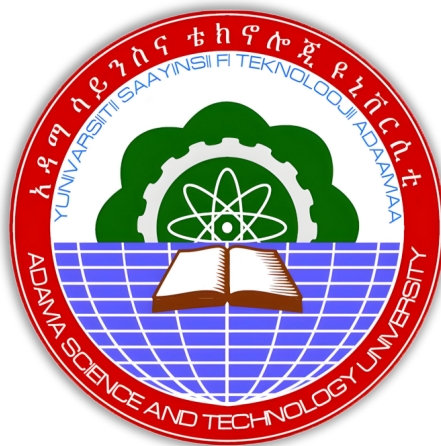


**Phytochemical Analysis, Antimicrobial, and Antioxidant
Activity Evaluation of *Datura stramonium* L. Leaf Extract
Against Selected Human Pathogens**



Moges Hidoto

**A Thesis Submitted to
The Department of Applied Biology
College of Applied Natural Science**

**Presented in Partial Fulfillment of the Requirement for the
Degree of Master of Science in Applied Biology (Applied
Microbiology)**

**School of Post-Graduate Studies
Adama Science and Technology University**

**Jun 2025
Adama, Ethiopia**

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**Advisor(s): Seid Mohammed (Ph.D.) Assoc. Prof.
Aman Dekebo (Ph.D.) Prof.**

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DECLARATION

I hereby declare that this Master Thesis entitled “**Phytochemical Analysis, Antimicrobial, and Antioxidant Activity Evaluation of *Datura stramonium* L. Leaf Extract Against Selected Human Pathogens**” is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Name of student

Signature

Date

RECOMMENDATION

We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled “**Phytochemical Analysis, Antimicrobial, and Antioxidant Activity Evaluation of *Datura stramonium* L. Leaf Extract Against Selected Human Pathogens**” prepared under our guidance by **Moges Hidoto**, submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Microbiology. Therefore, we recommend the submission of a revised version of the thesis to the department following the applicable procedures.

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Department Head

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Date

College Dean

Signature

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Graduate School Dean

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LIST OF ACRONYMS AND ABBREVIATIONS

AMR	Antimicrobial Resistance
ATCC	American-Type Culture Collections
BDM	Broth Dilution Method
CFU	Colony Forming Units
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EO	Essential Oil
GC	Gas Chromatography
HDT	Hydro-Distillation Techniques
IR	Infrared
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MHA	Mueller Hinton Agar
MHB	Mueller-Hinton Broth
MIC	Minimum Inhibitory Concentration
PDA	Potato Dexterosus Agar
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SLET	Solid Liquid Extraction Technique
TLC	Thin-Layer Chromatography
TMP	Traditional Medicinal Plants

ABSTRACT

*The alarming rise in newly emerging and re-emerging infectious diseases, coupled with the increasing prevalence of antimicrobial resistance underscores the urgent need to explore novel therapeutic agents derived from medicinal plants. The recent study aimed to investigate the antimicrobial, antioxidant activity and phytochemical constituents of *Datura stramonium* L. leaf extracts. The plant samples were collected, and leaves were dried in the shade and powdered. The powdered leaves were then macerated using petroleum ether, ethyl acetate, and methanol successively. Phytochemical screening was carried out using the standard procedures. The antimicrobial activity was carried out using paperdisc diffusion while antioxidant activities were determined using DPPH assay all tests were triplicates and data were analyzed using SPSS v23. The extraction process yielded 1.34, 1.75 and 7.51 percentage yield for pet. ether, ethyl acetate and methanol extracts respectively. The phytochemical analysis revealed that ethyl acetate extract was positive for all tested phytochemicals while steroids were missing in the methanol extract and flavonoids and phenols were absent from the petroleum extract. The GC-MS analysis leads to the identification of 25 compounds of which (2S,3S)-2-Methyl-3-(2-methyl-allyl)-cyclopentanone, 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-, 2-Hexenal and (3E)-3-Hexen-1-ol account for 60% of the total composition. The antioxidant activity revealed that the essential oil has the highest antioxidant activity with an IC_{50} value of 3.34 followed by the ethyl acetate and methanol extract with an IC_{50} value of 6.17 and 10.25 respectively. In the antimicrobial activity all extracts showed dose dependent activities. the pet. ether extract showed the highest activity on *S. aureus* (24.0 ± 0.0) while the lowest activity was observed on *E. faecium* (19.0 ± 0.0) at 200 mg/ml while the second highest activity was observed from the ethyl acetate extract against *P. aeruginosa* with an inhibition value of 22.5 ± 0.5 in mm. while the highest minimum inhibitory and bactericidal activity was observed from the essential oil with an MIC and MBC values of 3.125 and 6.25 respectively against *S. aureus* and *E. faecium*. Generally this study tried to demonstrate the medicinal potential of leaves of *D. stramonium* through the different activities that were carried out and the results reported although further activities were recommended to assess the full potential of the plant.*

Keywords: Antimicrobial activity, Crude extract, *Datura stramonium* L., Phytochemical screening

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Medicinal plants have been the primary source of medications in traditional medicine, as they are accessible, affordable, and reliable remedies due to their low toxicity and diverse pharmacological properties (Castronovo et al., 2021; Yetgin, 2024). Among these, *Datura stramonium* L., commonly known as Jimson weed, a member of the Solanaceae family, has a widespread use in traditional medicine for many years. This herbaceous plant, characterized by its height of 60-120 cm, branched structure, and pubescent leaf measuring 8-17 x 4-13 cm, thrives as a cosmopolitan weed in disturbed areas, waste grounds, fertile fields, and roadsides at altitudes of 600-2800 m (Mohammed et al., 2021). *D. stramonium* L. was originally from Tropical North America while now it is prevalent across Ethiopian regions, Eritrea, Sudan, Somalia, and various parts of tropical Africa, Europe, and Asia.

The pharmacological potential of *D. stramonium* L. is attributed to its rich phytochemical profile, which includes saponins, tannins, carbohydrates, proteins, steroids, flavonoids, alkaloids, phenols, and glycosides (Soni et al., 2012). Notably, the plant's leaves are a reservoir of secondary metabolites such as glycosides, phenols, lignins, saponins, sterols, and tannins, contributing to its analgesic, anti-asthmatic, and antimicrobial properties. The seeds further contain amino acids like alanine, glutamate, tyrosine, and phenylalanine, alongside tropane alkaloids, with hyoscine (scopolamine), hyoscyamine, and atropine being predominant (Myint et al., 2022; Sharma et al., 2021). These compounds underpin the plant's therapeutic applications, particularly in combating microbial infections and oxidative stress.

The global rise in antimicrobial resistance (AMR) poses a significant challenge to modern healthcare systems, driven by the genetic adaptability of bacteria to develop resistance against conventional antibiotics (Chinemerem Nwobodo et al., 2022; Uddin et al., 2021). This phenomenon, exacerbated by the emergence of multi-resistant bacterial strains, threatens the efficacy of surgical procedures, immunosuppressive treatments, and infection control measures, leading to increased morbidity and mortality. As resistance continues to evolve, there is an urgent need for alternative antimicrobial agents. Medicinal plants like *D.*

stramonium L. offer a promising avenue due to their bioactive compounds, which exhibit antibacterial, antifungal, antioxidant, and anti-infectious activities (Castronovo et al., 2021).

Despite the documented pharmacological potential of *D. stramonium* L., comprehensive studies on the phytochemical composition and antimicrobial efficacy of its leaf crude extracts remain limited, particularly in Ethiopia. Therefore, in order to evaluate the plant's bioactive constituents and their activity against human pathogens, this study aims to investigate the phytochemical profile, antibacterial, antifungal, and antioxidant activities of *D. stramonium* L. leaf extracts,

1.2. Statement of the problem

Ethiopia boasts a rich tradition of utilizing traditional medicine, deeply embedded in its diverse multi-ethnic, cultural, and linguistic landscape. However, the scarcity of comprehensive research on traditional medicinal plants (TMPs) has hindered their systematic integration into modern healthcare systems (Fentahun et al., 2017). Traditional medicine remains a widely accepted alternative for healthcare in Ethiopia, particularly in addressing the growing burden of infectious diseases. The alarming rise in newly emerging and re-emerging infectious diseases, coupled with the increasing prevalence of antimicrobial resistance (AMR), underscores the urgent need to explore novel therapeutic agents derived from medicinal plants (Batoool et al., 2020). Despite the development of numerous antibiotics by pharmaceutical industries over the past three decades, the rapid evolution of microbial resistance to these drugs poses a significant global health challenge, rendering conventional treatments less effective and exacerbating morbidity and mortality.

Among Ethiopia's traditional medicinal plants *D. stramonium* L. has been used for centuries in folklore medicine to treat various ailments. Despite its historical significance and reported pharmacological potential, *D. stramonium* L. remains underutilized in modern medical applications in Ethiopia due to a lack of scientific investigation into its phytochemical composition and therapeutic efficacy. The absence of detailed studies on its antimicrobial and antioxidant properties limits its recognition as a viable alternative to combat resistant pathogens. This study seeks to address this gap by investigating the phytochemical profile, antimicrobial, and antioxidant activities of *D. stramonium* L., thereby contributing to the development of effective, plant-based therapeutic agents.

1.3. Objective of study

1.3.1. General objective

- ❖ To analyze the chemical composition of the extracts of *D. stramonium* L. leaves part extract and evaluate their antimicrobial, and antioxidant activities against selected human pathogenic microorganisms.

1.3.2. Specific objectives

- ❖ To evaluate phytochemicals present in the leaves of *D. stramonium* L.
- ❖ To extract the essential oils of the leaves of *D. stramonium* L. using hydro-distillation techniques (HDT)
- ❖ To analyze the chemical composition of essential oils using GC-MS.
- ❖ To determine the antioxidant, antibacterial, and antifungal activities of *D. stramonium* L. essential oils and crude extracts against selected human pathogens.
- ❖ To determine the minimum inhibitory concentration (MIC), minimum bacteriocidal concentration (MBC), and minimum fungicidal concentration (MFC),

1.4. Scope of the study

The sampling area for this study was the Adama Science and Technology University. This research mainly focuses on the phytochemical investigation, and evaluation of the antimicrobial, and antifungal activity of *D. stramonium* L. leaf on selected human pathogens *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231 using disc diffusion assay and also its antioxidant activity using DPPH assay.

1.5. Significance of the study

The significance of this study is the exploration of the phytochemical composition, antioxidant, and antimicrobial properties of *D. stramonium* L., a plant indigenous to Ethiopia with a long-standing reputation for its medicinal potential. As a member of the Solanaceae family *D. stramonium* L. has been historically utilized in traditional medicine, making it imperative to scientifically validate its therapeutic applications. By conducting a phytochemical analysis, this research showed the bioactive phytochemicals present in *D.*

stramonium L. This analysis is crucial as it provides insights into the plant's therapeutic potential, which could pave the way for developing pharmaceutical or natural treatments derived from these bioactive substances. Previous studies have indicated that *D. stramonium* contains a variety of pharmacologically active compounds, including tropane alkaloids known for their anticholinergic properties, such as atropine and scopolamine, which have been linked to various health benefits and therapeutic uses (Batool et al., 2020).

Furthermore, this study established baseline information that is essential for future pharmacological research. By providing a systematic evaluation of the phytochemical constituents and their associated biological activities, this research served as a valuable resource for researchers interested in further investigations into the medicinal properties of *D. stramonium*. Such foundational work is critical in fostering greater understanding and utilization of traditional medicinal plants, particularly in combating contemporary health challenges like antibiotic resistance and emerging infectious diseases. Generally, the findings from this study enhances our understanding of *D. stramonium's* medicinal value but also contribute significantly to the broader field of ethnopharmacology by encouraging further research into traditional medicinal plants that hold promise for modern therapeutic applications.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Medicinal plants

For thousands of years, people have utilized medicinal plants and herbs to obtain a wide range of physiologically active substances. Generations of indigenous practitioners have employed the plant's crude material or its pure components to treat a wide range of diseases. These plants include a variety of bioactive components, such as phenolic compounds, terpenoids, and alkaloids, that have medicinal properties (Agidew, 2022; Stéphane et al., 2022). Some of the chemical constituents of medicinal plants that have a recognizable physiological effect on people are tannins, saponins, flavonoids, terpenoids, phenolic compounds, steroids, alkaloids, and carbonyl compounds. These compounds are mostly, or more precisely, products of secondary metabolism in living things. They are widely used in human therapy as well as veterinary, agricultural, and academic research. Several phytochemicals belonging to a variety of chemical classes have in vitro inhibitory effects on all microorganism kinds (Yadav & Agarwala, 2011). Medicinal herbs hold significant importance for Ethiopians and residents of other developing countries in Asia and Africa. These individuals highly value the use of medicinal plants, which serve as alternative or complementary remedies to compensate for the often-limited access to modern medical treatments. Moreover, the utilization of medicinal plants contributes to the enhancement of overall health and safety within the local communities. These plants play a crucial role in the daily lives of people and establish profound connections with diverse populations (Agidew, 2022).

The presence of phytochemical components such as tannins, phenolic compounds, flavonoids, caffeoylquinic acid, and terpenoids is closely associated with the antibacterial properties of plant extracts. Several plant extracts have been scientifically confirmed to possess antimicrobial characteristics and serve as antioxidants and preservatives. These antimicrobial agents have played significant roles in the domains of food and health products, primarily due to their non-toxic nature and high safety profiles. Likewise, the *Datura* plant primarily has phytochemicals including flavonoids, alkaloids, phenolic compounds, tannins, and cardiac glycosides and the seeds have also yielded a variety of

amino acids, such as glutamate, tyrosine, phenylalanine, and alanine. In the *Datura* species, tropane alkaloids are particularly prevalent. With differing amounts of indifferent plant sections, hyoscine [(-)-Scopolamine] is the primary tropane alkaloid, along with hyoscyamine and atropine (Myint et al., 2022; Sharma et al., 2021).

2.2. Botanical description of *D. stramonium* L.

2.2.1. Plant appearance

D. stramonium L. (Figure 1A) is a large and coarse shrub of about 3 to 4 feet in height. On rich soil, it may even reach a height of 6 feet. The root is large, and whitish in color, with a taproot system giving off many fibers. The stem is green or purple, hairless, cylindrical, erect and leafy, smooth, and branching repeatedly in a forked manner. leaves and a single, erect flower (Figure 1B) arise through the forks of the branches. The alternate leaves are ovate or ovate cordate in outline, but pinnately lobed. These lobes are somewhat shallow and point at their tips; there are usually 2 to 3 of these lobes on each side of the leaf blade. leaves are cauline and ramal, exstipulate, up to 8 cm long and 6 cm across, petiolate, simple, dissected, acute, glabrous, unicosted, and arranged in reticular venation.

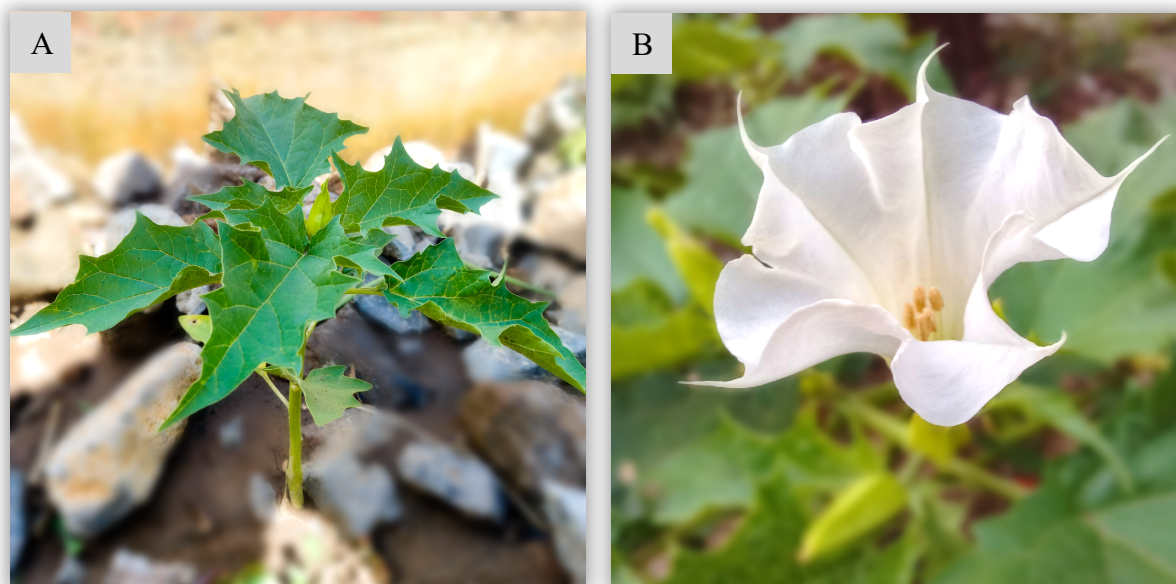


Figure 1: *D. stramonium* L. (Picture by Moges Hidoto, Adama Science and Technology University, Ethiopia, 2024). (A) areal part of the plant, (B) flower and reproductive part of the plant.

The upper surface is dark and grayish green, generally smooth, whereas the underside is paler, and when dry, minutely wrinkled. leaves, when bruised, exude a rank, heavy, and somewhat nauseating narcotic odor. The flowers are ebracteate, ebracteolate, pedicellate, actinomorphic, bisexual, complete, regular, and pentamerous, except fourthwhorland are hypogynous. They are sweets centered and can produce a stupor if breathed for a prolonged period. Each flower is replaced by a hard fruit that is dry and spiny, and spheroid-ovoid in shape. Underneath, each fruit is a truncated remnant of the calyx that curves sharply down. These fruits are initially green but become brown with maturity; they divide into four segments to release the seeds. The seeds are dull, irregular, and dark-colored; their surface may be pitted or slightly reticulated (Preissel & Preissel, 2002; Sanjita Das et al., 2012).

2.2.2. Geographical Distributions of *D. stramonium* L.

D. stramonium L. probably originated in Caspian Sea territories and spread to Europe in the first century. At present, it grows in waste places in Europe, Asia, America, and South Africa. *D. stramonium* L. is cultivated in Germany, France, Hungary, and South America and throughout the world. It is very common, especially in temperate climates. It spreads naturally in hot and temperate regions of the world, along roadsides, and in manure-rich animal shelters. It is found as a weed in dumpsites and wasteland in Europe (Mohammed et al., 2021). It occurs in most Ethiopian regions, and also in Eritrea, Sudan, Somalia, and throughout tropical Africa, Europe, and parts of Asia (Girmay, 2015). In Ethiopia, there are nearly 1000 medicinal plants that constitute about 10% of the entire flora available in the state (Abraham Bekele et al., 2015). For several years, the bulk of these plant materials have been employed by the local community as an alternative medicine to treat many diseases, even though most of them are not well Characterized scientifically (Baynesagne et al., 2017).

2.2.3. Habitat of *D. stramonium* L.

It is an annual upright herb of the family Solanaceae that is widely distributed in temperate and tropical regions. The whole *D. stramonium* L. plant contains alkaloids, which can cause poisoning when ingested by humans and livestock, especially the fruit and seeds. *D. stramonium* L. is larger in size and has a strong growth capacity; in particular, it has a considerable allelopathic inhibition effect on the growth of coexisting plants (Shobha Sondhia & Swain, 2002). As a malignant and harmful weed, *D. stramonium* L. mainly invades farmland and wasteland, has strong environmental adaptability and potential for

invasion, and is resistant to drought, soil infertility, and pollution (Trueman et al., 2010). *D. stramonium* L. is an invasive species whose range has expanded greatly in recent years in Romania (Niculescu, 2022) and studies have shown that, in 2024, the invaded area of *D. stramonium* L. may reach 130100 square kilometers in Liaoning Province, China (Wang et al., 2023). In addition to harming the biodiversity of the local ecosystem, it can also spread diseases and pests and pose a threat to the agricultural production, natural ecosystem, and human living environment of the invasion site. At present, studies on *D. stramonium* L. have mainly focused on its accumulation of heavy metals, allelopathy, and chemical compounds, but the species has only been mentioned in a very small number of regional invasive plant investigations (Ogunmoyole et al., 2019).

2.2.4. Ethno medicinal uses

Plant-derived drugs come into use in modern medicine through the use of plant material as indigenous cures in folklore or traditional systems of medicine. The leaves of *D. stramonium* are used for the relief of headaches and vapors of leaf infusion are used to relieve the pain of rheumatism and gout. The smoke from the burning leaf is inhaled for the relief of asthma and bronchitis. European remedy of *D. stramonium* for hemorrhoid is to steam the part over boiling water containing leaf. The fruit juice is applied to the scalp for the treatment of falling hair and dandruff. It is also applied to smooth painful wounds and sores. Seeds and leaves of *D. stramonium* L. are used to sedate hysterical and psychotic patients, and also to treat insomnia (Pandey et al., 2011). The growing plant works as an insect repellent, which protects neighboring plants from insects (Batool et al., 2020).

2.3. Phytochemistry

Phytochemical studies of *D. stramonium* L. have been conducted since the early 1930s. The major phytochemicals isolated from *D. stramonium* L. are tropane alkaloids, atropine, and scopolamine (Jakabova et al., 2012). The major phytochemicals isolated from *D. stramonium* are tropane alkaloids, atropine, and scopolamine. It is reported that the whole plant contains 0.26% alkaloids. The main alkaloids are hyoscyamine and hyoscyne (scopolamine). It also contains protein albumin and atropine. Atropine is formed from hyoscyamine by racemization. These alkaloids are usually present in the proportion of about two parts of hyoscyamine to one part of hyoscyne, but in young plants, hyoscyne is the predominant alkaloid. Ditiglyol esters of 3,6 dihydroxytropane and 3,6,7- trihydroxytropane

have been isolated from the roots in addition to hyoscyne, hyoscyamine, tropine, and pseudotropine. It also contains 6-hydroxy hyoscyamine, skimmianine, metelodine, acetyl derivatives of caffeic, p-coumaric, and ferulic acid, stigma sterol, campesterol, withanolide I, steroidal glycosides, daturataturins A and B, flavonoids, chrysin, quercetin, and their esters. With astramonolide and coumarins (umbelliferone and scopolin) are also present in the plant (Jagruoti and Bhalerao, 2018). The distribution of hyoscyamine and scopolamine in *D. stramonium* L. was studied. The production of hyoscyamine and scopolamine in *D. stramonium* L. has been investigated in the different plant parts, at different stages of their life cycle. The maximum contents were found in the stems and leaves of young plants, hyoscyamine being always the predominant component. These compounds were included in many pharmacopoeias because of their anticholinergic activities (Shagal et al., 2012). Protein and ash are more highly distributed in the seed coat than in the seed. In contrast, the fat, carbohydrate, and fiber contents of the seed were higher than the seed coat. The seeds also contained higher concentrations of phytate, tannin, and oxalate than the seed coat. In the seed coat calcium, iron, potassium, sodium, and phosphorus were higher than the seeds. Leaves extract contains different types of secondary metabolites such as glycosides, phenol, lignins, saponins, sterols, and tannins (Nain et al., 2013). The primary biologically active substances in *D. stramonium* L. are the alkaloids atropine and scopolamine (Ally & Mohanlall, 2020; Sisay et al., 2019).

2.4. Pharmacological Activity

2.4.1. Antiasthmatic Activity

The leaves of *D. stramonium* L. were also used as a sedative for psychotic patients and the treatment of insomnia. The liquid obtained from the flower of *D. stramonium* L. has been previously used in the treatment of an earache and was also noted for containing analgesic and anti-asthmatic activities (Dwivedi et al., 2019). Exposure of the fetus to *D. stramonium* L. when a mother uses it for asthma, will cause a continuous release of acetylcholine, resulting in the desensitization of nicotinic receptors, this could ultimately result in permanent damage to the fetus (Dwivedi et al., 2019). Atropine and scopolamine, tropane alkaloids in *Datura stramonium*, act as muscarinic receptor antagonists, blocking M2 receptors on airway smooth muscle and submucosal gland cells. This inhibits acetylcholine-mediated bronchoconstriction, dilating bronchial smooth muscle and relieving asthma symptoms (Pretorius & Marx, 2006).

2.4.2. Anticholinergic Activity

The alkaloids found in *D. stramonium*, are organic esters used clinically as anticholinergic agents. Jimson weed has been reported as a drug of abuse and has been involved in the accidental poisoning of humans and animals. Symptoms of acute Jimson weed poisoning included dryness of the mouth and extreme thirst, dryness of the skin, pupil dilation and impaired vision, urinary retention, rapid heartbeat, confusion, restlessness, hallucinations, and loss of consciousness. The anticholinergic syndrome results from the inhibition of central and peripheral muscarinic neurotransmission (Diker et al., 2007; Taha & Mahdi, 1984). Tropane alkaloids (atropine, hyoscyamine, scopolamine) competitively inhibit muscarinic acetylcholine receptors (M1-M5), preventing parasympathetic nerve impulses. This leads to reduced smooth muscle tone, decreased secretions, and pupil dilation, effective for conditions like Parkinson's and respiratory disorders (Pretorius & Marx, 2006).

2.4.3. Anticancer Activity

An integrated approach is needed to manage cancer using the growing body of knowledge gained through scientific developments. Thousands of herbal and traditional compounds are being screened worldwide to validate their use as anticancerous drugs. *D. stramonium* L. in a therapeutic dose of 0.05-0.10 g was used to cure cancer. An investigation by (Rajeshkanna et al., 2016) on the ethyl acetate flower extracts of *D. stramonium* L. for its anticancer activity against human liver cancer cells (HepG2) using the MTT assay exhibited anticancer activity comparable to that of the standard, Gemcitabine with a CTC50 value of 131.53 $\mu\text{g.mL}^{-1}$ (Rajeshkanna et al., 2016). Ethyl acetate leaf extracts and withanolides (e.g., 12 α -hydroxydaturametelin B, daturametelin B) exhibit cytotoxic effects, inducing cell-cycle arrest at the sub-G1 phase and apoptosis in cancer cells (e.g., gastric, leukemia, lung). They enhance immune-stimulatory activity, increasing IL-2 and IFN- γ secretion in lymphocytes (Nasr et al., 2022).

2.4.4. Anti-inflammatory activity

Coriandrum sativum (C. sativum), *D. stramonium* L., and *Azadirachta indica* (A. indica) are traditionally used in the treatment of inflammation. Ethanolic extracts of fruits of *C. sativum*, leaves of *D. stramonium* L. and *A. indica* was subjected to preliminary screening for anti-inflammatory activity in albino rats. All ethanolic extracts exhibited significant anti-inflammatory activity comparable to the standard drug diclofenac sodium against the

carrageenan-induced rat paw edema method. Among these plants, *A. indica* showed maximum anti-inflammatory activity per hour (Gupta Sonika et al., 2010). Phenolic and flavonoid compounds scavenge reactive oxygen species, reducing oxidative stress. Extracts inhibit nitric oxide production in activated macrophages and modulate pro-inflammatory cytokines (e.g., TNF- α , IL-6) via molecular docking, alleviating inflammation in hepatic injury models (Nasr et al., 2022).

2.4.5. Larvicide and mosquito repellent activities

Ethanollic extracts of leaves of *D. stramonium* L. were evaluated for larvicidal and mosquito-repellent activities against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The LD₅₀ values for larvicidal activity were found to be 86.25, 16.07, and 6.25 mg/L against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* respectively (Batoool et al., 2020). The ethanollic leaves extract of *D. stramonium* L. provided complete protection time (mosquito repellence) of 2.7, 71.7, and 117.7 min against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* at higher concentration (1%) (Swathi et al., 2012). *D. stramonium* exhibits significant larvicidal effects against *Tribolium castaneum* by reducing the growth rate and food consumption (Kamal et al., 2016). The ethanollic extracts of *D. stramonium* exhibit significant larvicidal and mosquito-repellent activities against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Batoool et al., 2020). Ethanollic leaf extracts disrupt mosquito larval enzymes through dephosphorylation by purified protein phosphatases (e.g., 30 kDa protein), causing mortality. Alkaloids and other metabolites deter oviposition and repel mosquitoes (e.g., *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*) (Swathi et al., 2012).

2.4.6. Antibacterial Activity

The methanol extracts of *D. stramonium* L. showed activity against Gram-positive bacteria in a dose-dependent manner. The leaf extract of *D. stramonium* L. exhibits antibacterial activity (Baynesagne et al., 2017). In 2017, a study by Baynesagne et al., evaluated *D. stramonium* L. extracts against clinical pathogenic isolates of bacteria. The extracts, which were prepared using a four-solvent system (petroleum ether, ethyl acetate, methanol, and water), displaced maximum antibacterial activity against *S. aureus* with a zone of inhibition of 18mm. The minimum antibacterial activity was displayed against *E. coli* with a zone of inhibition of 8.2 mm (Baynesagne et al., 2017). The antibacterial activity against the

microorganism strains of *P. aeruginosa*, in the alcoholic extract of the leaves *D. stramonium* L. was found to be most efficient and moderately effective against *K. pneumonia*, *S. aureus*, and *E. coli*. The leaf extract of *D. stramonium* L. Was found to exhibit excellent antifungal activity (Vaza & Bhalerao, 2018). Alkaloids, phenols, and flavonoids in ethanolic extracts disrupt bacterial cell membranes, particularly Gram-positive bacteria (e.g., *Staphylococcus aureus*), through dose-dependent inhibition. Minimal activity against Gram-negative bacteria (e.g., *Escherichia coli*) due to outer membrane barriers (Soni et al., 2012).

2.4.7. Antifungal Activity

Antifungal activity of a concoction brewed (mixture of several things) from *D. stramonium*, *Calotropis gigantea*, *A. indica* (neem), and cow manure (T1) followed by methanol-water (70/30v/v) extracts of *D. stramonium*, *Calotropis gigantea* and *A. indica* T2 against *Fusarium mangiferae*. The study proved that the concoction-brewed compost T1 is effective, expensive, easy to prepare, and constitutes a sustainable and eco-friendly approach to control floral malformation in mango when it is sprayed at the bud stage and again at the fruit set stage (Usha et al., 2009). Methanolic and chloroform extracts inhibit fungal growth (e.g., *Fusarium solani*, *Helianthus sporium*) by disrupting cell wall synthesis and membrane integrity, attributed to alkaloids and phenolic compounds. Activity varies by solvent and fungal species (Soni et al., 2012).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the study area

The plant sample used for this study was collected from Adama Science and Technology University Adama, Oromia Regional State, located around 92.2 kilometers southeast of Addis Ababa, Ethiopia. Adama is situated at an elevation of 1712 meters and coordinates are 8.54°N 39.27°E. The city is situated between the Great Rift Valley to the east and the foot of an escarpment to the west as indicated in Figure 2. It has an annual temperature of 22°C and 400–800 mm of rainfall. The overall area of the city is 9,616,399.5 m² (OWWDS, 2012).

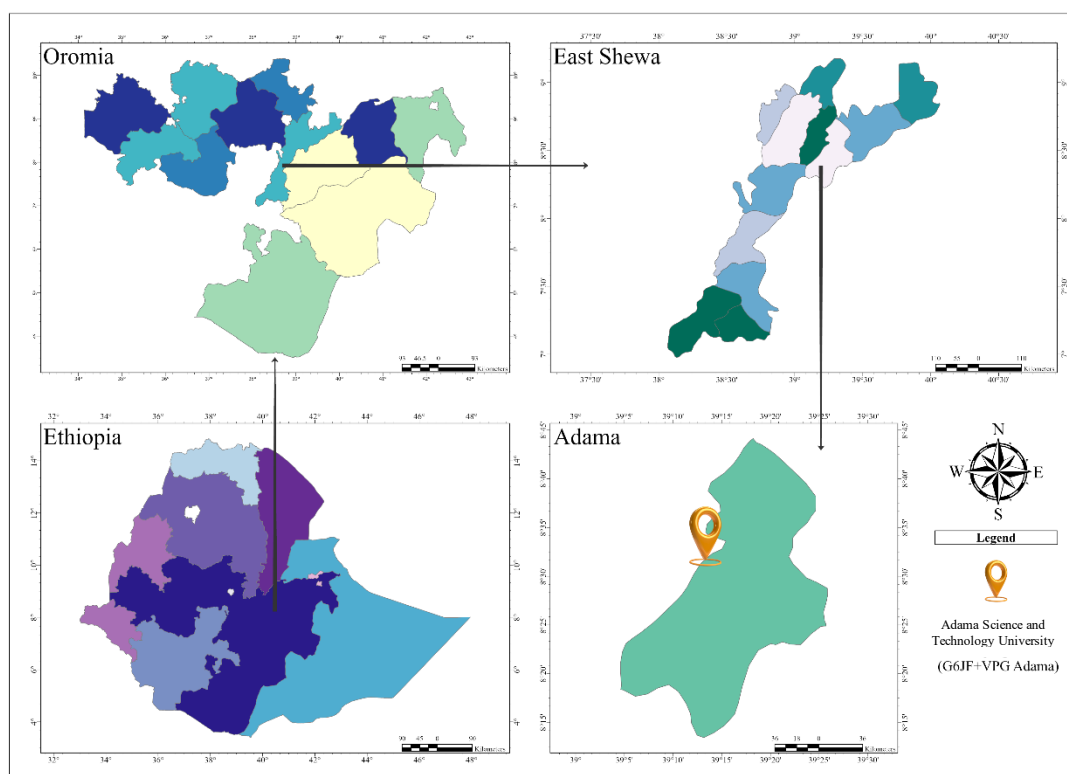


Figure 2: Map of Adama (ArcGIS Pro v.3.0.0)

3.2. Research Design

The research adopts a laboratory-based experimental design to investigate the phytochemical composition, antimicrobial, and antioxidant activities of *D. stramonium* L. leaf extract. This approach is suitable for controlled testing of the plant's effects on selected human pathogens

using the disc diffusion assay, and its antioxidant potential via the DPPH assay. The methodology involves collecting *D. stramonium* leaves from Adama Science and Technology University, followed by qualitative phytochemical analysis to identify alkaloids, flavonoids, and phenols. Antimicrobial activity was assessed by measuring inhibition zones in disc diffusion assays, with positive (ciprofloxacin/ketokonazol) and negative (DMSO soaked discs) controls for comparison. Antioxidant activity was evaluated by determining free radical scavenging capacity (IC₅₀) in the DPPH assay. Experiments were conducted in triplicate to ensure statistical reliability, with data analyzed using statistical tools ANOVA to determine significance. This design ensures precise, repeatable, and controlled evaluation of the plant's bioactivity.

3.3. Study samples and sampling techniques

3.3.1. Collection of Experimental Plant and Identification

The Mature leaves of *D. stramonium* L. used in this study were gathered from Adama Science and Technology University, Adama, Ethiopia on October 2024. To facilitate taxonomic identification, a voucher specimen was taken and pressed on a newspaper. The plant material was authenticated by a plant taxonomist Dr. Lemessa Kumsa at Adama Science and Technology University, Biology Department.

3.3.2. Sample Preparation and extraction

The solid-liquid extraction technique (SLET) was utilized as a general method for extraction. Plant samples were washed thoroughly under distilled water to remove dirt, dust, and insects, and dried at room temperature in a shaded area, without being exposed to direct sunlight to reduce the high moisture content over a period of approximately three weeks. Following the drying period, the dried plant materials were ground using an electronic grinder to obtain a fine powder of the plant. To achieve the total extraction of each plant material, a mixture of organic solvents and plant material was prepared in a ratio of 1:5 specifically, dried and ground plant material weighing 0.5 kg was extracted with 2.5 L of petroleum ether. The mixture was macerated at room temperature on a shaker for 72 h and then filtered using Whatman No.1 filter paper. The resulting filtrate was concentrated using a rotary evaporator. The residual plant material left after pet. ether extraction underwent further extraction using

ethyl acetate and methanol successively following the same procedure and the percentage of the yield of the crude extracts were calculated using equation 1 (Abubakar & Haque, 2020).

$$\text{Yield \%} = \frac{\text{The weight of the extract obtained}}{\text{Weight of plant sample}} \times 100 \quad (1)$$

3.4. Phytochemical analysis of *D. stramonium* L. leaf extract

Phytochemical examinations were carried out on *D. stramonium* L. extracts according to standard procedures.

Test for alkaloids: Wagner's test involves the addition of 1ml of Hydrochloric acid to 2 mg/ml of each extract, in addition to a few drops of Wagner's reagent. The detection of a yellow or brown precipitate served as an indication of the presence of alkaloids (Joshi et al, 2013).

Test for flavonoids: In shinoda test, 2 mg/ml of each extract was treated with 2 ml of 0.1 N sodium hydroxide solution and 0.1 N hydrochloric acid. Following this procedure, the presence of flavonoids was indicated by the color change from yellow to colorless in the solutions (Edema & Alaga, 2012).

Test for phenols: In ferric chloride test, 1 mg/ml of each extract was combined with 2 ml of distilled water, followed by the addition of five drops of 10% ferric chloride (w/v). The presence of phenols was indicated by the formation of blue or green colors (Sha'a et al., 2019).

Test for Saponins: For foam test, 1 mg/ml of each extract was vigorously mixed with a few drops of distilled water. The presence of saponins was indicated by the formation of persistent foam (Joshi et al., 2013).

Test for steroids: For salkowski's test, 2 ml of acetic anhydride was added to 2 mg/ml of each extract. Alongside, 2 ml of concentrated sulfuric acid was added. The test tube was then carefully inverted to form a distinct layer underneath. The test tube was observed for the appearance of a green coloration, which indicates the presence of steroids (Auwal et al., 2014).

Test for Tannins: For ferric chloride test, a few drops of a 10% Ferric chloride solution (w/v, light yellow) were added to 2 mg/ml of each extract. The appearance of a blackish-blue color served as an indication of the presence of tannins (Takaidza et al., 2018).

3.5. Essential oil extraction

To extract essential oils from *Datura stramonium*, 500 g of the leaf material was combined with 5000 mL of distilled water in a round-bottom flask and subjected to hydrodistillation for 4 hours using a Clevenger apparatus. The resulting distillate underwent liquid-liquid extraction with an equal volume of chloroform in a separatory funnel, and the chloroform layer was collected. To remove residual water, 5 g of anhydrous sodium sulfate (Na₂SO₄) was added to the chloroform layer, followed by filtration through Whatman No. 1 filter paper. The filtered solution was concentrated using a rotary evaporator, and the yield of the essential oil was calculated using equation 2 (Sisay et al., 2019).

$$\text{Extraction yield (\%)} = \frac{\text{Mass of concentrated oil}}{\text{A dry mass of plant powder used}} \times 100 \quad (2)$$

3.6. Antimicrobial Activity evaluation of leaf extracts of *D. stramonium* L.

3.6.1. Preparation of Inoculums

Standard pathogenic microbial strains, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231, were acquired from the Oromia Regional Laboratory, Adama, Ethiopia, and aseptically transported in an icebox to the Microbiology Laboratory at Adama Science and Technology University, where they were stored at 4°C. Bacterial strains were sub-cultured in Mueller-Hinton Broth (MHB), prepared per manufacturer guidelines and sterilized at 121°C and 0.11 MPa for 15 minutes, with cell concentrations adjusted to 1.5×10^8 CFU/mL using 0.8% NaCl solution, targeting an absorbance of 0.08–0.10 at 625 nm (0.5 McFarland standard, made by mixing 0.05 mL of 1% BaCl₂ with 9.95 mL of 1% H₂SO₄) (Muhammad and Sisay A, 2021). For the fungal strain, *C. albicans* was sub-cultured in Sabouraud Dextrose Broth (SDB), prepared and sterilized similarly, inoculated from a 24–48-hour Sabouraud Dextrose Agar culture, incubated at 30°C for 24 hours, and adjusted to $1-5 \times 10^6$ CFU/mL with 0.9% NaCl, matching the 0.5 McFarland standard's absorbance for consistency (Malik & Ahmed, 2016).

3.6.2. Paper Disk Diffusion Assay

Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were prepared according to manufacturer guidelines, with 25 mL of media poured per Petri dish, and Whatman No. 1 filter paper discs (6 mm diameter) were created using a puncher and sterilized at 121°C and 0.11 MPa for 15 minutes. Stock solutions of *D. stramonium* leaf extracts were prepared by dissolving 0.4 g of extract in 2 mL of 1% dimethyl sulfoxide (DMSO, prepared by mixing 1 mL DMSO with 99 mL deionized water) to yield a 200 mg/mL solution using equation 3 (Sambrook & Russell, 2001), which was serially diluted to 100, 50, and 25 mg/mL. Similarly, 100 mg of essential oil was dissolved in 2 mL of 1% DMSO and diluted to 100, 50, 25, and 12.5 mg/mL. Positive controls were prepared by dissolving 0.5 mg of powdered ciprofloxacin and ketoconazole tablets in 2 mL of 1% DMSO to obtain 0.25 mg/mL solutions for bacterial and fungal strains, respectively, while 1% DMSO-soaked discs served as the negative control. One hundred microliters of the standardized inoculum were spread evenly onto MHA or SDA plates using a cotton swab, and 1 mL of each extract or oil dilution was impregnated onto sterile discs, left for 30 minutes to ensure absorption, and placed on to the agar surface. After 30 minutes for diffusion, plates were incubated at 37°C for bacterial strains and 30°C for the fungal strain for 24 hours. The zones of inhibition (mm) were measured using a ruler (Cusack et al., 2019), with all tests performed in triplicate, and results reported as mean $\pm$ standard deviation using SPSS software (Version 27.0.1, IF026).

$$C_n = C_0 / 2^n \quad (3)$$

Where:

C_n = concentration at step n

C_0 = initial concentration

n = number of dilution steps

3.6.3. Determination of Minimum Inhibitory Concentration (MIC).

Mueller-Hinton Broth (MHB) and Sabouraud Dextrose Broth (SDB) were prepared according to manufacturer guidelines, with 5 mL of MHB or SDB dispensed per test tube and sterilized by autoclaving at 121°C and 0.11 MPa for 15 minutes. Stock solutions of crude extracts and essential oil were prepared at 50 mg/mL by dissolving 100 mg in 2 mL of 1% dimethyl sulfoxide (DMSO), followed by two-fold serial dilutions to obtain concentrations

of 50, 25, 12.5, 6.25, and 3.125 mg/mL. For bacterial strains, 5 mL of MHB was added to each test tube, inoculated with 100 μ L of standardized bacterial inoculum (1.5×10^8 CFU/mL), and supplemented with 1 mL of each extract and oil dilution; tubes were then incubated at 37°C for 24 hours. For the fungal strain, 5 mL of SDB was added to each test tube, inoculated with 100 μ L of standardized fungal inoculum ($1-5 \times 10^6$ CFU/mL), and supplemented with 1 mL of each dilution, followed by incubation at 30°C for 24 hours. The absorbance of each tube was measured at 600 nm using a spectrophotometer, and the minimum inhibitory concentration (MIC) was determined as the lowest concentration with an absorbance comparable to the negative control (broth without inoculum) (Trent et al, 2013).

3.6.4. Determination of Minimum bactericidal and fungicidal concentration (MBC and MFC)

To determine the minimum bactericidal concentration (MBC), 10 μ L samples were taken from test tubes with absorbance values at 600 nm close to the negative control and spread onto Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates using a sterilized glass rod; plates were then incubated at 37°C and 30°C for 24 hours, and the MBC and MFC was defined as the lowest concentration of crude extracts or essential oil that prevents visible growth of the organism (Trentin et al., 2013).

3.7. Antioxidant activity

Stock solutions of *D. stramonium* essential oil and crude extracts were prepared by dissolving 0.004 g of each sample in 2 mL of methanol (MeOH), followed by two-fold serial dilutions to obtain concentrations of 2000, 1000, 500, 250, and 125 μ g/mL. Ascorbic acid, used as a positive control, was also prepared similarly at the same concentrations. A 0.1 mM stock solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was made by dissolving 0.01 g of DPPH in 25 mL of MeOH, with its absorbance adjusted to 1.0 ± 0.1 at 517 nm. Then, 1 mL of 0.1 mM DPPH solution was mixed with 1 mL of each sample solution at each concentration and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a spectrophotometer, with measurements performed in triplicate. The absorbance of a DPPH solution in MeOH without sample served as the negative control. The percentage of DPPH radical scavenging activity was calculated for each sample concentration using equation 4 (Nayak et al., 2011).

$$\% \text{ DPPH scavenging} = \frac{\text{Control absorbance} - \text{extract absorbance}}{\text{Control absorbance}} \times 100 \quad (4)$$

3.8. Data analysis

The experimental results were expressed as mean $\pm$ standard deviation (SD) of three replicates. Where applicable, the data was subjected to SPSS version 26 and one-way, and the results were presented using tables and graphs. P values less than 0.05 were considered statistically significant.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

4.1. Yield of crude extracts

The lowest percentage yields of crude extract were found to be 1.34% for Pet. ether extracts, whereas the crude extract of methanol solvent gave the highest percentage yield (7.51%) against *D. stramonium* L. leaves extract (Table 1, Figure 3c). This could be due to methanol being more polar than ethyl acetate and Pet. ether solvents, as a similar result reported by (Nikam et al., 1996). In this study, ethyl acetate solvent (Table 1, Figure 3b) gave considerable amounts of percentage yields of crude extract for *D. stramonium* L. leaf extract. However, Pet. ether gave the lowest yield of crude extract (Table 1, Figure 3a). A previous study demonstrated (Flores-Villegas et al., 2019) that methanol solvent gave 9.7, 5.7, and 7.1%, extraction yields against root, stem, and leaf of *D. stramonium* L., respectively, which are comparable to our extraction yield (7.51%).

Table 1: Percentage yields of crude extract of *D. stramonium* L. leaf extract

Extracts	Weight of plant material (g)	Weight of plant extracts (g)	Yield (%)
Pet. ether	500	6.7	1.34
EtOAc	500	8.73	1.75
MeOH	500	37.54	7.51

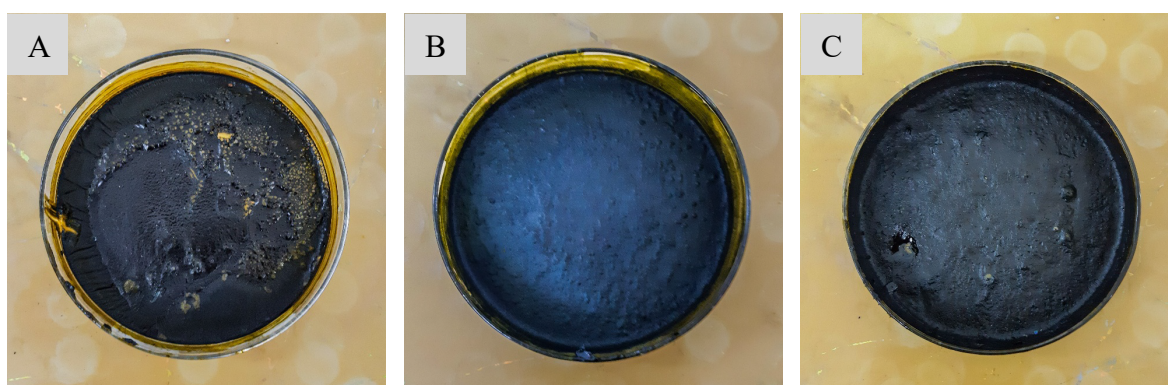


Figure 3: Crude extracts of leaves of *D. stramonium* L. (A) Petroleum extract, (B) Ethyl acetate extract, and (C) Methanol extract

4.2. Phytochemical analysis

The alkaloid, flavonoid, Phenol, Saponin, Steroid, and Tannin were among the phytochemical components detected against *D. stramonium L.* using Pet. ether, ethyl acetate, and methanol extracts (Table 2) (Appendix 1A, B & C). As displayed in Table 2, all the recent phytochemical components were positive against the crude extracts of ethyl acetate extracts. However, only steroids were predicted against Pet. ether crude extracts as illustrated in Table 2. In this study, steroid, however, was not detected for methanol extract against *D. stramonium L.* leaf crude extracts (Appendix 1A, B & C). In line with this study, phytochemical components such as alkaloids, glycosides, flavonoids, phenols, saponins, steroids tannins, and terpenoids were obtained against *D. stramonium L.* leaf extract (Baylie et al., 2023). However, in this study, these phytochemical components were found to be determined by the type of employed extracting solvents. As it was displayed in Table 2, some of them were present and some of them were absent while employing various extracting solvents.

Table 2: Phytochemical analysis of crude extracts

Phytochemicals	Plant Extracts		
	Pet. ether	EtOAc	MeOH
Alkaloid	☑	☑	☑
Flavonoid	☒	☑	☑
Phenol	☒	☑	☑
Saponin	☑	☑	☑
Steroid	☑	☑	☒
Tannin	☑	☑	☑

Key: ☑: indicates the presence of the phytochemical; ☒: indicates the absence of the phytochemicals

Other studies predicted metabolites such as acutumine, quinine, catechin, chlorogenic acid, cyanidin, formosanin, gallic acid, luteolin, quercetin, saponin, 3-carene, limonene, tannic acid, α -terpineol, and vanillic acid from the aqueous extract of *Datura stramonium L.* seeds (Joshua et al., 2022). The same metabolites can occur in other plant extracts. For instance, crude extracts of *Nerium oleander* and leaves were found to contain alkaloids, flavonoids, glycosides, tannic acid, steroids, triterpenoids, and saponins (Fatiqa Zafar et al., 2014). The same authors noticed alkaloids, glycosides, flavonoids, saponins steroids, tannic acid, and

triterpenoids from the leaf extract of *D. stramonium* L. Another study displayed that *Datura* leaf extract was found to contain alkaloids, saponins, and tannins but the flowers contained flavonoids and steroids (Mohamed et al., 2022).

4.3. GC-MS analysis of Essential oils (EOs)

In this study, a 0.03% yield of EOs was obtained (Appendix 2) from 500g leaves of *D. stramonium* L. which had been processed by hydro distillation for 4 h with 150 mg yield. In this study, various EOs were obtained from crude extracts of *D. stramonium* L. leaf. Some of the EOs which are higher compositions such as 2-Hexenal, (3E)-3-Hexen-1-ol, Cyclohexanol, Benzyl alcohol, Phenylacetaldehyde, 2-methoxy-4-vinyl-phenol, (2S,3S)-2-Methyl-3-(2-, 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-, Phytol, and Diisooctylphthalate were predicted using GC-MS analysis with various chemical formula, percentages, structures and RT (Table 3). As indicated in Table 3, other EOs with lest concentrations were detected from the crud extracts of *D. stramonium* L. leaf. Some of them are 1,5-Heptadien-4-one, 3,3,6-trimethyl-, 2-Pyridinamine, 2-Furancarboxaldehyde, Benzaldehyde, 1,5-Heptadien-4-one, 3,3,6-trimethyl-, Phenol, 4-cyclohexyl-, 1H-Indole, and others (Table 3). These EOs found to have various peaks of EOs as illustrated in Figure 4.

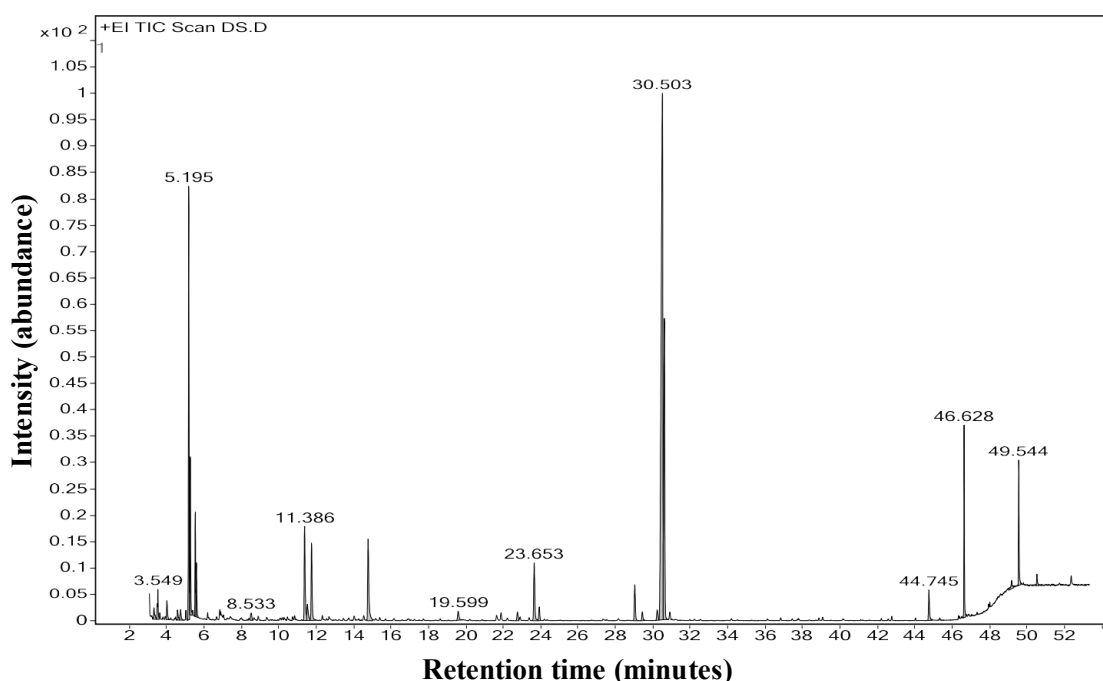


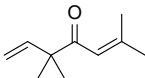
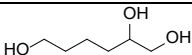
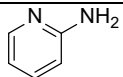
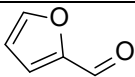
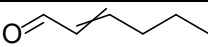
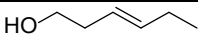
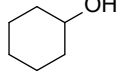
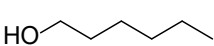
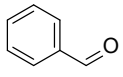
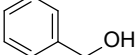
Figure 4: Gas chromatogram of essential oil of leaves of *D. stramonium* L.

These essential oils vary from place to place. For instance, study done on *D. stramonium*, which collected around Adama Science and Technology University found to contain EOs

such as 1,5-Heptadien-4-one, 3,3,6-trimethyl-, 2-Furancarboxaldehyde, (3E)-3-Hexen-1-ol, 1,5-Heptadien-4-one, 3,3,6-trimethyl-, Phenylacetaldehyde, 2-methoxy-4-vinyl-phenol, Phenol, 4-cyclohexyl-, 3-Hexyn-2-ol, 5-methyl-, 1-(Trimethylsilyl)-1-propyne, (2S,3S)-2-Methyl-3-(2-methyl-allyl)-cyclopentanone, 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-, Diisooctylphthalate and other (Table 3) based on GC-MS analysis.

However, for the same plants that collected from Solan (30.904486 °N, 77.096733 °E) region of Himachal Pradesh, India, EOs such as neophytadiene (Phytol acetate) (10.76%), β -damascenone (9.67%), and β -eudesmol (7.2%) (Chandan et al., 2020). These EOs are different from the recent results. This could be due to the growth of the same plant in various environments with different soil type, elevation, moisture, and ecological interaction as these are secondary metabolites (Saed-Moucheshi & Mozafari, 2022).

Table 3: Various essential oils obtained from the crude extracts of *D. stramonium* L. leaf

No	RT	Compound Name	Structure	Molecular formula	Composition (%)
1	3.34	1,5-Heptadien-4-one, 3,3,6-trimethyl-		C ₁₀ H ₁₆ O	0.53
2	3.55	1,2,6-Hexanetriol		C ₆ H ₁₄ O ₃	1.07
3	4.60	2-Pyridinamine		C ₅ H ₆ N ₂	0.36
4	4.76	2-Furancarboxaldehyde		C ₅ H ₄ O ₂	0.48
5	5.20	2-Hexenal		C ₆ H ₁₀ O	11.62
6	5.28	(3E)-3-Hexen-1-ol		C ₆ H ₁₂ O	4.37
7	5.55	Cyclohexanol		C ₆ H ₁₂ O	2.69
8	5.61	1-Hexanol		C ₆ H ₁₄ O	1.57
9	8.53	Benzaldehyde		C ₇ H ₆ O	0.41
10	11.39	Benzyl alcohol		C ₇ H ₈ O	3.90

11	11.54	1,5-Heptadien-4-one, 3,3,6-trimethyl-		C ₁₀ H ₁₆ O	0.85
12	11.76	Phenylacetaldehyde		C ₈ H ₈ O	3.24
13	14.78	Phenylethanol		C ₈ H ₁₀ O	4.15
14	19.60	4-Vinylphenol		C ₈ H ₈ O	0.54
15	22.76	1H-Indole		C ₈ H ₇ N	0.42
16	23.65	2-methoxy-4-vinyl-phenol		C ₉ H ₁₀ O ₂	2.68
17	23.93	Phenol, 4-cyclohexyl-		C ₁₂ H ₁₆ O	0.60
18	29.03	3-Hexyn-2-ol, 5-methyl-		C ₇ H ₁₂ O	1.59
19	29.43	1-(Trimethylsilyl)-1-propyne		C ₆ H ₁₂ Si	0.40
20	30.50	(2S,3S)-2-Methyl-3-(2-methyl-allyl)-cyclopentanone		C ₁₀ H ₁₆ O	36.18
22	30.61	2H-Pyran-2-one, 5,6-dihydro-6-pentyl-		C ₁₀ H ₁₆ O ₂	13.30
23	44.75	n-Hexadecanoic acid		C ₁₆ H ₃₂ O ₂	1.23
24	46.63	Phytol		C ₂₀ H ₄₀ O	4.25
25	49.54	Diisooctylphthalate		C ₂₄ H ₃₈ O ₄	3.04

D. stramonium L. is a valuable plant that rich therapeutic properties due to various secondary metabolites with a myriad of uses and applications (Soni et al., 2012). These secondary metabolites are presented in various percentages against *D. stramonium*. For instance, (2S,3S)-2-Methyl-3-(2-methyl-allyl)-cyclopentanone, 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-, 2-Hexenal, and others extracted and identified from the crude extracts of *D. stramonium* L. using GC-MS tools. Using the same techniques metabolites such as 3,7-dihydroxy-6-propionyloxytropene, 6,7-dehydro-3-tigloyloxytropene, 3-tigloyloxy-6,7-epoxytropene, 3,7-dihydroxy-6-(2'-methylbutyryloxy)tropene, 6,7-dehydroapoptropine, 3-(3'-methoxytropoyloxy)tropene, 3-tigloyloxy-6-isobutyryloxy-7-hydroxytropene, 3-tropoyloxy-6-isobutyryloxytropene, 3β-tropoyloxy-6β-isovaleroyloxytropene were reported

from *D. stramonium* L. (El Bazaoui et al., 2011). The same authors further extracted metabolite-like alkaloids cyclopropine, dihydroaposcopolamine, 6,7-dehydrohyoscyamine, and 4'-hydroxylitorine for the first time for the genus *Datura*. The study conducted on *D. stramonium* L. exhibited that ethanol extracts of *Datura* leaves were found to contain Butanol, 3-methyl (79.76%), Toluene (6.14%), and Phytol (3.9%) (Mohamed et al., 2022). However, the flower extract contains formic acid, and 3-methylbut-2-yl ester (82.22%) (Mohamed et al., 2022), which is the main component reported using a similar identification tool against *D. stramonium* L.

4.4. Antioxidant activity of crude extracts and essential oil of *D. stramonium* L. leaf.

The *in vitro* antioxidant activity showed that the essential oil exhibited better-scavenging potential than the others. Accordingly, the essential oil displayed the highest DPPH radical scavenging activity with IC_{50} values of 3.34 $\mu\text{g/mL}$ (Table 4). The scavenging activities were also not too far from ascorbic acid (IC_{50} : 0.28 $\mu\text{g/mL}$). The ethyl acetate and methanol extracts also revealed good scavenging activities with IC_{50} values of 6.17 and 10.25 $\mu\text{g/mL}$, respectively. While the petroleum ether extract showed the lowest activity with IC_{50} value of 100.09 $\mu\text{g/mL}$.

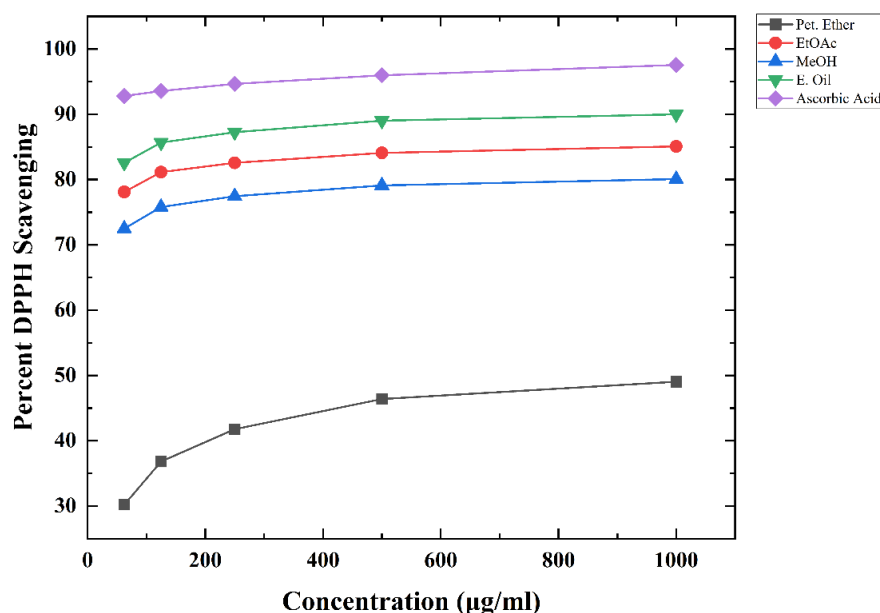


Figure 5: DPPH % radical scavenging activity of crude extracts against the control ascorbic acid

Table 4: Antioxidants of crude extracts of *D. stramonium* L. leaf

Concentration (µg/mL)	-ve control	Extracts								+ve Control	
	DPPH in MeOH	Pet. ether		EtOAc		MeOH		E. Oil		Ascorbic acid	
	Mean Abs.	Mean Abs.	%RSA	Mean Abs.	%RSA	Mean Abs.	%RSA	Mean Abs.	%RSA	Mean Abs.	%RSA
1000	1.009	0.523	49.16	0.160	84.14	0.210	79.19	0.110	89.10	0.03	97.03
500	1.009	0.550	48.17	0.170	83.15	0.220	78.19	0.120	88.11	0.05	95.04
250	1.009	0.597	45.49	0.185	81.67	0.237	76.51	0.138	86.32	0.06	94.05
125	1.009	0.647	40.83	0.199	80.28	0.253	74.93	0.154	84.74	0.07	93.06
62.5	1.009	0.713	35.88	0.230	77.21	0.287	71.56	0.185	81.67	0.08	92.07
IC₅₀		100.09		6.17		10.25		3.34		0.28	

Key: Abs.; absorbance, %RSA; percent radical scavenging activity, -ve; negative, +ve; positive

The exceptional scavenging activity of the essential oil (IC₅₀: 3.34 µg/mL), which closely approaches that of ascorbic acid (IC₅₀: 0.28 µg/mL), aligns with previous findings on *Datura stramonium* essential oils. For instance, Kumar et al. (2018) reported that essential oils from *Datura stramonium* leaves possess strong antioxidant properties, attributed to volatile compounds such as terpenoids, phenolics, and alkaloids. However, their study reported a slightly higher IC₅₀ value of 5.8 µg/mL, possibly due to differences in extraction techniques (e.g., steam distillation vs. hydrodistillation) or variations in plant chemotype influenced by environmental factors (Kumar et al., 2018). Similarly, Sharma and Gupta (2020) noted IC₅₀ values ranging from 4 to 10 µg/mL for essential oils, suggesting that the lower IC₅₀ value in this study may reflect a higher concentration of bioactive compounds, potentially enhancing its suitability for pharmaceutical or nutraceutical applications.

The ethyl acetate and methanol extracts, with IC₅₀ values of 6.17 µg/mL and 10.25 µg/mL, respectively, are consistent with prior research demonstrating that polar solvents effectively extract antioxidant compounds from *Datura stramonium* leaves. For example, Patel et al. (2019) reported an IC₅₀ value of 9.2 µg/mL for methanol extracts, which is comparable to the 10.25 µg/mL observed here, indicating reliable extraction of phenolic acids and flavonoids (Patel et al., 2019). The ethyl acetate extract's stronger activity compared to methanol aligns with findings by Singh and Verma (2021), who reported an IC₅₀ of 7.5 µg/mL for ethyl acetate extracts, attributing this to the selective extraction of less polar phenolics (Singh & Verma, 2021). The slightly better performance of the ethyl acetate extract in this study may suggest a richer phenolic profile or optimized extraction conditions.

In contrast, the petroleum ether extract's poor scavenging activity (IC₅₀: 100.09 µg/mL) corroborates previous studies reporting limited antioxidant potential for non-polar extracts. Kumar et al. (2018) observed an IC₅₀ value of 120 µg/mL for petroleum ether extracts, attributing this to the predominance of lipophilic compounds like waxes and fatty acids, which lack significant radical-scavenging properties (Kumar et al., 2018). Similarly, Sharma and Gupta (2020) reported IC₅₀ values exceeding 100 µg/mL for non-polar extracts, reinforcing that such fractions are less suitable for antioxidant applications (Sharma & Gupta, 2020).

4.5. Antimicrobial susceptibility test

The crude extract of methanol, ethyl acetate, and Pet. ether of *D. stramonium* L. employed to affect the growth of *E. coli* 25922^T (ATCC), *P. aeruginosa* 27853^T (ATCC), *S. aureus* 25923^T (ATCC), *E. faecalis* 29212^T (ATCC), and *C. albicans* 10231^T (ATCC) (Table 5, Figure 6). The lowest inhibition zone was recorded for *E. faecium* 19434^T (ATCC) (11±0.00 mm) at 25 mg/ml and the highest inhibition zone was detected for *S. aureus* (ATCC 25923) (24±0.00 mm) against crude extract of Pet. ether of *D. stramonium* L. (Table 5) at 200 mg/ml. Considerable amounts of inhibition zone were also noticed against *S. aureus* 25923^T (ATCC) (at 200 mg/ml, 23±0.00 mm), and *C. albicans* 10231^T (ATCC) (Appendix 3) (at 200 mg/ml, 21.5±0.00 mm) due to the same crude extracts. One-way ANOVA exhibited that mostly very significant variation was detected against zone of inhibition (mm) due to Pet. ether extracts of *D. stramonium* L. at various concentrations against these pathogens (p<0.05) (Appendix 3) with different mean, SD, SE, upper bound, lower bound, minimum, maximum, values.

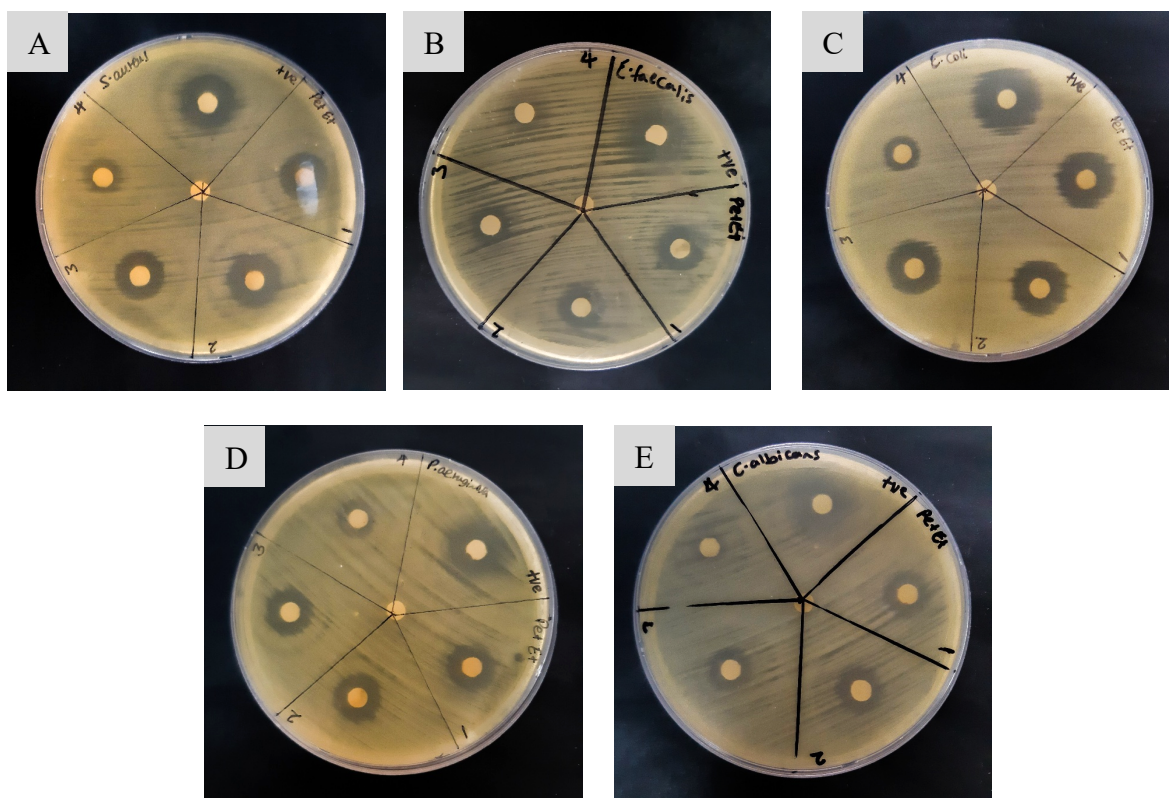


Figure 6: Antimicrobial activity of Crude extracts of Pet. ether of *D. stramonium* L. against (a) *S. aureus* 25923^T (ATCC), (b) *E. faecalis* 29212^T (ATCC), (c) *E. coli* 25922^T (ATCC), (d) *P. aeruginosa* 27853^T (ATCC), and (e) *C. albicans* 10231^T (ATCC)

The inhibition of these pathogens could be due to the presence of certain metabolites of *D. stramonium* L. such as alkaloids, glycosides, flavonoids, phenols, saponins, steroid and tannins. These metabolites might have been employed to target various pathogenic cellular structures like cell walls, cell membranes, chromosomes, proteins, ribosomes, and other structures. Similarly, the study confirmed that tannin targets the synthesis of pathogenic nucleic acids along with phytoalexins (Huang et al., 2024). Other studies demonstrated that saponins, which were obtained from *Chenopodium quinoa* found to target cell wall formation and disrupt cytoplasmic membrane and membrane proteins, which finally resulted in leakage of the cell contents (Dong et al., 2020). Further studies confirmed that alkaloids can disrupt the cell membrane of bacterial pathogens due to their toxicity (Matsuura & Fett-Neto, 2015), and steroids can affect the cell membrane of pathogens, specifically by interacting with specific intracellular receptors of pathogens (Tuohimaa et al., 1996).

Table 5: The mean values of the inhibition zone of crude extracts of *D. stramonium* L. leaf, which was prepared using Pet. ether, EtOAc, MeOH, and Cipro./Keto. against various pathogenic strains at various concentration

Extracts	Concentration (mg/ml)	Inhibition Value (mm)				
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecium</i>	<i>C. albicans</i>
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Pet. ether	200	22.0±0.0	23.0±0.0	24.0±0.0	19.0±0.0	21.5±0.5
	100	20.5±0.5	19.5±0.5	21.0±0.0	16.5±0.5	19.5±0.5
	50	17.0±0.0	18.0±0.0	18.5±0.5	14.5±0.5	16.5±0.5
	25	15.0±0.0	13.5±0.5	16.0±0.0	11.0±0.0	14.5±0.5
	Cipro. / Keto. (0.25)	24.5±0.5	25.5±0.5	25.5±0.5	25.0±0.0	24.5±0.5
	-ve control	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
EtOAc	200	21.5±0.5	22.5±0.5	22.0±0.0	19.0±1.0	23.5±0.5
	100	18.0±0.0	19.5±0.5	20.0±0.0	16.5±0.5	21.5±0.5
	50	14.5±0.5	18.5±0.5	16.5±0.5	15.5±0.5	19.5±0.5
	25	13.5±0.5	16.5±0.5	14.5±0.5	13.5±0.5	17.0±0.0
	Cipro. / Keto. (0.25)	23.0±0.0	25.0±0.0	24.5±0.5	24.0±0.0	24.5±0.5
	-ve control	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
MeOH	200	19.5±0.5	20.5±0.5	21.0±0.0	21.5±0.5	23.5±0.5
	100	16.5±0.5	19.0±0.0	18.5±0.5	18.5±0.5	20.0±0.0
	50	12.5±0.5	16.5±0.5	16.0±0.0	15.5±0.5	17.0±0.0
	25	11.0±0.0	13.5±0.5	11.5±0.5	12.0±0.0	14.5±0.5
	Cipro. / Keto. (0.25)	23.5±0.5	24.5±0.5	23.5±0.5	23.0±0.0	23.5±0.5
	-ve control	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
E. oil	100	21.5±0.5	22.5±0.5	21.5±0.5	21.0±0.0	21.5±0.5
	50	19.5±0.5	20.5±0.5	19.5±0.5	18.5±0.5	20.5±0.5
	25	17.0±0.0	18.0±0.0	17.5±0.5	17.5±0.5	18.5±0.5
	12.5	15.0±0.0	16.5±0.5	14.5±0.5	15.5±0.5	17.0±0.0
	Cipro. / Keto. (0.25)	23.0±0.0	23.5±0.5	24.5±0.5	23.5±0.5	23.0±0.0
	-ve control	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0

Key: Cipro.: Ciprofloxacin used for the bacterial test strains; **Keto.:** Ketoconazol used for the fungal strain; **-ve control:** DMSO soaked filter paper

The inhibition of the recent bacterial strains could be also due to the presence of essential oils which are predicted by GC-MS as indicated in Table 3. These EOs and other compounds such as 1H-Indole, 4-Vinylphenol, Phenylethanol, 3-Hexyn-2-ol, 5-methyl-, Diisooctylphthalate, Phytol, Phenol, 4-cyclohexyl-, 2-methoxy-4-vinyl-phenol, Benzyl alcohol, 1-Hexanol, Cyclohexanol, and others can affect the cellular structures of the recent

pathogens. These metabolites can be used as ant fungi, anti-cancer, anti-inflammation, and anti-parasites. For instance, a previous study showed that 1-Hexanol was found to inhibit the growth of Gram-negative pathogens (Kyoui et al., 2023), Cyclohexanol which was obtained from *Amphirosellinia nigrospora* JS-1675 inhibits the growth of bacterial and fungi pathogens (Nguyen et al., 2019), 2-Methoxy-4-vinyl phenol (2M4VP) can act as anticancer, and anti-inflammation (Kim et al., 2019), and thiophene-2-yl)-1H-indole and its derivatives found to act as anti-cancers against HCT-116 cell proliferation via cell cycle arrest and down regulation of miR-25 (Abdelazeem et al., 2024).

The minimum and maximum inhibition zones were noticed for the crude extract of EtOAc against *E. coli* 25922^T (ATCC) and *C. albicans* 10231^T (ATCC) at 25 and 200 mg/ml with 13.5 ±0.5 and 23.05±0.5 mm. Crude extract of EtOAc at 200 mg/ml displayed a larger zone of inhibition against *P. aeruginosa* 27853^T (ATCC) followed by the same crude extract and its concentration against *S. aureus* 25923^T (ATCC) with 22±0.0 mm zone of inhibition. In line with this study, a previous study demonstrated that Ethyl acetate leaf extracts of *D. stramonium* L. and *D. inoxia* exhibited and/or acted as cytotoxicity against cell lines, anticancer, antileukemic, and others (Nasir et al., 2020). The same authors further reported that the ethyl acetate extract of these medicinal plants acts as a cytotoxicity against PC-3, MDA-MB 231, and MCF-7 cell lines with percent inhibition at 20 µg/ml final concentration. *D. stramonium* L. extraction solvents such as water, ethanol, 2-propanol, n-butanol, propanone, butanone, 3-methyl-2-pentanone, dichloromethane, xylene, and toluene found to inhibit the growth of bacteria, and fungi, and other organisms like herbivores, invertebrates, and insects (Vega-Ceja et al., 2022). As indicated in Table 5, the zone of inhibition against these standard strains due to crude extract of ethyl acetate is larger than Pet. ether extracts.

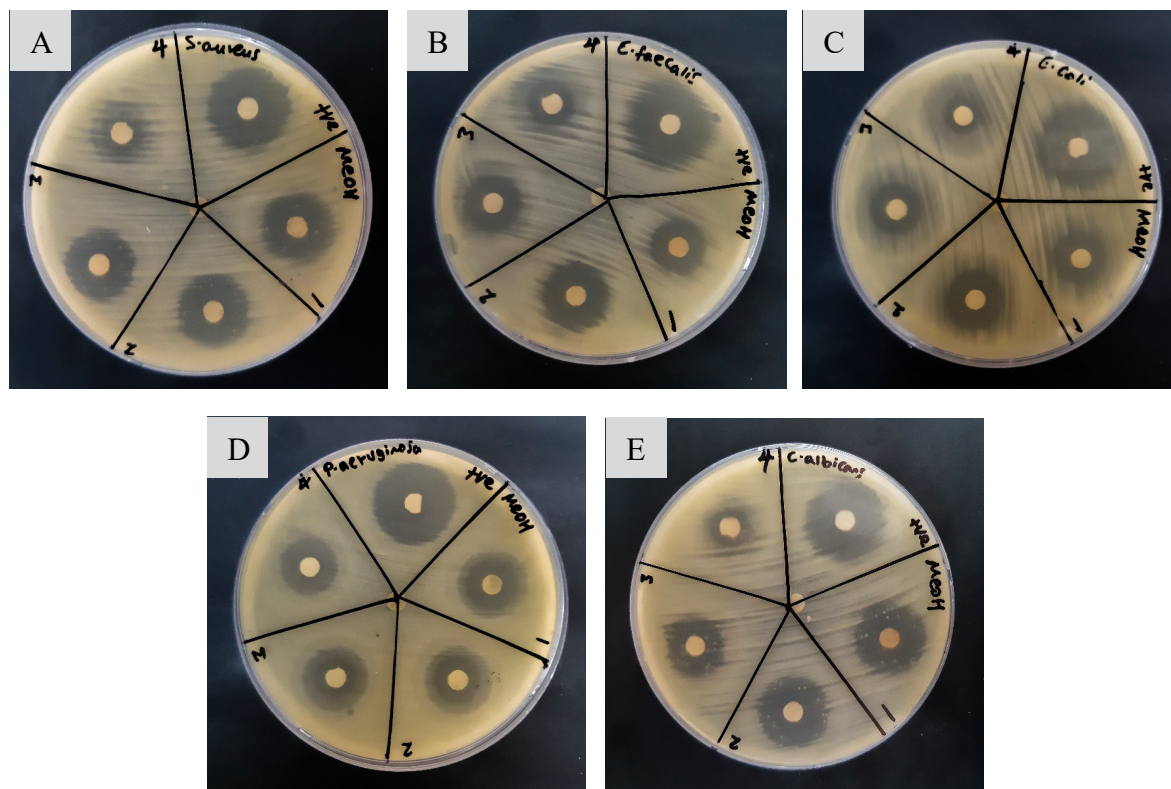


Figure 7: Antimicrobial activity of Crude extracts of MeOH of *D. stramonium* L. against (a) *S. aureus* 25923^T (ATCC), (b) *E. faecalis* 29212^T (ATCC), (c) *E. coli* 25922^T (ATCC), (d) *P. aeruginosa* 27853^T (ATCC), and (e) *C. albicans* 10231^T (ATCC)

The standard antibiotics, Cipro. gave the maximum zone of inhibition against all test strains (Table 5, Appendix 3) ranging from 24.50 ± 0.5 to 25.500 ± 0.5 mm. Great significant variations were documented among these zones of inhibition for crude extracts of Pet. ether, EtOAc, & MeOH solvents and Cipro. for these strains ($p=0.000$) (Appendix 3). However, no variations were noticed among Cip against *E. faecium* 19434^T (ATCC) at 200 mg/ml and Cip against *E. coli* 25922^T (ATCC) at 200 mg/ml ($p=0.174$), Cip against *E. faecium* 19434^T (ATCC) at 200 mg/ml & Cip against *P. aeruginosa* 27853^T (ATCC) at 200 mg/ml ($p=0.174$), Cipro. against *E. faecium* 19434^T (ATCC) at 200 mg/ml & Cip against *S. aureus* 25923^T (ATCC) at 200 mg/ml ($p=0.174$), and Cip against *E. faecium* 19434^T (ATCC) at 200 mg/ml & Cip against *C. albicans* 10231^T (ATCC) at 200 mg/ml (Appendix 3). As predicted by One-Way-ANOVA, of LSD and Post Hoc test at 0.05, confidence intervals, no variations were predicted between Crude extract of EtOAc against *S. aureus* 25923^T (ATCC) at 200 mg/ml & Crude extract of EtOAc against *E. coli* (ATCC 25922^T) at 200 mg/ml, and Crude extract of EtOAc against *S. aureus* 25923^T (ATCC) at 200 mg/ml &

Crude extract of EtOAc against *P. aeruginosa* 27853^T (ATCC) at 200 mg/ml ($p=0.174$) (Appendix 3).

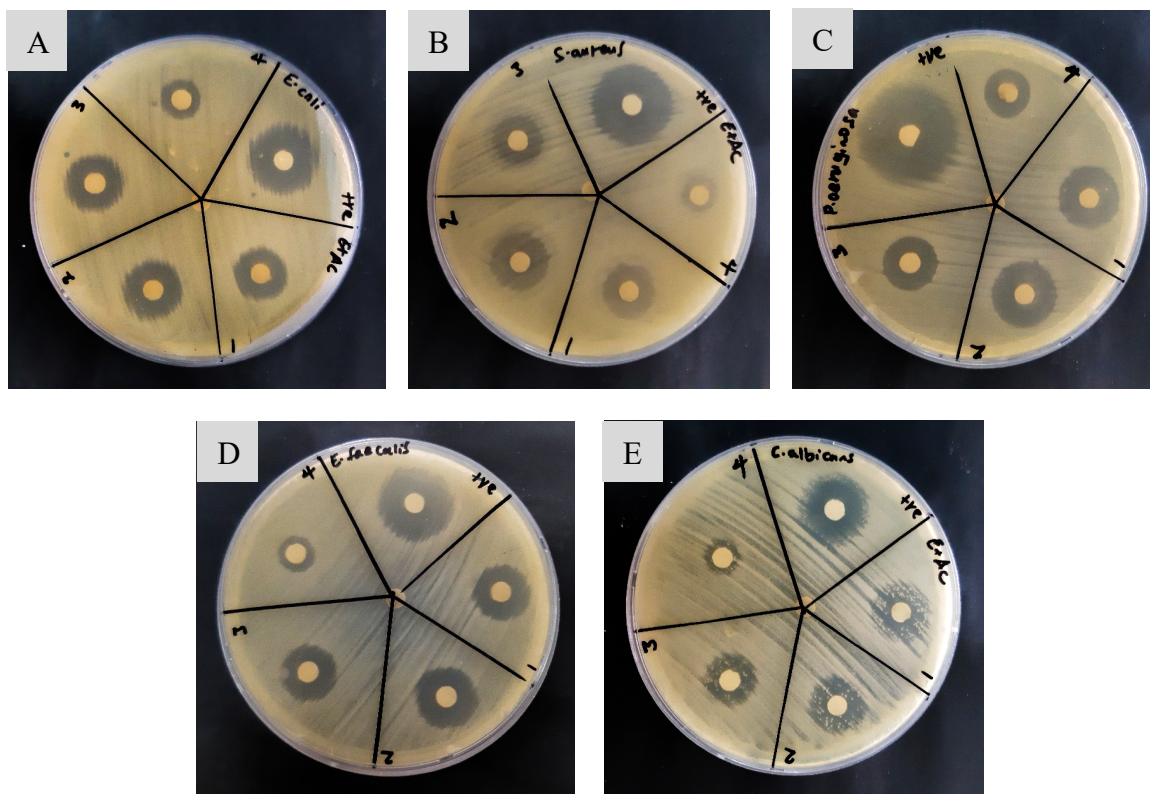


Figure 8: Antimicrobial activity of Crude extracts of Ethyl acetate of *D. stramonium* L. against (a) *E. coli* 25922^T (ATCC), (b) *S. aureus* 25923^T (ATCC), (c) *P. aeruginosa* 27853^T (ATCC), and (d) *E. faecalis* 29212^T (ATCC), (e) *C. albicans* 10231^T (ATCC)

The crude extracts of MeOH leaf extract of *D. stramonium* L. displayed an inhibitory effect against *E. coli* 25922^T (ATCC), *S. aureus* 25923^T (ATCC), *P. aeruginosa* 27853^T (ATCC), and *E. faecalis* 29212^T (ATCC), and *C. albicans* 10231^T (ATCC) strains (Figure 8, Table 5). Crude extracts of MeOH leaf extract displayed the lowest (minimum) inhibition zone at 25 mg/ml against *E. coli* 25922^T (ATCC) (11 ± 0.0 mm) indicating that these strains could develop resistance against lower doses of crude extracts. The type of Gram reaction could be the determinant factor for the resistivity of bioactive compounds. Previous studies also demonstrated that Gram-negative bacteria can efflux drugs and other bioactive compounds than Gram-positive pathogenic bacteria species (Uddin et al., 2021) due to the presence of unique structures like efflux pump proteins making the strain more drug resistant. However, the highest zone of inhibition due to crude compounds of MeOH leaf extracts was obtained for *C. albicans* 10231^T (ATCC) with 23.5 ± 0.5 mm and followed by *E. faecalis* 29212^T (ATCC) with 21.5 ± 0.5 mm for the same concentration of crude compound (Table 5,

Appendix 3). A considerable zone of inhibition due to crude compounds of MeOH leaf extract was noticed for *S. aureus* 25923^T (ATCC) at 200 mg/ml with 21.0±0.0 mm (Table 5, Appendix 3). Similarly, a previous study (Arage et al., 2022) demonstrated that the methanol extract of *Solanum anguivi* exhibited better activity against most bacterial strains, like *Salmonella enterica* serovar *Typhimurium*. The same authors further illustrated that the seed extract of *D. stramonium* L. displayed an inhibitory effect on most test organisms. Very significant ($P=0.000$) and non-significant ($P>0.05$) variations were predicted among the crude extracts of Pet. ether, EtOAc, MeOH, and Cipro. at various concentrations (i.e. 200, 100, 50, and 25 mg/ml) against the recent standard strains in terms of zone of inhibition (mm). (Appendix 3).

The EOs found to inhibit the growth of Gram-positive, Gram-negative, and fungi pathogens (Table 5). The EOs of *D. stramonium* L. may target the microbial cell wall, cell membranes, or other cellular structures. In line with this study, the *D. stramonium* L extracts used to inhibit the growth of *K. pneumonia* (16 mm by chloroform seed extract), *B. subtilis* (24 mm by aqueous seed extract), *E. coli* (13 mm by chloroform seed extract), *S. aureus* (14 mm by methanol seed extract), *P. aeruginosa* (27 mm by methanol seed extract), *E. coli* (16mm by ether seed extract), and *B. subtilis* (12 mm by chloroform seed extract) (Powar & A, 2016). In the present study, considerable inhibitions on pathogens were observed for EOs than other extract compounds against *D. stramonium* L. leaf extract as indicated in Table 5.

The lowest inhibition zone (14.5±0.5 mm at 12.5 mg/ml) was observed for *S. aureus* 25923^T (ATCC), whereas the highest zone of inhibition was noticed for *P. aeruginosa* 27853^T (ATCC) (22.5±0.5 mm at 100 mg/ml) due to EOs obtained from *D. stramonium* L. leaf extract. These inhibitions might be due to the presence of EOs such as 1,5-Heptadien-4-one, 3,3,6-trimethyl-, 2-Furancarboxaldehyde, (3E)-3-Hexen-1-ol, 1,5-Heptadien-4-one, 3,3,6-trimethyl-, (2S,3S)-2-Methyl-3-(2-methyl-allyl)-cyclopentanone, 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-, Diisooctylphthalate and other (Table 5). EOs such as α -pinene (51.21%), germacrene-B (4.80%), and Δ -cadinene (2.56%), which obtained from *Juniperus polycarpus* var. *turcomanica* leaves found to display inhibiting effect on *Alternaria alternata*, *Colletotrichum trichellum*, *Curvularia fallax*, *Cytospora sacchari*, *Fusarium oxysporum*, and *Macrophomina phaseolina* (Mehdizadeh et al., 2020).

4.5.1. Minimum Inhibitory and Bactericidal Concentration (MIC and MBC)

The least MBC detected for *E. faecalis* 29212^T (ATCC) and *S. aureus* 25923^T (ATCC) against EtOAc, MeOH (Table 6) of which 6.25 mg/ml. However, the highest MBC showed by Pet. ether leaf extract for *S. aureus* 25923^T (ATCC) and *P. aeruginosa* 27853^T (ATCC) with 50 mg/ml (Table 6). Higher MBCs were also documented for *E. coli* 25922^T (ATCC), *E. faecalis* 29212^T (ATCC), *P. aeruginosa* 27853^T (ATCC), *S. aureus* 25923^T (ATCC) at 25 mg/ml for these solvents, Pet. ether, MeOH, and EtOAc. Similarly, the 80% methanol extract of the fruit of *S. anguivi* exhibited minimum inhibitory concentration and minimum bactericidal concentration at 1.3 mg/mL and 1.95 mg/mL, respectively (Arage et al., 2022). From the same plants, MIC and MBC were found to range from 6.25 to 12.5 mg/ml (Baynesagne et al., 2017). For instance, maximum antibacterial activity reported for *Staphylococcus aureus* 25923^T (ATCC) (18.2±2.1 mm) using chloroform extract of *D. stramonium* L. and the minimum antibacterial activity was recorded against *Escherichia coli* clinical isolate (8.2±1.8 mm) using acetone extract (Baynesagne et al., 2017).

4.5.2. Minimum Inhibitory and Fungicidal Concentration (MIC and MFC)

Various MFCs were recorded against the recent pathogenic strain, *C. albicans* 10231^T (ATCC) (Table 6). The lowest and highest MFC detected against EOs (12.5 mg/ml) and Pet. ether crude compound (50 mg/ml) as indicated in Table 6 for *C. albicans* 10231^T (ATCC). The same amounts of MIC (12.5 mg/ml) were recorded against crude compounds of EtOAc and MeOH for *D. stramonium* L. leaf extract.

Table 6: MIC, MBC and MFC of crude extracts of *D. stramonium* L. leaf extract

Extracts	MIC, MBC and MFC (mg/mL)									
	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>		<i>E. faecium</i>		<i>C. albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Pet. ether	25	50	25	50	12.5	25	12.5	25	25	50
EtOAc	12.5	25	12.5	25	6.25	12.5	6.25	12.5	12.5	25
MeOH	12.5	25	12.5	25	6.25	12.5	6.25	12.5	12.5	25
E. Oil	6.25	12.5	6.25	12.5	3.125	6.25	3.125	6.25	6.25	12.5

Previous study exhibited that the EOs displayed antifungal activity with the MIC and MFC values against various *candida* species such as *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and *C. auris* CDC B11903 (Ruiz-Duran et al., 2023). The same authors recorded 563 to 750 µg/mL against *C. albicans* ATCC 10231 & *C. parapsilosis* ATCC 22019 and 375 µg/mL for various EOs, of which less concentration than the recent our EOs concentration. This could be due to the effect of various environmental factors that affect the production of EOs, which are determined by certain on and off gene expression. For instance EOs produce enzymes encoded by certain genes such as geranyl diphosphate synthase (GPPS), (1-Deoxy d-xylulose-5-phosphate synthase (DXS), β- caryophyllene synthase (CPS), isopentenyl diphosphate isomerase (IPPI), limonene synthase (LS), and menthofuran synthase (MFS) reported for *Mentha aquatica* L., which contributed for terpenoid biosynthesis (Chegeni et al., 2022).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this study, the following points were concluded. The percentage yield of crude extracts of *D. stramonium* L., leaf was determined and found to be 1.34 % for Pet. ether, 1.75 % for EtOAc, and 7.51% for MeOH. Phytochemical components were evaluated against the leaf extract of *D. stramonium* L. and recognized to be Alkaloid, flavonoid, phenol, saponin, steroid, and tannin. The GC-MS analysis was performed against the crude compounds of *D. stramonium* L., leaf extract and the EOS such as 1,5-Heptadien-4-one, 1-(Trimethylsilyl)-1-propyne, 2-Furancarboxaldehyde, (3E)-3-Hexen-1-ol, 3,3,6-trimethyl-, 1,5-Heptadien-4-one, 3,3,6-trimethyl-, Diisooctylphthalate, 2-methoxy-4-vinyl-phenol, Phenylacetaldehyde, 4-cyclohexyl-, 5-methyl-, (2S,3S)-2-Methyl-3-(2-methyl-allyl)-cyclopentanone, 3-Hexyn-2-ol, 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-, Phenol, and other were predicted. These metabolites are used to give various peaks. The antioxidants were evaluated and a range of 35.88 to 49.16 for Pet. ether, 77.21 to 84% for EtOAc, 71.56 to 79.19 % for MeOH, and 81.67 to 89.10 % EOs were obtained. Antimicrobial activities were carried out against *E. coli* 25922^T (ATCC), *E. faecium* 19434^T (ATCC), *P. aeruginosa* 27853^T (ATCC), *S. aureus* 25923^T (ATCC), and *C. albicans* 10231^T (ATCC) and 11+0.0 to 24+0.0 mm zone of inhibitions were recorded. Various MIC, MBC, and MFC were recorded and 6.25 to 50 of MBC, 6.25 to 25 of MIC, and 12.5 to 25 of MFC ranges were obtained. Hence, *D. stramonium* could be a good source of bioactive compounds.

5.2. Recommendations

The recent study on crude leaf extracts of *D. stramonium* L. have demonstrated antibacterial, antioxidants, and antifungal potential of the plant and could be a promising medicinal plant for potential pathogens controls but further studies are required to know the full potential of the plant. and as a result, the following recommendations are made:

- ❖ The use of different solvents, optimization and utilization of different extraction methods
- ❖ Carrying out more *in vitro* and *in vivo* biological activities
- ❖ Extraction of the active compounds using different chromatographic techniques
- ❖ Characterization of pure compounds using FTIR and NMR

6. REFERENCES

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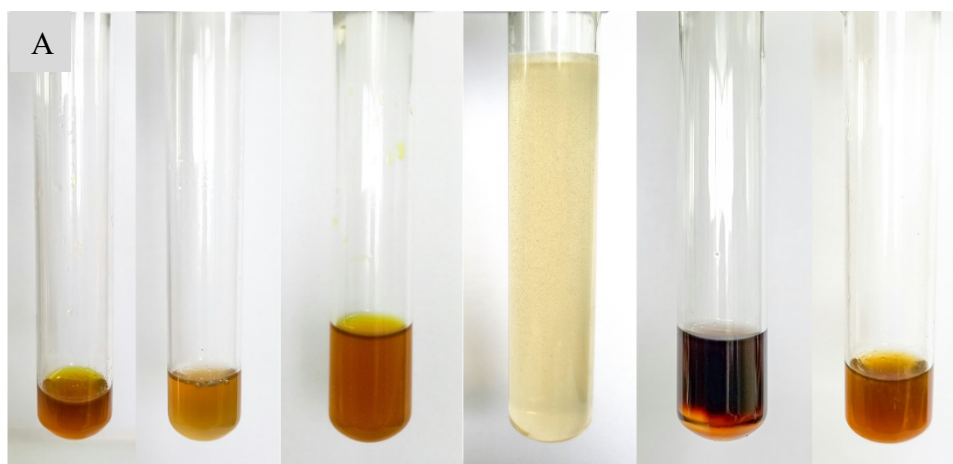
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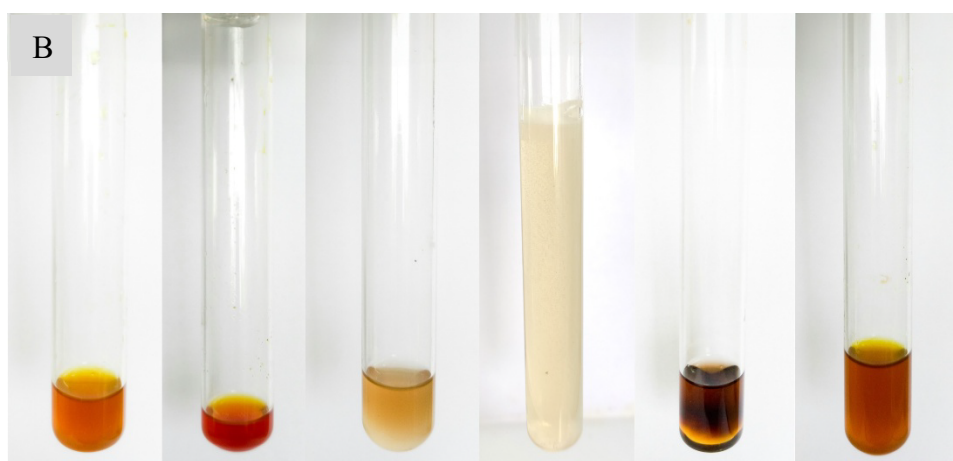
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7. APPENDICES

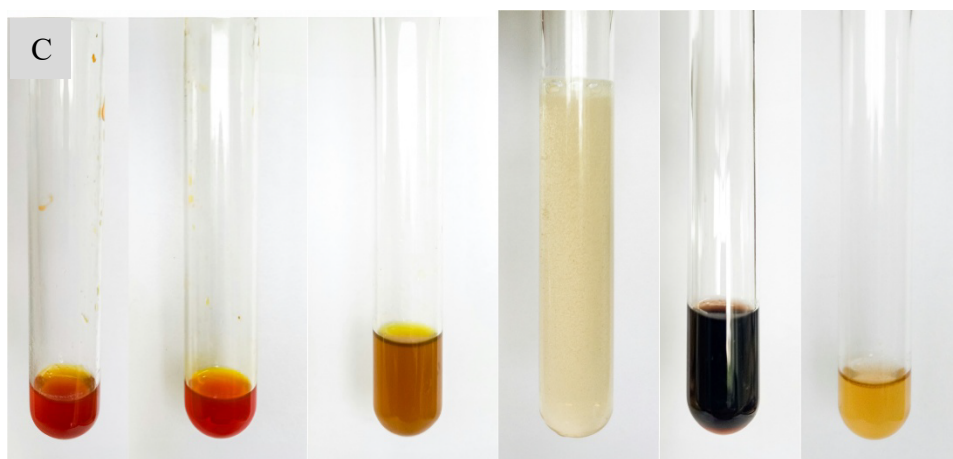
Appendix 1: Phytochemical analysis of crude extracts of *D. stramonium*. (A) Petroleum ether extract, (B) Ethyl acetate extract, (C) Methanol extract



Alkaloid Flavonoid Phenol Saponin Steroid Tannin



Alkaloid Flavonoic Phenol Saponin Steroid Tannin



Alkaloid Flavonoid Phenol Saponin Steroid Tannin

Appendix 2: Essential oil of *D. stramonium*



Appendix 3: One Way ANOVA for Pet. ether crude extract of *D. stramonium* against various pathogens and their multiple comparisons at 0.05% confidence interval (LSD)

Crude extract of Pet. ether against various pathogens		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Crude extract of Pet. ether against <i>E. coli</i> (ATCC 25922)	Crude extract of PE against <i>P. aeruginosa</i> (ATCC 27853 ^T)	-1.000*	.289	.001	-1.58	-.42
	Crude extract of PE against <i>S. aureus</i> (ATCC 25923 ^T)	-2.000*	.289	.000	-2.58	-1.42
	Crude extract of PE against <i>E. faecium</i> (ATCC 19434 ^T)	3.000*	.289	.000	2.42	3.58
	Crude extract of PE against <i>C. albicans</i> (ATCC 10231 ^T)	.500	.289	.091	-.08	1.08
Crude extract of Pet. ether against <i>P. aeruginosa</i> (ATCC 27853)	Crude extract of PE against <i>E. coli</i> (ATCC 25922 ^T)	1.000*	.289	.001	.42	1.58
	Crude extract of PE against <i>S. aureus</i> (ATCC 25923 ^T)	-1.000*	.289	.001	-1.58	-.42
	Crude extract of PE against <i>E. faecium</i> (ATCC 19434 ^T)	4.000*	.289	.000	3.42	4.58
	Crude extract of PE against <i>C. albicans</i> (ATCC 10231 ^T)	1.500*	.289	.000	.92	2.08