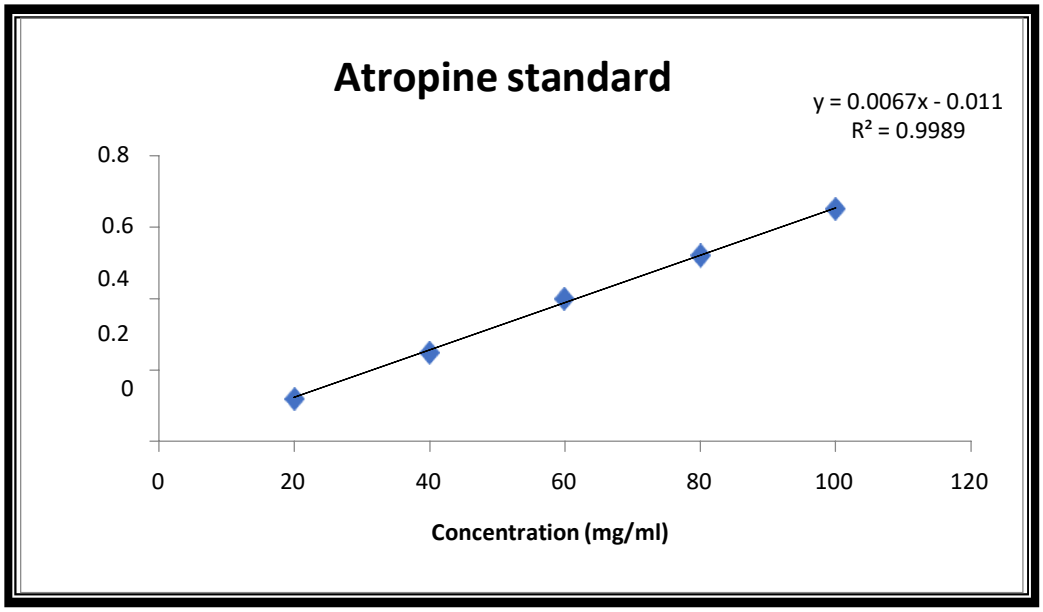

Fig.1: Standard calibration curve of Atropine for estimation of alkaloids

Absorbance of standard compound (Atropine) at 470nm is shown in **Table 5.** and **Fig.1**. The total alkaloids were stated in mg atropine equivalent using the standard curve equation Y= 0.0067X, R^2= 0.9989, where Y= absorbance at 470nm, X= total alkaloids in the extract. Total alkaloids content in methanol extract of the selected plant parts is provided in **Table 5**.

4.2 Saponins

Table 6.

Quantification of Saponins

S. No.	Sample	Quantity (mg/g)
1.	MF-Flower	0.0133±0.06*
2.	CF-Fruit	0.065±0.0057*

*Values are expressed as mean±SD of three replicates

Total saponins content in methanol extract of the selected plant parts is provided in **Table 6**.

4.3**Flavonoids**

Table 7

Absorbance of standard compound (Quercetin) and extracts for estimaton of flavonoids

S. No.	Standard/extract	Concentration (mg/ml)	Absorbance at 510 nm (O.D.)
1.	Quercetin	0.781	0.249
		1.562	0.387
		3.125	0.412
		6.25	0.525
		12.5	0.605
		25.00	0.82
		50.00	1.09
		100.00	1.8
2.	MF-Flower	0.781±.008*	0.24
3.	CF-Fruit	11.7±0.66*	0.54

*Values are expressed as mean±SD of three replicates

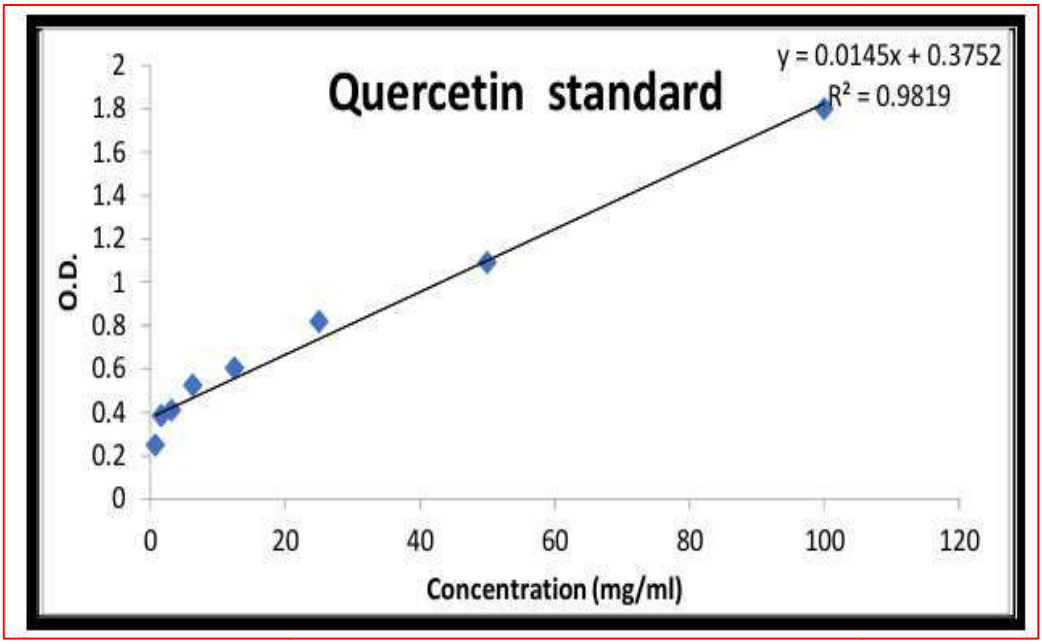

Fig. 2. Standard calibration curve of Quercetin for estimation of flavonoids

Absorbance of standard compound (Quercetin) at 510nm is shown in **Table 7.** and **Fig. 2**. The the measurement of total flavonoids was mg Quercetin equivalent using the standard curve equation Y= 0.0145X, R^2= 0.9819, where Y= absorbance at 510nm, X= total flavonoids in the extract. Total flavonoids content in methanol extract of the selected plant parts is provided in **Table 7.**

4.4 Tannins

Table 8.
Absorbance of standard compound (Gallic acid) and extracts for estimation of tannins

S.No.	Standard/extract	Concentration (mg/ml)	Absorbance at 640nm (O.D.)
1.	Gallic acid	2	0.081
		4	0.194
		6	0.269
		8	0.385
		10	0.501
2.	MF-Flower	3.2±0.24*	0.145
3.	CF-Fruit	11.9±0.58*	0.678

*Values are expressed as mean±SD of three replicates

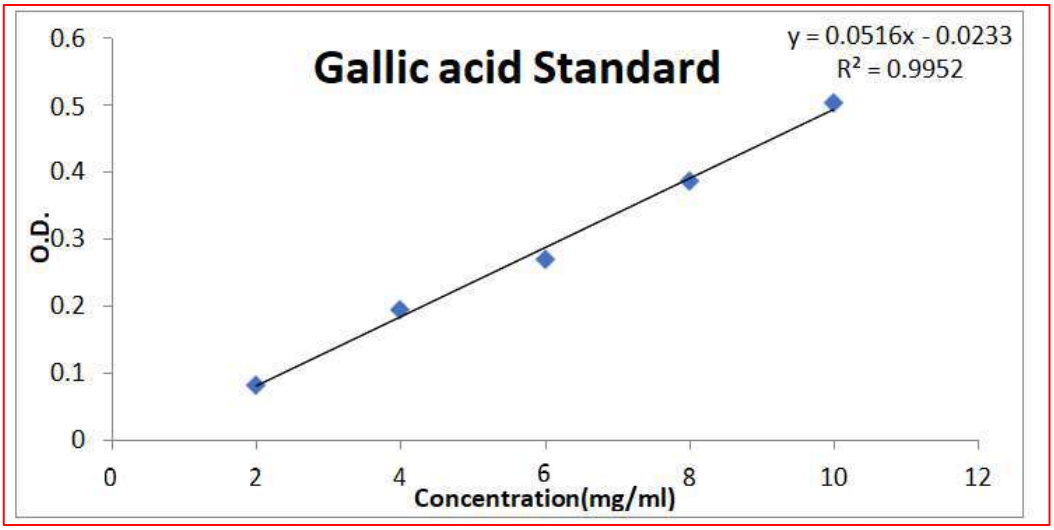

Fig. 3. Standard calibration curve of Gallic acid for estimation of tannins

Absorbance of standard compound (gallic acid) at 640nm is shown in **Table 8.** and **Fig. 3**. The total tannins were stated in milligrams of gallic acid precursor using the standard curve equation Y= 0.0516X, R^2= 0.9952, where Y= absorbance at 640nm, X= total tannins in the extract. Total tannins content in methanol extract of the selected plant parts is provided in **Table 8**.

4.5 Steroids

Table 9.

Absorbance of standard and extracts for estimation of steroids

S.No.	Standard/extract	Concentration (µg/ml)	Absorbance at 780 nm (O.D.)
1.	Standard	50	0.17
		100	0.27
		150	0.38
		200	0.45
		250	0.54
2.	MF-Flower	1286±12.8*	1.2
3.	CF-Fruit	916±8.17*	0.83

*Values are expressed as mean±SD of three replicates

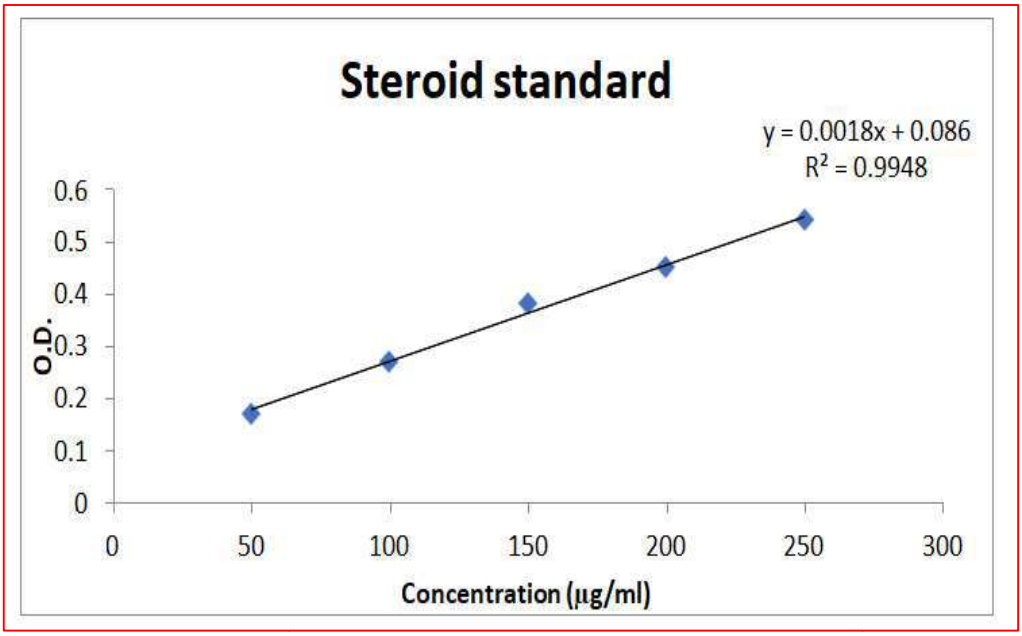

Fig. 4. Standard calibration curve of standard for estimation of steroids

Absorbance of standard compound at 780nm is shown in **Table 9.** and **Fig. 4**. The total steroids were stated in mg standard equivalent using the standard curve equation Y= 0.0018X, R^2= 0.9948, where Y= absorbance at 780nm, X= total steroids in the extract. Total steroids content in methanol extract of the selected plant parts is provided in **Table 9**.

4.6 Total Phenolic Content

Table 10.

Absorbance of standard compound (Gallic acid) and extracts for estimation of total phenolic contect

S.No.	Standard/extract	Concentration (mg/ml)	Absorbance at 765 nm (O.D.)
1.	Gallic acid	2	0.081
		4	0.194
		6	0.269
		8	0.385
		10	0.501
2.	MF-Flower	3.3±0.34*	0.15
3.	CF-Fruit	6.2±0.54*	0.294

*Values are expressed as mean±SD of three replicates

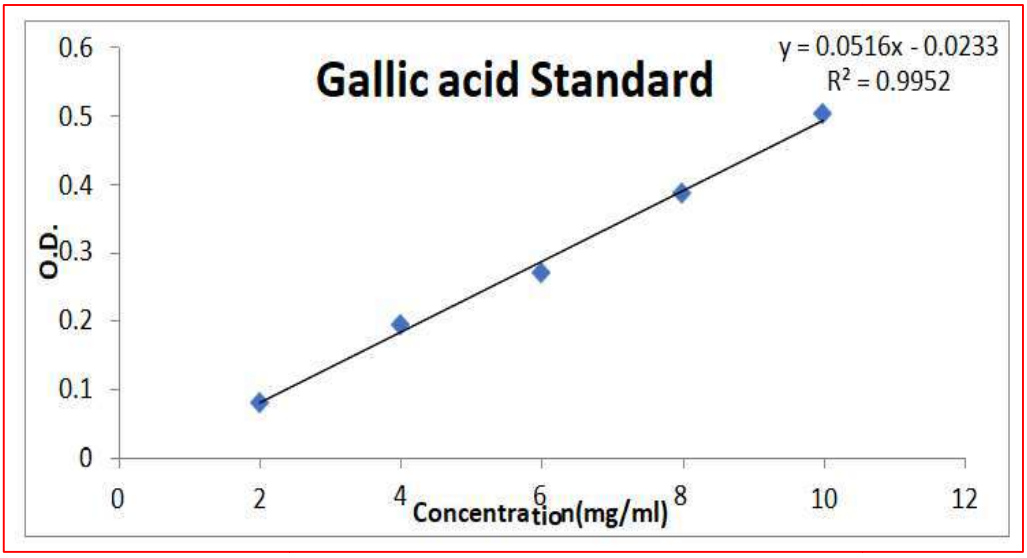

Fig. 5. Standard calibration curve of Gallic acid for estimation of total phenolic contect

Absorbance of standard compound (Gallic acid) at 765nm is shown in **Table 10.** and **Fig. 5**. The measurement of total phenolic content was mg standard (Gallic acid) equivalent using the standard curve equation Y= 0.0516X, R^2= 0.9952, where Y= absorbance at 765nm, X= total total amount of phenolics in the extract. The total amount of phenolics in methanol extract of the selected plant parts is provided in **Table 10**.

4.7 Carbohydrates

Table 11
Absorbance of standard compound (Glucose) and extracts for estimation of carbohydrates

S.No.	Standard/extract	Concentration (mg/ml)	Absorbance at 490 nm (O.D.)
1.	Glucose	2	0.081
		4	0.157
		6	0.269
		8	0.361
		10	0.501
2.	MF-Flower	12.4±0.54*	0.61
3.	CF-Fruit	10.5±0.81*	0.51

*Values are expressed as mean±SD of three replicates

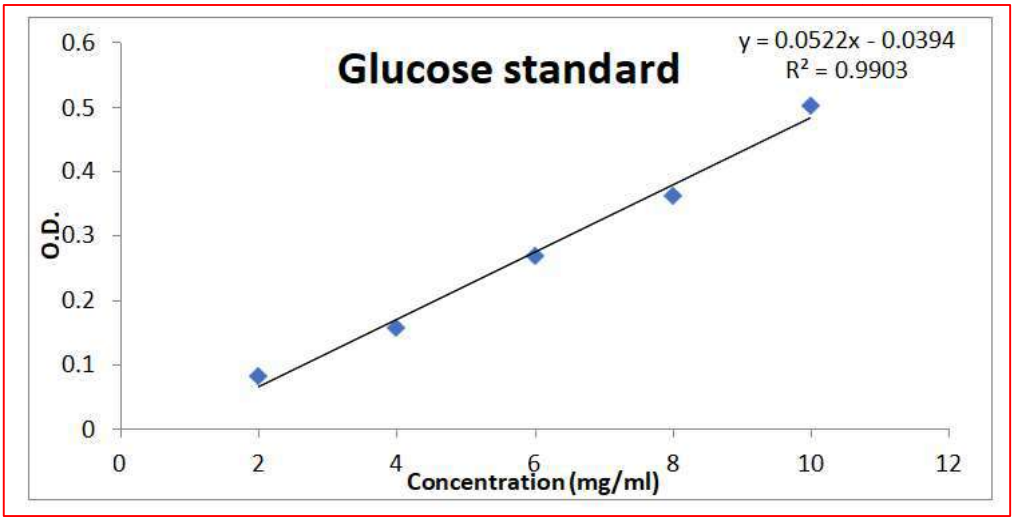

Fig. 6. Standard calibration curve of Glucose for estimation of carbohydrates

Absorbance of standard compound (Glucose) at 490nm is shown in **Table 11.** and **Fig. 6**. The total carbohydrates were stated in mg glucose equivalent using the standard curve equation Y= 0.0522X, R^2= 0.9903, where Y= absorbance at 490nm, X= total carbohydrates in the extract. Total carbohydrates content in methanol extract of the selected plant parts is provided in **Table 11**.

4.8 Proteins

Table 12.
Absorbance of standard compound (BSA) and extracts for estimation of proteins

S.No.	Standard/extract	Concentration (mg/ml)	Absorbance at 660 nm (O.D.)
1.	BSA	0.2	0.110
		0.4	0.212
		0.6	0.290
		0.8	0.301
		1.0	0.552
2.	MF-Flower	1.03±0.10*	0.559
3.	CF-Fruit	1.69±0.13*	0.92

*Values are expressed as mean±SD of three replicates

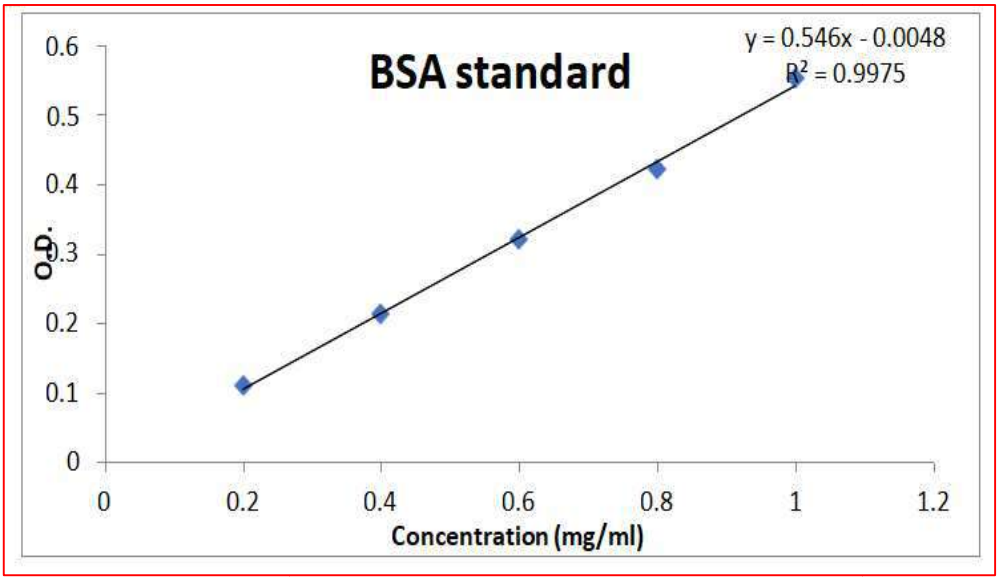

Fig. 7. Standard calibration curve of BSA for estimation of proteins

Absorbance of standard compound (BSA) at 660nm is shown in **Table 12.** and **Fig. 7**. The total proteins were stated in mg glucose equivalent using the standard curve equation Y= 0.546X, R^2= 0.9975, where Y= absorbance at 660nm, X= total proteins in the extract. Total proteins content in methanol extract of the selected plant parts is provided in **Table 12**.

4.9 Determination of Percentage Lipid

Table 13.

Determination of Percentage Lipid

S. No.	Sample	Percentage Lipid
1.	MF-Flower	0.5%
2.	CF-Fruit	1%

Total Percentage Lipid in methanol extract of the selected plant parts are provided in **Table 13**.

4.10 Quantification of Terpenoids

Table 14.

Quantification of terpenoids

S. No.	Sample 1	Quantity (gm)
1.	MF-Flower	1.3±0.20*
2.	CF-Fruit	1.86±0.16*

*Values are expressed as mean±SD of three replicates

Total terpenoids in methanol extract of the selected plant parts are provided in **Table 14**.

4.11 Estimation of total glucosinolate content by spectrophotometric method

Table 15

Absorbance of standard compound and extracts for estimation of total glucosinolate

Sr. No.	Standard/extract	Concentration (µmol/g)	Absorbance at 425 nm (O.D.)
1.	Standard	13.00742	0.097656
		24.61484	0.195313
		47.82969	0.390625
		94.25938	0.78125
		187.1188	1.5625
		372.8375	3.125
		744.275	6.25
		1487.15	12.5
2.	MF-Flower	75.88±0.86*	0.593
3.	CF-Fruit	28.37±0.51*	0.216

*Values are expressed as mean±SD of three replicates

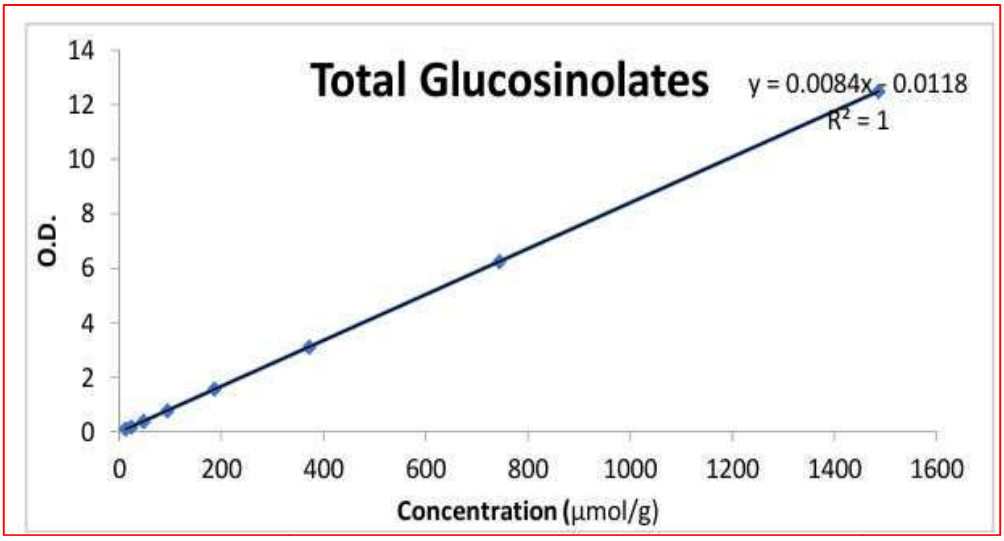

Figure 8. Standard calibration curve of standard for estimation of total glusinolate

Absorbance of standard compound at 425nm is presented in **Table 15.** and **Fig. 8**. The total glucosinolate content were expressed as mg standard equivalent using the standard curve equation Y= 0.0084X, R^2= 1.0, where Y= absorbance at 425nm, X= total glucosinolate content in the extract. **Table 15** shows the total amount of glucosinolate present in the extracted methanol of the chosen plant sections.

4.12 Quantitative Analysis of Total Anthraquinones

Table 16.
Absorbance of standard compound and extracts for estimation for total anthraquinones

S.No.	Standard/extract	Concentration (µg/ml)	Absorbance at 515 nm (O.D.)
1.	Standard	5	0.25
		10	0.32
		15	0.48
		20	0.57
		25	0.69
2.	MF-Flower	19.90±0.31*	0.561
3.	CF-Fruit	33.60±0.71*	0.869

*Values are expressed as mean±SD of three replicates

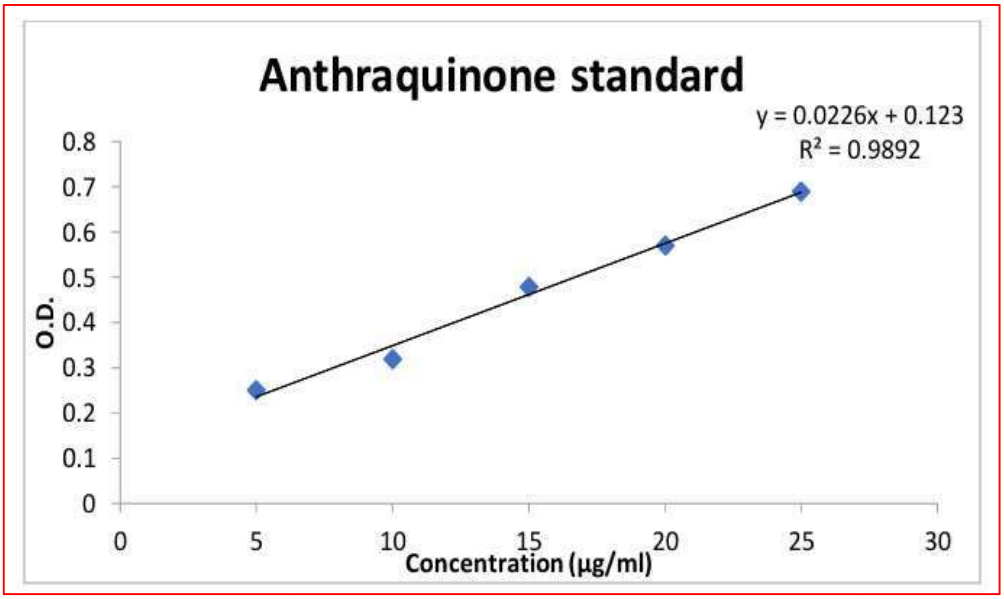

Fig. 9. Standard calibration curve of standard for estimation of total anthraquinones

Absorbance of standard compound at 515nm is presented in **Table 16.** and **Fig. 9.** The total anthraquinones content were expressed as mg standard equivalent using the standard curve equation Y= 0.0226X, R^2= 0.9892, where Y= absorbance at 515nm, X= total anthraquinones content in the extract. Total anthraquinones content in methanol extract of the selected plant parts is provided in **Table 16**.

3. ANTIMICROBIAL ANALYSIS

3.1 Antimicrobial Activity of Crude Extracts of O*xystelma esculentum* R.Br.

Oxystelma esculentum's antibacterial and antifungal characteristics were investigated in this work. Using methanol as a solvent, extracts were made from various sections of O. *esculentum* R.Br., specifically the fruits and flowers. The crude extracts were assessed for their antifungal and antibacterial properties against *Aspergillus flavus* and *Candida albicans*, as well as against the gram-negative and gram-positive bacteria *Staphylococcus aureus* and *Escherichia coli*, respectively. The zone of inhibition's diameter was determined in millimeters using the disc diffusion method. The disc's edge to the inner border of the surrounding pathogens was used to measure the inhibitory zone. In this experiment, every assay was run three times.

3.1.1 Antimicrobial Activity of Crude Extracts of Fruits of *Oxystelma esculentum* R.Br.

Table 17 presents the results of testing the *Oxystelma esculentum* R.Br. fruit methanol extract against two bacteria and two fungi. The extract's mean zone of inhibition for bacteria varied from 9.80±0.28 to 22.5±0.35mm. The extract's mean zone of inhibition for fungus varied from 6.8±0.28 to 12.8±0.25mm. In relation to bacteria, the figures of MIC and MBC ranged from 21.50 up to 250µg/mL and 38.25 to 500µg/mL, respectively. For fungi, the corresponding MIC and MFC figures were 500–500µg/mL and 1000–1000µg/mL. When it came to *Staphylococcus aureus*, the bacteria had the biggest mean zone of inhibition (22.5±0.35mm at 1000µg/disc), and the lowest MIC (21.50µg/mL) and MBC (38.25µg/mL) values were noted.

Aspergillus flavus had the largest mean zone of inhibition (12.8±0.25mm at 1000µg/disc) among all fungi. When it came to *Aspergillus flavus*, the lowest the values of MFC (1000µg/mL) and MIC (500µg/mL) were recorded. The bacteria were positively controlled by ciprofloxacin (5µg/disc), and the mean zone for inhibition varied from 24.0±0.65mm to 28.8±0.65mm. When amphotericin-B (100 units/disc) was used as the positive control for *Candida albicans* and ketoconazole (10µg/disc) was used as the positive control for *Aspergillus flavus*, the mean zones of inhibition were 14.5±0.50mm and 16.50±0.80mm, respectively. Fungi exhibited the lowest zone of inhibition (6.8±0.28mm) against *Candida albicans*, whereas bacteria exhibited the lowest activity (9.80±0.28mm at 250µg/disc) against *Escherichia coli*.

3.1.2 Antimicrobial Activity of Crude Extracts of Flowers of *Oxystelma esculentum* R.Br.

Oxystelma esculentum R.Br. flowers were used in the current investigation to screen their methanol extract against microbial strains. The findings of screening *O. esculentum's* methanol extract against strains that both fungi and bacteria were displayed in **Table 18**. The extract's imply bacterial mean zone of inhibition varied from 9.50±0.43 to 19.8±0.25mm. The range of the fungus's mean zone of inhibition was 8.70±0.28 to 12.5±0.50mm. The ranges of values for the bacterial MBC and MIC were 125 to 1000µg/mL and 62.50 to 500µg/mL,

respectively. For fungus, the values of MIC and MFC varied among 500 and 500µg/mL. and 1000 to 1000µg/mL, respectively.

In terms of microorganisms, *Staphylococcus aureus* produced the minimum MIC (62.50µg/mL), MBC (125µg/mL), and maximum mean zone of inhibition (19.80±0.25mm at 1000 µg/disc). *Aspergillus flavus* exhibited the largest 12.50±0.50mm was the mean zone of inhibition at 1000µg/disc among all fungi. Against investigated fungal strains, 500µg/mL MIC and 1000µg/mL MFC values were noted. As a positive control for microorganisms, the mean zone of inhibition varied from 30.50±0.50mm to 29.80±0.56mm when ciprofloxacin (5µg/disc) was used. Amphotericin-B (100 units/disc) and ketoconazole (10µg/disc) served as the positive controls for *Aspergillus* and *Candida*, respectively. The mean inhibition zones varied from 17.5±0.50 to 21.0±0.76mm. The study found that the lowest possible area of inhibition (8.7±0.28mm) for fungi was reported against *Candida albicans*, whereas the bacteria that exhibited the least motility (9.5±0.43mm at 250µg/disc) was *Escherichia coli*.

The current study involved screening *Oxystelma esculentum* R.Br. in methanol extract of fruits and flowers as an antifungal and antibacterial properties against strains of both bacteria that are both Gram-positive and Gram-negative fungus. In contrast to other extracts, O. *esculentum* methanol extracts fruits showed the greatest average inhibition zone (22.5±0.35 mm at 1000 µg/disc) against a *Staphylococcus aureus* strain. Extracts of O. *esculentum* in methanol fruits showed the lowest possible minimum bactericidal values (38.25 µg/mL) and minimum inhibitory concentrations (21.50 µg/mL) in opposition to *S. aureus*. When applied to *Aspergillus flavus*, the methanol extract of *O. esculentum* fruits exhibited the lowest zone of inhibition (7.3±0.25 mm at 250 µg/disc).

On the other hand, the greatest average zone of inhibition (12.5±0.50 mm at 1000 µg/disc) against *Candida albicans* in fungus was observed in the methanol extract of *O. esculentum* flowers. The lowest amount of MFC (1000 µg/mL) and MIC (500 µg/mL) against *Aspergillus flavus* and *Candida albicans* were found with methanol extracts of *O. esculentum* fruits and flowers.

Table 17. Antibacterial And Antifungal Activities of Methanol Extract of Fruits of *Oxystelma esculentum* R.Br.

Microorganisms	Average zone of inhibition[a] (mm)[b]			Ciprofloxacin (5µg/disc)/ Amphotericin-B (100units/disc)/ /Ketoconazole (10µg/disc)	MIC (µg/mL)	MBC/MFC (µg/mL)
	The Concentrations of the disc (µg/mL)					
	250	500	1000			
Staphylococcus aureus	17.5±0.25*	20.25±0.3*	22.5±0.35*	28.8±0.65	21.5	38.25
Escherichia coli	9.8±0.28	12.3±0.26	13.3±0.28	24.0±0.65	250	500
Candida albicans	6.8±0.28	7.5±0.35	9.6±0.25	14.5±0.50	500	1000
Aspergillus flavus	7.3±0.25	9.8±0.50	11.8±0.25	16.50±0.80	500	1000

a- a- The zone of inhibition's diameter (mm), which includes the disc's 6 mm diameter

b- the three tests' means; - the standard deviation - $P < 0.05$ for statistical significance

Table 18. Antibacterial And Antifungal Activities of Methanol Extract of Flowers of *Oxystelma esculentum* R.Br

Microorganisms	Average zone of inhibition[a] (mm)[b]			Ciprofloxacin (5µg/disc)/ Amphotericin-B(100units/disc)/ Ketoconazole (10µg/disc)	MIC (µg/mL)	MBC/MFC (µg/mL)
	The Concentrations of the disc (µg/mL)					
	250	500	1000			
Staphylococcus aureus	14.5±0.20*	16.55±0.50*	19.8±0.25*	30.5±0.50	62.5	125
Escherichia coli	9.5±0.43	11.7±0.25	13.8±0.56	29.80±0.56	500	1000
Candida albicans	8.7±0.28	10.5±0.26	12.5±0.50	17.5±0.50	500	1000
Aspergillus flavus	8.8±0.25	10.5±0.28	12.3±0.50	21.0±0.76	500	1000

a- The zone of inhibition's diameter (mm), which includes the disc's 6 mm diameter

b- the three tests' means; - the standard deviation - $P < 0.05$ for statistical significance

4. ANTIOXIDANT ANALYSIS

4.1. DPPH Radical Scavenging Activity

The outcomes depicted in **Fig. 10. & Fig. 11.** illustrate the DPPH• (2,2-diphenyl-1-picrylhydrazyl) Efficient scavenging of radicals by different extracts from the flowers and fruits of *O. esculentum*, alongside the activity of the radical scavenger positive control (Ascorbic acid). The methanol extracts from flowers of *O. esculentum* demonstrated the highest DPPH activity. The IC50 values for methanol extracts obtained from the fruits and flowers were determined to be 205µg/mL, 232 µg/mL respectively. In comparison, the IC50 value for L-ascorbic acid was discovered to be 170 µg/mL.

4.2. ABTS·+ Scavenging Effects

The ABTS·+ radical scavenging activity results for various parts of *Oxystelam esculentum* R.Br. using methanol exctract along with the control which is positive (ascorbic acid), are shown in **Fig. 12**. **& Fig. 13.** Methanol extract obtained from the flowers exhibited the most potent ABTS·+ scavenging effects. The IC50 amounts for methanol extracts from the fruits and flowers, as well as for Butylated Hydroxytoluene (BHT) acid, were determined to be 223µg/mL, 245 µg/mL, and 173 µg/mL, respectively.

4.3. Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity results using methanol extract from various parts of *Oxystelma esculentum* R. Br, along with the affirmative benchmark, are depicted in **Fig. 14 & Fig. 15.** Methanol extract derived from the fruits demonstrated the maximum level superoxide anion activity. The IC50 values for methanol extracts from the fruits and flowers were determined to be 305 µg/mL, 320 µg/mL respectively. In comparison, the IC50 amount for ascorbic acid was discovered to be 262 µg/mL.

4.4. Nitric Oxide Radical Scavenging Activity

The results for Nitric oxide radical scavenging activity using methanol extract from various parts of *Oxystelma esculentum* R. Br, alongside the affirmative benchmark Ascorbic acid, are illustrated in **Fig. 16 & Fig. 17**. Methanol extracts from fruits from this plant exhibited the maximum level antioxidant activity. The IC50 values for extracts of methanol from the fruits and

flowers of *O. esculentum* were determined to be 281 µg/mL, 315 µg/mL, respectively. In comparison, the IC50 amount for ascorbic acid was discovered to be 161 µg/mL.

4.5. Hydrogen Peroxide Scavenging Effects

The findings of the scavenging of hydrogen peroxide using methanol extract from various parts of *Oxystelma esculentum* R. Br, in addition to the affirmative benchmark, are displayed in **Fig. 18 & Fig. 19**. Methanol extracts from the fruits displayed the most significant action of hydrogen peroxide. The IC50 values for extracts of methanol from the fruits and flowers were determined to be 292 µg/mL, 321 µg/mL, respectively. In comparison, the IC50 amount for EDTA was discovered to be 219 µg/mL.

4.6. Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was conducted on methanol extract from several *Oxystelma esculentum* R. Br and the outcomes are depicted in **Fig. 20. & Fig. 21.** Vitamin C served as the constructive model. The most favorable Using methanol extract, hydroxyl radical scavenging activity was demonstrated from the fruits. The IC50 values for methanol extracts from the fruits and flowers were determined to be 374 µg/mL, 405 µg/mL respectively. In comparison, the IC50 amount for Vitamin C was obtained to be 227 µg/mL.

4.7. Ferric Reducing Antioxidant Power Assay

The results for antioxidant that reduces ferric iron power, using methanol extract from different *Oxystelma esculentum* R. Br, along with the affirmative benchmark Gallic acid, is displayed in **Fig.22. & Fig. 23**. Methanol extracts from the fruits of *O. esculentum* displayed the most intense action. The methanol extract IC50 values from the fruits and flowers were determined to be 295 µg/mL, 345 µg/mL, respectively. In comparison, the IC50 amount for Gallic acid was discovered to be 185 µg/mL.

4.8. Total Antioxidant Activity

The evaluation of the overall level of antioxidant activity was conducted methanol extracts from the flowers and fruits of *Oxystelma esculentum* R.Br. The outcomes are displayed in **Fig. 24. & Fig. 25**, with Ascorbic acid serving as the beneficial standard. The highest the plant's methanol extract flowers showed

complete antioxidant activity. The methanol extract IC50 values from the fruits and flowers of this botanical were determined to be 275 µg/mL, 321 µg/mL respectively. In comparison, the IC50 amount for L-ascorbic acid was determined to be 186 µg/mL.

Table 19

Summary of antioxidant crude extracts' potential as fruits and flowers of *Oxystelma esculentum* R.Br.

S.No.	Antioxidant activity against	IC50 Value (µg/mL)	
		Fruit extract	Flower extract
1.	DPPH radicle	205	232
2.	ABTS·+	223	245
3.	Superoxide radicle	305	320
4.	Nitric oxide radicle	281	315
5.	Hydrogen peroxide	292	321
6.	Hydroxyl radicle	374	405
7.	Ferric reduction evaluates for antioxidant power	295	345
8.	Total antioxidant	275	321

Sumary of antioxidant crude extracts' potential as fruits and flowers about *Oxystelma esculentum* R.Br. is given in **Table 19**. IC50 values are expressed in µg/mL.

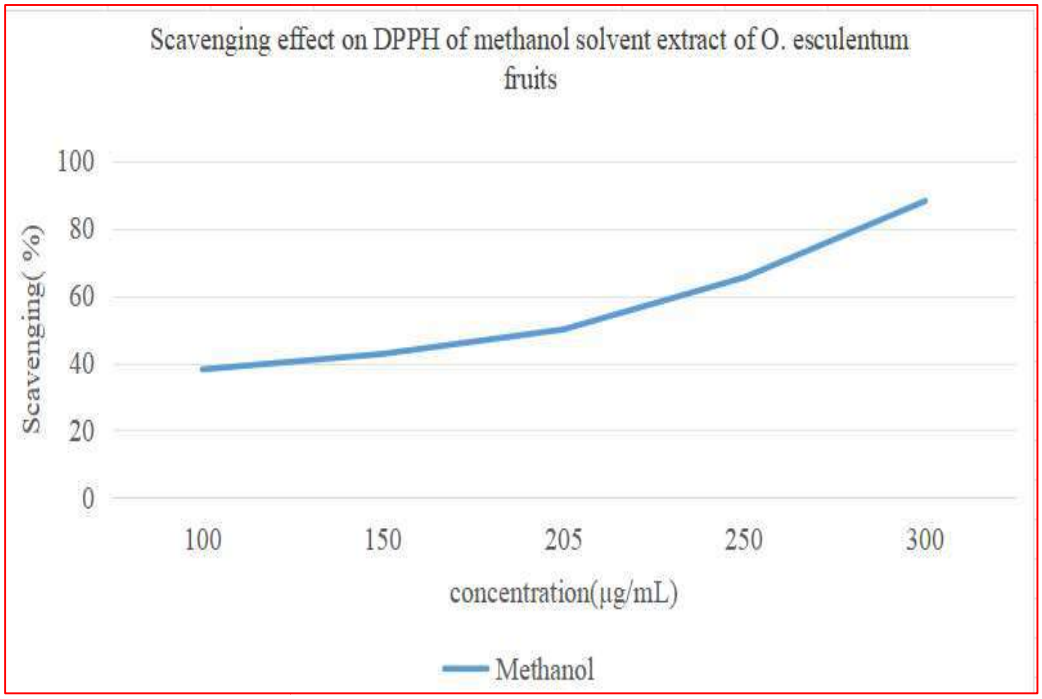

Fig. 10. Scavenging effect of O. esculentum fruit methanol solvent extract on DPPH

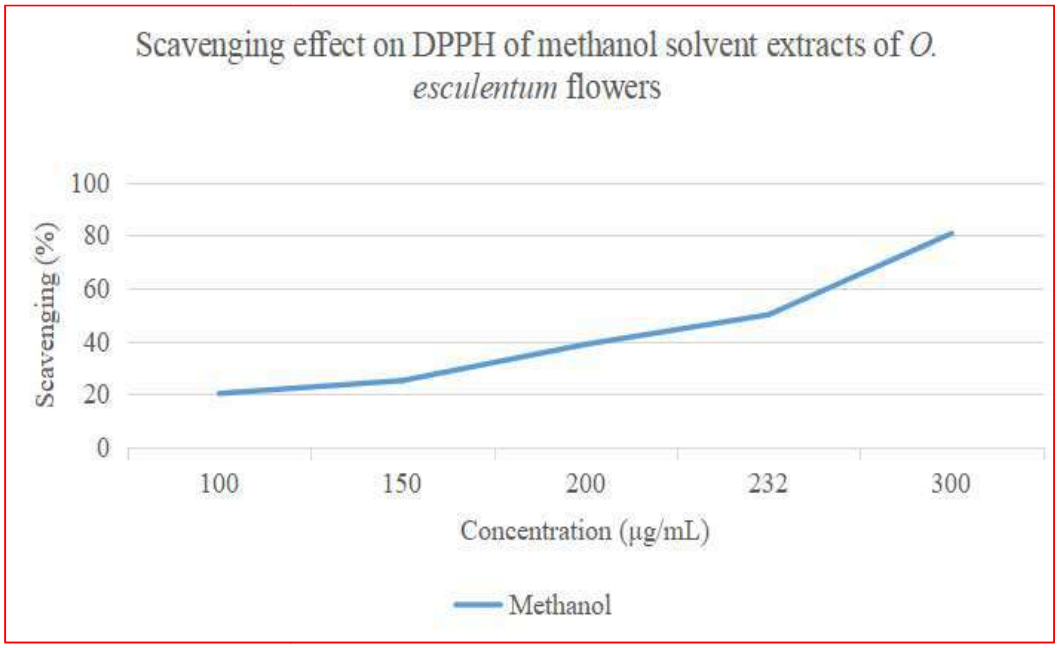

Fig. 11. Scavenging effect on DPPH of methanol solvent extract of *O. esculentum* flowers

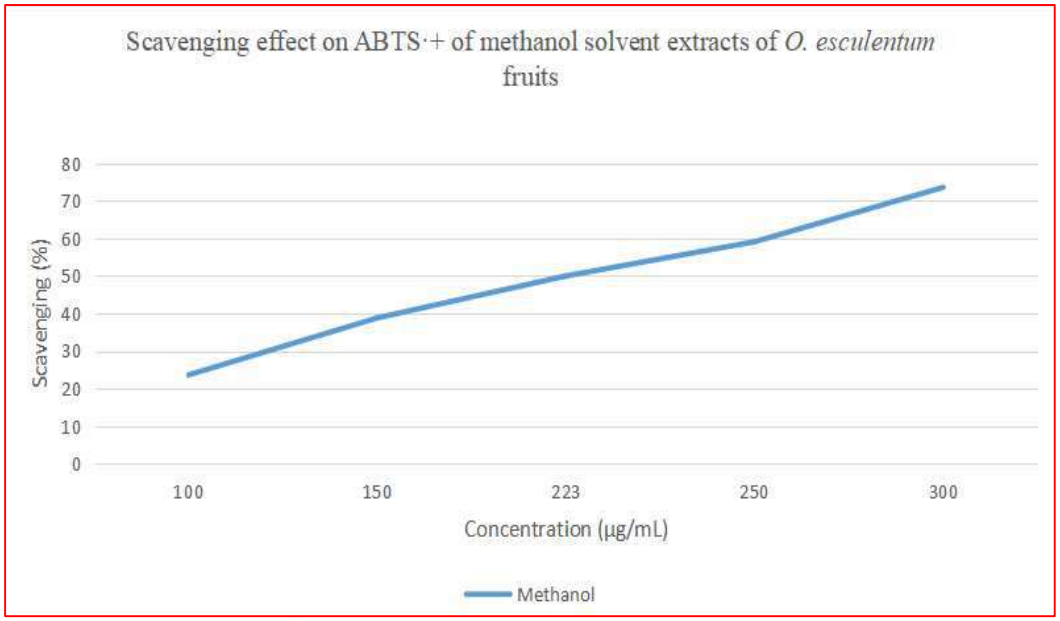

Fig. 12. Scavenging effect on ABTS·+ of methanol solvent extract of *O. esculentum* fruits

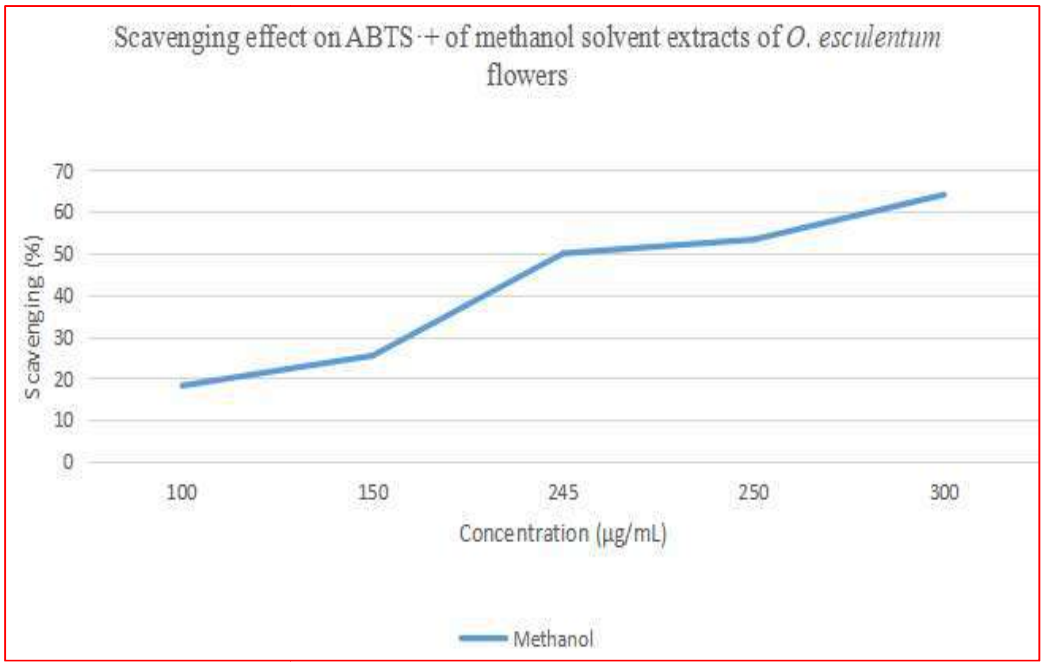

Fig. 13. Scavenging effect on ABTS·+ of methanol solvent extract of *O. esculentum* flowers

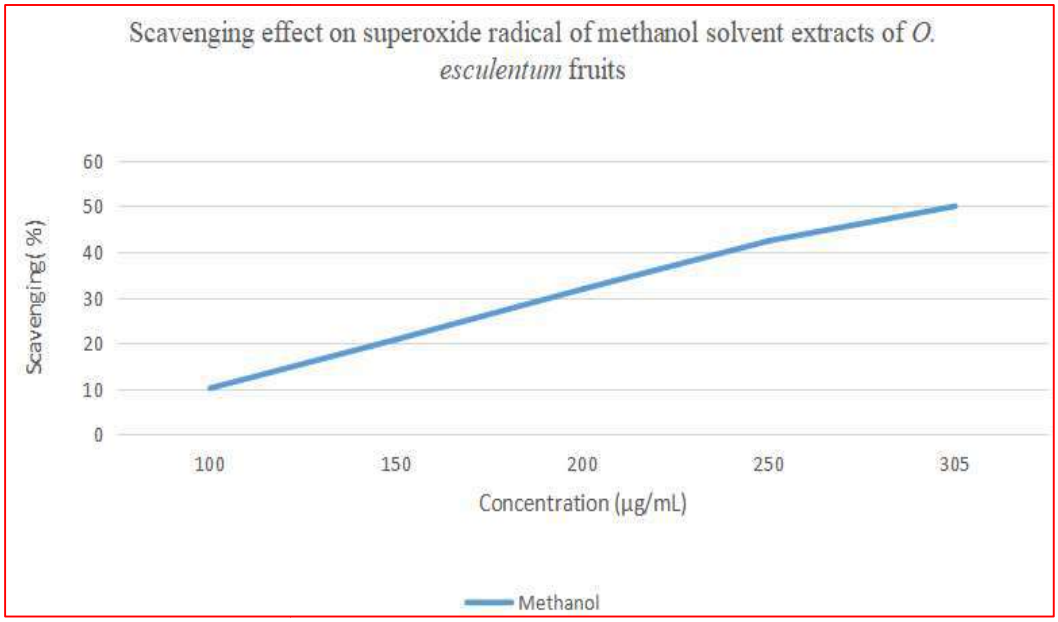

Fig. 14. Scavenging effect on superoxide radical of methanol solvent extract of *O. esculentum* fruits

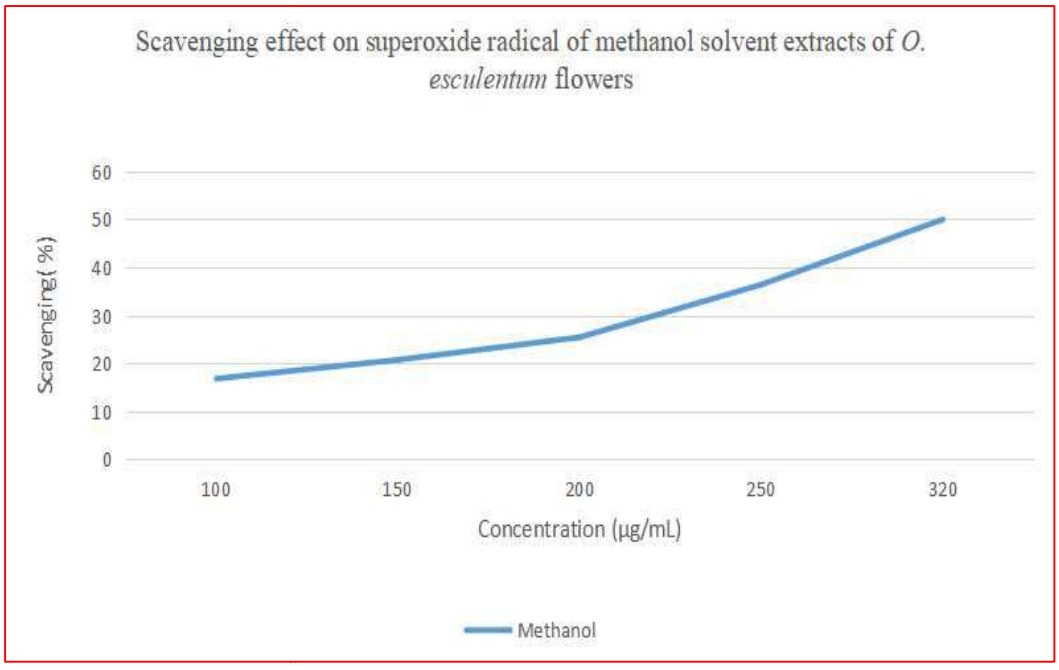

Fig. 15. Scavenging effect on superoxide radical of methanol solvent extract of *O. esculentum* flowers

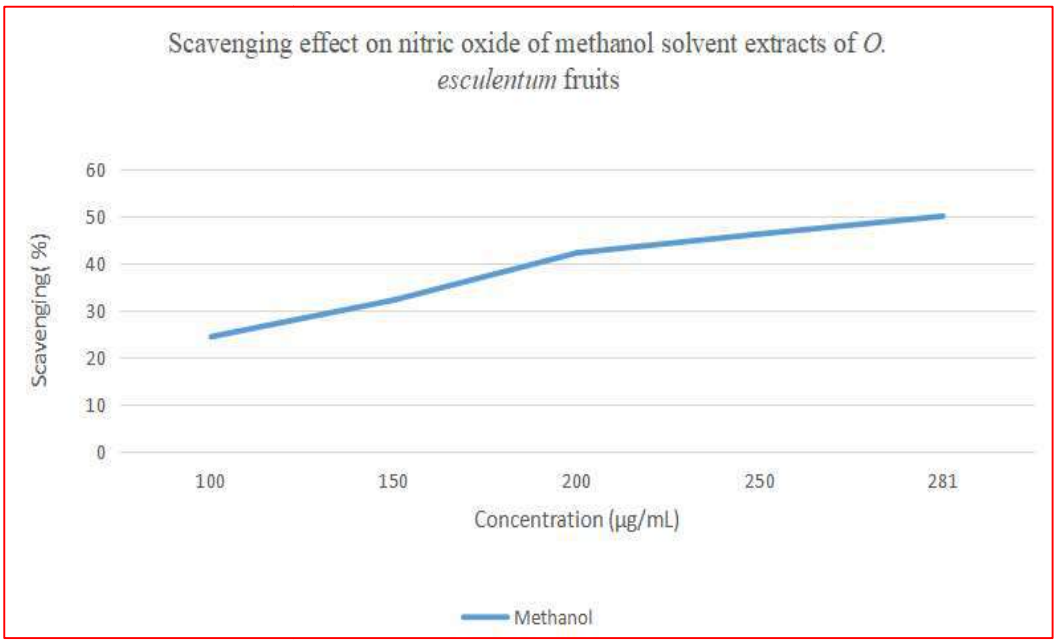

Fig. 16. Scavenging effect on nitric oxide of methanol solvent extract of *O. esculentum* fruits

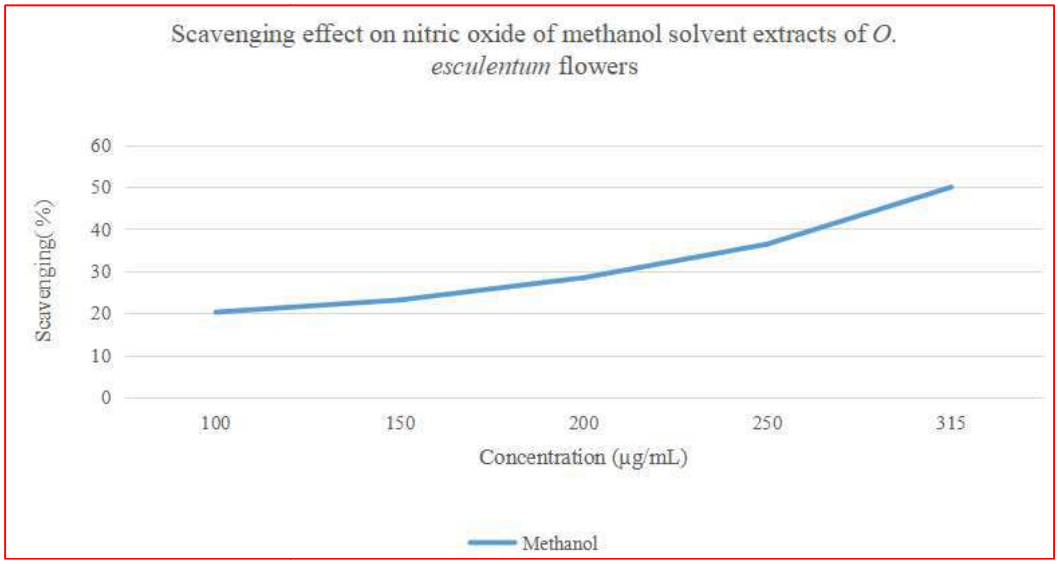

Fig. 17. Scavenging effect on nitric oxide of methanol solvent extract of *O. esculentum* flowers

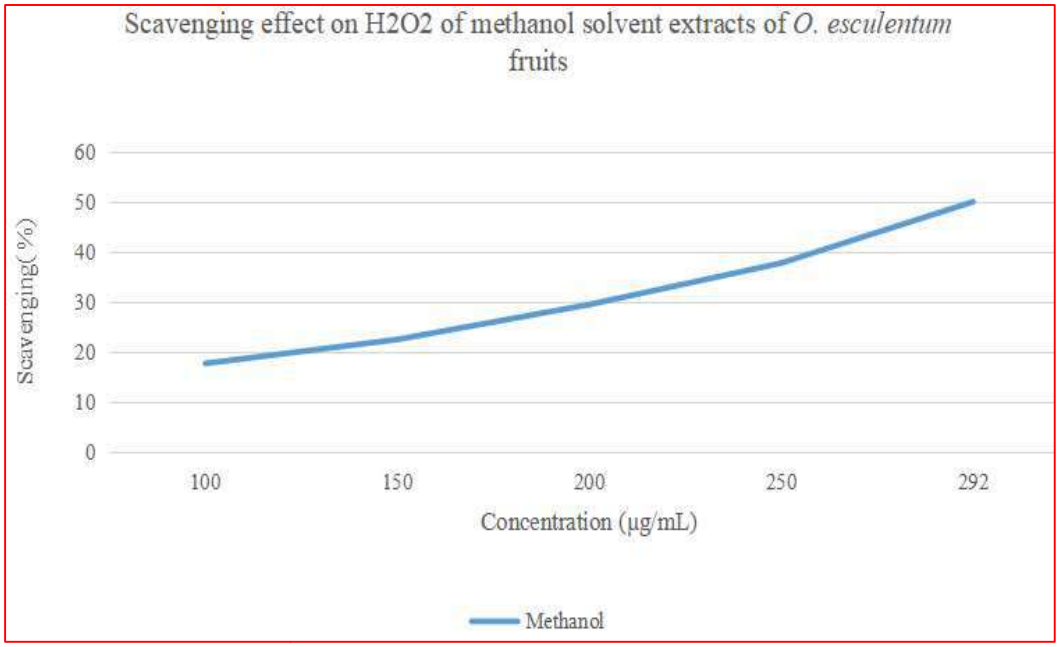

Fig. 18. Scavenging effect on H_2O_2 of methanol solvent extract of *O. esculentum* fruits

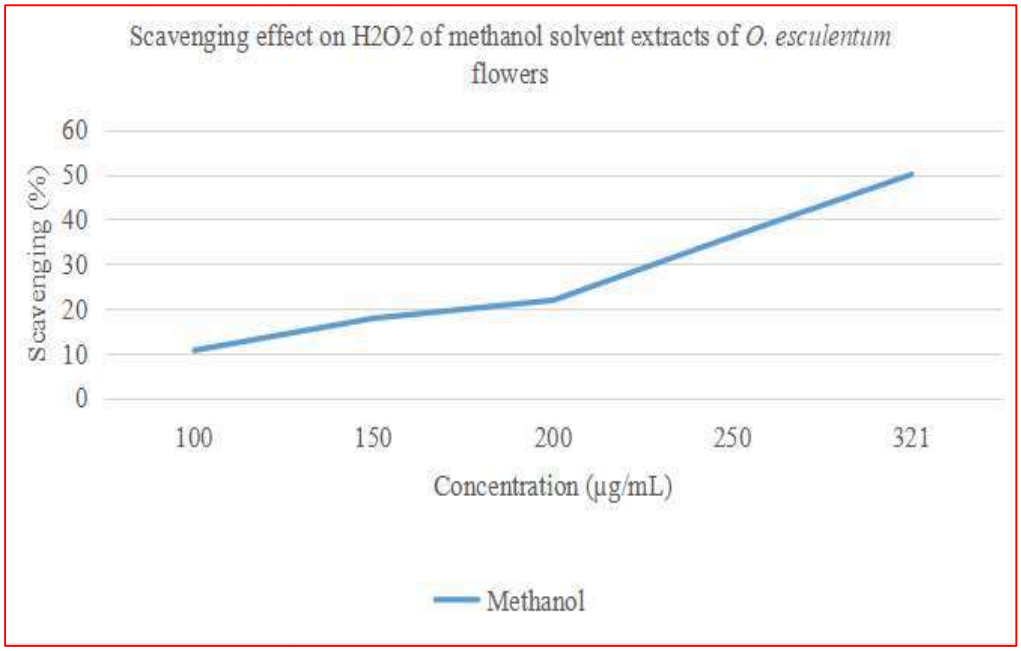

Fig. 19. Scavenging effect on H_2O_2 of methanol solvent extract of *O. esculentum* flowers

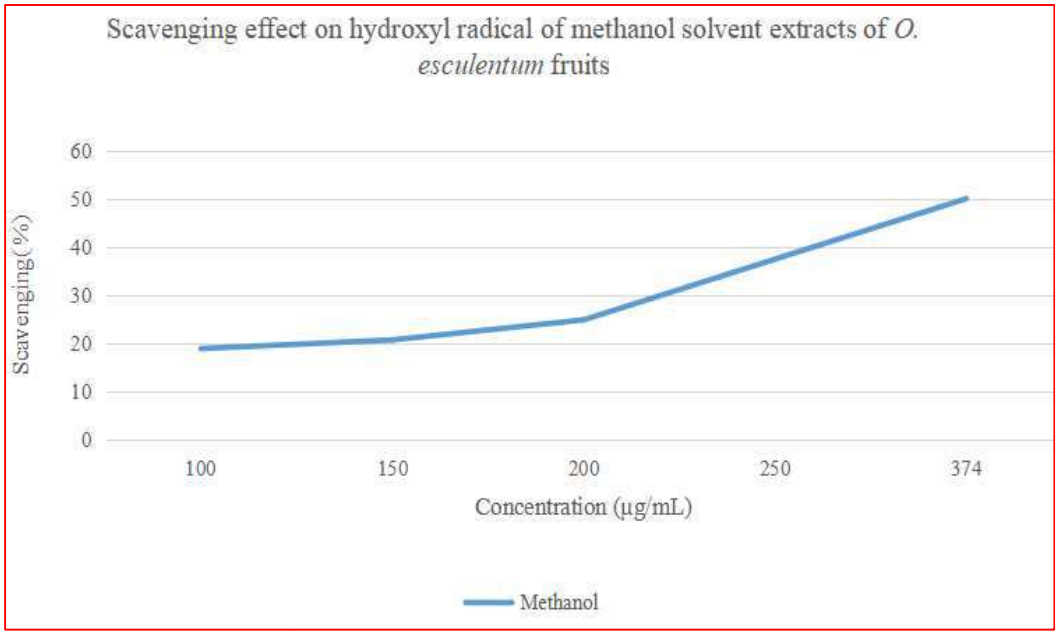

Fig. 20. Scavenging effect on hydroxyl radical of methanol solvent extract of *O. esculentum* fruits

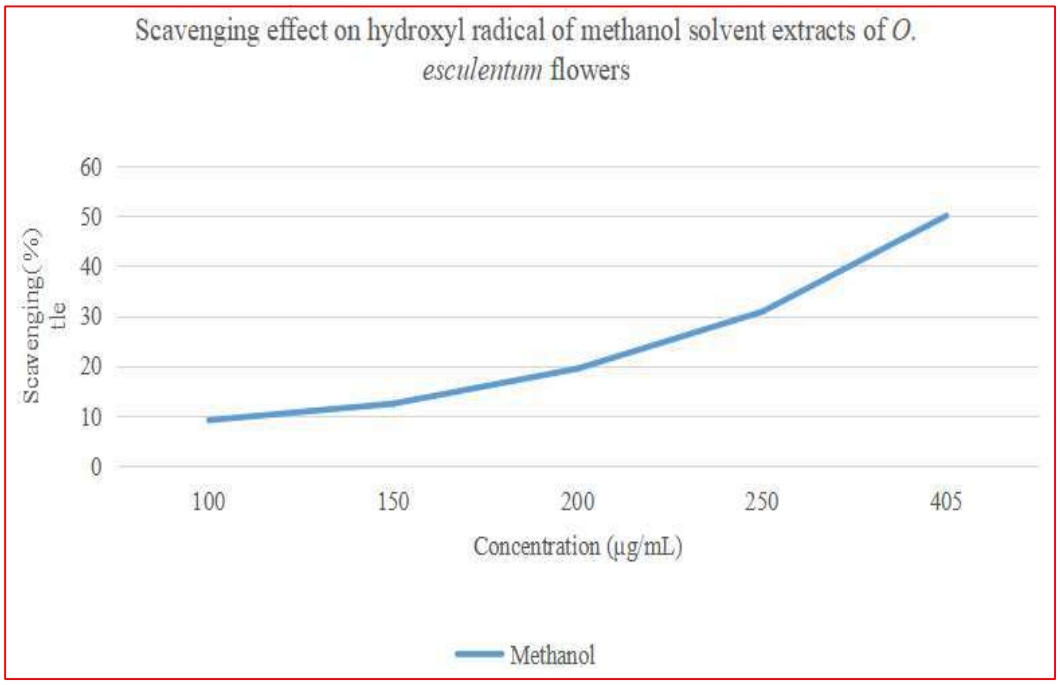

Fig. 21. Scavenging effect on hydroxyl radical of methanol solvent extract of *O. esculentum* flowers

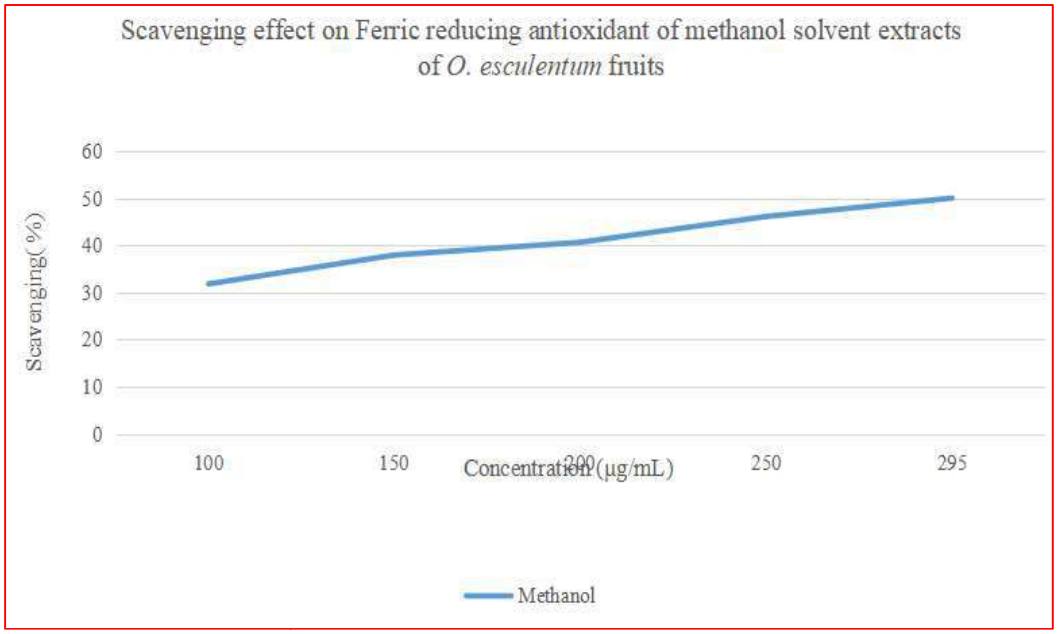

Fig. 22. Scavenging effect on ferric reducing antioxidants of methanol solvent extract of *O. esculentum* fruits

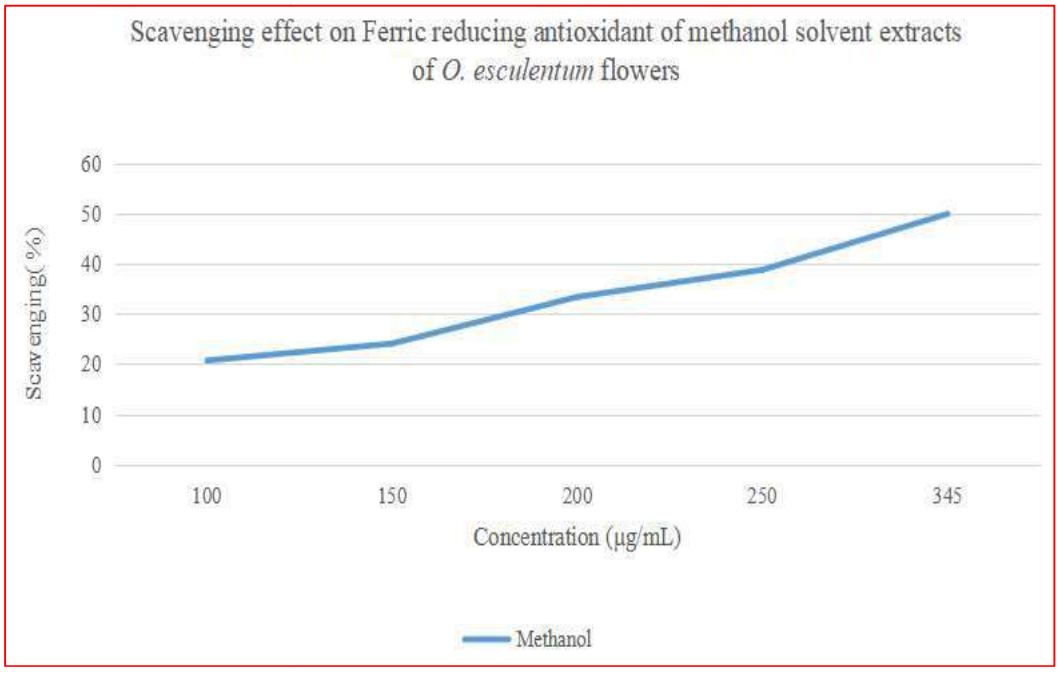

Fig. 23. Scavenging effect on ferric reducing antioxidants of methanol solvent extract of *O. esculentum* flowers

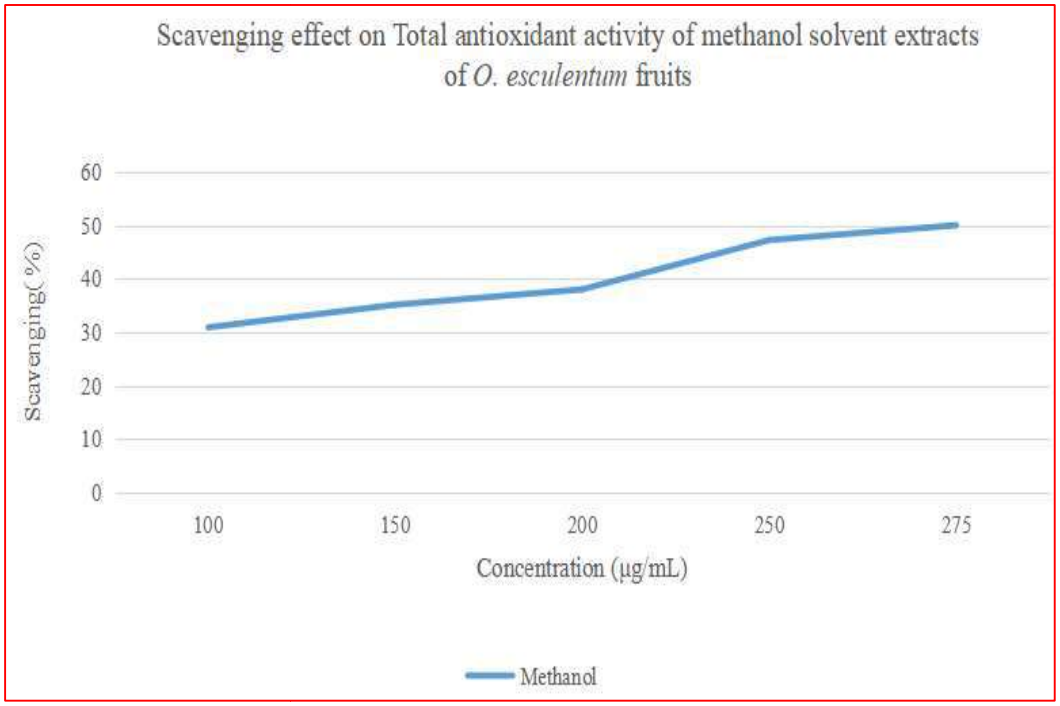

Fig. 24. Scavenging effect on total antioxidant activity of methanol solvent extract of *O. esculentum* fruits

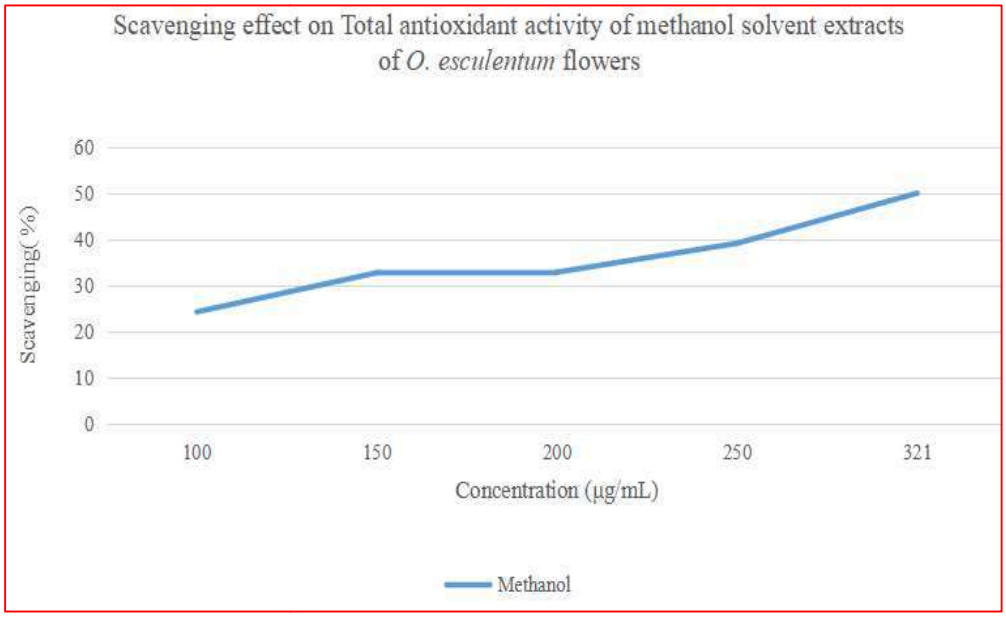

Fig. 25. Scavenging effect on total antioxidant activity of methanol solvent extract of *O. esculentum* flowers

4. FTIR ANALYSIS

Chromatography FTIR examination of the extract of methanol from fruits of *Oxystelma esculentum* R. Br showed sixteen significant peaks were found, and the components that matched the peaks were identified as follows. The FTIR examination of *Oxystelma esculentum* R.Br. fruits demonstrated the existence of Alcohols, Alkanes, Esters,Amide I & II, Aromatic compounds, Phenol, Phosphate-I, Sulfoxide, Alkenes and Alkyl halides, exhibiting prominent peaks at 3349, 2916, 2849, 2321, 1725, 1639, 1551, 1459, 1409, 1323, 1244, 1036, 888, 718, 612, 557 $cm.^{-1}$ **(Fig. 26).**

FTIR chromatogram for a methanol extract of flowers of *Oxystelma esculentum* R.Br. showed the existence of fourteen the following was the determination of the principal peaks and the components related to the peaks. The FTIR analysis of *Oxystelma esculentum* R. Br flowers demonstrated the existence of Alcohols, Alkanes, Alkynes, Allene, Esters, Amide I & II, Aromatic compounds, Phosphate-I, Sulfoxide and Alkyl halides with strong peaks at3779, 3280, 2921, 2855, 2313, 2112, 1925, 1730, 1637, 1558, 1406, 1241, 1022, 530 $cm.^{-1}$ **(Fig. 27).**

The findings mentioned above lead to the conclusion that *Oxystelma esculentum* R.Br. has high concentrations of alcohols, alkanes, amides I and II, phosphate-I, sulfoxide, and alkyl halides. The current research shows that different regions of the plants have varied concentrations of biomolecules. Further study in the biological the therapeutic properties of plants and the analysis of their phytochemicals is possible thanks to this effort.

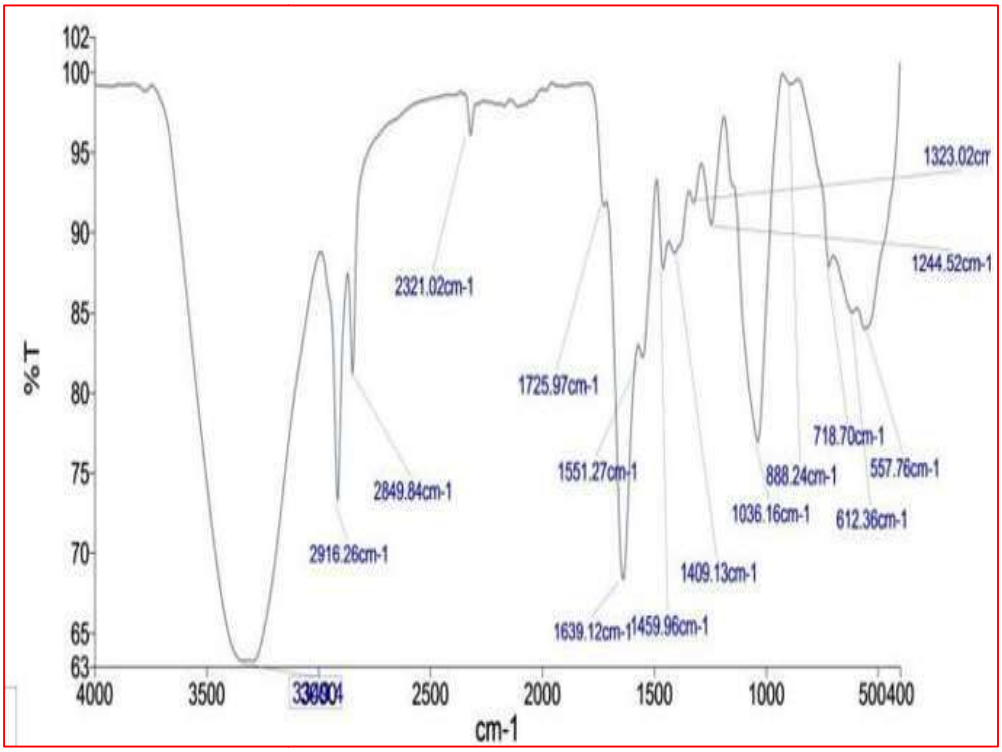

Fig. 26. FTIR spectrum of ME extract of fruit of *O. esculentum*

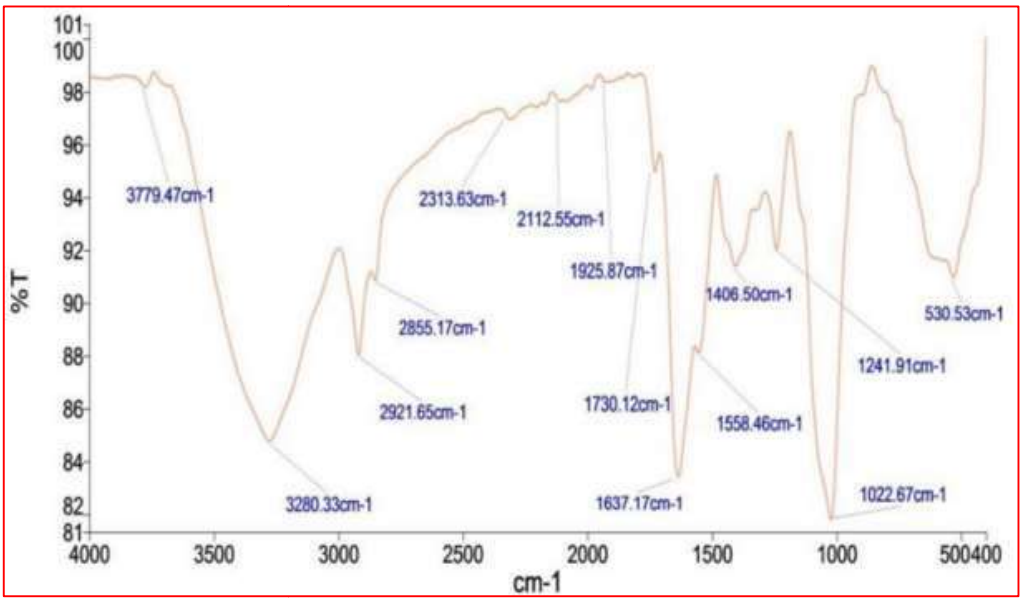

Fig. 27. FTIR spectrum of ME extract of flower of *O. esculentum*

5. GC-MS ANALYSIS

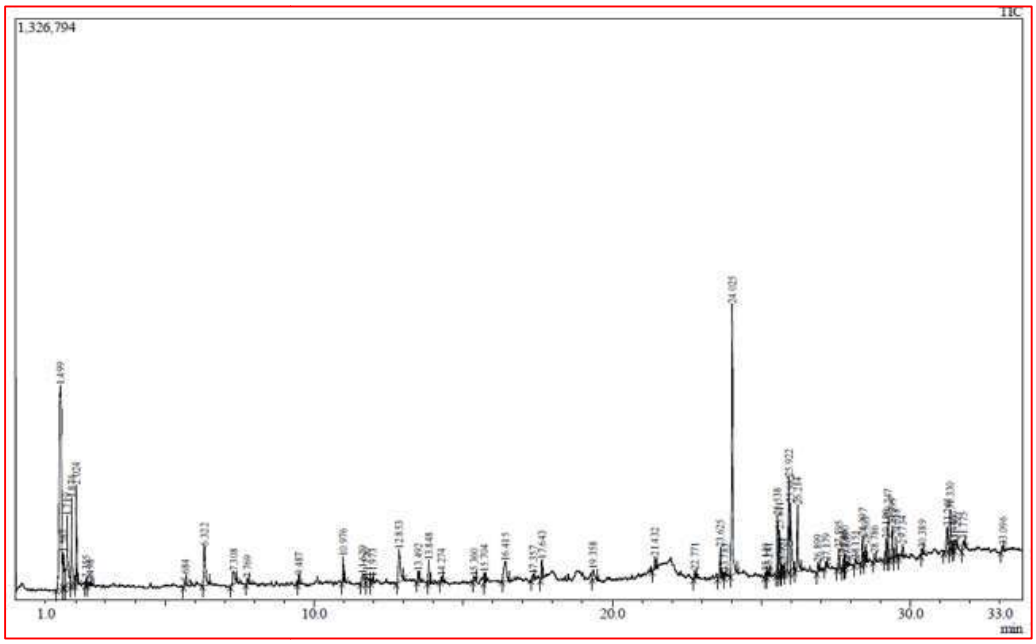

Fig. 28. GC-MS characteristics of methanol fraction of fruits of *O. esculentum*

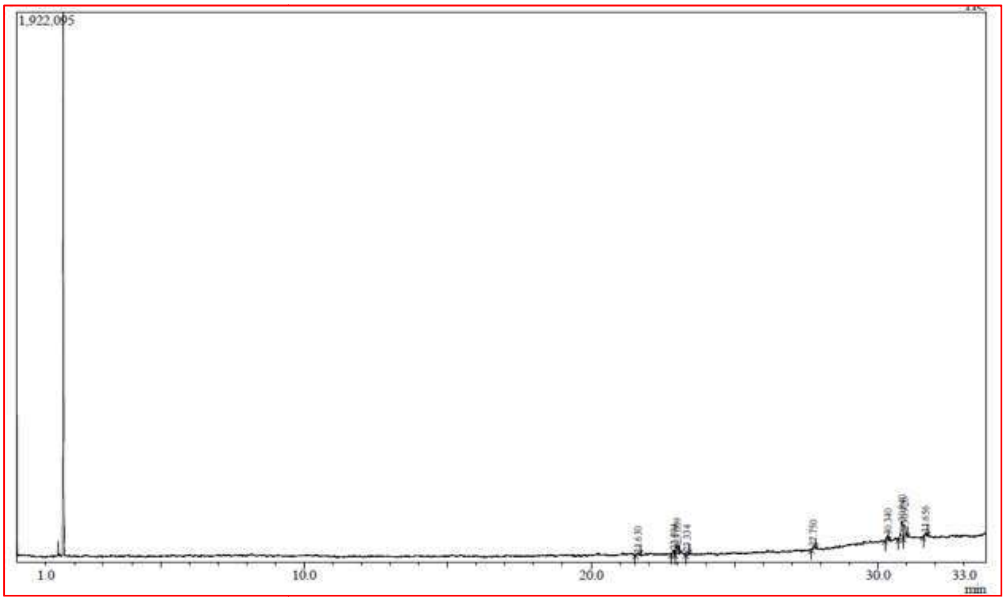

Fig. 29. GC-MS characteristics of methanol fraction of flowers of *O. esculentum*

The methanol extracts of fruits of *O. esculentum* were further subjected to carry out GC-MS characterization due the highest activity against tested microbes. The compounds were identified in the crude extract such as 2-

Chloroethyl methyl sulfoxide, Acetic acid, methyl ester, Trichloromethane, n-Hexadecanoic acid,9(E), 11(E)-Conjugated linoleic acid, 6-Octadecenoic acid, (Z)- etc. Similarlay, the methanol extracts of flowers of *O. esculentum* showed presence of 1-Heptacosanol, beta. -Sitosterol acetate, 9-Octadecen-1-ol, acetate, (Z), Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite,dl-.alpha.-Tocopherol compounds in GC-MS characterization. (**Fig. 28 & Fig. 29**)

6. HPLC ANALYSIS

The HPLC analyses of methanol extract of fruits and flowers of *Oxystelma esculentum* R.Br. was performed and results are shown in **Fig. 30. & Fig. 31.**

Oxystelma esculentum R.Br. fruits and flowers were subjected to an HPLC methanol extract. The results indicated that the main peak, which corresponds to flavonoids at a wavelength of 270 nm, occurred at 2.563 RT and 2.566 RT respectively. By measuring the retention time of the sample against the standard (quercetin) and by mixing a known concentration of the pure standard into the extract and comparing the peak heights that resulted, the presence of flavonoids was verified. Potential synergistic effects of the extract's deducted flavanoids and antioxidant activities could be observed.

Flavonoids were found to be the main peak; some minor peaks may have been caused by steroids, terpenoids, phenolics, etc. Plant flavonoids are important bioactive substances that have demonstrated antibacterial properties.Wide-ranging biological activities have been demonstrated for it, such as hepatoprotective, antiviral, antiallergic, anti-inflammatory, antimutagenic, antiproliferative, and antithrombotic effects (Bors and Saran, 1987; Middleton and Kandaswami, 1994; Robak and Gryglewski, 1988; Wall, 1992; Chang and Kinghorn, 2001).

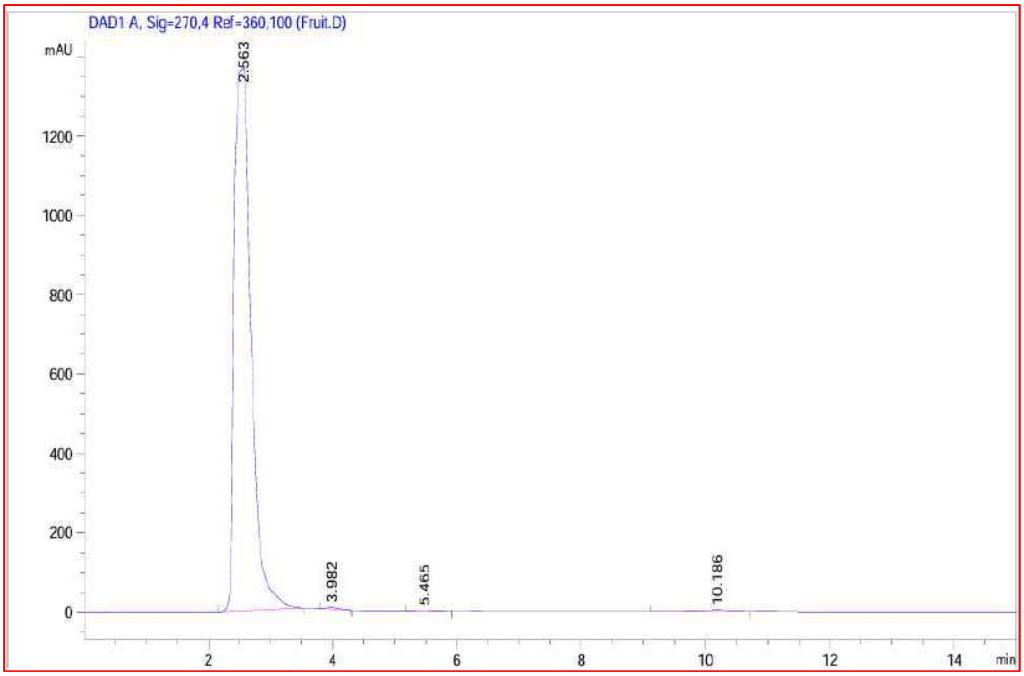

Fig. 30. HPLC spectrum of methanol extract of fruits of *O. esculentum*

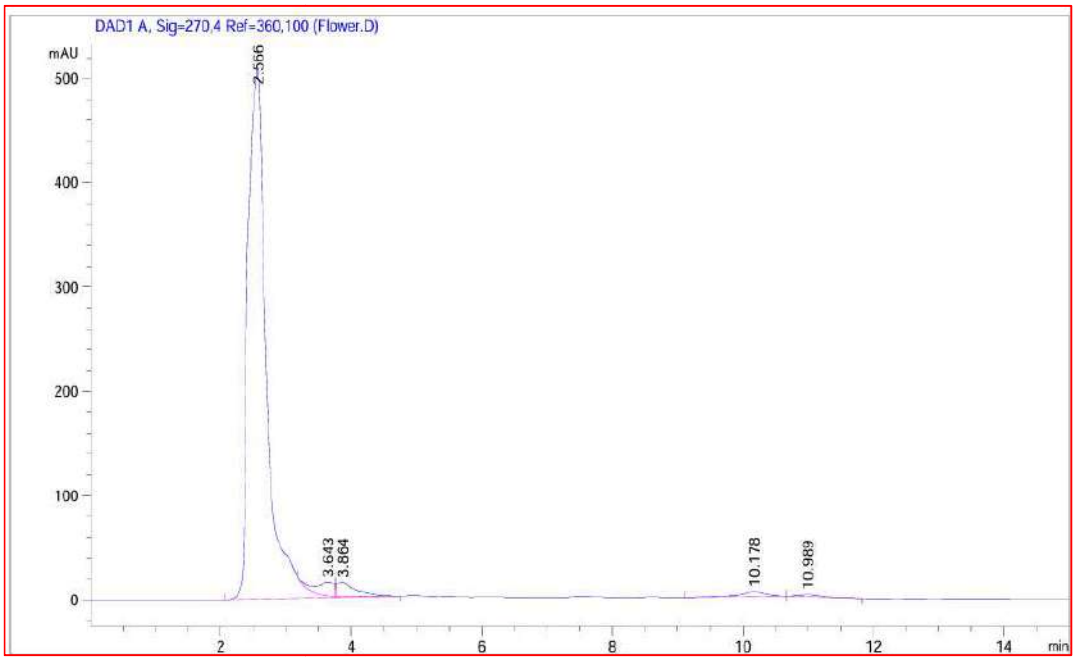

Fig. 31. HPLC spectrum of methanol extract of flowers of *O. esculentum*

CHAPTER - V

DISCUSSION

CHAPTER - V
DISCUSSION

Herbal treatments have been used for ages to cure a wide range of infectious disorders in human history. In many underdeveloped nations, plant materials are still widely used as therapeutic medicines in primary healthcare today (Zakaria, 1991). For the majority of people on the planet, plants remain their primary source of medication (Hamburger and Hostettmann, 1991).

Due to the frequently insufficient availability of modern treatment, the low cost and effectiveness of medicinal plants, as well as cultural beliefs and preferences, most developing nations have billions of people using medicinal plants (Shanley and Luz, 2003; Sheldon *et al*., 1997). Many biologically active substances found in plants have been demonstrated to have antibacterial qualities (Cowan, 1999).

For thousands of years, traditional treatment in most parts of the world has included plant-derived medications, and interest in plants as sources of agents to combat microbial infections is growing (Chariandy *et al*., 1999). A significant therapeutic resource for the treatment of illnesses, particularly in developing nations, is the utilization of medicinal plants (Freitas, 1999).

The current research involved conducting a phytochemical analysis on different solvent extracts of *Oxystelma esculentum* R.Br. The methanol extracts from the fruits of *Oxystelma esculentum* R.Br exhibited significant levels of phytochemicals including alkaloids, tannins and flavonoids. Similarly, the acetone extracts from the fruits of this plant revealed a notable presence of alkaloids, cardiac glycosides, flavonoids and carbohydrates. Likewise, the chloroform extracts from the fruit part displayed strong levels of alkaloids, ketones, cardiac glycosides, flavonoids, and carbohydrates.

Alkaloids possess various pharmacological effects, including antibacterial, antifungal, antihypertensive, anti-arrhythmic, anti-malarial, and anticancer properties (Wink *et al*., 1998; Molyneux *et al*., 1996).

Flavonoids represent a widely distributed group of natural pigments found in vascular plants, contributing significantly to the coloring of nature. They have historically played significant roles in medicinal treatments, showcasing diverse biological activities such as cardiovascular protection (Cook and Samman, 1996), antioxidant effects (Robak and Gryglewski, 1988), anticancer potential (Manach *et al*., 1996), as well as anti-inflammatory and antimicrobial properties (Harborne and Williams, 2000). Its antimicrobial potential has been proposed for developing new food preservatives and therapies against microbial infections due to increasing microbial resistance to antibiotics (Daglia, 2012). Numerous studies have highlighted its antioxidant, anti-inflammatory, antimicrobial, vascular activities, and other medicinal benefits, with reports on their antimicrobial activities (Baez *et al*., 1999; Xu and Lee, 2001; Ogundipe *et al*., 2001). Investigations into the antimicrobial properties of flavonoids have indicated significant activity against various strains of bacteria such as *Staphylococcus aureus*, *Streptococcus faecalis*, and *Escherichia coli* (Chattopadhyay *et al*., 2001).

Saponins, another important collection of organic substances, show a variety of pharmacological effects, including cardiac, antifungal, antimicrobial, anti-inflammatory, and cytotoxic activities (Rao and Gurfinkel, 2000; Wallace, 2004; Liby *et al*., 2007). These compounds are commonly present in therapeutic herbs used in conventional medicine (Sharma and Paliwal, 2013) and have been looked into for developing new natural medicines, validating the efficacy of traditional herbal remedies (Waller and Yamasaki, 1995).

Tannins have been reported to possess a range of pharmacological characteristics, such as anti-diabetic, anti-inflammatory, antibacterial, and antitumor effects. Certain tannins have also shown selective inhibition of HIV replication and have been used as diuretics. Plant-derived tannins are well-known for their pharmacological benefits (Haslem, 1989).

Cardiac glycosides, present in both plants and amphibians as secondary compounds, are commonly found throughout nature and possess cardiovascular effects.

Plant steroids demonstrate a diverse range of medicinal, pharmaceutical, and agrochemical effects, including anti-tumor, immunosuppressive, hepatoprotective, antibacterial, regulation of plant growth hormones, sex hormone properties, antihelminthic, cytotoxic, and cardiotonic activities.

Recent pharmacological studies have revealed that anthraquinones exhibit numerous potent actions, like anticancer, anti-inflammatory, anti-injury, antibacterial, anti-osteoporosis, as well as antioxidative properties (Li and Jiang, 2018).

In the quantitative phytochemical analysis, alkaloids, saponins, flavonoids, tannins, steroids, total Phenolic Content, carbohydrates, proteins, lipids (%), terpenoids, total glucosinolate and total anthraquinones content has been assessed in the two parts, i.e., fruit and flower methanol extracts of the medicinal plant.

The total alkaloids, saponins, flavonoids, tannins, steroids, total phenolic content, carbohydrates, proteins, percentage lipids, terpenoids, total glucosinolate and total anthraquinones content in the powdered fruits of the chosen plant was determined to be 94.1±0.81 mg/ml, 0.065±0.0057 mg/g, 11.7±0.66 mg/ml, 11.9±0.58 mg/ml, 916±8.17 µg/ml, 6.2±0.54 mg/ml, 10.5±0.81 mg/ml, 10.5±0.81 mg/ml, 1.69±0.13 mg/ml, 1.00%, 1.86±0.16 gm/10ml, 28.37±0.51µmol/gm, 33.60±0.71 µg/ml, respectively, using quantitative analysis.

Similarly, analyzing the powdered flowers of the chosen plant, the total alkaloids, saponins, flavonoids, tannins, steroids, total phenolic content, carbohydrates, proteins, percentage lipids, terpenoids, total glucosinolate and total anthraquinones content was estimated to be 183.5±0.32 mg/ml, 0.0133±0.06 mg/g, 0.781±.008 mg/ml, 3.2±0.24 mg/ml, 1286±12.8 µg/ml, 3.3±0.34 mg/ml, 12.4±0.54 mg/ml, 1.03±0.10 mg/ml, 0.50%, 1.3±0.20 gm/10ml, 75.88±0.86 µmol/gm, 19.90±0.31µg/ml, respectively.

The extracts containing methanol of the fruits and flowers of *Oxystelma esculentum* R.Br. was tested due to its antifungal and antibacterial qualities against the gram-positive bacterium *Staphylococcus aureus*, the gram-negative bacterium *Escherichia coli* and two fungal strains, including *Candida albicans* and *Aspergillus flavus*. The investigation's findings revealed differential zones of inhibition against the examined microorganisms. The methanol extract of fruits

from *O. esculentum* was shown to have the highest mean zone of inhibition (22.5±0.35mm at 1000µg/disc) against *Staphylococcus aureus*. An antibacterial agent's presence in the crude extracts may be the reason of their maximum antibacterial activity (Barbour *et al*., 2004).

Our findings are in line with those of Anpin Raja *et al*. (2011), who observed that the *Cyclea peltata* extracts containing methanol demonstrated antibacterial action against three pathogens, including *Streptococcus pyogenes, Proteus vulgaris* and *Escherichia coli,* with inhibition zones of 12 mm, 10 mm and 9 mm, respectively. With inhibition zones of 15 mm and 12 mm, respectively.

The ethanol extract of *Curcuma longa* was tested by Chakraborty *et al*. (2014). This extract showed the highest antibacterial activity when compared to other extracts, which was demonstrated by the zone of inhibition against *Propteus mirabilis*, which was 20.67±0.59 mm. Similar results were reported by Jayasree *et al*.(2014) using *Azadirachta indica* leaf extracts dissolved in ethanol. They demonstrated inhibitory zones of 16mm against *Escherichia coli*, 15 mm against *Klebsiella pneumoniae*, 14 mm against *pseudomonas aeruginosa* and 20 mm against *Staphylococcus aureus*.

With an inhibitory zone of 19 mm, the *Cardiospermum halicacabum* acetone leaf extract demonstrated strong antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus* (Krishna Moorthy Naik *et al*., 2015). El Gendy *et al*. (2015) examined the antibacterial activity of *Origanum syriacum* essential oils and found that *Staphylococcus aureus* was the organism with the biggest inhibition zone (32±4.0 mm) and *Enterococcus faecalis* had the smallest inhibition zone (9±0.01 mm).

In the current study, the minimum inhibitory and minimum bactericidal/fungicidal concentrations ranged from 21.50-500 and 38.25-1000 µg/mL, respectively. Minimum inhibitory concentration and minimum bactericidal concentration (21.50 and 38.25 µg/mL, respectively) were determined to be desirable (lowest values).

Using different plant extracts, various researchers discovered the same trend and interesting MIC values. The cyclohexane extract of *Monodora myristica* fruits and the ethyl acetate extract of *Albizia gummifera* stem bark had higher

Efforts to deal with *Candida albicans* and *Staphylococcus aureus*, with MIC values of 6.3 µg/mL. (Mbosso *et al*., 2010). The bark and stems of *Bolusanthus speciosus* showed good efficacy against *Escherichia coli* and *Staphylococcus aureus* with MIC values ranging from 0.098 to 0.012 mg/mL in the chloroform and ethyl acetate extracts. The best efficacy against *Candida albicans* was shown by the chloroform extract of *Bolusanthus speciosus* bark, with MIC and MFC values of 0.012 mg/mL. (Mulaudzi *et al*., 2011). Previous research regarding the extract of methanol of *Piper cubeba* demonstrated strong antimicrobial potential with MIC values against *Aspergillus niger* (12.8 mg/mL), *Candida albicans* (1.6 mg/mL), *Escherichia coli* (3.2 mg/mL), *Pseudomonas aeruginosa* (6.4 mg/mL), and *Staphylococcus aureus* (1.6 mg/mL), which are in line with our findings (Rukayadi *et al*., 2013).

The dichloromethane and methanol (1:1) extract of *Lippia javanica*, which yielded the lowest MIC of 0.25 mg/mL against *Staphylococcus aureus*, showed the similar pattern, according to Hubsch *et al*. (2014). In contrast, the ethanol extracts of *Centaurea nigra*, *Arctium minus* and *Taraxacum officinale* had MIC values against the bacteria ranging from 250 to 500 µg/mL. The ethanol extract of *Centaurea scabiosa* had the lowest MIC values against *Staphylococcus aureus* (187.5 µg/mL), MRSA (187.5 µg/mL) and *Bacillus cereus* (250 µg/mL) (Kenny *et al*., 2014).

Candida albicans, which has a worldwide distribution due to its role as an opportunistic pathogen in AIDS patients, had the highest susceptibility among the fungal strains studied (De Pavia *et al*., 2003: Nolte *et al*., 1997). It is a frequent commensal of the human gastrointestinal and urogenital tracts, as well as the cause of Candiasis in women (Black, 1996). Singh and Karnwal (2006) discovered that *Cassia fistula* leaf extract possessed efficaciousness against *Candida albicans* and yielded similar substantial results. *Curtisia dentata*, *Terminalia sambesiaca* and T. *phanerophlebia* extracts demonstrated the maximum activity against *Candida albicans*, with a minimal inhibitory concentration of 0.1 mg/mL. (Shai *et al*., 2008). Bhalodia *et al*. (2011) discovered a remarkable suppression of *Candida albicans* in a chloroform extract of *Cassia fistula* seeds. Muthukumaran *et al*. (2011) discovered that *Cassia auriculata*

extract produced larger zones of inhibition against *Candida* sp. than *Moringa tinctoria* crude extract. Similarly, Mansourian *et al*. (2014) discovered that *Syzygium aromaticum* had superior anti candidal efficacy, with an inhibitory zone diameter of 29.62 mm. A sesquiterpene, xanthanolide, 4-(2-methybutyryl)-4H-tomentosin, was isolated from the *Carpesium macrocephalum* entire plant and demonstrated antifungal activity (MIC50>256 µg/mL) (Xie *et al*., 2015).

The frequency of microbial infections is steadily increasing as a consequence of the rise of bacteria that are resistant to synthetic antimicrobial drugs. Many research using ethnomedicinal plants and their active components against bacteria, fungus, algae, and viruses have been conducted (Mickymaray *et al*., 2016; Casciaro *et al*., 2019). Devakumar *et al*. (2016) discovered that S. *cumini* and S. *jambos* leaves in acetone extract had high antifungal efficacy against A. *niger*, C. *albicans*, C. *neofromans* and T. *rubrum*. Aqueous extract, however, showed medium range fungicidal efficacy. Elfadil *et al*. (2016) isolated an antibacterial component from S. *cumini* leaves and tested its efficacy against S. *aureus*, E. *coli*, P. *aeruginosa* and B. *subtilis* using petroleum ether, methanol, and water at varying doses (15, 10 and 5%). They claimed that the best extraction solvent was methanolic extract. According to Sumithra and Kumar (2016), an agar well diffusion experiment revealed that the extract of methanol from S. *calophyllifolium* leaf exhibited broad spectrum antibacterial activity in comparison to other solvent extracts.

Using S. *cumini* leaves as a source, Imran and Khan (2017) extracted bioactive chemicals and discovered that petroleum ether as well as extracts from ethanol had the best antibacterial activity. Hasanuzzaman and Islam (2018) assessed the antibacterial effectiveness of a methanolic root extract of the Bangladeshi S. *cumini* plant against six gram-positive and six gram-negative bacteria. A significant inhibitory zone (16 mm) against *Sarcina lutea* and *Shigella dysenteriae* was seen at a concentration of 200 µg. According to Sumangala *et al*. (2019), S. *travancorium*'s methanolic extract has a 10 mm inhibitory zone for *Staphylococcus aureus*, an 8 mm zone for *Salmonella typhi* and 7 mm zone for *Klebsiella pneumoniae*. Twelve pathogenic pathogens along with two benchmark bacterial strains were used to test the antibacterial properties of four distinct plants

extracts. A test of the antibacterial abilities of *Cinnamomum tamala*, *Artemisia vulgaris*, and *Oxalis corniculata* and *Ageratina adenophora*'s methanolic extracts was conducted. *O. corniculata* extract illustrated the greatest potential, with zones of inhibition (ZOI) of 17 millimeters, 13 millimeters, 16 millimeters, 11 millimeters, and 12 millimeters, respectively, against E. *coli*, S. *Typhi*, MDR S. *Typhi*, K. *pneumoniae*, and C. *koseri*. The greatest MIC was similarly demonstrated by *Oxalis corniculata* against the test organisms. S. *aureus* was successfully combated by the methanolic extract of *Artemisia vulgaris*, *Cinnamomum tamala* and *Ageratina adenophora*. *Rhizopus* species were likewise resistant to the antifungal effects of *Ageratina adenophora* (Manandhar *et al*., 2019).

In 2021, Rafiu *et al*. investigated the antibacterial effects of *Lannea egregia* stem bark on six gram-negative, eight gram-positive and five fungus strains. Hexane, DCM, ethanol and methanol among other organic solvents, were used to create extracts. According to the study mentioned above, the extract prepared using DCM solvent has a better minimum inhibitory zone at a focus of 100 mg/ml than other solvents. For P. *aeruginosa*, DCM extract of bark on stems exhibits a 23 mm inhibition zone at 100 mg/ml, a 24 mm inhibition zone against S. *aureus* and a 22 mm inhibition zone against S. *aureus.* 100 mg/ml of the stem bark DCM extract has antifungal properties and produced an 18mm zone of inhibition against *Penicillium* species. A 22 mm zone of inhibition against *Candida albicans* was seen in an extract of stem bark in hexane at 100 mg/ml. 100 mg/ml of the stem bark DCM extract demonstrated an 18 mm zone of inhibition against A. *niger*.

Wasilewska *et al*. (2022), In their research, natural reducing agents found in the extracts of apples, oranges, potatoes, red pepper, white onion, garlic, and radish were used to create Ag nanoparticles utilizing green synthesis techniques. S. *aureus*, B. *cereus*, E. *coli* and C. *krusei* were used as test organisms to determine each nanoparticle's antibacterial capabilities, which were expressed as the minimal inhibitory concentration. The findings indicated that distinct particles with varying antibacterial activity were found in each extract, ranging in size from 9 to 302 nm, according to the data. According to research, plant extracts offer Ag

nanoparticles particular capabilities, however the highest antibacterial properties are demonstrated by nanoparticles made using potato extract.

Twelve steroid-based hydrazones were assessed as antibacterial agents in silico by Merlani *et al*. in 2023. The results of the experimental analysis showed that, with the exception of B. *cereus*, all substances have low to moderate antibacterial activity. Eleven compounds appeared to be the most effective, with MIC and MBC of 0.75 and 1.5 mg/mL, respectively. When compounds were compared to ampicillin, which lacked bacteriostatic or bactericidal properties, their antibacterial efficacy against the three resistant strains of MRSA, E. *coli* and P. *aeruginosa* was found to be superior. Although each chemical had a distinct sensitivity to the studied fungi, they all showed good antifungal activity with MICs of 0.37-1.50 mg/mL and MFCs of 1.50-3.00 mg/mL.

In the current investigation, the *Oxystelma esculentum* R.Br. fruit methanol extract had the best antibacterial activity. It is also advised to conduct chemical and medicinal studies on this plant to use it commercially as an antibacterial agent.

In this current study, *Oxystelma esculentum* R.Br. methanol extract was employed to assess its efficacy in various antioxidant assays, including hydrogen peroxide scavenging, DPPH free radical scavenging, superoxide anion radical scavenging, nitric oxide radical scavenging, and Ferric Reducing Antioxidant Power. The fruit extract exhibited the highest level of antioxidant activity, with the flower extract following as the next most potent.

Numerous studies have explored the antioxidant potential of medicinal plants, aligning with the focus of research. Shyura *et al*. (2005) conducted a study examining the DPPH radical-scavenging activity of various plants, including *Ludwigia octovalvis*, *Vitis thunbergii*, *Rubus parvifolius*, *Lindernia anagallis*, and *Zanthoxylum nitidum*. They also observed superoxide anion scavenging activity in extracts from *Ludwigia octovalvis*, *Vitis thunbergii*, *Prunella vulgaris*, *Saurauia oldhamii*, and *Rubus parvifolius*.

Katalinic *et al.* (2006) found the highest phenol content in *Melissae folium*. These antioxidants play a crucial role in safeguarding the human body against oxidative damage, as noted by Silva *et al*. (2007). It's worth noting that

commonly used synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole, have been associated with adverse effects, including liver damage and carcinogenicity, as reported by Politeo *et al*. (2007).

Desmodium gangeticum showed the highest antioxidant activity, with descending order of strength observed in *Amaranthus caudatus*, *Desmodium nigrum*, *Piper longum*, *Eclipta alba*, and *Ocimum sanctum*, as reported by Prakash *et al.* in 2009. The greatest reduction in DPPH was recorded for H. *indicus* (77.0%), followed by P. *zeylanica* (73.41%), A. *calamus* (20.88%), and H. *antidysenterica* (20.06%) extracts at a concentration of 100 µg/mL, according to Zahin *et al.* in 2009. Ashafa *et al.* in 2010, demonstrated that the acetone extracts of *Felicia mauricata* were found to possess substantial total antioxidant activity, because of the gallic acid present.

In their study, Chanda *et al*. in 2011 also demonstrated the antioxidant potential of twelve medicinal plants. They found that the methanol and acetone extracts of *Paltophorum ferrugineum* and *Triumfetta rotundifolia* exhibited the most significant DPPH free radical scavenging activity. Additionally, *Buchanania lanzan*'s methanol and acetone extracts displayed the highest hydroxyl radical scavenging activity, while the methanol and acetone extracts of *Paltophorum ferrugineum* showed superior superoxide anion radical scavenging activity compared to others. These findings align with previous reports by Chatterjee *et al*. (2012), Har and Ismail (2012), who noted similar antioxidant properties in root extracts of *Syzygium cumini*, seed extracts of *Eugenia jambolana*, and leaf extracts of *S. polyanthum*, respectively. Furthermore, the results from Chanda *et al*.'s study are consistent with those of Ho *et al.* (2012), who identified *Rotala rotundifolia* as having the highest antioxidant capacity, followed by *Juncus effusus* var. decipiens, *Cyperus iria*, *Salix warburgii*, and *Kyllinga brevifolia*.

Recent studies have associated free radicals with various diseases, including cancer, heart disease, aging, cataracts, and damage to the central nervous system. Cancer preventive medications, as suggested by Asimi *et al*. in 2013, can mitigate the harmful effects of these free radicals by reducing oxidation levels and protecting cells. Natural remedies that enhance cell resilience are employed to prevent and address conditions related to oxidative stress, like

diabetes, Alzheimer's disease, atherosclerosis, stroke, and cancer, as indicated by research conducted by Devasagayam *et al*. in 2004 and Howlader *et al*. in 2012.

In research conducted by Hossain *et al*. in 2013, similar findings were reported for the fruits of *Datura metel*. The results revealed that the ethyl acetate extract exhibited the highest percentage of total phenolics at 60.26%, surpassing the percentages found in hexane, chloroform, butanol, and methanol extracts. Additionally, Kaur and Mondal research in 2014 highlighted the antioxidant capacity of various plants. They noted that *Citrus aurantifolia* had the maximum level of activity at 87.05%, followed by *Ocimum sanctum* at 81.80%, *Catharanthus roseus* at 71.4%, and *Cassia fistula* at 77.8%.

In their study, Wansi *et al*. in 2010 evaluated that the methanol extract obtained from the outer layer of the stalk of *Klainedoxa gabonensis* using the DPPH assay exhibited substantial antioxidant activity, as indicated by high antioxidant values. Similarly, Pawar *et al*. in 2015 claimed the solutions consisted of ethanol and chloroform of *Asteracantha longifolia* demonstrated greater scavenging effects on DPPH radicals compared to the standard antioxidant, suggesting their potent antioxidant properties.

Antioxidants are substances that play a crucial role in breaking the chain reaction of oxidative damage in the body. They function by scavenging various types of free radicals, including hydroxyl, alkoxyl, and peroxyl radicals, as well as singlet oxygen and nitric oxide. Additionally, antioxidants help decompose hydroperoxides and can chelate metal ions, further mitigating oxidative stress (Carocho and Ferreira, 2013). Numerous studies have acknowledged the antioxidant properties of various plants and their constituents. Earlier research has demonstrated that the consumption of antioxidant-rich fruits and vegetables can reduce oxidative stress and its associated health conditions (Rimm *et al*., 1996).

Rajalakshmi *et al*. (2018) noted that the ethanolic extract of *S. travancoricum* exhibited the highest DPPH scavenging activity, showing a 43.44% inhibition. Jothiramshekar *et al*. (2014) found that *Syzygium calophyllifolium* demonstrated a 66.42% inhibition of DPPH radicals, while *Euphorbia cotinifolia* showed a 21.34% inhibition. Kamsala *et al*. (2015) demonstrated a dose-dependent DPPH reducing capacity in the *S. anacardium*

extract. Among the various extracts, the removal of water displayed the highest action of DPPH radical scavenging, followed by ethanol, with the lowest recognized in the hexane extract. Solankar *et al*. (2018) also observed an rise in DPPH scavenging activity with higher concentrations of *S. anacardium* extract.

The results of the FTIR study of the aforementioned plant indicate that *Oxystelma esculentum* R.Br. has high concentrations of alcohols, alkanes, amides I & II, phosphate-I, sulfoxide, and alkyl halides. The current research shows that different parts of the plant have varied concentrations of biomolecules.

The extracts made with methanol from *Oxystelma esculentum* R.Br. fruits showed the highest effectiveness against the investigated microorganisms, GC-MS characterization was performed on them. The following chemicals were found in the crude extract- 2-Chloroethylmethyl sulfoxide, Acetic acid, methyl ester, Trichloromethane, n-Hexadecanoic acid, 9(E), 11(E)-Conjugated linoleic acid, 6-Octadecenoic acid,(Z)- etc. The methanol extracts of flowers of the plant showed presence of 1-Heptacosanol, beta. -Sitosterol acetate, 9-Octadecen-1-ol, acetate, (Z), Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite,dl-.alpha.-Tocopherol compounds in GC-MS characterization.

Oxystelma esculentum R.Br. fruits and flowers were subjected to an HPLC methanol a portion. The findings showed that the main peak, which corresponds to flavonoids at a wavelength of 270 nm, occurred at 2.563 RT and 2.566 RT respectively. Flavonoids were found to be the main peak; some minor peaks may have been caused by steroids, terpenoids, phenolics, etc. Plant flavonoids are significant bioactive compounds that have been shown to have antibacterial properties.

CHAPTER – VI

CONCLUSION

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CONCLUSION

Plants are an abundant supply of several medications since they generate a wide variety of bioactive chemicals. The majority of pharmaceuticals utilized in conventional medical systems these days come from natural sources or are semi-synthetic derivatives of natural materials. Thus, screening conventionally manufactured natural materials makes sense in the drug development process. A significant portion of newly developed antibiotics that are brought to market are derived from natural resources, and about 20% of every plant has served as the subject of biological or pharmacological testing.

Most often, medicinal herbs are utilized in the pharmaceutical industry due to the variety of compounds found in them that applied to the procedure of both infectious and chronic illnesses. The World Health Organization (WHO) states that the greatest place to find a broad variety of medications is from medicinal plants. Phytochemicals are a class of compounds that give plants their therapeutic potential and have a particular physiological impact on human bodies. These plant-based compounds were found in naturopathic and botanical remedies that were utilized to treat the illness. These compounds, which lack nutritional value, provide protective or disease-preventive properties. Screening medicinal plants for bioactive chemicals becomes necessary in order to provide the groundwork for future pharmacological research.

A preliminary phytochemical screening of the various extracts of *Oxystelma esculentum* R.Br. fruits and flowers was carried out in this study, and the findings showed the presence of numerous substances such as alkaloids, terpenoids, glycosides, steroids, flavonoids, phenolics, tannins, and saponins. Strong phytochemicals like alkaloids, terpenoids, phenolics, saponins, and flavonoids were found in the methanol extracts of fruits in the qualitative phytochemical screening, followed by flowers. Fruit methanol extracts responded better in statistical phytochemical evaluations than flower extracts.

Oxystelma esculentum R.Br. was examined for antifungal and antibacterial properties. Using methanol, extracts from distinct regions of *O. esculentum*,

including the fruits and flowers. The antibacterial activity of the extract has been assessed against both gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. Antifungal effects of *Aspergillus flavus* and *Candida albicans* were also assessed. The inhibition zone's diameter was assessed using the disc diffusion method.

The methanol extract of *O. esculentum* R.Br fruits had the highest overall zone of inhibition against *Staphylococcus aureus* (22.5±0.35 mm at 1000 µg/disc) when compared to other tested strains. The minimum bactericidal concentration (MBC) (38.25 µg/mL) and minimum inhibitory concentration (MIC) (21.50 µg/mL) were found in *O. esculentum* methanol extracts. A methanol isolate of *O. esculentum* R.Br flowers showed the lowest zone of inhibition (9.5±0.43 mm at 250 µg/disc) against *E. coli*.

Concerning fungi, the methanol extract of *O. esculentum* R.Br flowers showed the highest zone of inhibition (12.5±0.50 mm at 1000 µg/disc) against *Candida albicans*. When it came to *Aspergillus flavus* and *Candida albicans*, methanol extracts from *O. esculentum* R.Br fruits and flowers exhibited the lowest possible MIC (500 µg/mL) and MFC (1000 µg/mL) values.

Ciprofloxacin (10 µg/disc) was utilized as an inhibitory agent for bacteria, and the mean zone of inhibition varied from 24.0±0.65 mm to 29.80±0.56 mm. The mean zone of inhibition varied from 14.50±0.50 to 21.0±0.76 mm, with amphotericin-B (100 units/disc) and ketaconazole (5 µg/disc) serving as positive controls for *Aspergillus flavus* and *Candida albicans*, respectively.

Several fractions of the unrefined extracts of *Oxystelma esculentum* R.Br. were put to the test to determine whether they were capable to scavenge radicals against DPPH, ABTS, hydrogen peroxide, superoxide, and nitric oxide radicals in addition to being able to for reduce ferric oxide radicals and their overall antioxidant activity. Fruit extracts in methanol demonstrated higher levels of tested antioxidants than flower extracts.

The FTIR analysis revealed that *Oxystelma esculentum* R.Br. has high concentrations of alcohols, alkanes, amides I and II, phosphate-I, sulfoxide, and alkyl halides.

In GC-MS characterization, 2-Chloroethyl methyl sulfoxide, Acetic acid, methyl ester, Trichloromethane, n-Hexadecanoic acid,9(E), 11(E)-Conjugated linoleic acid, 6-Octadecenoic acid, (Z)-, 1-Heptacosanol, beta. -Sitosterol acetate, 9-Octadecen-1-ol, acetate, (Z), Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite,dl-.alpha.-Tocopherol compounds were identified.

In HPLC analysis of the plant, flavonoids which shows significant antibacterial properties, were found to be the main peak; some minor peaks may have been caused by steroids, terpenoids, phenolics, etc.

The study concluded by demonstrating the effectiveness of *Oxystelma esculentum* R.Br.'s antibacterial and antioxidant qualities and supporting the plant's potential as a source of complementary and alternative medicine. Antioxidants in crude extracts cause oxidative damage to components to be delayed or prevented. Antioxidants may therefore help to regulate the quantity of free radicals. Therefore, using natural products especially the fruits of *Oxystelma esculentum* R.Br. might be a new way to get antibacterial and antioxidant properties. It has been demonstrated that natural compounds have less unintended reactions with the environment and fewer adverse effects.

SUMMARY

SUMMARY

Oxystelma esculentum R.Br. is an important medicinal plant belongs to Asclepiadaceae family and commonly known as 'Jaldudhi'. It is a perennial twiner found throughout the plains of the Indian sub-continent near water-logged areas. This plant is native to China, South Asia, Southeast Asia, Northeastern Africa, and South-west Asia. Plant genus derives its name from two words: Oxys (sharp) and Stelma (crown), which describe the acute lobes of the corolla. Characters such as a milky latex, climber, opposite leaves, pointed leaf tip, white (outside) and pink (inside) coloured flowers, pointed petal tips with hairy edges, presence of pollinia, and smooth follicular fruit with many hairy tiny seeds can be used to identify it in the field. The plant is used as laxative, antiseptic, depurative, anthelmintic, antiulcer, aphrodisiac, hepato protective and useful in leucoderma and bronchitis. Decoction of plant is used in ulcer, sore-throat and itches. Milky juice is used as galactogogue, antiperiodic, antiulcer and as a vulnerary. Leaves are used as antiperiodic. Its root is prescribed in jaundice. Fruit is bitter, tonic, expectorant, anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent.

The plant collection was done in Baran district of Rajasthan. Baran is located at 24°25′N to 25°25′N latitude and 76°12′E to 77°26′ E Longitude. It is surrounded by three Rivers Kalisindh, Parvati and Parban. The city is situated on the border of Rajasthan and Madhya Pradesh. The average annual rainfall in the district is 895.2 mm. It has an average elevation of 262 metres (859 ft). January is the coldest month with the average daily maximum temperature of 24.3°C and the average daily minimum temperature of 10.6°C.

The present study was carried out with the following objectives:

1. Selection, authentication and to prepare crude extracts from the fruits and flowers of the plant.
2. Preliminary phytochemical investigation of the plant.
3. To assess the antibacterial and antifungal activities of the crude extracts of flowers and fruits of the plant.
4. To determine the extent of inhibitory zone, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and

Minimum Fungicidal Concentration (MFC) of all the extracts.

5. To check the antioxidant potential of the plant parts (flowers and fruits) and to study their free radical scavenging effects.
6. To separate and identify the compounds from various extracts using GC-MS, HPLC and FT-IR.

The important observations and results emanating from the thesis can be summarized as follows:

1. Qualitative Phytochemical Screening

Table 1. Preliminary phytochemical analysis of medicinal plant using methanol extracts

S. No.	Parameters	Sample Fruit	Sample Flower
1	Alkaloid- Dragendorff reagent	++	+
2	Alkaloid- Mayers reagent	+	-
3	Tannins- Gallic	++	-
4	Saponin	+	+
5	Reducing sugar	+	-
6	Free reducing sugar-Fehling solution	-	-
7	Ketones	+	-
8	Pentoses	+	-
9	Phlobatannins	+	-
10	Cardia Glycosides	+	-
11	Steroids- Libermann test	-	+
12	Steroids- Salkowsk's test	+	-
13	Flavanoids- Shibita test	++	+
14	Flavanoids- Pew's test	+	-
15	Anthraquinones	-	-
16	Carbohydrates- Molish test	+	-
17	Carbohydrates- Barfoed test	+	+

(+) = Present; (-) = Absent; ++ = Strong

The crude methanol fruit extract of the plant was tested for qualitative assay for phytochemicals, of which alkaloids, flavonoids and tannins were strongly positive. Whereas other phytochemicals such as saponins, reducing

sugars, ketones, pentoses, phlobatannins, cardia glycosides, carbohydrates and steroids also showed their presence. Similarly, flower extract of the plant showed presence of alkaloids, saponins, Steroids, flavanoids and carbohydrates.

Table 2. Preliminary phytochemical analysis of medicinal plant using acetone extracts

S. No.	Parameters	Sample Fruit	Sample Flower
1.	Alkaloid- Dragendorff reagent	++	+
2.	Alkaloid- Mayers reagent	++	-
3.	Tannins- Gallic	+	+
4.	Saponin	+	+
5.	Reducing sugar	+	-
6.	Free reducing sugar- Fehling solution	+	-
7.	Ketones	+	-
8.	Pentoses	+	+
9.	Phlobatannins	+	-
10.	Cardia Glycosides	++	+
11.	Steroids- Libermann test	-	+
12.	Steroids- Salkowsk's test	+	-
13.	Flavanoids- Shibita test	++	+
14.	Flavanoids- Pew's test	++	+
15.	Anthraquinones	-	+
16.	Carbohydrates- Molish test	+	-
17.	Carbohydrates- Barfoed test	++	+

(+) = Present; (-) = Absent; ++ = Strong

The crude acetone fruit extract of the plant was tested for qualitative assay for phytochemicals, of which alkaloids, flavonoids, cardia glycosides and carbohydrates were strongly positive. Whereas other phytochemicals such as tannins, saponins, reducing sugars, free reducing sugars, ketones, pentoses, phlobatannins and steroids, also showed their presence. Similarly, flower extract

of the plant showed presence of alkaloids, tannins, Saponins, pentoses, cardia glycosides, steroids, flavanoids, anthraquinones and carbohydrates.

Table 3. Preliminary phytochemical analysis of medicinal plant using chloroform extracts

S. No.	Parameters	Sample Fruit	Sample Flower
1.	Alkaloid- Dragendorff reagent	+	+
2.	Alkaloid- Mayers reagent	++	-
3.	Tannins- Gallic	+	++
4.	Saponin	+	+
5.	Reducing sugar	+	+
6.	Free reducing sugar- Fehling solution	-	-
7.	Ketones	++	-
8.	Pentoses	+	+
9.	Phlobatannins	+	-
10.	Cardia Glycosides	++	++
11.	Steroids- Libermann test	-	+
12.	Steroids- Salkowsk's test	+	-
13.	Flavanoids- Shibita test	+	+
14.	Flavanoids- Pew's test	++	+
15.	Anthraquinones	+	-
16.	Carbohydrates- Molish test	+	+
17.	Carbohydrates- Barfoed test	++	+

(+) = Present; (-) = Absent; ++ = Strong

The crude chloroform fruit extract of the plant was tested for qualitative assay for phytochemicals, of which alkaloids, ketones, cardia glycoside, flavonoids and carbohydrates were strongly positive. Whereas other phytochemicals such as tannins, saponins, reducing sugars, pentoses, phlobatannins, steroids, anthraquinones also showed their presence. Similarly, flower extract of the plant showed strong presence of tannins and cardia glycosides. alkaloids, saponins, reducing sugars, pentoses, steroids, flavanoids

and carbohydrates were also present in the extract.

2. Quantatative Phytochemical Screening

Table 4. Summary of preliminary quantative phytochemical analysis of medicinal plant

S. No.	Parameters	Sample CF-Fruit (Mean ± S.D)*	Sample MF-Flower (Mean ± S.D)*
1.	Alkaloids	94.1±0.81 mg/ml	183.5±0.32 mg/ml
2.	Saponins	0.065±0.0057 mg/g	0.0133±0.06 mg/g
3.	Flavonoids	11.7±0.66 mg/ml	0.781±.008 mg/ml
4.	Tannins	11.9±0.58 mg/ml	3.2±0.24 mg/ml
5.	Steroids	916±8.17 µg/ml	1286±12.8 µg/ml
6.	Total Phenolic Content	6.2±0.54 mg/ml	3.3±0.34 mg/ml
7.	Carbohydrates	10.5±0.81 mg/ml	12.4±0.54 mg/ml
8.	Proteins	1.69±0.13 mg/ml	1.03±0.10 mg/ml
9.	Lipids (%)	1.00%	0.50%
10.	Terpenoids	1.86±0.16 gm/10ml	1.3±0.20 gm/10 ml
11.	Total glucosinolate	28.37±0.51 µmol/gm	75.88±0.86 µmol/gm
12.	Total Anthraquinones	33.60±0.71 µg/ml	19.90±0.31 µg/ml

The current research involved conducting a phytochemical analysis on different solvent extracts of *Oxystelma esculentum* R.Br. The methanol extracts from the fruits of *Oxystelma esculentum* R.Br. exhibited significant levels of phytochemicals including alkaloids, tannins and flavonoids. Similarly, the acetone extracts from the fruits of this plant revealed a notable presence of alkaloids, cardiac glycosides, flavonoids and carbohydrates. Likewise, the chloroform extracts from the fruit part displayed strong levels of alkaloids, ketones, cardiac glycosides, flavonoids, and carbohydrates.

The total alkaloids, saponins, flavonoids, tannins, steroids, total Phenolic Content, carbohydrates, proteins, lipids(%), terpenoids, total glucosinolate and total anthraquinones content in the powdered fruits of the chosen plant was

determined to be 94.1±0.81mg/ml, 0.065±0.0057mg/g, 11.7±0.66mg/ml, 11.9±0.58mg/ml, 916±8.17 µg/ml, 6.2±0.54mg/ml, 10.5±0.81mg/ml, 10.5±0.81mg/ml, 1.69±0.13mg/ml, 1.00%, 1.86±0.16gm/10ml, 28.37±0.51µmol/gm, 33.60±0.71µg/ml, respectively, using quantitative analysis.

Similarly, analyzing the powdered flowers of the chosen plant, the total alkaloids, saponins, flavonoids, tannins, steroids, total phenolic content, carbohydrates, proteins, lipids(%), terpenoids, total glucosinolate and total anthraquinones content was estimated to be 183.5±0.32mg/ml, 0.0133±0.06mg/g, 0.781±.008mg/ml, 3.2±0.24mg/ml, 1286±12.8µg/ml, 3.3±0.34mg/ml, 12.4±0.54mg/ml, 1.03±0.10mg/ml, 0.50%, 1.3±0.20 gm/10ml, 75.88±0.86µmol/gm, 19.90±0.31µg/ml, respectively.

3. Antimicrobial activity of crude extracts of fruits and flowers of *Oxystelma esculentum* R.Br.

Table 5. Antibacterial and antifungal activities of methanol extract of fruits of *Oxystelma esculentum* R.Br.

Microorganisms	Mean zone of inhibitiona (mm)b			Ciprofloxacin (5µg/disc)/ Amphotericin-B(100units/disc)/ Ketoconazole (10µg/disc)	MIC (µg/mL)	MBC/MFC (µg/mL)
	Concentrations of the disc (µg/mL)					
	250	500	1000			
Staphylococcus aureus	17.5±0.25*	20.25±0.3*	22.5±0.35*	28.8±0.65	21.5	38.25
Escherichia coli	9.8±0.28	12.3±0.26	13.3±0.28	24.0±0.65	250	500
Candida albicans	6.8±0.28	7.5±0.35	9.6±0.25	14.5±0.50	500	1000
Aspergillus flavus	7.3±0.25	9.8±0.50	11.8±0.25	16.50±0.80	500	1000

a-diameter of zone of inhibition (mm) including the disc diameter of 6 mm

b-mean of three assays;- standard deviation.*– Statistical significance at $P < 0.05$

Table 6. Antibacterial and antifungal activities of methanol extract of flowers of *Oxystelma esculentum* R.Br.

Microorganisms	Mean zone of inhibitiona (mm)b			Ciprofloxacin (5µg/disc)/ Amphotericin-B(100units/disc)/ Ketoconazole (10µg/disc)	MIC (µg/mL)	MBC/MFC (µg/mL)
	Concentrations of the disc (µg/mL)					
	250	500	1000			
Staphylococcus aureus	14.5±0.20*	16.55±0.50*	19.8±0.25*	30.5±0.50	62.5	125
Escherichia coli	9.5±0.43	11.7±0.25	13.8±0.56	29.80±0.56	500	1000
Candida albicans	8.7±0.28	10.5±0.26	12.5±0.50	17.5±0.50	500	1000
Aspergillus flavus	8.8±0.25	10.5±0.28	12.3±0.50	21.0±0.76	500	1000

a-diameter of zone of inhibition (mm) including the disc diameter of 6 mm

b-mean of three assays; - standard deviation. * – Statistical significance at $P < 0.05$

The methanol extract of fruits of *Oxystelma esculentum* R.Br. was tested against two bacteria and two fungi and the results are presented in **Table 5**. The mean zone of inhibition exhibited by the extract for bacteria ranged from 9.80±0.28 to 22.5±0.35 mm. For fungi, the mean zone of inhibition formed by the extract ranged between 6.8±0.28 and 12.8±0.25 mm. The MIC and MBC values for bacteria were from 21.50 to 250 µg/mL and 38.25 and 500 µg/mL respectively. The MIC and MFC values for fungi were 500 to 500 µg/mL and 1000 to 1000 µg/mL respectively. The highest mean zone of inhibition for bacteria (22.5±0.35 mm at 1000 µg/disc), lowest MIC (21.50 µg/mL) and MBC (38.25 µg/mL) values were recorded against *Staphylococcus aureus*.

The highest mean zone of inhibition for fungi (12.8±0.25 mm at 1000 µg/disc) was observed against *Aspergillus flavus*. The lowest MIC (500 µg/mL) and MFC (1000 µg/mL) values were observed against *Aspergillus flavus*. Ciprofloxacin (5 µg/disc) was used as positive control for bacteria and the mean zones of inhibition were from 24.0±0.65 mm to 28.8±0.65mm. Amphotericin-B (100 units/disc) was used as positive control for *Candida albicans* and Ketoconazole (10 µg/disc) was used as positive control for *Aspergillus flavus* and the mean zones of inhibition were between 14.5±0.50

mm and 16.50±0.80 mm. For bacteria, the lowest activity (9.80±0.28 mm at 250 µg/disc) was recorded against *Escherichia coli* and for fungi, the lowest zone of inhibition (6.8±0.28 mm) was observed against *Candida albicans*.

In the present study, methanol extract of flowers of *Oxystelma esculentum* R.Br. against microbial strains were screened and the results are tabulated in **Table 6**. The It showed the results of methanol extract of *O. esculentum* was screened against bacterial and fungal strains. The mean zone of inhibition exhibited by the extract for bacteria ranged from 9.50±0.43 to 19.8±0.25 mm. For fungi, the mean zone of inhibition ranged between 8.70±0.28 and 12.5±0.50 mm. The MIC and MBC values for bacteria were between 62.50 and 500 µg/mL and 125 and1000 µg/mL respectively. The MIC and MFC values for fungi were between 500 and 500 µg/mL and 1000 and 1000 µg/mL respectively.

For bacteria, the highest mean zone of inhibition (19.80±0.25 mm at 1000 µg/disc), lowest MIC (62.50 µg/mL) and MBC (125 µg/mL) values were obtained against *Staphylococcus aureus*. The highest mean zone of inhibition for fungi (12.50±0.50 mm at 1000 µg/disc) was observed against *Aspergillus flavus*. The MIC values of 500 µg/mL and MFC values of 1000 µg/mL were recorded against tested fungal strains. Ciprofloxacin (5 µg/disc) was used as positive control for bacteria and the mean zones of inhibition were from 30.50±0.50 mm and 29.80±0.56 mm. Amphotericin-B (100 units/disc) for *Candida albicans* and Ketoconazole (10 µg/disc) for *Aspergillus* were used as the positive controls and the mean zones of inhibition were between 17.5±0.50 and 21.0±0.76 mm. For bacteria, the lowest activity (9.5±0.43 mm at 250 µg/disc) was recorded against *Escherichia coli* and for fungi, the lowest zone of inhibition (8.7±0.28 mm) was observed against *Candida albicans.*

In the present investigation, methanol extract of fruits and flowers of *Oxystelma esculentum* R.Br. were screened for antibacterial and antifungal activities against the strains of Gram-positive and Gram-negative bacteria and fungi. Among the extracts tested, the highest mean zone of inhibition (22.5±0.35 mm at 1000 µg/disc) was observed with methanol extracts of fruits of *O. esculentum* R.Br. against a strain of *Staphylococcus aureus* when

compared to others.The lowest minimum inhibitory concentration (21.50 µg/mL) and minimum bactericidal concentrations (38.25 µg/mL) were observed with methanol extracts of fruits of *O. esculentum* R.Br. against S. *aureus*. The lowest zone of inhibition (7.3±0.25 mm at 250 µg/disc) was observed in methanol extract of fruits of *O. esculentum* R.Br. against *Aspergillus flavus*.

Where as in fungi, the highest mean zone of inhibition (12.5±0.50 mm at 1000 µg/disc) was recorded with methanol extract of flowers of *O. esculentum* R.Br. against *Candida albicans*. The lowest values of MIC (500 µg/mL) and MFC (1000 µg/mL) were recorded with methanol extracts of fruits and flowers of *O. esculentum* R.Br.against *Aspergillus flavus* and *Candida albicans*.

In the present study, the *Oxystelma esculentum* R.Br. fruit methanol extract had the best antibacterial activity. It is also advised to conduct chemical and medicinal studies on this plant to use it commercially as an antibacterial agent.

4. Antioxidant activity of crude extracts of fruits and flowers of *Oxystelma esculentum* R.Br.

Table 7. Sumary of antioxidant activity of crude extracts of fruits and flowers of *Oxystelma esculentum* R.Br.

S.No.	Antioxidant activity against	IC50 Value (µg/mL)	
		Fruit extract	Flower extract
1.	DPPH radicle	205	232
2.	ABTS·+	223	245
3.	Superoxide radicle	305	320
4.	Nitric oxide radicle	281	315
5.	Hydrogen peroxide	292	321
6.	Hydroxyl radicle	374	405
7.	Ferric reducing antioxidant power assay	295	345
8.	Total antioxidant	275	321

In this current study, the methanol extract of *Oxystelma esculentum* R.Br. was employed to assess its efficacy in various antioxidant assays, including DPPH free radical scavenging, hydrogen peroxide scavenging, superoxide anion radical

scavenging, nitric oxide radical scavenging, and Ferric Reducing Antioxidant Power. The fruit extract exhibited the highest level of antioxidant activity, with the flower extract following as the next most potent.

5. FTIR spectral data interpretation

The FTIR spectrum of fruit and flower extracts (prepared in methanol solvent) of *Oxystelma esculentum* R. Br. are given in **Fig. 1 & Fig. 2**.

Fig.1 FTIR spectrum of ME extract of fruit of *Oxystelma esculentum* R. Br.

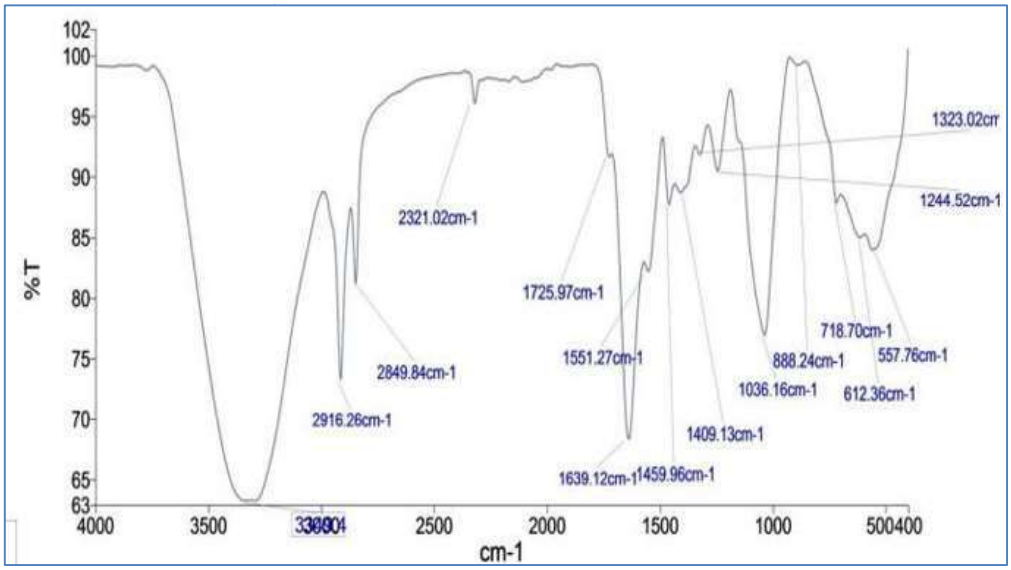
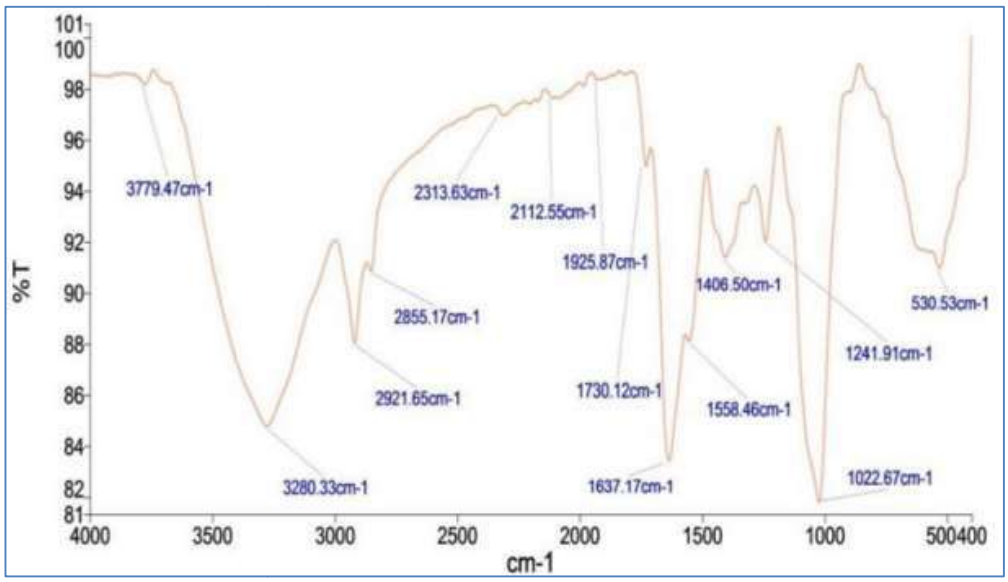


Fig.2 FTIR spectrum of ME extract of flower of *Oxystelma esculentum* R. Br.



Chromatography FTIR analysis of the methanol extract of fruits of *Oxystelma esculentum* R. Br showed the presence of sixteen major peaks and the components corresponding to the peaks were determined as follows. The FTIR analysis of *Oxystelma esculentum* R. Br fruits proved the presence of Alcohols, Alkanes, Esters,Amide I & II, Aromatic compounds, Phenol, Phosphate-I, Sulfoxide, Alkenes and Alkyl halides which shows major peaks at 3349, 2916, 2849, 2321, 1725, 1639, 1551, 1459, 1409, 1323, 1244, 1036, 888, 718, 612, 557 $cm.^{-1}$

Chromatogram FTIR analysis of the methanol extract of flowers of *Oxystelma esculentum* R. Br showed the presence of fourteen major peaks and the components corresponding to the peaks were determined as follows. The FTIR analysis of *Oxystelma esculentum* R. Br flowers proved the presence of Alcohols, Alkanes, Alkynes, Allene, Esters, Amide I & II, Aromatic compounds, Phosphate-I, Sulfoxide and Alkyl halides which shows major peaks at 3779, 3280, 2921, 2855, 2313, 2112, 1925, 1730, 1637, 1558, 1406, 1241, 1022, 530 $cm.^{-1}$

The findings mentioned above lead to the conclusion that *Oxystelma esculentum* R. Br. has high concentrations of alcohols, alkanes, amides I and II, phosphate-I, sulfoxide, and alkyl halides. The current research shows that different regions of the plants have varied concentrations of biomolecules. Further study in the biological activity of medicinal plants and the analysis of their phytochemicals is possible thanks to this effort.

6. GC-MS characteristics of methanol fraction of fruits and flowers of *Oxystelma esculentum* R. Br.

The methanol extracts of fruits of *O. esculentum* was further subjected to carry out GC-MS characterization due the highest activity against tested microbes. The compounds were identified in the crude extract such as 2-Chloroethyl methyl sulfoxide, Acetic acid, methyl ester, Trichloromethane, n-Hexadecanoic acid,9(E), 11(E)-Conjugated linoleic acid, 6-Octadecenoic acid, (Z)- etc. Similarlay, The methanol extracts of flowers of O. esculentum showed presence of 1-Heptacosanol, .beta.-Sitosterol acetate, 9-Octadecen-1-ol, acetate,(Z), Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite,dl-.alpha.-Tocopherol compounds in GC-MS characterization. (**Table 8 & Table 9**).

Fig. 3. GC-MS characteristics of methanol fraction of fruits of *Oxystelma esculentum* R.Br.

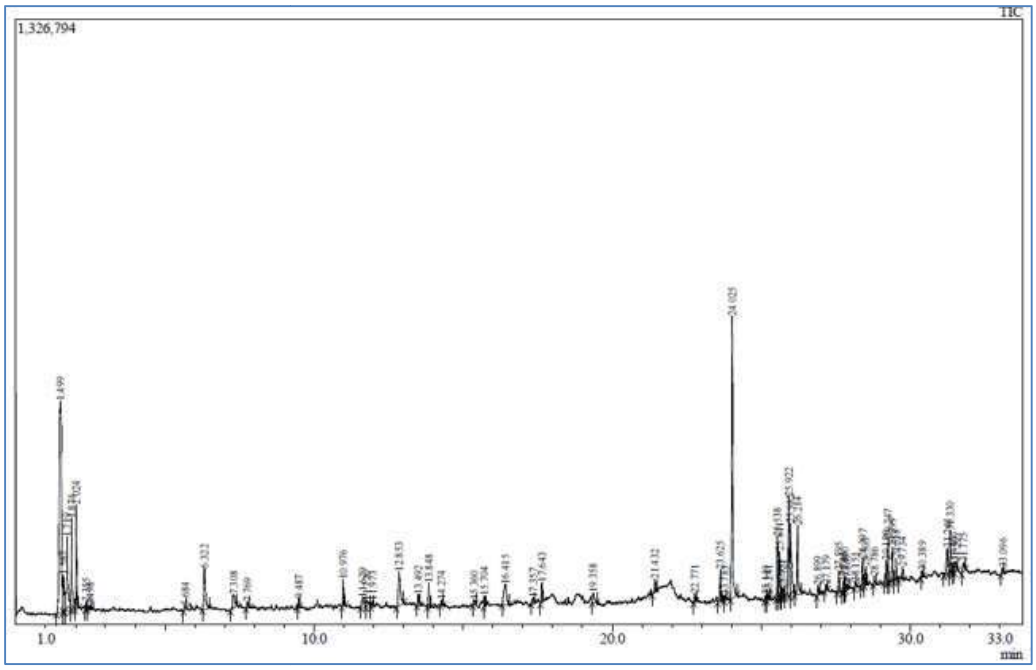
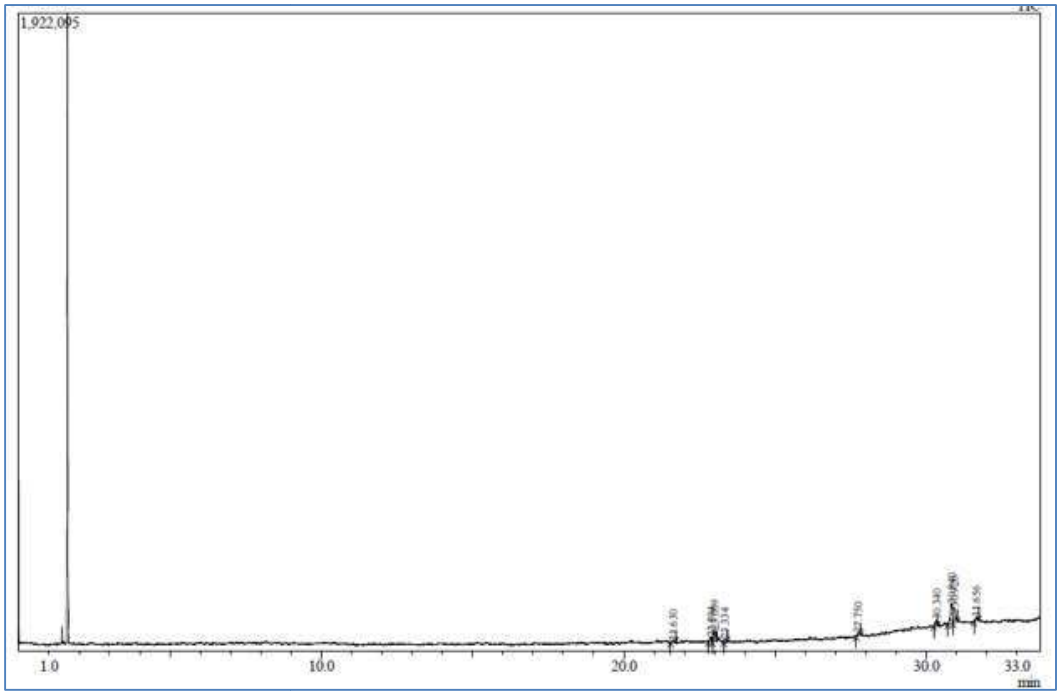


Fig. 4. GC-MS characteristics of methanol fraction of flowers of *Oxystelma esculentum* R.Br.



7. HPLC analyses

The HPLC analyses of methanol extract of fruits and flowers of *Oxystelma esculentum* R.Br. was performed and results are shown in **Fig. 5 & Fig. 6.**

Fig. 5. HPLC spectrum of methanol extract of fruits of *Oxystelma esculentum* R.Br.

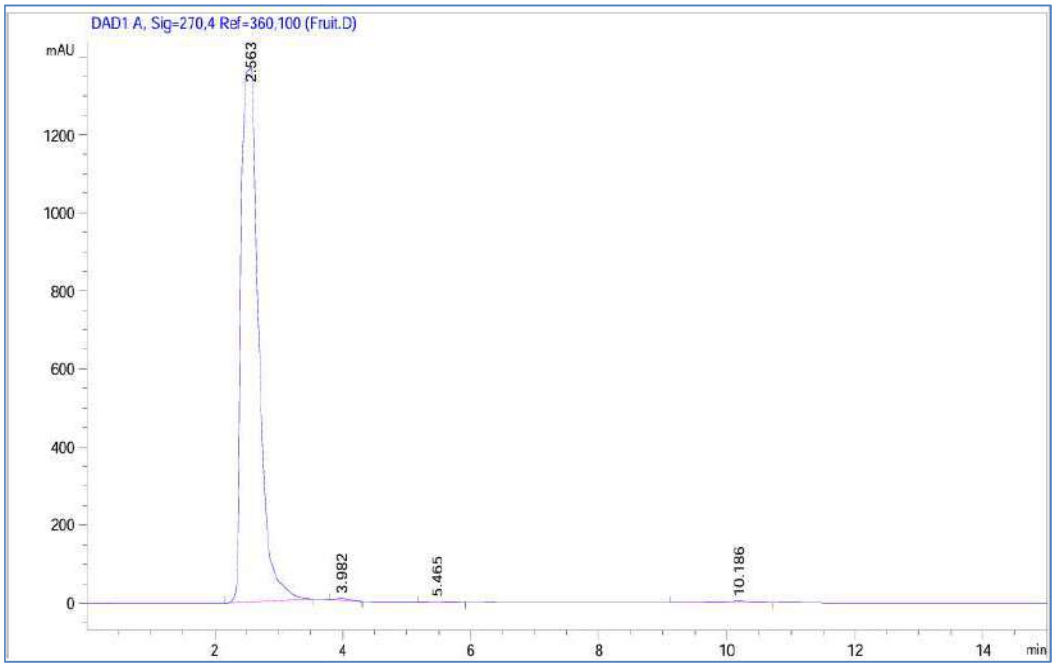
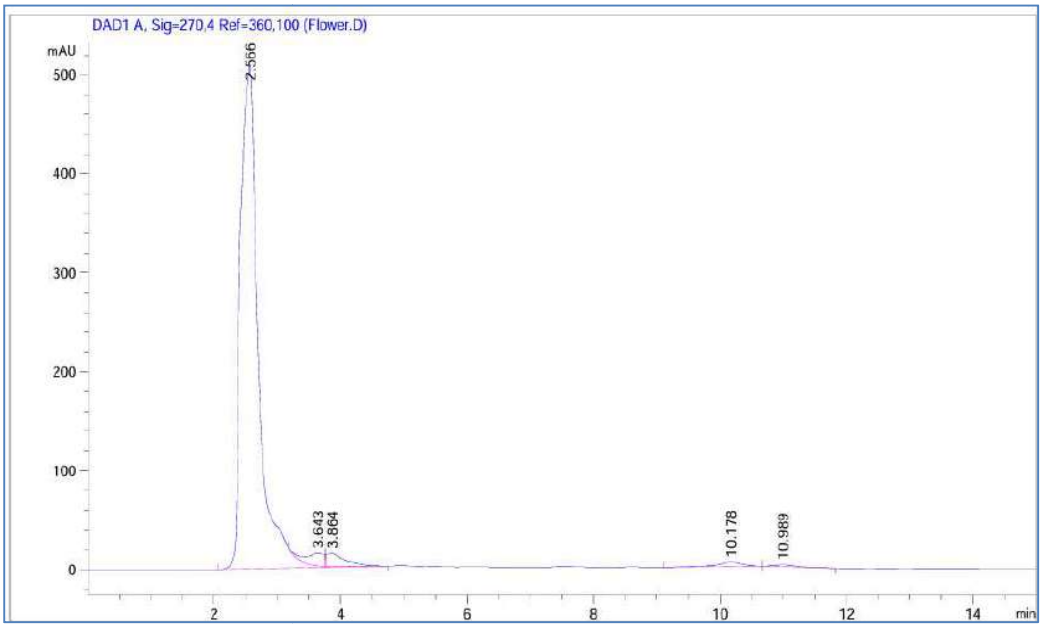


Fig. 5. HPLC spectrum of methanol extract of flowers of *Oxystelma esculentum* R.Br.



Oxystelma esculentum R.Br. fruits and flowers were subjected to an HPLC methanol extract. The results indicated that the main peak, which corresponds to flavonoids at a wavelength of 270 nm, occurred at 2.563 RT and 2.566 RT respectively. By measuring the retention time of the sample against the standard (quercetin) and by mixing a known concentration of the pure standard into the extract and comparing the peak heights that resulted, the presence of flavonoids was verified. Potential synergistic effects of the extract's deducted flavanoids and antioxidant activities could be observed.

Flavonoids were found to be the main peak; some minor peaks may have been caused by steroids, terpenoids, phenolics, etc. Plant flavonoids are significant bioactive compounds that have been shown to have antibacterial properties.Wide-ranging biological activities have been demonstrated for it, such as hepatoprotective, antiviral, antiallergic, anti-inflammatory, antimutagenic, antiproliferative, and antithrombotic effects (Bors and Saran, 1987; Middleton and Kandaswami, 1994; Robak and Gryglewski, 1988; Wall, 1992; Chang and Kinghorn, 2001).

Significance of proposed research work

1. The proposed study has provided actual data of the phytochemicals (flavonoids, Alkaloids, glycosides, steroids, tannin, terpenoids etc.) present in the studied plant. The anti diuretic, anti-inflammatory, anti analgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites.

2. The phytochemical analysis of the selected plant is also important and has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

3. Proposed study will provide more attention towards conservation, cultivation for effective utilization of the selected plant with balancing threat to the biodiversity.

4. The given research proposal is try to discover hidden pharmacological activities present in selected medicinal plant which is used as traditional/ethno medicine by tribes.

5. This research work again tries to isolate the active compound(s) from the plant which is responsible for such pharmacological activities. It will also makes the addition of one more herbal drug for effectively commercialize and to export.

In conclusion, the study proved the efficiency of antimicrobial and antioxidants properties of *Oxystelma esculentum* R.Br. and advocates the potentiality of the plant as a source of alternative medicine. Presence of antioxidants in the crude extracts leads to delay or prevent oxidative damage to compounds. Therefore, antioxidants may serve to control the level of free radicals. So, use of natural products, especially fruits of *Oxystelma esculentum* R.Br. may be considered as a new source of natural antioxidant and antimicrobial agents. Natural substances have been proved to have fewer side effects and less unwanted reactions with the environment.

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RESEARCH PAPERS

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Section B: Herbal Chemistry

Research Article

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A Review on Phyto chemical, Antimicrobial and Antioxidant properties in medicinal plant *Oxystelma esculentum* R. Br.

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Abstract: Numerous plants that are frequently encountered and described in works on traditional Indian medicine have not been properly studied. It is required to evaluate, standardize, record, and patent these plants in a systematic manner. One of these understudied plants is Oxystelma esculentum R. Br. (Family - Asclepiadaceae), also known as "Jaldudhi." It offers a wide range of therapeutic potential that is crucial for treating diseases of the modern day like cancer, hepatitis, kidney disorders, disorders linked to stress and microbial infections. Cardenolides and pregnane glycosides, which are easily derived from this plant and can serve as precursors of numerous therapeutically significant chemicals, are two highly important groups of phytoconstituents that are present in it. Future research on this plant will be crucial for creating formulations or semi-synthetic compounds, conducting pre-clinical or clinical trials, and fractionating medicinal phytoconstituents based on bioactivity. The current review illuminates the research that has been done on this plant so far and offers insightful guidelines for the work that can be done in the future. It is based on a thorough literature search of reputable books, scientific websites, and high-impact journals.

Keywords: Asclepiadaceae, Cardenolides, Diuretic, Jaldudhi, Oxystelma esculentum, phytoconstituents, bioactivity.

1.INTRODUCTION

The common Ayurvedic plant known as "Jaldudhi," Oxystelma esculentum R. Br. (Family - Asclepiadaceae), has not been thoroughly studied. Cardenolides and pregnane glycosides, two key groups of therapeutically significant phytoconstituents, are only found in a few numbers of plants. According to reports [1-4], *O. esculentum* has effective therapeutic effects against a variety of modern diseases. The current review, which is based on a thorough examination of credible books, websites and journals, comments on the research that has been done so far on this plant and offers suggestions for further study. *Oxystelma secamone Linn., Periploca esculenta Roxb., Periploca secamone Linn., Sarcostemma secamone Bennet, Sarcostemma esculentum Linn., Asclepias rosea R. Br* are some synonyms of the plant [5,6]. It is called Dugdhika in Sanskrit, Dudhlata in hindi, Jaldudhi in Gujrati, Khirai in Bengali, Dudhika in Marathi, Dudipala in Telugu and Usipallai in Tamil. It is found in the plains, usually near water, and lower hills of India, Ceylon, and Java. This plant can also be found in China and Indonesia.

2.MORPHOLOGY OF THE PLANT

The plant genus is named after two words: Oxys (sharp) and Stelma (crown), which describe the corolla's acute lobes. It is a twining herb or undershrub with a cylindrical, glabrous, long, slender, and heavily branched stem. Simple, opposite, dorsiventral, deciduous, linear lanceolate leaves with acute apex and symmetrical base, with long and slender petiole. Inflorescence is Racemose, subumbellate cyme or solitary [7]. Flowers are drooping, pedunculate, lateral subumbellate, or racemose flowered cymes that are widely open, white with purple veins, 2.5-3cm in diameter. The calyx is pentasepalous, connate, glabrous, oblong-lanceolate, acute, and glandular. The corolla is pentapetalous, connate, 2.5cm wide, glabrous, saucer-shaped, broadly rotated, lobed half-way down with a densely pubescent corolline corolla and a double corona [8]. The corolla lobes are triangular, acute, ciliate, purple veined, valvate at the base, and briefly overlap to the right. Androecium has five stamens that are adnate near the base of the corolla and have connate filaments, anthers with inflexed membranous deltoid tips, and waxy, pendulous, elongate-clavate, solitary pollen in each cell. The gynoecium is bicarpellary with a truncate or convex style apex and a depressed or sub-convex stigma. Follicles are 4.5-7.5cm long, often solitary, ovate-lanceolate, glabrous with an acute apex and numerous black, broadly ovate seeds. *Oxystelma esculentum* var. wallichii is another *Oxystelma* species that is uncommon in India. Its follicles are shorter, 2.5-4cm long, oblong, and rounded at both ends.Its follicles are the only morphological distinction between the two types[9,10].

2.1. Uses of the plant: It has spermatogenic, diuretic, laxative, antitussive, anthelmintic and antileprotic properties. It increases the fertility of women. The entire plant is used for leucoderma and bronchitis as well as a diuretic, laxative, antibacterial, anthelmintic, antiulcer, aphrodisiac and hepatoprotective properties. Plant decoction is applied to ulcers, sore throats and itchiness. Galactogogue, antiperiodic, antiulcer and vulnerary are all uses the milky juice. When treating jaundice, the root is utilized traditionally. Fruit functions as an anthelmintic, expectorant, and bitter tonic. Fruit juice is administered to children as an astringent and is used in the treatment of leucoderma, gonorrhea, cough,

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and muscle soreness [11,12].

3.PHYTOCHEMICAL REVIEW

The phytochemical investigations of the leaves of *Oxystelma esculentum* showed the presence of alkaloids, terpenoids, glycosides, steroids, flavonoids, phenolics, tannins and saponins in the petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts. Strong phytochemicals like alkaloids, terpenoids, phenolics, saponins and flavonoids were present in the methanol extracts. Alkaloids and phenolics were present in the petroleum ether extracts, alkaloids, terpenoids and phenolics were present in the chloroform extracts, flavonoids, tannins, terpenoids and phenolics were present in the ethyl acetate extracts, and saponins and terpenoids were present in the acetone extracts [13].

All of the examined phytochemicals, excluding tannins were present in the various *Oxystelma esculentum* stem extracts. Strong alkaloids, terpenoids, glycosides, steroids, flavonoids, phenolics, and saponins were present in the methanol and acetone extracts. The petroleum ether extracts solely contained phenolics, whereas the chloroform extracts also contained alkaloids and terpenoids. The ethyl acetate extracts revealed flavonoids, glycosides, phenolics, and terpenoids [14].

Oxystelma esculentum's root contains alkaloids, flavonoids, phenolics, glycosides, saponins, terpenoids, and steroids, which were all detected in the various extracts of the plant. Strong amounts of terpenoids, flavonoids, phenolics, and saponins were present in the methanol extracts. Alkaloids, phenolics, and terpenoids were present in the ethyl acetate extracts; terpenoids and alkaloids were present in the chloroform extracts; and only flavonoids were present in the petroleum ether extracts. The methanol extracts of these plant's leaves shown more potent phytochemicals than the others among the various solvent extracts [15].

3.1. Antimicrobial Activities: The leaves, stem, and root of *Oxystelma esculentum* were extracted using petroleum ether, chloroform, ethyl acetate, acetone, and methanol, and their antibacterial and antifungal properties were tested against strains of both Gram-positive and Gram-negative bacteria and fungi. When compared to all the extracts among the tested strains, methanol extracts of leaves from *Oxystelma esculentum* showed the highest mean zone of inhibition (23.5±0.25 mm at 1000 µg/disc) against a strain of *Staphylococcus aureus* [16-18]. Methanol extracts of leaves from *Oxystelma esculentum* were shown to have the lowest minimum inhibitory concentration (15.62 µg/mL) and mininum bactericidal concentration (31.25 µg/mL) against *S. aureus*. The petroleum ether extract of the root of *Oxystelma esculentum* against *Escherichia coli* and the chloroform and acetone extracts of the root of *Oxystelma esculentum* against *Proteus vulgaris* showed the lowest zone of inhibition (7.0±0.50 mm at 250 µg/disc). In contrast, a methanol extract of the leaves of *Oxystelma esculentum* was shown to have the largest mean zone of inhibition (12.8±0.28 mm at 1000 µg/disc) when used to treat *Aspergillus flavus*. With ethyl acetate, acetone, and methanol extracts of leaves of *Oxystelma esculentum* against *Aspergillus flavus* and methanol extracts of leaves of *O. esculentum* against *Aspergillus niger*, the lowest values of MIC (250 µg/mL) and MFC (500 µg/mL) were found [16-20].

3.2. Antioxidant Activities: The highest DPPH activity was seen in methanol extracts of *O. esculentum*'s leaves, stem, and root, followed by ethyl acetate, acetone, petroleum ether, and chloroform. L-ascorbic acid, leaf, stem, and root methanol extracts were found to have IC50 values of 121, 232, 255, and 170

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µg/mL, respectively. The highest ABTS+ scavenging effects were demonstrated by methanol extracts of the leaves, stem, and root, followed by ethyl acetate, acetone, petroleum ether, and chloroform. 210, 230, 263 and 173 µg/mL, respectively, were the IC50 values of methanol extracts of leaf, stem, root, and buttylated hydroxyl toluene (BHT) acid [16-18].

The highest levels of superoxide anion activity were seen in leaf, stem, and root extracts of methanol. methanol extracts' IC50 values for the leaf, stem, and root were 286 µg/mL, 304 µg/mL, 326 µg/mL, and 262 µg/mL, respectively. The highest hydrogen peroxide activity was seen in the leaves, stem, and roots of plants extracted in methanol. Methanol extracts of the leaf, stem, root, and EDTA had IC50 values of 232, 378, 406, and 219 µg/mL, respectively.

Different extracts from different sections of *Oxystelma esculentum* were tested for their ability to scavenge hydroxyl radicals. The positive control used was vitamin C. Leaf, stem, and root extracts in methanol demonstrated the beneficial hydroxyl radical scavenging activity. It was discovered that the IC50 values for methanol extracts of leaf, stem, root, and vitamin C were 345, 434, 406, and 227 µg/mL, respectively [16-18].

The findings of ferric reducing antioxidant activity of petroleum ether, chloroform, ethyl acetate, acetone, and methanol extracts of various sections of *Oxystelma esculentum* and positive standard (Gallic acid) were assessed. The most active extracts were *O. esculentum* methanol extracts. The IC50 values of methanol extracts of leaf, stem, root, and gallic acid were 255, 360, 384, and 185 µg/mL, respectively. The methanol extract of leaves from *Oxystelma esculentum* showed the highest levels of flavonoids (6.38 ±0.50 mg/g extract) and total phenol (1.67±0.22 mg/g extract). However, chloroform extracts of the root have the lowest concentrations of phenols (0.02 mg/g extract) and flavonoids (0.41 mg/g extract)[19,20].

O. esculentum extracts in methanol showed the highest level of overall antioxidant activity, followed by extracts in ethyl acetate, acetone, petroleum ether, and chloroform. The IC50 values for this plant's leaf, stem, root, and L-ascorbic acid were 213, 300, 353, and 186 µg/mL, respectively.

4.DISCUSSION

One of the rare species, *Oxystelma esculentum* has cardenolides and pregnane glycosides, which are easily and cheaply extracted from this plant. These phytoconstituents can also serve as the building blocks for a variety of other therapeutically significant chemicals. Health conditions include cancer, hepatitis, stress-related diseases, urinary disorders, and bacterial infections have become important global issues as a result of the changing climate and lifestyle. Many of these disorders have been said to respond well to the treatment provided by this plant. A complete assessment, standardization, documenting, and patenting of this plant may be made possible by the current review. The human race could benefit greatly from thorough pharmacognostic, phytochemical, pre-clinical, clinical, and formulation-based study on this plant.

5.CONCLUSION

Given the current findings, it is possible to draw the conclusion that *Oxystelma esculentum* R.Br. contains a variety of active secondary metabolites, including alkaloids, anthraquinones, catechins, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, tannins, and terpenoids. The methanol and

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chloroform extracts demonstrated the greatest concentration of chemicals among the four distinct extracts. Hexane and benzene extracts both revealed the existence of four compounds in addition to the presence of seven compounds in methanol and chloroform extracts. With the help of this report, active secondary metabolites may be isolated and evaluated for their bioactivity and efficacy.

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Section A: Greeen Chemistry

Research Article

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Identification and comparison of biomolecules in medicinal plant *Oxystelma esculentum* R. Br. by using FTIR

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Abstract: The purpose of this work is to use the concept of metabolic fingerprinting through the use of Fourier Transform Infrared technology to comprehend the composition, chemical structure and discrimination of biomolecules in medicinal plant *Oxystelma esculentum* R. Br. To distinguish and identify specific functional groups found in this medicinal plant belonging to the family Asclepiadaceae, IR spectra in the mid-infrared region (4000-400 cm^{-1}) was utilised. The presence of O-H, C=O, C-H, C=C, S=O, C-CL were noted. Alkanes, alkenes, alcohols, esters, sulfoxides, Amide-I, Halo compounds are all a result of these bonding structures. A sensitive and useful test for the identification and comparison of biomolecules in medicinal plant was utilised in the current investigation and it was called FTIR Spectroscopy. The characteristic peak values and their functional groups were found using the FTIR technique on a spectrophotometer instrument. The present study's findings led to the creation of an FTIR spectrum profile for the medicinally significant plants of *Oxystelma esculentum* R. Br.

Key words: FTIR, biomolecules, *Oxystelma esculentum* R. Br., Asclepiadaceae.

1.INTRODUCTION

Since most medicinal plants have fewer negative effects than synthetic medications, they are employed as alternative treatments for many human and animal ailments. Identification of the chemical makeup of the phytochemicals found in medicinal plants will reveal some details about the many functional groups in charge of their therapeutic effects.

Iqbal Ahamad *et al.* [1] used infrared spectroscopy to identify significant categories of chemicals as the most active fraction of extracts from four plants. The bioactive group of compounds in the dry leaf powder of *Calotropis gigantea* were tested by FTIR analysis by Ramamoorthi and Kannan[2].Using FTIR spectroscopy, Kareru *et al.*[3] found saponins in the crude dry powder of 11 different plants. *Eclipta alba* and *Eclipta prostrata* leaf, stem, and root powder samples were subjected to FTIR spectroscopic investigation by Muruganantham *et al.* [4]

Gauravkumar *et al.* [5] performed an FTIR analysis on the aqueous methanolic leaf extracts of *Bauhinia racemosa* to look for phytochemical components (2010). Using a spectroscopic approach, Ragavendran *et al.* [6] identified the functional groups in several *Aerva lanata* samples. Using FTIR spectroscopic technique, Thangarajan Starlin *et al.* [7] identified the elements and functional groups in the ethanol extract of the entire *Ichnocarpus frutescens* plant. *Ampelocissus latifolia* leaf extract was subjected to an FTIR spectroscopic examination by Paraj A. Ashok kumar *et al.* [8] done Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. FT-IR analysis of methanol fraction of leaves of *Oxystelma esculentum* was carried out by Savitha *et al.* [9].

The functional groups of the fruits and flowers of *Oxystelma esculentum* R. Br. have not yet been analysed using FTIR, according to a review of the literature. In order to better understand the functional groups of phytoactive chemicals found in the fruit and floral extract in methanol of this medicinal plant, an analysis of their functional groups is attempted in the current work.

2.MATERIALS AND METHODS

2.1 Collection of plant: Fruit and flower parts of the medicinal plant *Oxystelma esculentum* R. Br. (Family: Asclepiadaceae) were collected from Babji nagar, near Shivpuri bypass, Baran district, India. Identification of the plant species was done with the help of Dr. K. M. Meena, Professor of Botany, Govt. College, Baran (Raj.).These cleaned plant parts were shade dried and placed in polythene bags. The Herbaria of these plants were kept in the Department of Botany, JDB Govt Girls College Kota, (Rajasthan). The plant parts were shade dried at room temperature in a clean environment to avoid contamination for 14 days and powdered in a domestic grinder. The powdered samples were stored in air tight glass bottles at room temperature for further analysis.

2.2 Preparation of extracts: A mechanical grinder was used to powder the sun-dried fruits and flowers of the plant (20°C). Each powder was weighed at 20 grammes, 150 cc of solvent was added, and storage was allowed for three days. Whatman No. 1 filter paper was used to filter the extract, and the filtrate was then collected. Supernatants were obtained after the residue had been extracted twice more (with a three-day gap between each extraction). The supernatants were collected and evaporated until the volume was decreased to 150 ml (at room temperature, 28±1°C). For subsequent study, extracts of each plant powder were made using the solvent methanol (ME) and stored in airtight vials.

2.3. Fourier Transform Infrared Spectrophotometer (FTIR): Perhaps the most effective tool for determining the kinds of chemical bonds (functional groups) present in compounds is the Fourier

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Transform Infrared Spectrophotometer (FTIR). As can be seen in the annotated spectrum, the wavelength of light absorbed is indicative of the chemical bond. The chemical bonds in a molecule can be identified by analysing the infrared absorption spectra. FTIR analysis was conducted using dried powdered methanol solvent extracts of each plant material. To create translucent sample discs, 100 mg of KBr pellet and 10 mg of the dried extract powder were combined. Each plant specimen's powdered sample was placed into a Shimadzu IR Affinity 1 FTIR spectroscope, which has a scan range of 400 to 4000 cm^{-1} and a resolution of cm^{-1}.

3.Results and Discussion

The FTIR spectrum of fruit and flower extracts (prepared in methanol solvent) of *Oxystelma esculentum* R. Br. are given in **Fig 1 & 2**. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the leaf and flower extracts (prepared in methanol solvent) of *Oxystelma esculentum* R. Br. are presented in **Tables 1 & 2**.

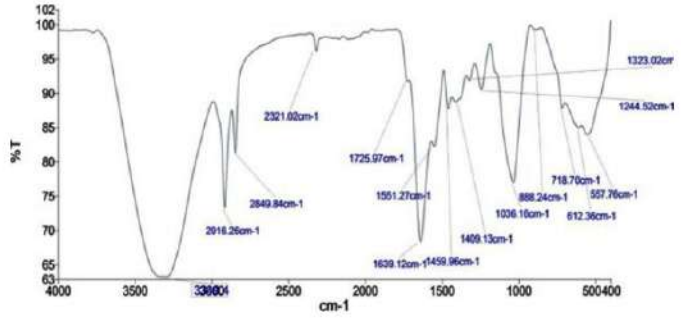

Fig.1: FTIR spectrum of ME extract of fruit of *Oxystelma esculentum* R. Br

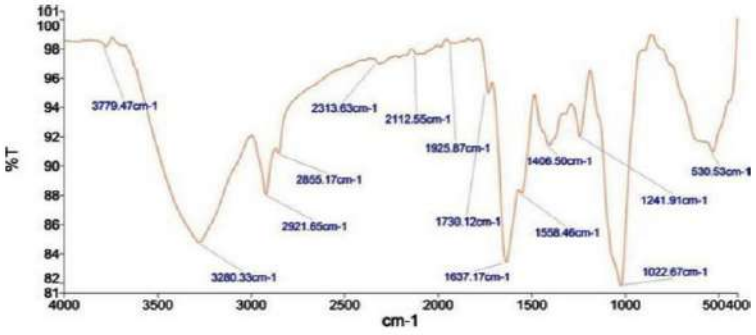

Fig.2: FTIR spectrum of ME extract of flower of *Oxystelma esculentum* R. Br

3.1. **FTIR spectral data interpretation**

3.1.1. Methanol (ME) extract of fruits: Chromatogram FTIR analysis of the methanol extract of

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fruits of *Oxystelma esculentum* R. Br **(Table 1)** showed the presence of sixteen major peaks and the components corresponding to the peaks were determined as follows. The FTIR analysis of *Oxystelma esculentum* R. Br fruits proved the presence of Alcohols, Alkanes, Esters,Amide I & II, Aromatic compounds, Phenol, Phosphate-I, Sulfoxide, Alkenes and Alkyl halides which shows major peaks at 3349, 2916, 2849, 2321, 1725, 1639, 1551, 1459, 1409, 1323, 1244, 1036, 888, 718, 612, 557 cm^{-1}

Table1: FTIR spectral peak values and functional groups obtained for the fruit extractin methanol solvent of *Oxystelma esculentum* R. Br.

S. No.	Peak (cm^{-1})	Intensity	Bond	Type of Vibration	Functional group assignment	Group freq. (cm^{-1})
1.	3349	Strong & Broad	O–H	Stretching	Alcohols	3550 – 3200
2.	2916	Strong	C-H	Stretching	Alkanes	2990 – 2850
3.	2849	Medium	C-H	Stretching	Alkanes	2990 – 2850
4.	2321	Weak	C-H	Stretching	Alkanes	2990 – 2850
5.	1725	Weak	C=O	Stretching	Esters	1730-1715
6.	1639	Strong	C=O	Stretching	Amide I	1700-1630
7.	1551	Weak	N-H	Stretching	Amide II	1640-1550
8.	1459	Medium	C=C	Stretching	Aromatic	1625 – 1440
9.	1409	Weak	C=C	Stretching	Aromatic	1625 – 1440
10.	1323	Weak	O-H	bending	phenol	1390-1310
11.	1244	Medium	PO^{-2}	Stretching	phosphate I	1245-1244
12.	1036	Strong & Sharp	S=O	Stretching	sulfoxide	1070-1030
13.	888	Weak	=C–H	Bending	Alkenes	1000-650
14.	718	Weak	=C–H	Bending	Alkenes	1000-650
15.	612	Medium	C-Cl	Stretching	Alkyl halides	< 600 – 840
16.	557	Strong & Broad	C-I	Stretching	Alkyl halides	< 600

3.1.2. Methanol (ME) extract of flowers: Chromatogram FTIR analysis of the methanol extract of flowers of *Oxystelma esculentum* R. Br **(Table 2)** showed the presence of fourteen major peaks and the components corresponding to the peaks were determined as follows. The FTIR analysis of *Oxystelma esculentum* R. Br flowers proved the presence of Alcohols, Alkanes, Alkynes, Allene, Esters, Amide I & II, Aromatic compounds, Phosphate-I, Sulfoxide and Alkyl halides which shows major peaks at 3779, 3280, 2921, 2855, 2313, 2112, 1925, 1730, 1637, 1558, 1406, 1241, 1022, 530 cm^{-1}

Table2: FTIR spectral peak values and functional groups obtained for the flower extractin methanol solvent of *Oxystelma esculentum* R. Br.

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S. No.	Peak (cm^{-1})	Intensity	Bond	Type of Vibration	Functional group assignment	Group freq. (cm^{-1})
1.	3779	Weak	O-H	Stretching	Alcohols	3790-3584
2.	3280	Strong & Broad	O-H	Stretching	Alcohols	3550 – 3200
3.	2921	Medium	C-H	Stretching	Alkanes	2990 – 2850
4.	2855	Weak	C-H	Stretching	Alkanes	2990 – 2850
5.	2313	Weak	C-H	Stretching	Alkanes	2990 – 2850
6.	2112	Weak	C=C	Stretching	Alkynes	2250 – 2100
7.	1925	Weak	C=C=C	Stretching	Allene	2000-1900
8.	1730	Weak	C=O	Stretching	Esters	1730-1715
9.	1637	Strong & Sharp	C=O	Stretching	Amide	1700 – 1630
10.	1558	Weak	N-H	Stretching	Amide II	1640-1550
11.	1406	Medium	C=C	Stretching	Aromatic	1625 – 1440
12.	1241	Medium	PO^{-2}	Stretching	phosphate I	1245-1244
13.	1022	Strong & Sharp	S=O	Stretching	sulfoxide	1070-1030
14.	530	Strong & Broad	C-I	Stretching	Alkyl halidess	< 600

4.CONCLUSIONS

The findings mentioned above lead to the conclusion that Oxystelma esculentum R. Br. has high concentrations of alcohols, alkanes, amides I and II, phosphate-I, sulfoxide, and alkyl halides. The current research shows that different regions of the plants have varied concentrations of biomolecules. Further study in the biological activity of medicinal plants and the analysis of their phytochemicals is possible thanks to this effort. The biomolecular makeup of cells can be detected using Fourier transform infrared spectroscopy, which has been shown to be a trustworthy and sensitive technique. In the current work, we looked at the potential of FTIR spectroscopy for quick and simple differentiation between different functional groups that are in charge of medicinal characteristics.

With fewer side effects, herbal medications have been shown to be just as effective as synthetic ones. An effective method for analysing herbs and deciphering the structures of the chemicals, as well as for the quantitative examination of medications, is infrared spectroscopy.

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He/She presented a paper entitled Renewable Energy

Dr. R.C. Agrawal	Dr. Maha Singh	Dr. S.S. Dhayal	Dr. Prenita Gupta	Mr. Vivek Singh Maharia
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