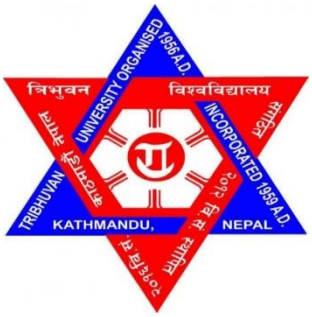
BIOLOGICAL ACTIVITIES OF *EUPHORBIA HIRTA* LINN. EXTRACT AND ITS COMPREHENSIVE SPECTROSCOPIC ANALYSIS



A THESIS SUBMITTED TO THE

DEPARTMENT OF CHEMISTRY

BIRENDRA MULTIPLE CAMPUS

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN CHEMISTRY

SUBMITTED BY:

Sachin Silwal

EXAMS ROLL NO: Chem 1656/075

TU REGISTRATION NO: 5-2-19-421-2014

OCTOBER 2023

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DECLARATION

I declare that this dissertation entitled **"Biological activities of Euphorbia Hirta Linn extract and its comprehensive spectroscopic analysis,"** are my own research work. This work has not been published or accepted and submitted for any degree award. Plagiarism checked at Birendra Multiple Campus Library also confirmed that the work is original and genuine.

Mr. Sachin Silwal

October 2023

RECOMMENDATION

The dissertation entitles **"Biological activities of Euphorbia Hirta Linn extract and its comprehensive spectroscopic analysis," is** submitted by **Mr. Sachin Silwal** for the partial fulfillment of M.Sc. degree in Chemistry at Birendra Multiple Campus. The entire work is completed under our supervision. All the reports presented here are his finding. We confidently recommend this thesis for final evaluation.

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FOREWORD

The thesis work **"Biological activities of Euphorbia Hirta Linn extract and its comprehensive spectroscopic analysis,"** submitted by **Sachin Silwal** as a part of M.Sc. Coursework in Chemistry at Birendra Multiple Campus is carried out under my supervision. Any part of this thesis work has not been submitted for any other degree award.

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LETTER OF APPROVAL

Date: October 11, 2023

On the recommendation of **Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)** and **Bodh Babu Bhattarai, Ph.D. (Asst. Prof.)**, this M.Sc. thesis submitted by **Sachin Silwal** entitled "**Biological activities of Euphorbia Hirta Linn extract and its comprehensive spectroscopic analysis**," is forwarded by Department of Chemistry, Birendra Multiple Campus (BMC) to the office of Dean, IOST, T.U.

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Date: October 11, 2023

This dissertation entitled "**Biological activities of Euphorbia Hirta Linn extract and its comprehensive spectroscopic analysis,**" by **Sachin Silwal**, under the supervision of ***Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)*** and ***Bodh Babu Bhattarai, Ph.D. (Asst. Prof.)*** Department of Chemistry, Tribhuvan University is submitted for the partial fulfillment of the Master of Science (M.Sc.) degree in Chemistry. It is importantly note that this work has not been previously presented to any other institution for pursue any degree.

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ABSTRACT

Biological activities of *Euphorbia Hirta* Linn. extract and its comprehensive spectroscopic analysis was carried out using its methanolic extract. *Euphorbia Hirta* has been used since a long ago as a traditional therapy in many diseases. Hence, biological activities of the plant extract were assayed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is sustainable and affordable approach for the measurement of antioxidant properties. Plant extract was assayed on five biological samples, i.e. *coli, K. pneumonia, P. aeruginosa, B. subtilis* and *S. aureus*. In all biological samples, growth of the biological specimens was inhibited significantly. Total phenolic content (TFC), Total flavonoid content (TFC) and total antioxidant activity (TAA) was assayed using double beam UV-visible spectrophotometer. The analysis of the plant extract revealed the antioxidant, antimicrobial activities of selected plant part. The HR-LCMS analysis of the sample disclosed the phytochemical composition of selected plants parts.

The gummy crude extract of the *Euphorbia Hirta* Linn. was found to contain diverse range of chemical compounds. A Soxhlet extractor was used to prepare the plant extract from which 18.284% extract was obtained from the dried sample of the leaves. The TPC, TFC, TAA and IC_{50} DPPH content in methanolic extract was found 52.22 ± 0.249 mg GAE/g DE, 258.04±2.001 mg RE/g, 166.47±0.231 mg AAE/g and 44.0419 µg/ml simultaneously. Notably, most effective antibacterial activity was reported in *E. coli* in which the extract displayed the inhibition zone of 15.330 ±1.530 mm. The HR-LCMS analysis of the crude extract was found to contain 117 compounds (39 unidentified and 78 identified compounds). Among them, some of these were found very potential for the medicinal aspects, i.e., Lentiginosine, Flurandrenolide, Polidocanol, Irinotecan, Glyurallin B, Hyperoside, Cynaroside, Pibutidine, Syzginin B, Capvipetin D, Ganoderic acid K, Ginsenoside-F_3, Paramethansone, Medicanine etc. The specific characteristics of the molecules make them useful for the treatment of different diseases, i.e. HIV, various types of skin related diseases (skin allergies, itching, swelling etc.), removal of spider veins, typical cancers like colon and rectal, liver, lung and carnival. Besides this, these molecules also work on viral (potential SARC-COV-2 binder) and bacterial infection. Other important aspects of the molecules are: antihypertensive agent, immunomodulating, anti-inflammatory agent, vasodilator agent etc. Whereas 25 compounds are bio-logically active.

Additionally, HR-LCMS inspection unveiled that the plant extract contains various classes of compounds like terpenoids, alkaloids, flavonoids, steroids and tannins. These are active metabolites and are also potential biomarkers for the exceptional biological properties.

To sum up, the extraction of the molecules from the plant as in its natural form depends on the polarity of solvent used in the process. Therefore, this study recommends to extract the molecules in the solvent of different polarity and characterizes them to get all information of the biological molecules.

Keywords: Phytochemical screening, Antioxidant activity, Antimicrobial Activity, DPPH, LCMS spectra analysis.

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LIST OF SYMBOLS

°C	Degree Celsius
A	Alpha
β	Beta
γ	Gamma
M	Mu
%	Percentage
µL	Microliter
µg/mL	Microgram per milliliter

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
ºC	Degree Celsius
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
Cm	Centimeter
DMSO	Dimethyl Sulphoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
D/W	Distilled Water
EC50	Effective concentration, 50%
FCR	Folin-Ciocalteu Reagent
FDA	Food and drug Administration
Fe (II)	Ferrous
g	Gram
g/L	Gram per Liter
GAC	Gallic acid concentration
GAE	Gallic Acid Equivalent
h	Hours
IC50	Inhibitory Concentration for 50% inhibition
kg	kilogram
L	Liter
LC-MS	Liquid chromatography - mass spectrometry

M	Meter
Mcg	microgram
MeOH	Methanol
MHA	Muller Hinton Agar
MHB	Muller Hinton Broth
mg	Milligram
ml	Milliliter
Mm	Millimeter
Mm	Milli-molar
mol.wt.	molecular weight
nm	Nanometer
ppm	Parts per million
RE	Rutin Equivalent
rpm	Revolution per minute
RT	Room Temperature
SD	Standard Deviation
TAC	Total antioxidant capacity
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV	Ultraviolet
V/v	volume by volume
w/v	weight by volume
WHO	World Health Organization
ZOI	Zone of Inhibition

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1 CHAPTER I: INTRODUCTION

1.1 Background

Nature have been recognized and used as medicines by ancient cultures all around the world. More than 80% of the world population relies almost entirely on the plant medicine for primary health care (WHO, 2000). According to WHO, traditional medicine is "Sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences in indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2013). All the pharmaceutical dynamic composites have been emerged from the natural product chemistry (Harvey, 2008). In general, last four decades showed more than 50% of medicinal drugs are close up or far away from plants (Newman & Cragg, 2016). Nepal is well known for its prosperous biodiversity having area of 147,181 km² lies in between China and India. The altitude variation starts from almost sea level (~70 meter) to the top of the world (8848 meter) having climate difference, varied ecological habitats, rich off flora and fauna life. According to the report, Nepal covers 0.1% globe landmass out of which 3.2% of flora and 1.2% of fauna respectively (GON, 2014). The country has its total 6973 plants species out of which 10% (700) are of medicinal kind (Schippmann et al., 2002), Which are distinct with specific adaptation features.

Biodiversity has direct benefits to the mankind in the form of food, medicine, dietary supplements, chemical potential for synthetic and pharmaceutical resources, timber, ornamental plants and animals (Balunas & Kinghom, 2005). The major concern regarding natural products is to move to action by their fruitful uses in drugs, dyes, polymers, oils, perfume, wax, glues and fibers (Gewali & Awale, 2008). It plays vital roles in maintaining human and animal health. A wide variety of plants and funguses are used as medicines, essential vitamins and painkillers etc. The medicinal potency of these plants lies on the phytochemical constituents present therein. These phytochemicals exhibit specific pharmacological effect on our body. They are natural compound found in medicinal plants, vegetables and fruits in conjunction with nutrients and dietary fibers. The main theme of

this project is to explore central key constituents present in natural product (plant extract) and their medicinal activities.

1.2 Natural Products & Drugs

All chemical constituents obtained from the natures (living organisms) are known as Natural products (NPs). It has been predicted that only 10% of biodiversity was evaluated having medicinal features (Dias et al., 2012). The study conducted in the era between 1970-1980, people are engaged to cure different types of diseases in human using western pharmaceutical medicine and plant derived active ingredients (Newman et al., 2003). Later on, pharmaceuticals research progress seems quite slow (Koechn et al., 2005). People have been directly or indirectly benefited from natural products because of its easy availability, economic cause, oral prescriptions from the local people and lesser side effect on human health. Natural products encompass a broader range of chemical diversity compared to synthetic small molecules libraries (Lachance et al., 2012). In early 18^{th} century, Anton Von Storck alarmed the toxic function of aconite & colchicum which helped in the 19^{th} century to strongly participate in exploring the medicinal plants and so on. Historically, a 21-year-old German pharmacist's Friedrich Sertuner successfully isolated the morphine, a pure active compound from the poppy plant, *Papaver somniferum*, which was derived from the opium obtained on cutting seed pods of poppy plant and used as analgesic and sleep-inducing agent (Atanasova, et al., 2015). The significant expansion of the pharmaceutical drug research was held at the end of Second World War and primarily driven by prominent finding of penicillin. This expansion encompassed extensive screening of microorganism to identify novel antibiotics (Li & Vederas, 2009). By 1990, approximately 80% of medication comprised either natural products or derivatives inspired by them (Li & Vederas, 2009). Some of the important clinical medicines derived from plants are given below:

Table 1-1: List of some plant-derived drugs and their therapeutic actions

S. N	Plant species	Drugs	Therapeutics	References.
1	*Papaver somniferum* L	Apomorphine hydrochloride (Apokyn®)	Alzheimer's disease	(Dias et al., 2012)
2	Catharanthus roses (L.) G. Don	Vinblastine Vincristine	Anti-cancer	(Anand et al., 2019; Dias et al., 2012)
3	Cannabis sativa L	Cannabidiol	Epilepsy-dystonia, Parkinson's disease	(Anand et al., 2019; Dias et al., 2012)
4	Taxus brevifolia Nutt. & Taxus chinensis Pilg.) Rehde	Paclitaxel	Anticancer	Dias et al., 2012)
5	Artemisia annua L	Artemisinin Quinine	Antimalarial	(Numonov et al., 2019)
6	Digitalis purpurea L	Digoxin Digitoxin	Heart	(Whayne, 2018)
8	Solanum xanthocarpum	Lovastatin & Analogs	Lipid control Cholesterol	D0I:10.1080/14786419.2015.1016938

Source: Different articles and journals data

Plant-based drugs helped us to increase our life expectancy. The intermediate end products formed in plant through cellular metabolism are called metabolites, which generate as a result of enzymatic catalysis processes. Those metabolites which are necessary for plant growth and development are primary metabolites whereas number of small molecules outgrowth by the plants, which are not required for their growth and development (i.e. non-essential chemical constituents) but have an important character in defense properties against pathogens, insects and predators are called secondary metabolites. The concentration of secondary metabolites varies from plant to plant because of variation of soil type, pH, temperature (bioenvironmental variation of location), irrigation quality, soil microbes etc. The key constituent present in plant (i.e. Secondary metabolites) plays

significant role to maintain healthy immune for prosperous care of a lot of illness. As a result, crude extract or semi-synthetic form was focused for the finding of modern medicines (Zafar et al., 2021). The diverse range of bioactive metabolites, i.e. terpenoids, alkaloids, flavonoids, glycosides, steroids, and phenolic compounds, present in the plant extract had made remarkable attention for making effective plant extract and played significant role in the development of pharmaceuticals (Ouerghemmi et al., 2017). Extensive active constituents are underway to explore from diverse source, including both terrestrial and marine environment (Harvey et al., 2015). Unfortunately, the prioritization of harnessing the therapeutics potential and usage of these plant-based compounds has been lacking, which hampers the growth of quality life (Dhami, 2008). Now a day, researchers are focusing in the pursuit of unique antimicrobial properties. Scientist are actively involved in both exploration of new natural product and refinement of exciting natural product classes, capitalizing and advancement on biosynthetic engineering (Lešnik et al., 2015), total synthesis (Kling et al., 2015) and semi synthetic approaches (Shaeer et al., 2019; Smith et al., 2018).So that, natural product possesses distinct features compared to conventional synthetic molecules, presenting both benefit and threat within the drug uncovering procedure (Atanosoy et al., 2021).Undoubtedly, natural products have played and will continue to play a significant action as valuable reservoirs for discovery of unique pharmaceutical compounds (Lahlou, 2007).

1.3 Oxidative stress and Antioxidant

Oxygen is potent oxidizing agent. It easily units with variety elements, compounds and generates oxides in the process. It exists in the airspace in its lowest energy state as stable triplicates biradicals ($^3O_2$), participates in step wise deduct procedure (Taslimi & Gulçin 2018; Rezai et al., 2018). Ground state O_2 exhibits two parallel spins situated in different antibonding orbitals. These spins restrict its capability to receive a pair of electrons from an electron donor. In contrast, redox reactions are vital metabolic process within the living organism, facilitating the exchange of electron between various entities. All these reaction procedures happen in our biological system as a series of chemical chain reaction known as oxidation process. The utilization of the oxygen derived from the air ultimately leads for the production of ATP, a vital energy source for the organism (Gulcin et al., 2012). A radical species will be produced when an electron flow in the biological system becomes unpaired

(single electron). This seems major challenge for the biological system. Unstable molecules features are atoms, molecules, or ions that possess radical elements which are extremely unsteady and prone to engage in chemical reaction with other molecules. Oxygen, nitrogen and sulfur are the potent element that can produces free radicals in the biological system and have negative impact on living organism (Gulcin et al., 2020).

The metabolic by products formed in biological (cellular) process are commonly known as reactive species i.e., reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant cell that generates oxygen radical and their derivatives are called as reactive oxygen species (ROS). Reactive oxygen species consist of both free radical and non-free radical oxygenated molecules, (i.e., superoxide anion, O_2 •−; hydroperoxyl radical, HO_2•; alkoxy radical, RO•; and hydroxyl radical, •OH; hydrogen peroxide, H_2O_2 and singlet oxygen, $^1O_2$) (Hasanuzzaman et al., 2021 and Pioschi et al., 2015).

Reactive nitrogen species (RNS), along with reactive oxygen species (ROS), including iron, copper, and sulfur species, are greet in biological systems (Halliwell & Gutteridge, 1992). The primary cellular sites for ROS generation are chloroplasts, mitochondria, peroxisomes, apoplast, and plasma membranes (Singh et al., 2019). Key enzymatic reactions catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase (MPO), and xanthine oxidoreductase (XOR) contribute significantly to ROS production in living cells (Bayir, 2005). These ROS are involved in michondrial respiratory chain reactions, prostaglandin synthesis, phagocytosis, and the cytochrome 450 system (Pizzino et al, 2017).Similarly reactive nitrogen species (RNS) such as nitric oxide (NO) and peroxynitrite ($ONOO^-$) are present in the cardiovascular, nervous, and immune systems and have been implicated in various pathological conditions, including neurodegenerative disorders (Parkinson, Alzheimer, Huntington's disease and amyotrophic lateral sclerosis), emphysema, cardiovascular and inflammatory diseases, cataracts and cancer (López-Alarcóna et al., 2013). The presence of both ROS and RNS highlights their significance in physiological processes and their involvement in the development of several diseases. The increase in free radical can cause Oxidative stress i.e., cell death due to damage of carbohydrates, proteins, lipids, RNA and DNA (Isik et al. 2017; Krawczyk, 2019). When the production of ROS and RSN species surpasses the cellular defense mechanism, i.e., antioxidant capacity, it leads the condition known as oxidative stress. According to Helmut Sies, imbalance between the number of antioxidant and free radical

is the cause of oxidative damage in biological systems (Forman & Zhang, 2021). Thus, oxidative stress is thought to be main cause of various illnesses. ROS have high reactivity due to their uncoupled electrons presence in their molecular structure, engaging in interaction with innumerous cellular macromolecules like carbohydrates, proteins, nucleic acids and lipids which result in the influence of their functionality (Birben et al., 2012). Oxidative stress plays a notable role in the development of inflammatory diseases such as cancer, autism, cardiovascular diseases, Alzheimer's, aging and cataracts (Rahaman, 2007). The advancement of proteomics, genomics and metabolomics in the future will facilitate a complete understanding of a biological network for cellular response to oxidative stress.

In biological cells, presence of free radicals is neutralized by special substance either accepting or donating electrons or by active hydrogen atom called antioxidant which help to prevent or delay of cell damage or oxidative stress. Antioxidants are featured as "any substance that retards, inhibits or eliminates oxidative damage to a specific target molecule" (Halliwell, 2000). Some antioxidants like resveratrol and curcumin are known to enhance cardiovascular health (Banez et al., 2020) & polyphenols have ability to reduce the risk of cancer (Shahidi & Amibigainpalan, 2015). Both internal and external source fulfill these antioxidant (Khalid et al., 2014). The human body itself employs strategies to combat oxidative stress and free radicals through utilizing enzymatic (e.g., CAT, GPX and SOD) and non-enzymatic (i.e. gluthathione, co-enzyme Q10, Lipoic acid and L-arginine) endogenous antioxidant. Additionally, exogenous antioxidants from plants or animals' sources are introduced into the human body through diet or natural supplement (Pizzno et al., 2017). The antioxidants compounds are synthesized as secondary metabolites in plants. The non-nutritive chemicals constituent found in plants offer array of health benefits and plays a significant role in disease prevention (Ahmed et al., 2014).

Synthetic antioxidants like BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole), TBHQ (Tertiary butylhydroquinone), PG (Propylene glycol), etc., are not recommended for pharmacotherapeutic uses due to their potential health hazards. Plant-based antioxidant diet is supposed to be the supplementary options for treatment. The antioxidant compound must have the following two characteristics:

- It should effectively inhibit the substrate's oxidation even in small concentration relative to oxidizable substrate.

- Antioxidant should prevent the chain propagation reactions of polyphenol radical (Hallwell, 2007).

The aim of this study is to evaluate the quantative analysis of different antioxidant's present in Euphorbia Hirta plant and the properties of efficient antioxidant.

1.4 Anti-microbial Activity

The rise of antimicrobial resistance has paid attention on efficacy of currently available medicine. Frequent use of antibiotics, malpractice in antibiotics use and overdose has contributed to emergence of drug resistance strain. All bacteria possess an inherent capacity to develop drug resistance through mutations in their chromosomal genes, enabling them to withstand specific antibiotics (Blair et al., 2015). In general, anti-biotics resistance pathogens ensue significant illness and death rate, imposing a substantial economic burden on health care system. In our environment, multidrug resistance occurs through the contaminated soil, air and water as well as the waste generated from hospital, clinics, university research lab, livestock farms and many more sources that contain numerous antimicrobials in which the bacterial growth continues through mutation to acclimate in the changing environment and proliferate multi-drug resistant (MDR) bacteria (Khare et al., 2021). Antimicrobial resistance is the global issues of public health. Various multi-drug resistance strains such *as enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species* and others are posing several clinical issues on both human and animal populations. Studies on pharmacology & traditional medicine showed that fresh crude plant extract have high antimicrobial activity. Plant extract can be prepared in cheap cost and have quality treatment due to low rate of side effect. Plant parts such as root, bulbs, rhizome, steam, bark, flowers, fruits and seeds contain different kinds of phytochemical that may exhibit antimicrobial properties (Karalija et al., 2018). In Nepal, the actual extent of antimicrobial resistance data remains unclear due to insufficient research, lack of government policies and scientific publication (Dahal & Chaudhary, 2018). To address this, world health organization (WHO) organize a series of coordination efforts to prevent transmission and support the research to investigate innovative strategies against infection (Barbieri et al., 2017). Varity of bioactive compound derived from plants have been extensively used in combating broad spectrum of pathogens. Approximately 500,000 species within the plant kingdom have been investigated for their antimicrobial properties (Mickymaray, 2019).

Researchers have shown significant interest in screening traditional plant extracts to discover novel bioactive compounds that can effectively treat microbial infections.

1.5 Research (Exploration Goal) Objective

Objectives:

The objectives of this research are as follows:

i. To collect Euphorbia Hirta Linn plant sample.
ii. To prepare the methanolic extract of plant using Soxhlet extractor.
iii. To estimate the amount of total phenolic content (TPC) and total flavonoids content of the crude extract.
iv. To analyze the biological properties of the plant extract on the Pathogenic bacteria.
v. To determine antioxidants properties of extract.
vi. To make chemical profiling of the plant extract using HR-LC-MS.

1.6 Rationale

Herbal plants played a pivotal role as traditional healers, addressing wide array of therapeutic remedies from ancient times. Now a day's huge number of natural products is applied in synthesis of modern medicine. The therapeutics characters of plant likely to be originated from antipyretics, antimicrobial and antioxidant effect (Cowrn et al., 1999). These features are actually coming from secondary metabolites which resolve several diseases. According to International Union for Conservation of Nature (IUCN), numerous significant plant floras are going to be endangered because of widespread exploration, habitats loss, deforestation and inadequate awareness of their values. This situation alarms the need of their conservation (Ramakrishnan et al., 2017). Proper strategies planning, scientific management & application practice, engagement of governmental bodies and scientific documentation are must necessary parts for understanding medicinal & pharmaceutical use in development of medicinal drugs. Contemporary research is mainly concentrated on screening of plant and analysis of their important properties regarding healing of different diseases and toxicity. This research will focus to discover the biological assay of plant extract, evaluation of phytochemical properties and quantitative investigation of their medicinal activities. The sophisticated instrumental analysis will help to understand and elaborate the potential role in identifying drug suitable for curing various illnesses.

2 CHAPTER II: LITERATURE REVIEW

Literature Review

Nepal has rich diversity of indigenous herbal and toxic flora. Local people of the particular area utilized the local medicinal plant to address the different health issues and ailments. Medicinal plants are used traditionally for rituals, ceremonials, spirituals, and dietary, pharmaceuticals as well as nutraceuticals applications. The deep rooted traditional medicinal knowledge is intertwined in different ethnic groups inhabiting particularly in villages (Thorsen & Pouliot, 2016) The traditionally used medicinal plants have substantial biological properties, i.e. antimicrobial, antioxidant, antidiabetics, anticancer, antipyretic, analgesics and many more actions. Hit and trial methods are utilized in herbal plants by the local people to solve their health-related issues. Recently, synthetic chemistry, retrosynthetic chemistry and combinatorial chemistry has been utilized for production of allopathic medicines along with the development of the industry. However, these drugs not only have some side effects but also increases hazardous in the environment. Therefore, medicinal plant and plant-based products or extract serve to compelling and promising alternative for therapeutic uses.

The majority of medicinal plants still need investigation to disclose their hidden potential, i.e. Medicinal activities. There is no enough research in the medicinal plants of different regions of Nepal. However, very few scientific publications of scholars documented the characteristics of few medicinal plants. Extensive literature review on *Euphorbia Hirta* is carried out using google scholar, PubMed, X-MOL, Scopus, CAS, Scifinder, Reaxys, Semantic Scholar, Base, Core etc.

2.1 Introduction

Herbal medicinal products are derived from secondary metabolites synthesized by plant from primary metabolites. The growing concern over antimicrobial resistance and drug side effect has led to increase interest in herbal medicine as an alternative to synthesize drugs. Medicinal plants are endemic and are the potential source of many natural antioxidant (Exarchou et al., 2002). Medicinal value of the plant relies on the content of the bioactive compound such as alkaloids, tennis, flavonoids and phenolic compounds. This study focuses on assessing the chemical and phytochemical parameters and antioxidants activity of E. Hirta. This review is conducted on E. Hirta under the following headings;

General Overview of plant

Euphorbiaceae is the third largest genius of flowering plants and belongs to a family spurge. It contains around 300 genera and over 7500 species. They are mostly distributed in tropical and subtropical regions. Euphorbiaceae exhibits wide range of ecological adaptations along with varied characteristics including succulence, drought tolerance and some species contains toxic compound. Some species also have economical & ornamental value, while other can be poisons and should handle with care. This diversity and adaptability make it an interesting subject to study for botanist or plant enthusiasts.

One of the well-known genus of Euphorbiaceae is Euphorbia, which has varied characteristics such as succulent and non-succulent plants called "Euphorbias". Euphorbia has more than 1600 species (Basyal et al., 2021). In Nepal, Euphorbia Hirta Linn is promising examples among the 1600 species of Euphorbia. Locally, Euphorbia Hirta is also known as "Asthma Plant" or "Snakeweed" or "Dudhi Jhar" in Nepali. This is small annual herbaceous plant native to tropical and subtropical region in the world.

Scientific classification of *E. Hirta L.*

Table 2-1 Taxonomy of Euphorbia Hirta

Euphorbia Hirta	
Kingdom	Plantae
Sub kingdom:	Viridaeplantae
Infrakingdom:	Straptophyta
Phylum:	Magnoliophyte
Division:	Tracheophyte
Subdivision:	Spermatophyte
Infra-subdivision:	Angiosperms

Class:	Magnoliopsida
Order:	Malpighias
Family:	Euphorbiaceous
Genus:	Euphorbia
Species:	Euphorbia Hirta
Botanical Name:	Euphorbia Hirta Linn
Common Name:	Asthma plant
Synonym:	Euphorbia capitata lann, Euphorbia pilulifera jacq, chamaesycehirta (l.) millsp [Al-Snafi & AE Asha, 2017]

Source: From Different articles and journals, book

Morphology

E. Hirta L. is a rhizomatous reddish-green or purple hairy slender-steamed, erect or ascending herb of about 60 cm long. The foliage is about 1-2.5 cm in length. Generally, Leaves exhibits elliptical in shapes and are arranged oppositely with each other, featuring sub-sessile, scattered edge and displaying dipper green color on their upper surface, while the lower surface appear pale in color (Roland Nâg-Tiéro Meda et al., 2023; Basma, et al., 2011).

Habit and Habitat

E. Hirta L. stands as an annual flock medicinal herb renowned for its diverse therapeutic attributes, thriving across multiple tropical continents encompassing Asia, America and Africa (Nacoulma, 1996). It is widely distributed in plane tropical and sub-tropical region having banks of watercourses mostly in roadside, rice field, open grasslands, and pathways (Mamun-Or-Rashid, et al., 2013;). They are found in wetland with slightly dry condition up to 2000m altitude from sea level.

Ethnobotany and Pharmacological Properties

Euphorbia Hirta, commonly known as "Asthma plant" is traditionally used as a medicinal herb across the globe reflects its biological and pharmacological properties. Its significance as traditional medicine is still evident in various regions.

In China, the plant healing properties were first documented in the "Ling Nan Yao Lu". E. Hirta has widespread traditional use and was included in Chinese pharmacopeia in 1977. It has been employed to address skin ulcer and body swelling. Indian traditional medicine system employs E. Hirta in treating worm infections in children, as well as dysentery, digestive problem, jaundice, tumors, gonorrhea, pimples, in constipation (K.K. Kirtikar & Basu, 1991). Its leaves are used for the treatment of kidney stones, cold, syphilis, cough, bronchial infection, asthma and fevers. Additionally, it has been used as an antidote and pain reliever for scorpion stings and snake bites (Yuet et al., 2013). Indonesian widely uses it to care gastrointestinal issue, respiratory diseases, skin aliments and connectives (Kumar S & Kumar D., 2010). *E. Hirta* L. has been employed as remedy for vomiting, diarrhea and as anti-venom against snakebite (Amos Samkumar et al., 2019). E. Hirta plants are applied for ear diseases, sore treatments, wound healing, (Tuhin et al., 2017), edema (Johonson et al., 1999), gastrointestinal and skin diseases (Rodríguez-Pérez et al., 2019).

Pharmacological investigation has demonstrated that *Euphorbia Hirta* extract exhibits broad spectrum as antimicrobial, antioxidant, sedative anxiolytic, antiepileptic, anti-inflammatory, Anti-asthematic, antipyretic, analgesic, anti-diabetic, antihistaminic, anticancer, gastrointestinal, immunological, hepatoprotective, anti-parasitic, glactogenic, angiotensin converting enzyme inhibiting and anti-dipsogenic activities (Al-Sanif, 2017). Previous studies have disclosed some active constituents of *Euphorbia Hirta* and their pharmacological action. Earlier work on *E. Hirta* also make the comprehensive listing of some active molecules present in its extract along with their specific properties (Uddin et al., 2019; Ghosh et al., 2019; Kumar S & Kumar D., 2010).

Chemical Constituents of E. Hirta L.

The examination of leaf extract through phytochemicals analysis unveiled the existence of various compounds including carbohydrates, steroids, reducing sugar, alkaloids, saponins, tennis, proteins, fats, oils, mucilage, coumarins, carotenoids, anthraquinones and

chlorophyll (Haleshappa et al., 2020). Six compounds were successfully extracted from E. Hirta leaves and characterized as gallic acid, myricitrin, quercetin, 2,4,6-tri-O-galloyl-D-glucose, 1,2,3,4, 6-penta-O-galloyl-beta-D-glucose and 3,4-di-O-galloylquinic acid [Chen L., 1991]. Further ten more compounds were detected within the methanolic leaf extract of E. Hirta. These compounds were identified as methyl 14-methylpentadecanoate, 17-carboxyheptadec-9-en-1-ylium, 2-amino-3-sulfanylpropanoic acid, 2,3,5-trimethyl-1 H-pyrrole; niacin or nicotinic acid, 4-amino-4-oxobut-2-enoic acid, S-methyl-L-cysteine, palmitic acid and chloromorpholin-4-ium (Igwe et al., 2016). Successfully isolation of Afzelin (I), quercitrin (II) and myricitrin (III) from methanolic extract of E. Hirta make attention to the researchers (Liu Y et al., 2007).

Antioxidant Activity of E. Hirta L.

The antioxidant activity of leaves extract showed more pronounced effect on the assessment of DPPH scavenging activity relative to its blossoms, roots and steams. The relative IC_{50} values of different parts of the plant were: leaves 0.803 mg/mL, roots 0.989 mg/mL, flowers 0.972 mg/mL, steam 1.358 mg/mL. Notably, the reference compound butylated hydroxy toluene (BHT) exhibited 0.794 mg/mL in response to the plant extract. The reducing power exhibited by the leaf decoction of *E. Hirta* was observed to be contingent on its concentration, mirroring the behaviors of ascorbic acid. Further analysis of methanol decoction of *E. Hirta* leaves through phytochemical testing unveiled the several bioactive constituents such as phenolic compounds, alkaloids, flavonoids, terpenoids, tannins, steroids and reducing sugar. *E. Hirta* may have potent antioxidant properties, according to the reports (Basma et al., 2011). Methyl-3-(3,5-ditertbutyl-4-hydroxyphenyl) propionate, a hydroxyphenyl carboxylic acid esters shows antioxidant activity by DPPH test. *Euphorbia Hirta* plant displayed notable values of total Phenolic Content (TPC). Total Flavonoid Contents (TFC) and DPPH scavenging activity was observed as 109.86±1.38 mg (GAE/g DE), 18.92±1.33 mg (QE/g DE) respectively whereas IC_{50} was measured 17.26±1.33 µg/mL (Ngan Tran, et al., 2020).

Antimicrobial Activity of *E. Hirta* L.

The antimicrobial efficacy of the methanolic extract derived from various parts of *E. Hirta*, i.e. leaves, steam, flowers, and roots, was systematically assessed against a panel of eight bacteria encompass four Gram positive strain (*S. aureus*, *Mycobacterium species*, *B. subtilis*, and *B. thuringenis*) and four Gram -negative strains (*E. coli, K. pneumoniae, S. typhi*, and *Proteus mirabilis*). Remarkably, the decotation prepared from the leaves exhibited a substantial zone of inhibition against all tested microorganism, underscoring its potent antibacterial activity (Kumari & Pandey, 2017). Crude extract of *E. Hirta* in methanol, hexane, and distilled water were tested against five bacterial strains (*E. coli, bk. Pneumoniae, S. dysentery, S. typhi* and *P. mirabilis*) using the well diffusion method and it was observed that the aqueous extract demonstrated the highest zone of inhibition against all bacteria, followed by the methanol and hexane extract (Abubakar, 2009).

2.2 Study conducted in Nepal

The antibacterial properties of both water and methanol extract from *E. Hirta* have been reported in different studies (Mahmood, 2009 and Vijaya et al., 1995). Additionally, these extracts have demonstrated effectiveness as expectorants (Adeddapo et al., 2005) and bronchodilators (Joshi, 2011). This finding aligns with traditional use of plant in treating respiratory ailments (Kuwar et al., 2010). The quantitative assessment of the ethyl acetate extract of E. Hirta showed that total phenolic (TPC) and total flavonoid (TFC) was found as 288.10 mg GAE/g, and 29.36 mg QE/g respectively. Its DPPH radical scavenging activity exhibits as an IC_{50} value of 32.23 µg/mL suppressing the standard ascorbic acid (IC_{50} =32.23 µg/mL ($p<0.05$)) (Basyal et al., 2021).

Moreover, the methanol extract of *E. Hirta* demonstrated notable antimicrobial properties exhibiting zone of inhibition (ZOI) of 10 mm against *S. aureus*, 12mm against *B. subtilis*, 10 mm against *S. Typhi* and 10 mm against *E. coli* (Sharma, 2020). The overall study of the *E. Hirta* disclosed that it is a potential source of potent antioxidant and antimicrobial agent.

3 CHAPTER III: MATERIALS AND METHODS

3.1 Chemicals and equipment

All chemicals i.e., standard and reagents used in this work were of analytical grade with high purity and distilled water (DW) was collected from small scale glass distillation set present in the chemistry laboratory of Birendra Multiple campus. DPPH (Sigma-Aldrich's. St. Louis, USA), DMSO (Merck Life Science Mumbai India), ascorbic acid (Merck Mumbai India), absolute methanol (Merck Mumbai India), Sodium carbonate (Merck Mumbai India), Sodium Hydroxide (Merck Mumbai India), Sodium Hydroxide (Merck Mumbai India), Ferric chloride hexahydrate (Merck Mumbai India), Sodium acetate (Merck Mumbai India),Potassium acetate (Merck Mumbai India), Hydrochloric acid (Thermo-Fisher Scientific India, Pvt. Ltd.), Sulphuric Acid (Thermo-Fisher Scientific India), Ethanol (Thermo-Fisher Scientific India), aluminum trichloride (Thermo-Fisher Scientific India) and double distilled water was brought from local vendor. Besides these, chemicals like Gallic acid (Loba Chemie Pvt. Ltd., Mumbai India), Rutin (Loba Chemie, Mumbai India), Nutrient Agar (Hi-media Pvt. Ltd, Mumbai India), Muller Hinton Agar (Hi-media, Mumbai India) and other analytical reagents were used without further purification.

Borosilicate glass wares were used to perform the chemical test and preparation of sample solution in order to avoid the contamination. The equipment and instruments used in this thesis work were Digital weight balance(S.N. AE9YZ251 Adam Equipment Co. Ltd. UK), Electric grinder (BAJAJ EASY 500 Mixer GRINDER, 410153, S.N. P101 1115 97468), Soxhlet (Omsons Glassware, Germany), Hot air oven (Italy, Torre de Picenardi,(CR), model PANACEAA 430, N Series15810, Rotatory Evaporator (IKA MV 10, HB Digital, Staufen, Germany), Digital water bath (Clifton, Nickel etrol Ltd, England S.N. 45508), Refrigerator,0.2-micronnylon Syringe filter and No.1 Whatman filter paper along with these, colorimetric measurement was carried out using double beam UV-visible spectrophotometer (T80+, PG Instruments, UK) (S.N. 28-1885-01-0118) at Birendra Multiple Campus, Chitwan, Nepal) as in convergence with 1cm quartz cuvette. Phytochemical profiling was performed with the help of Centre for Research in Nanotechnology and Science (CRNTS), Sophisticated Analytical Instrument Facility

(SAIF), Indian Institute of Technology, Bombay, 400076, India using HR-LCMS instrument.

Software

MS Power Point, MS word and MS Excel were used to interpret and analyses all information regarding this research. iThenticate (a facility of Turnitin software) is used for plagiarism check and Mendeley for reference manger. The software Origin Lab Origin pro was used for the analysis of the data.

3.2 Plant Extract

Collection of plant *(Sampling and study area)*

The Samples were collected at flowering stage in mid-summer 25th July to 5th August 2022 (around 10 days) from Khairahani-10 (Kumroj) &11 (Sundi, Gaidha, Pidrahni) Chitwan, Bagmati Province (No.03) Nepal. It was collected from two different GPS point (27˚34'15" N 84˚35'29" E) and elevation at 185 m to 200m from sea level in southern plain part and collected in clean Gripper bag.

Identification of the Plant

For the identification and authentication of the plant specimen, it was sent to the office of National Herbarium & Plant Laboratories (NHPL) Godawari-05, Lalitpur, Nepal in August immediately after collection.

Drying, grinding and storage of plant sample

All the collected plant parts were properly washed in running tap water to remove any coarse materials and finally rinse with distilled water. The plant parts were chopped into a small piece using stainless steel scissor and shed dried at ambient room temperature. Sample were protected from sunlight and left them to dry for 3-4 weeks at room temperature (24-30ºC) until the constant weight of samples were obtained. When the constant weight of the samples after drying were conformed, it was sealed in neat and clean zipper bag (polythene bag) and kept inside desiccator until extraction in order to avoid moisture absorption.

Preparation of methanolic plant extracts / (*Extraction Procedure)*

Dried plant sample was pulverized into powder using electric blender. Subsequently Coarse powder will pass through 60- mesh size screen /sieved to ensure homogenous powder size. The dried powder form will store in air tight glass container and put away from sunlight in cool and dry place at 4ºC (in refrigerator) until further use.

The coarsely powdered samples were packed into the thimble 25mm × 80 mm (100g) and extracted with Soxhlet extractor using polar solvent i.e., methanol (300 ml) (Chandrana et al., 2017). The process was continued till the color changes from dark green to light green for complete extraction (or about 96 hours). Boiling point of solvent in Soxhlet was set according to the boiling point of solvents mentioned in polarity index table.

Filtration of the crude extract

Thus, obtained crude extract was then filtered by using Whatman No.1 (0.45µm) filter paper to detach all the unwanted solid particles through simple filtration.

Solvent Evaporation

The methanol extract was concentrated in a rotary evaporator at 650mbar and 40˚C and then dried in hot water bath.

Storage of dry extract

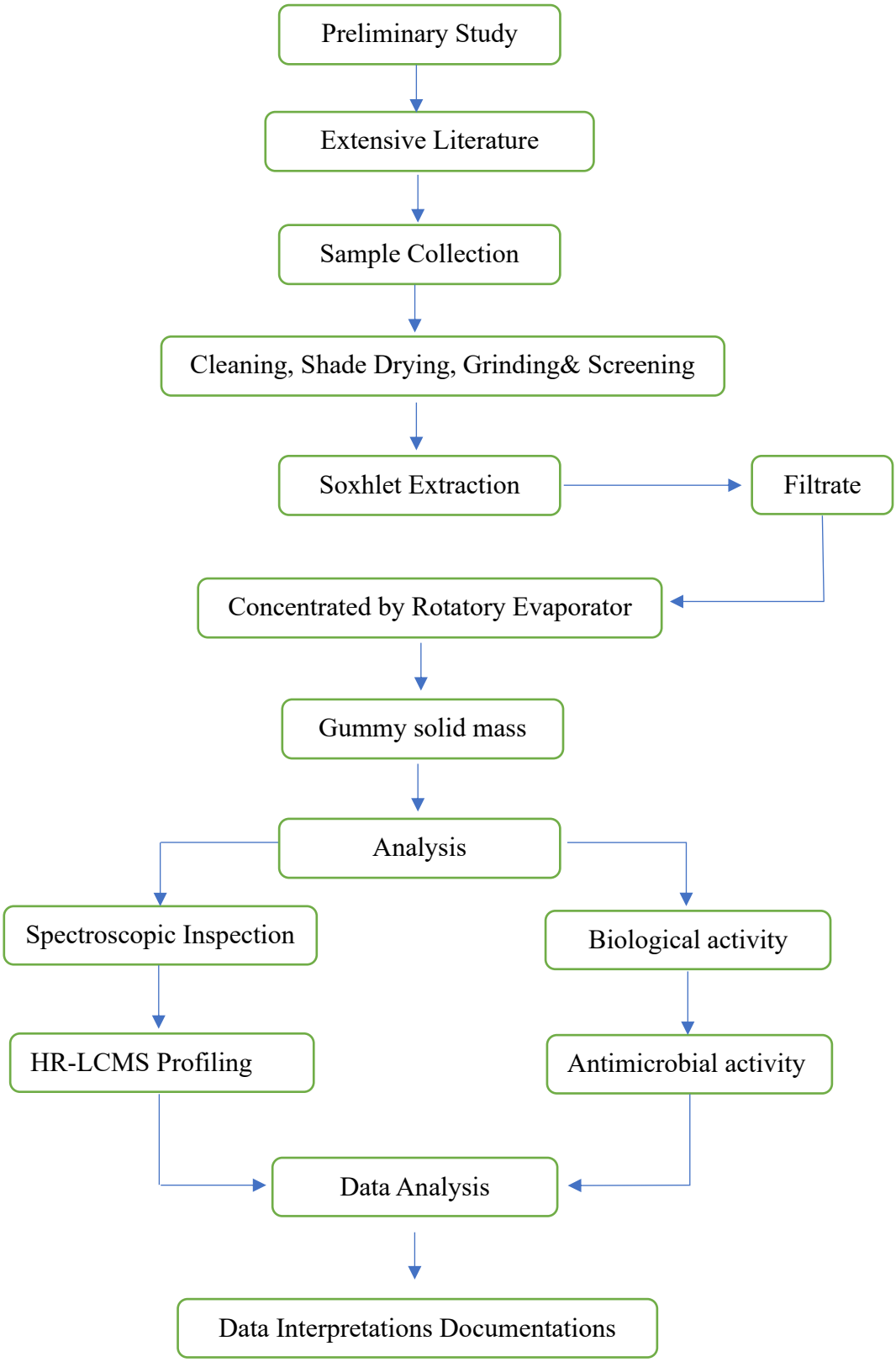
The gummy mass of plant extract obtained after evaporation was stored in the amber color glass bottle, sealed air tight using thin parafilm tape and labeled them with appropriate codes and stored at -4℃ until analysis. A part of this sample was sent to Centre for Research in Nanotechnology and Science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay, 400076, India for HR-LC-MS profiling.

Percentage yield of methanolic extract:

The estimated % yield of the plant extract was calculated using the following formula:

$$\text{Percentage Yield (\%)} = \frac{Weight\ of\ crude\ extract}{Weight\ of\ the\ powdered\ plant\ sample\ taken} \times 100$$

The schematic diagram for showing the methodology of the research:



Scheme 1: Flow chart for extraction, screening, and analysis

3.3 Qualitative Phytochemical screening

All the procedure and test of phytochemical screening were presented in supportive documents at last.

3.4 Quantitative analysis

Antimicrobial Activity

The agar well diffusion procedure was applied for antibacterial activity. The efficacy of the plant extract against the bacterial activity was determined by zone of inhibition (Daoud et al., 2019). The experiment was carried out using standard protocols given as clinical laboratory standard institute 2018 guidelines.

Collection of Test Organism

All the standard pathogenic bacteria were collected from the Bharatpur Hospital Chitwan Nepal. Nutrient Agar was used for the best growth of microorganism.

Preparation of Stock/ Working Solution

50 mg/mL of stock solution was prepared by dissolving 50 mg of plant extract into 1mL of 10% DMSO solution. Then the solution was kept inside in closed vial or Eppendorf tube in refrigerator at -4˚C for further use.

Assessing and Comprehensive analysis of Antimicrobial Property

Mueller Hinton Agar (MHA) plates, previously prepared were dried to eliminate excess moisture on the agar surface. A sterile cotton swab was immersed in the prepared inoculum (Appendix-IV) and any surplus inoculum was removed by gently pressing and rotating the swab against the upper inner way of tubes, just above the liquid level. The plates being rotated at 60-degree angle after each swabbing pass. Finally, swabbing was performed along the edge of agar surface. Then the inoculated plates were covered with lids and left to dry inside the laminar flow to avoid cross contamination.

After drying the inoculated agar plate, the sterile cork borer having 7 mm of diameter was employed to punch the well on the inoculated agar plate and mark accurately. Subsequently, 80µL plant stock solution was loaded gently into the respective wells using micropipette. DMSO (10%) solvent was used as control and different antibiotics were used as positive control. The plates having control, sample and antibiotics were closed with lid and left about 45 minutes to diffuse the extract.

Plates were kept in the incubator in upright position at 37˚C for about 18-24 hours. After appropriate incubation, the plates were examined for a clear zone of inhibition (i.e., a clear zone devoid of bacterial growth) surrounding each well. The zone of inhibition was measured using ruler, the mean value was reported to estimate the potency of the plant extract or antibacterial substance.

Quantification of Total Phenolic Content

General Protocol for Total Phenolic Content

Total phenol content was estimated by Folin-Ciocalteu method, (Zuorro & Lavechia., 2012). In concise, 5 mL of 0.1 M HCl, 195 µL of Folin-Ciocalteu reagent and 200 mL of aliquot or target liquid were combined with in the graduated glass vial. To this mixture, 20% Na_2CO_3 was added until a final volume of 10 mL. The vial was shaken vigorously and placed undisturbed at ambient room temperature (25˚C) in no light environment for about an hour. Following an hour incubation, the absorbance having λmax at 525 nm was determined using a colorimeter (**T80+, PG** Instruments, UK). Total phenolic content was quantified in milligram of gallic acid equivalent (mg GAE/g Sample DE) utilizing the calibration curve generated with gallic acid as reference standard.

Measurement of Total Phenolic Content (TPC)

Standard calibration curve was constructed and the concentration of phenolics was calculated from the equation of a straight line. The total phenol content was measured by using the formula;

$$TPC(C) = \frac{c \times v}{m} \ldots\ldots\ldots\ldots (1)$$

Where, C = Total phenol content mg GAE/g DE

c = concentration of gallic acid from curve (mg/mL)

V=volume of extract (mL)

m= weight of plant extract (g)

Standard calibration curve was constructed and the concentration of total phenolic compounds was calculated from the equation of a straight line.

Statistical analysis

The mean absorbance values, derived from triplicates measurement of each concentration were calculated. These values were utilized to determine the linear correlation coefficient and establishing the regression equation.

$Y = mx + c$ ……(2)

Where, Y= absorbance of extract

m = slope of curve

x = concentration of extract

c = intercept

By the use of this regression equation, concentration of extracts was determined in gallic acid equivalent/ gram.

Determination of Total Flavonoid Content

General Protocol:

The determination of total flavonoid content was conducted using calorimetric method (Gu et al., 2014). Aliquot of 0.3 mL of rutin, 0.09 mL of aqueous (5%) $NaNO_2$ followed by 1.5 ml of distilled water. After thorough agitation for 5 minutes, 0.18 mL of a 10% $AlCl_3.6\ H_2O$ solution was incorporated. An additional blending period of 6 minutes was ensued before the addition of 0.6 mL of 1M NaOH solution. Finally distilled water was added to make final volume of 3 mL. The double beam UV- visible spectrophotometer was used to measure the absorbance λmax of 510 nm. Rutin was taken as standard reference for the determination of the λmax value. Total flavonoid content was reported and expressed as rutin equivalent per g of sample in dry extract (DE) basis.

Measurement of Total Flavonoid Content (TFC)

The measurement and statistical analysis were carried out by the similar procedure as the previously mentioned in TPC section.

Quantitative phytochemical analysis (Antioxidant Activity)

Phosphomolybdenum assay [The total antioxidant activity (TAA)]

General protocol:

The estimation of total antioxidant capacity of plant extract was carried out utilizing the phosphomolybdate method (Prieto et al., 1999). In succinct, 0.1 mL aliquot of sample was introduced by employing 1mL of reagent solution composed of equal combination of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Subsequently, reaction volume was securely capped and placed in water bath having temperature of 95˚C for 90 minutes. Then the sample was gradually cooled to room temperature, absorbance of resulting reaction mixture was measured at 695nm.

Blank solution = reagent + solvent

DPPH free radical scavenging assay

General Protocol for DPPH Assay

Various concentrations of plant extract (0.3 mL) were mixed with a methanolic solution of DPPH radicals having concentration of 0.1 mM at a volume of 2.7 mL, the resulting mixture was vigorously shaken and left undisturbed for 60 minutes in dark environment until stable absorption values were achieved. To assess the reduction of DPPH radicals, the absorbance was measured at 517 nm wavelength (Barros et al., 2008).

Measurement of DPPH free radical scavenging activity

The radicals scavenging activity (RAS) to plant extract was quantified using the formula:

$$\textbf{Percentage Scavenging} = \frac{\textbf{Absorbance of Contol} - \textbf{Absorbance of Sample}}{\textbf{Absorbance of Sample}} \times \mathbf{10}$$

$$\%\boldsymbol{RSA} = \frac{(\boldsymbol{ADPPH} - \boldsymbol{AS})}{\boldsymbol{ADPPH}} \times \mathbf{100}$$

Ascorbic acid was used as reference standard for the determination of DPPH free radical scavenging activity. The concentration of extract necessary to attain 50% radical scavenging **(IC_{50})** was ascertained by constructing the graph that correlates % RSA with varying concentration of extract which helped to determine the antioxidant parameters.

3.5 Statistical analysis

All the statistical analysis was carried out using MS excel software. Three replicate (n = 3) sample data were meticulously collected and outcomes were subsequently presented as mean value accompanied by its corresponding standard deviation (mean ± SD). Comparing the MS/MS fragmentation patterns with those spectral documented in existing literature or deposited spectral library like Pub Chem, Dictionary of Natural Products 2, Che Spider (http://mona.fiehnlab.ucdavis.edu/; http://www. massbank.eu/; https://metlin.scripps.edu) and acquired in equipment's with ESI sources and experiments type CID (collision inducted dissociation).

3.6 High Resolution Liquid Chromatography – Mass Spectrometry

Chemical inspection of methanolic extract was carried out by using HR-LCMS spectroscope model G6550A -i funnel -Q-TOF_S system having 0.01% mass resolution. This experimental analysis was conducted at the Centre for Research in Nanotechnology and Science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF) located at IIT (Powai) Bombay, India. It includes a hipper sampler (G4226A model), binary pump, column compartment, Q-TOF having dual ionization mode ESI along with Agilent Jet System. The sample had an ancillary speed 100µL/mL, flush factor 5µL and injection volume 5µL and time of acquisition was set for 30 minutes, whereas the initial 2 minutes dedicated to the flow of solvent.

Chromatographic separation was accomplished by using ZORBAX EclipseC_{18} (150 × 2.1 mm, 5µm) column along with mobile phase (eluent solvent) A (0.1% formic acid in Milli-Q water) and B (Acetonitrile). This acquisition method set as minimum mass ranges from 50 Dalton (m/z) to maximum 1200 Dalton (m/z). All the data obtained was analyzed and interpreted by Agilent Mass Hunter Software. Complete information regarding the spectroscopic method and process is present in appendix I.

4 CHAPTER IV: RESULTS AND DISCUSSION

4.1 Percentage yield of sample extracts

The % yields of the extract obtained from the dry weight of the sample of Euphorbia Hirta L. using methanol as a solvent is tabulated below.

Table 4-1: Physical characteristics and percentage yield of plant extract

Sample Plant	Colour	Dry weight of Plant (g)	Weight of extract (g)	Percentage Yield (%)
E. Hirta L.	Brown-Green	135	24.683	18.2837

Phytochemical screening

Different class of compounds were detected &observed by preliminary phytochemical screening. It is accomplished on the basis of the procedure given by (Ciulei, 2003) and the result is mentioned below on the table.

Table 4-2: Phytochemical screening of methanolic extract of leaves of *Euphorbia Hirta L.*

S.N.	Phytochemicals	Test name	Reference	Observation	Result			
					T_1	T_2	T_3	Remarks
1	Alkaloids	Mayer's	White/Pale yellow ppt.	No turbid	-	-	-	Absence
		Wagner's	Yellow /brown ppt	No ppt	-	-	-	Absence
		Hager's	Brown ppt	No ppt	-	-	-	Absence
2	Flavonoids	Pew's	Red color	Rose red	+	+	+	Presence
		Sodium hydroxide	Yellow ppt	Yellow ppt	+	+	+	Presence
		Lead acetate	Yellow ppt	Yellow ppt	+	+	+	Presence

3	Phenols	Ferric chloride	Bluish Black /Greenish Yellow colour	Green color	+	+	+	Presence
		Liebermann's			+	+	+	Presence
		Lead acetate	White ppt	White ppt	+	+	+	Presence
4	Tannins	Ferric chloride	Brownish green colour	Bluish green	+	+	+	Presence
		Dichromate	Brown ppt	Yellowish	+	+	+	Presence
		Gelatins	White ppt.	White ppt	+	+	+	Presence
5	Quinones		Green colour	No color formation	-	-	-	Absence
	Anthro-quinones				-	-	-	Absence
6	Saponins	Forth	Forth formation	Forth appeared	+	+	+	Presence
		Foam	Foam formation	Foam appeared	+	+	+	Presence
7	Cardiac Glycosides	Modified Bruntrager's	Rose pink color	Dark rose color	+	+	+	Presence
		Killer-Kilani	The Reddish-brown ring at the junction of two solvents	Brown ring interference	+	+	+	Presence
		Legal's	Pink color	No color	-	-	-	Absence
8	Coumarins		Yellow color	Yellow	+	+	+	Presence
9	Phobatannins		Red ppt	No	-	-	-	Absence

10	Carbohydrates	Molisch's test	Violet ring at the junction	Yellow	-	-	-	Absence
		Bendict's test	Brick red ppt	No changes	-	-	-	Absence
		Fehling's test	Red/Brick red ppt.	No changes	-	-	-	Absence
11	Proteins and Amino acids	Millon's test	Flesh red ppt	Red ppt	+	+	+	Presence
		Ninhydrins test	Blue colour (Amino acids)	White color	-	-	-	Absence
		Xanthoproteic	Yellow colour (Proteins)	No changes	-	-	-	Absence
12	Terpenoids		Radish brown	No changes	-	-	-	Absence
13	Gums & Resins	Precipitation	ppt formation	No changes	-	-	-	Absence
14	Oils& fats	Oil strain	Oil strain s		+	+	+	Presence

Phytochemical screening test of methanolic extract of plant is found slightly different from the data found in literature review. Soil composition, pH of soil, altitude of the vegetation, sample collection season and environmental conditions may vary the chemical composition in the plant. The amount of chemicals present in the extract also depends on the extraction procedure, solvent purity and grades of the chemicals.

Antibacterial Screening Analysis

Methanolic plant extract shows the significant antibacterial activity against the different pathogenic bacteria, both gram negative and gram-positive bacterial resistance with plant extract. 80 µL of 10 % DMSO was used as negative control. The efficacy of the extract against the different bacterial strain is presented in bar graph below.

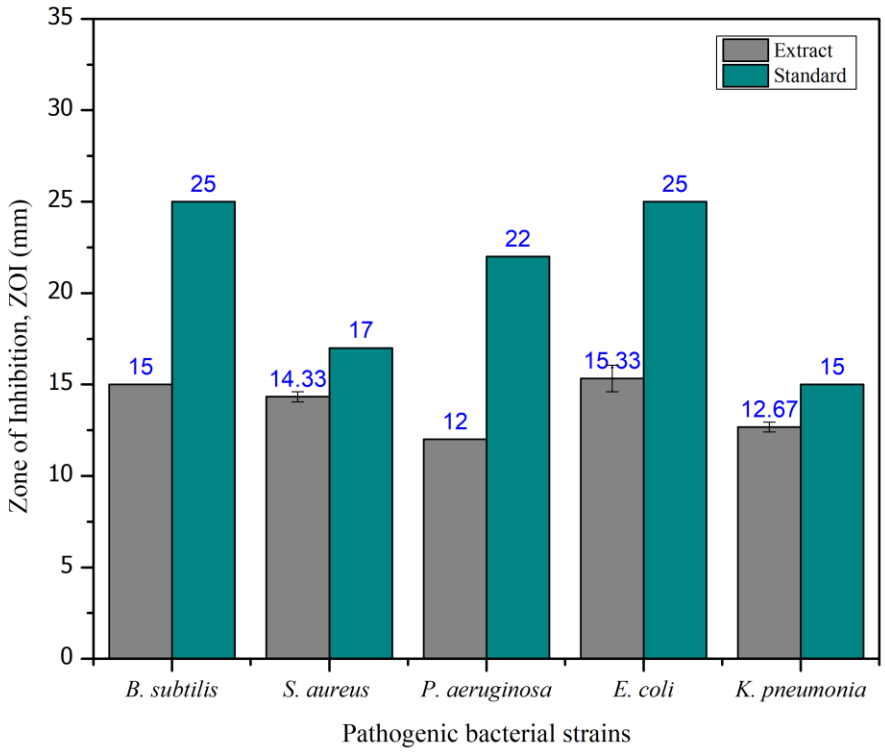


Figure 1*:*Antibacterial screening shown by methanolic plant extract

(Note: 15µg Erythromycin *(K. pneumonia, and B. Subtills, Escherichia coli*)*,)*, 10µg Gentamycin (P. *aeruginosa*), 30 µg Vancomycin (*S. aureus)* PC stands Positive controls (standard drug), NC stands Negative control (Solvent used i.e., DMSO) N= Negative result or No activity. (-Ve) = gram negative bacteria and (+Ve) = gram positive pathogens.)

Tabulated information on graph below at index VI.

4.2 Estimation of total phenolic content

Total phenols content was estimated using the Gallic acid as standard. The calibration of the spectrophotometer is carried by using different concentration (1µg/mL, 2µg/mL, 4µg/mL, 8µg/mL, 16µg/mL, 32µg/mL) of standards (Gallic acid). The construction of calibration curve of the instruments between absorbance and various concentrations of standard at 765nm wavelength is illustrated bellow: -

Construction of calibration curve

The numerical data provided in APPENDIX - VI.

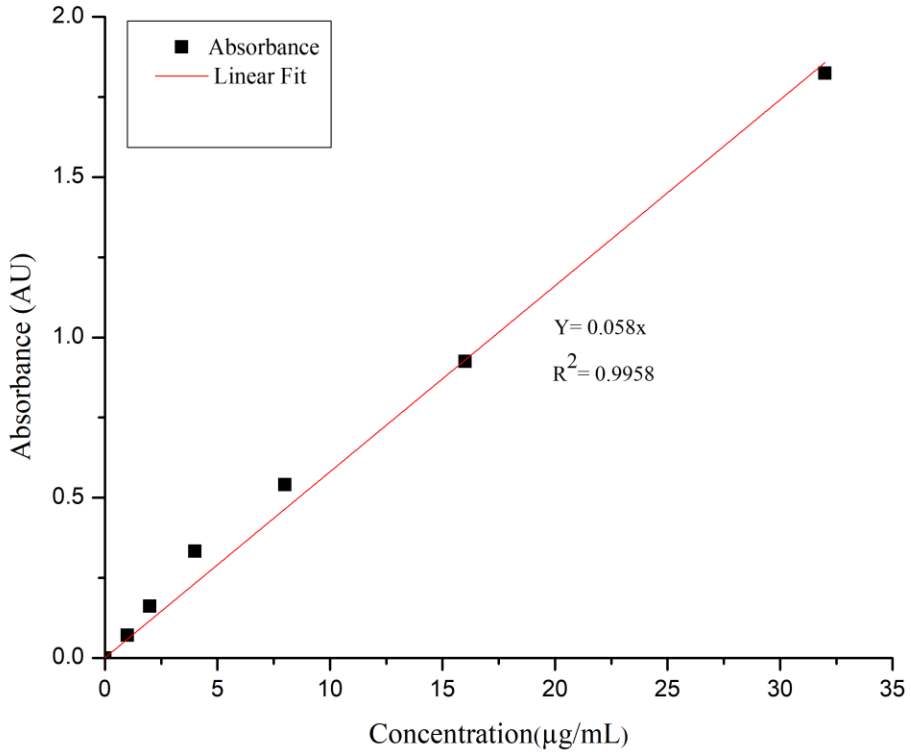


Figure 2:Calibration curve of Gallic acid in determining total phenolic content

Calculation of total Phenolic content in plant extracts

Total phenolic content of plant extract was estimated by using the standard calibration curve of Gallic acid using the regression equation Y=0.058x, R^2 =0.9958obtained from Excel software sheet.

Here,

"y" represents the absorbance;

"x" denotes the concentration of Gallic acid (GAE) in microgram per milliliters (µg/mL)

"m" crosses ponds to the slope of calibration curve s = 0.0558

"c" designates the y intercept of the curve = 0.0

Thus, total phenolic content was estimated by using simple equation TPC= x(V/m) and is expressed in mg GAE Equ. /g dry weight of the extract.

Table 9: Total Phenolic Content (TPC) of methanolic plant extracts

S. N	Concentration	Absorbance			Total phenolic Content			Mean ± SD
		A_1	A_2	A_3	C_1	C_2	C_3	
1	250 µg/ml	0.761	0.753	0.758	52.4	51.98	52.28	52.22± 0.249

4.3 Estimation of total flavonoid content

Determination of total flavonoid content in methanolic plant extract was carried out by using Aluminium Chloride Colorimetry Assay at 515 nm wavelength. A standard calibration curve of Rutin was plotted for the calculations of total flavonoid content of Euphorbia Hirta Linn leaves extract. And the numerical data of absorbance of different concentration of standard Rutin is shown in Appendix VI.

Construction of calibration curve

The absorbance curve for standard Rutin is shown in Figure as;

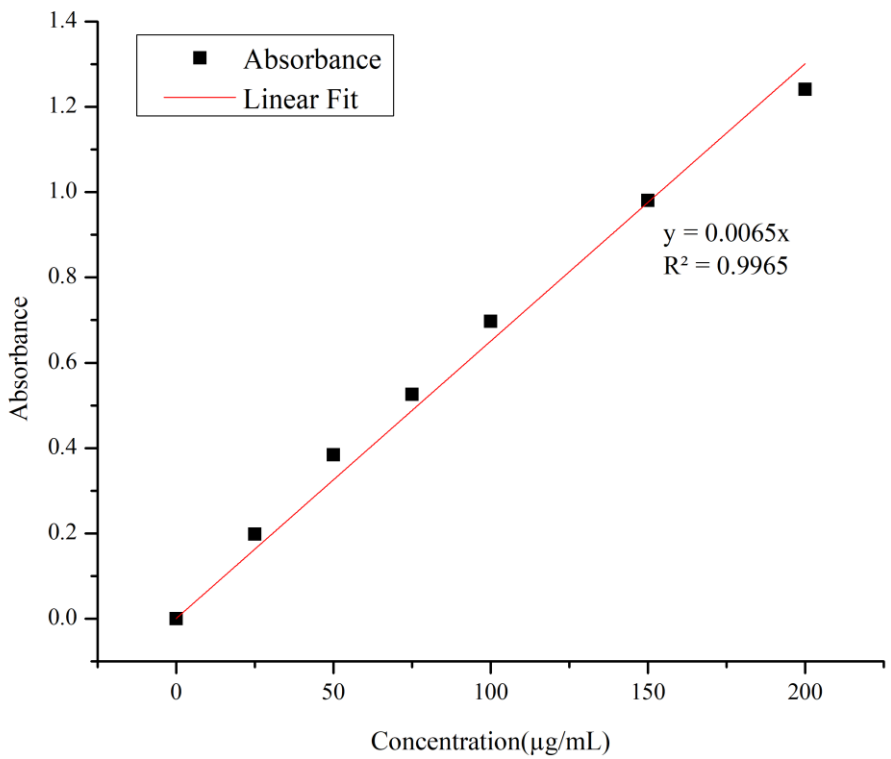


Figure 3:A Calibration curve for Rutin is essential in calculation of total flavonoid content

Calculation of total flavonoid content in plant extract

Thus, generated regression equations between absorbance and amount of Rutin was Y=0.0065x (R^2 = 0.9965). Hence the TFC was estimated by using by the formula x (V/m) and expressed as mg QE/g of extract in dry weight. All the determination procedure is similar to that of TPC process.

The TFC of methanolic extract was found to be 258.04±2.001mg RE/g dry extract (DE).

4.4 Antioxidant activity

4.4.1 Phosphomolybdenum assay (TAC)

A higher absorbance confirms higher antioxidants property.

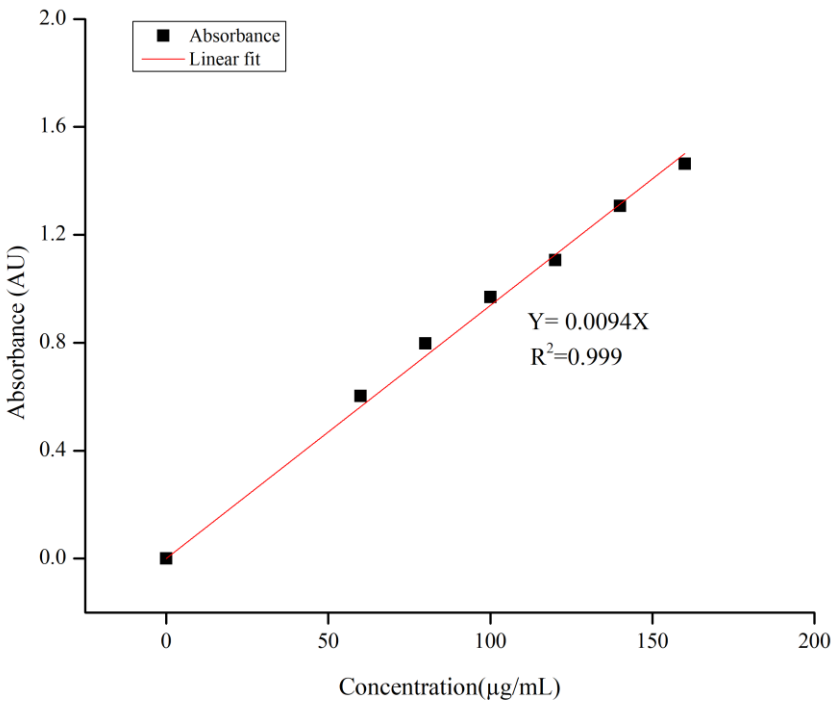


Figure 4 Variation of absorbance with concentration for standard Ascorbic acid

Total antioxidant activity was estimated as166.47 ± 0.231 mg AAE/g DE.

4.4.2 DPPH free radical scavenging Activity

All the sample solutions exhibited varying degree of antioxidant activity. They have different extent of scavenging ability to scavenge free radicals generated by DPPH. The

scavenging capacity of free radicals by the methanolic extract is presented in Table; APPENDIX - VI.

The relation between antioxidant potential and the IC_{50} value is inversely correlated and this value can be determined through logarithmic regression of the percentage of inhibition against antioxidant activity. A lower the IC_{50} value signify higher the level of antioxidant activity. Graphical representation of scavenging activity in-terms of % inhibition of plant and standard on DPPH free radical as is presented in the given graph bellow as: -

Construction of calibration curve

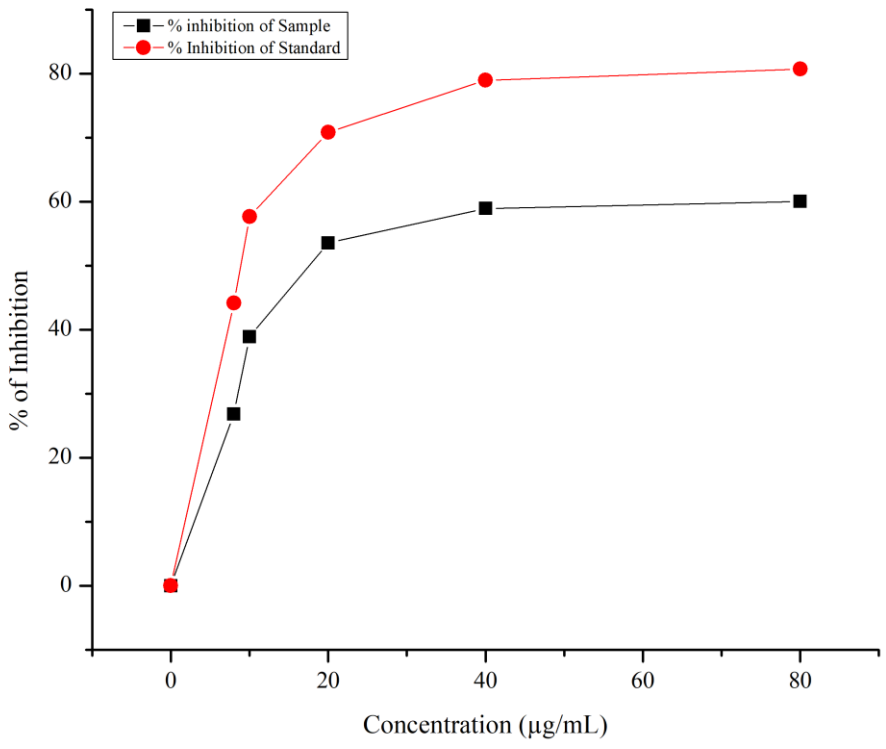


Figure 5: Graphical representation of the DPPH assay

A plot of % free radical scavenging of methanolic extracts Euphorbia Hirta linn and Ascorbic acid as standard gives DPPH scavenging is 18.930 µg/mL

The IC_{50} value of methanolic extract of E. Hirta Linn and ascorbic acid were shown in Appendices III and are found to be 44.0419 µg/mL.

4.5 Liquid Chromatography – Mass Spectrometry Analysis

The methanolic extract of Euphorbia Hirta Linn was subjected to HR-LCMS analysis. HR-LCMS analysis is conducted in both positive and negative modes which revealed the presence of 117 distinct compounds. Among them, 78 compounds are successfully identified, while the remaining 39 compounds needs to be characterization. The comprehensive list of detected compounds can be found in Appendices I.

The compounds were separated using the LC column (ZORBAX Eclipse C_{18} (150 × 2.1 mm, 5µm)), and subsequently analysed using mass spectrometer. The chromatograms of the molecules present in the methanolic extract and their Mass spectra capture under positive and negative modes of ionization are illustrated in figure 5 and 6 respectively.

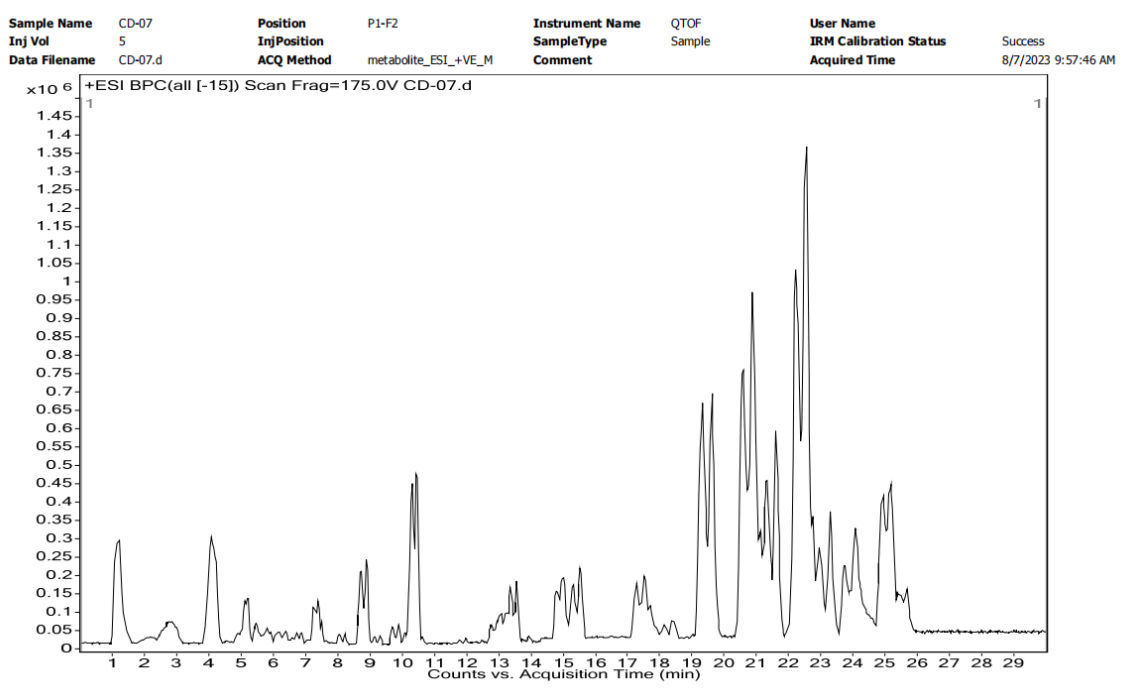


Figure 6*:* Chromatogram of methanolic extract of sample in positive ESI mode of Mass spectrometer.

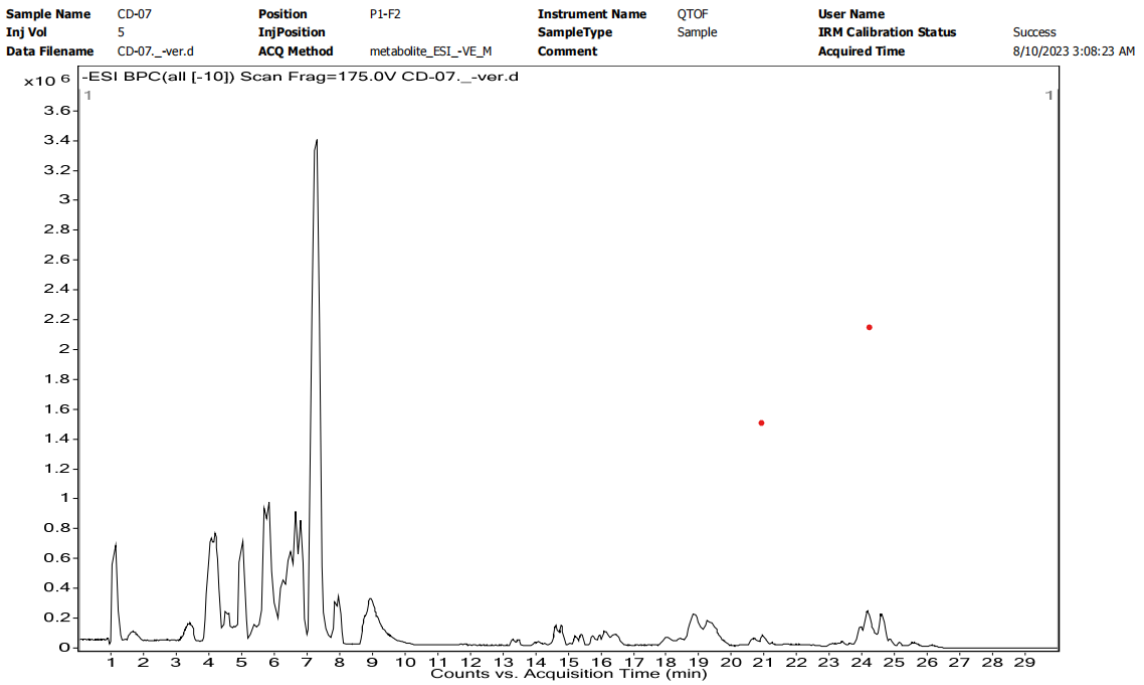


Figure 7*:* Chromatogram of methanolic extract in negative ESI modes of Mass spectrometer

Table 4-3: Secondary metabolites identified from *Euphorbia Hirta* Linn through mass spectrometer

S.N	Putative Compound	Retention Time (Min)	Mass	Molecular Formula	DB Diff (ppm)	Chemical Class
1	Lentiginosine	1.233 (+ ESI)	157.1087	$C_8 H_{15} N O_2$	10.01	Alkaloids
2	Flurandrenolide	5.458 (+ ESI)	436.2262	$C_{24} H_{33} F O_6$	-0.27	Steroid
3	Polidocanol	18.121(+ ESI)	582.4317	$C_{30} H_{62} O_{10}$	4.47	Glycerides
4	Irinotecan	19.862(+ ESI)	586.2787	$C_{33} H_{38} N_4 O_6$	0.68	Enzyme
5	Glyurallin B	5.043 (-ESI)	422.1745	$C_{25} H_{26} O_6$	-3.66	Flavonoid
6	Hyperoside	6.514 (-ESI)	464.1015	$C_{21} H_{20} O_{12}$	-13.01	Flavonoid

7	Cynaroside	7.263(-ESI)	448.1069	C_{21} H_{20} O_{11}	-14.06	Flavonoid

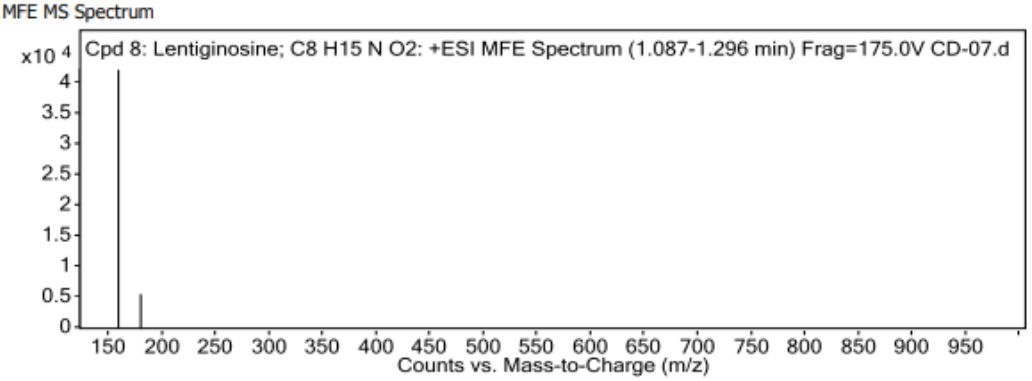
Few Potential bioactive molecules present in the plant extract which is analysed using HR-LCMS are illustrated below.

Lentiginose

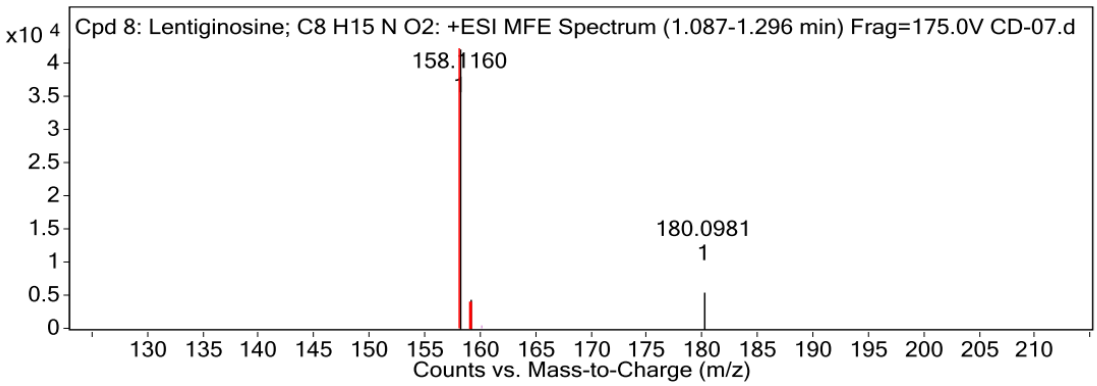
HR-LCMS data of the methanolic extract of E Hirta shows that Lentiginose has retention time of 1.23 minutes. It is polyhydroxylated indolizide alkaloid. It has ability to inhibit glycosidase enzyme. Glycosidase enzyme is involved in breakdown of complex sugar (glycon) by inhibiting glycosidase Lentiginosine which interfere glycon processing and influence biological process. Its characteristic has been found to inhibit amyl glycosidase. This molecule has characteristics as to suppress the Human immune deficiency virus. (Heravi et al., 2020).

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 8: Lentiginosine; C8 H15 N O2	**Lentiginosine**	158.116	1.233	Find by Molecular Feature	157.1087

MFE MS Spectrum



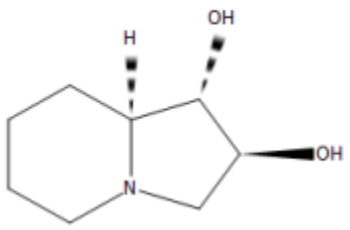
MFE MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
158.116	1	42149.66	C8 H15 N O2	(M+H)+
159.119	1	4655.25	C8 H15 N O2	(M+H)+
180.0981	1	5543.23		(M+Na)+

Compound Structure

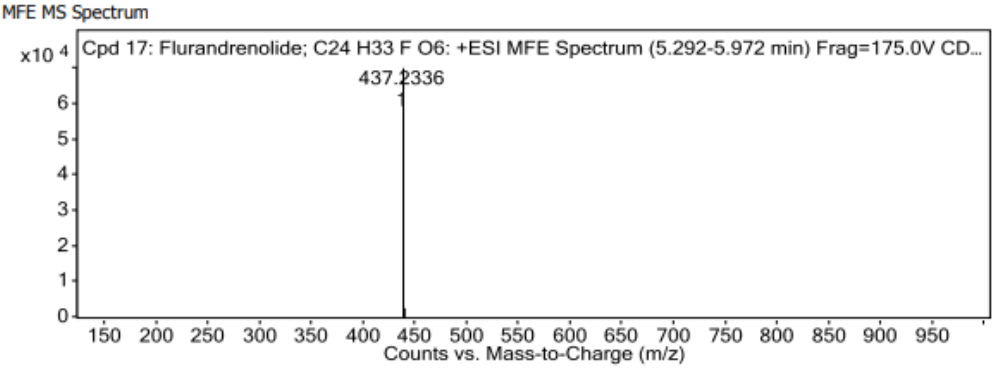


Flurandrenolide

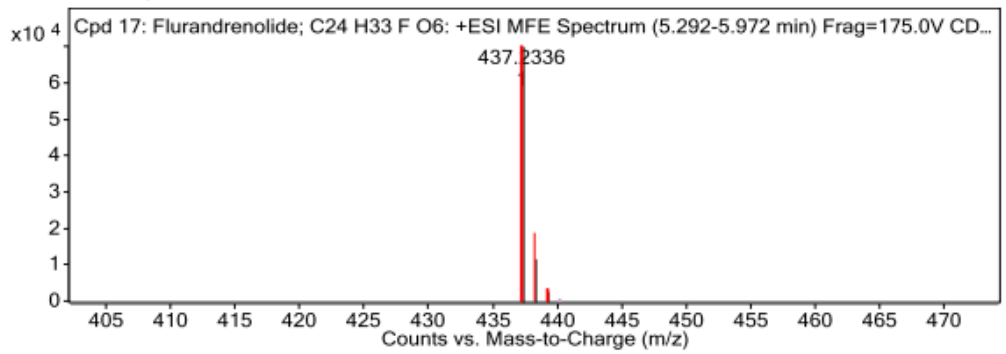
The Chromatogram having retention time of 5.45 minutes in HR-LCMS is of Flurandrenolide. The methanolic extract of E. Hirta contains substantial amount of this molecules (C_{24} H_{33} F O_6). This molecule is used as a corticosteroid drug to treat skin psoriasis (Krueger et al., 1998). The United States pharmacopoeia had already approved this compound as drug to care for skin disorder. Flurandrenolide is used in creams, foams, ointments and tape to direct applied on skin to remove itching, redness, rash, allergies & inflammation associated with situation like eczema, dermatitis, psoriasis plaques, swelling etc. due to its anti-inflammatory, anti-parotic and vasso-constructiveness action.

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 17: Flurandrenolide; C24 H33 F O6	**Flurandrenolide**	437.2336	5.458	Find by Molecular Feature	436.2262

MFE MS Spectrum



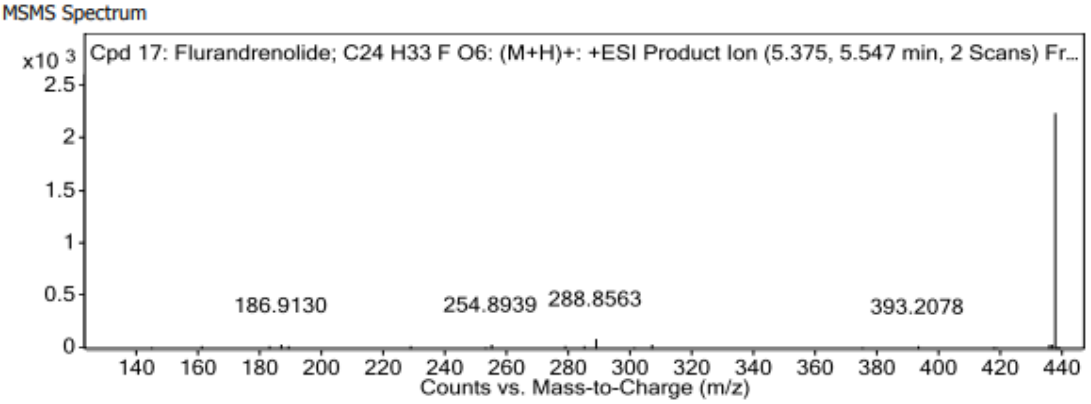
MFE MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
437.2336	1	70089.04	C24 H33 F O6	(M+H)+
438.2368	1	11882.64	C24 H33 F O6	(M+H)+
439.2389	1	2975.52	C24 H33 F O6	(M+H)+

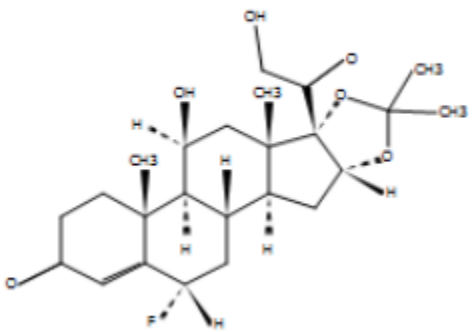
MSMS Spectrum



MS/MS Spectrum Peak List

m/z	z	Abund
161.0062	1	28.64
186.913	1	36.75
254.8939	1	43.01
284.8847	1	32.34
288.8563	1	98.65
306.8671	1	45.53
435.2188	1	44.48
436.1967	1	34.79
437.189	1	39.04
437.2337	1	2250.67

Compound Structure

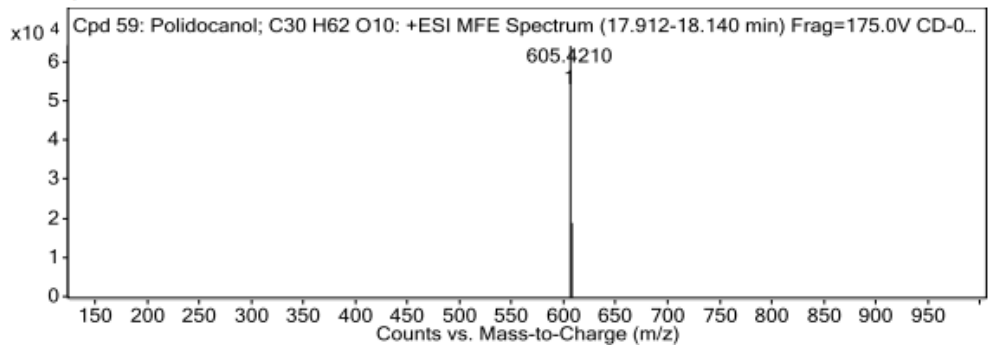


Polidocanol

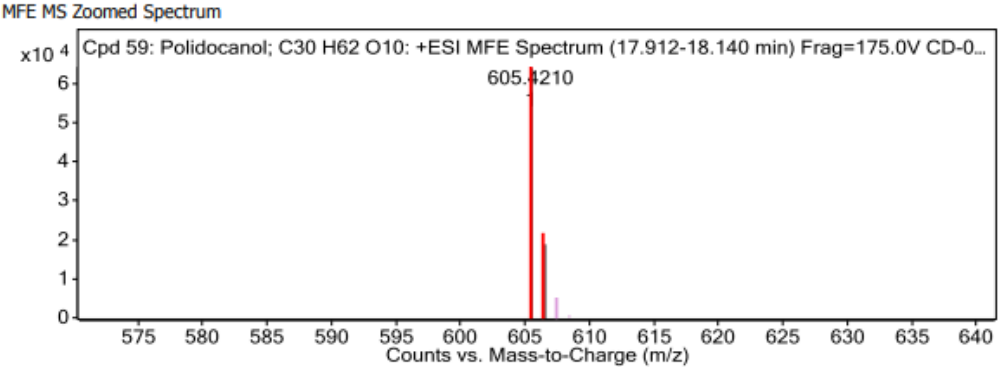
The open chain molecule Polidocanol [C_{12} H_{25} $(OCH_2CH_2)_n$ OH] has mean molecular weight of approximately 600(n = 9) is found in the methanolic extract of E. Hirta at retention time of 18.12 minute. It is long chain fatty alcohol used as novel therapeutics medicine. It acts as sclerosing agent to treat varicose veins and spider veins (saphenous veins). Polidocanol is also known as local aesthetic and it also acts as anti-itching agent and it is also used as ingredient in ointments (Kiguchi et al., 2018).

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 59: Polidocanol; C30 H62 O10	**Polidocanol**	605.421	18.121	Find by Molecular Feature	582.4317

MFE MS Spectrum

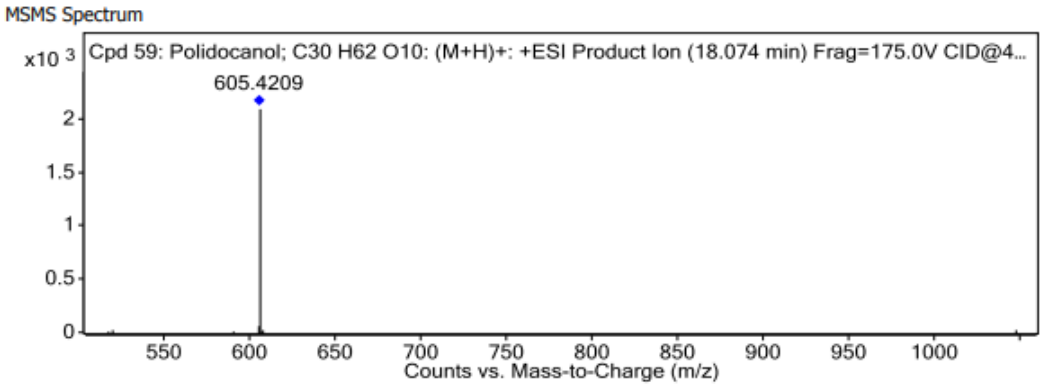


MFE MS Zoomed Spectrum



MS Spectrum Peak List

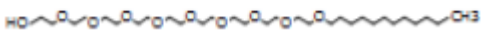
m/z	z	Abund	Formula	Ion
605.421	1	64158.89	C30 H62 O10	(M+H)+
606.424	1	19028.67	C30 H62 O10	(M+H)+



MS/MS Spectrum Peak List

m/z	z	Abund
517.3642	1	23.02
519.3026	1	40.91
589.9504	1	20.63
605.3237	1	80.92
605.4209	1	2103.2
605.6408	1	16.17
605.8546	1	20.4
606.9013	1	19
607.2033	1	40.67
1046.6697	1	35.13

Compound Structure

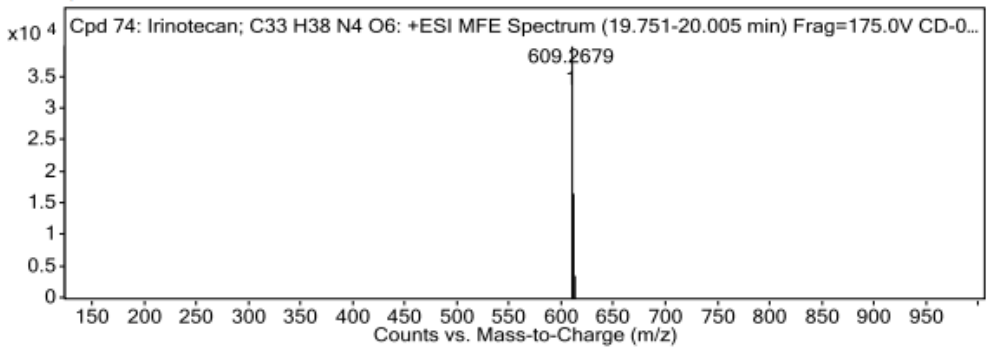


Irinotecan

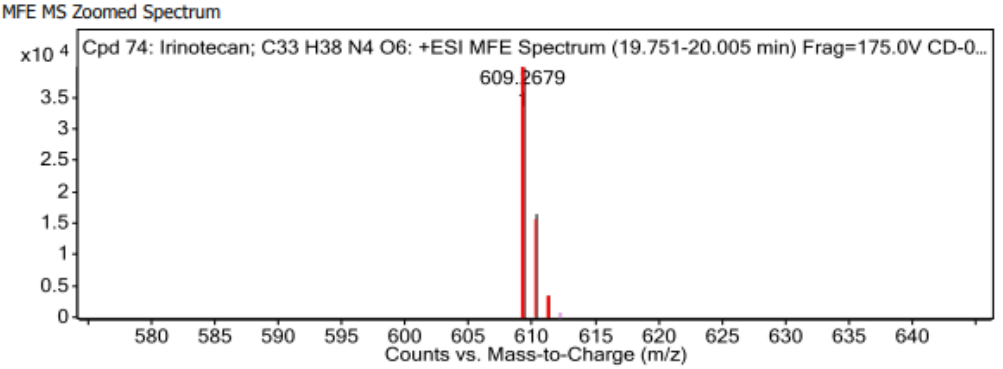
Irinotecan has complex structure. Its presence in the methanolic extract of E. Hirta is proved by its chromatogram present at retention time of 18.12 minute. It is on thelist of WHO essential medicines. It is made from the natural compound camptothecin which is found in the Chinese ornamental tree Camptotheca acuminate. Irinotecan is first approved as cancer treatment medicine for solid tumours in 1994. Irinotecan triggers topoisomerase I (Topo 1) inhibition, the active SN-38 traps the enzyme on DNA molecules forming 'cleavable complex'. As a result, DNA loses its super coils and breaks DNA strand (i.e. DNA cleavage). The breakage of DNA strands cannot proceed replication & transcription in cancer cell (Bailly, 2019).

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 74: Irinotecan; C33 H38 N4 O6	**Irinotecan**	609.2679	19.862	Find by Molecular Feature	586.2787

MFE MS Spectrum



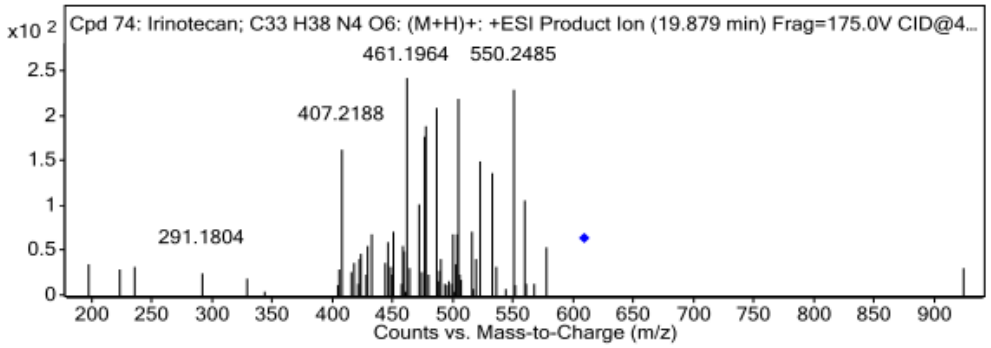
MFE MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
609.2679	1	39841.57	C33 H38 N4 O6	(M+H)+
610.271	1	16535.76	C33 H38 N4 O6	(M+H)+
611.2747	1	3789.8	C33 H38 N4 O6	(M+H)+

MSMS Spectrum

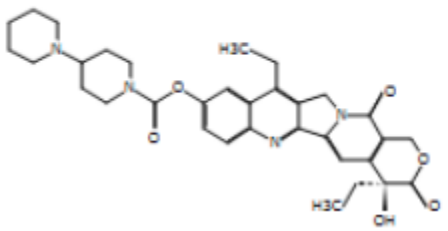


MS/MS Spectrum Peak List

m/z	z	Abund
407.2188	1	163.84

461.1964	1	243.04
475.2104	1	178.09
477.2182	1	189.91
486.2024	1	210.33
503.2436	1	220.73
521.254	1	150.09
531.2367	1	137.27
549.2446	1	136.25
550.2485	1	230.1

Compound Structure

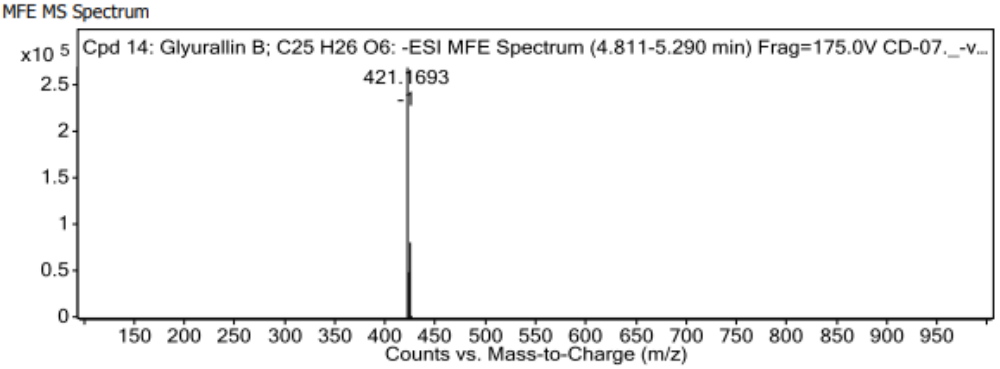


Glyurallin B

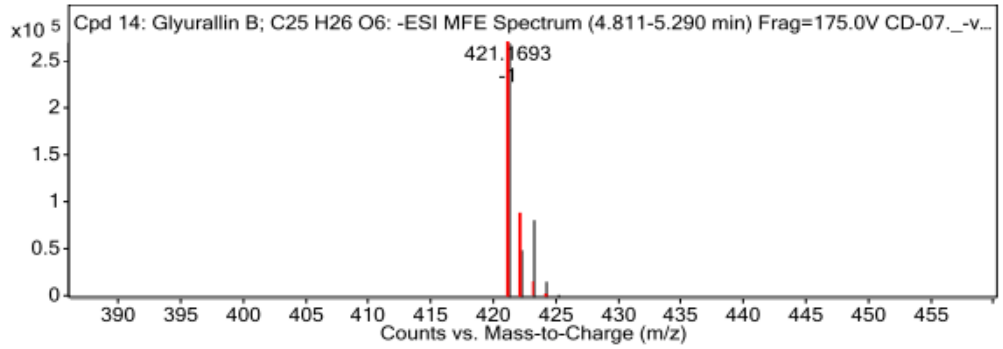
Glyurallin B has the molecular formula of $C_{25}H_{26}O_6$ appears at retention time of 5.04 minutes in HR-LCMS analysis of methanolic extract of E. Hirta in negative ESI mode. It exhibits $ABTs^+$ radical scavenging activity and anti-inflammatory properties, including the inhibition of reactive oxygen species (ROS) production and reduction of nitric oxide (NO), inter leukin -6 (IL-6) and prostaglandin E_2 (PGE_2) in LPS (Lipopolysaccharide) induced macro-phage cell (Fu, et al., 2013). The traditional use of this plant as anti-inflammatory medicine is supported by its presence in the plant extract.

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 14: Glyurallin B; C25 H26 O6	**Glyurallin B**	421.1693	5.043	Find by Molecular Feature	422.1745

MFE MS Spectrum



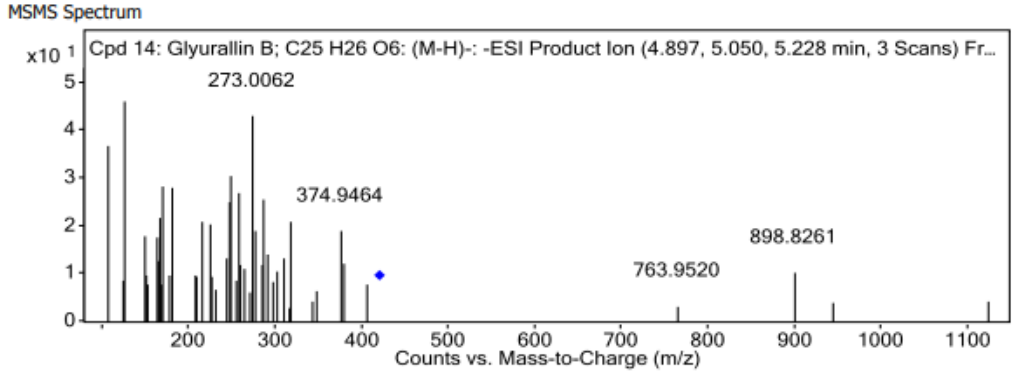
MFE MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
421.1693	-1	269945.56	C25 H26 O6	(M-H)-
422.1722	-1	49685.29	C25 H26 O6	(M-H)-
423.167	-1	82050.34	C25 H26 O6	(M-H)-
424.17	-1	16395.05	C25 H26 O6	(M-H)-
425.1743	-1	2990.16	C25 H26 O6	(M-H)-

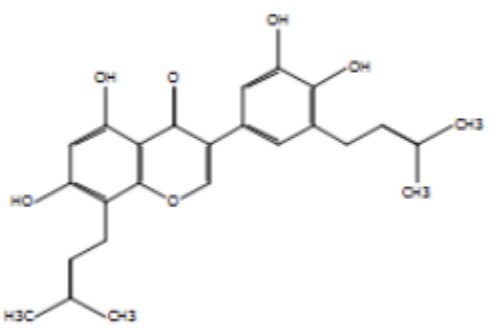
MSMS Spectrum



MS/MS Spectrum Peak List

m/z	z	Abund
107.0144	1	36.77
125.0283	1	46.04
169.0152	1	28.29
179.9979	1	24.54
180.0198	1	28.21
247.0133	1	25.19
249.0434	1	30.48
257.1189	1	27.07
273.0062	1	43.1
286.0409	1	25.59

Compound Structure



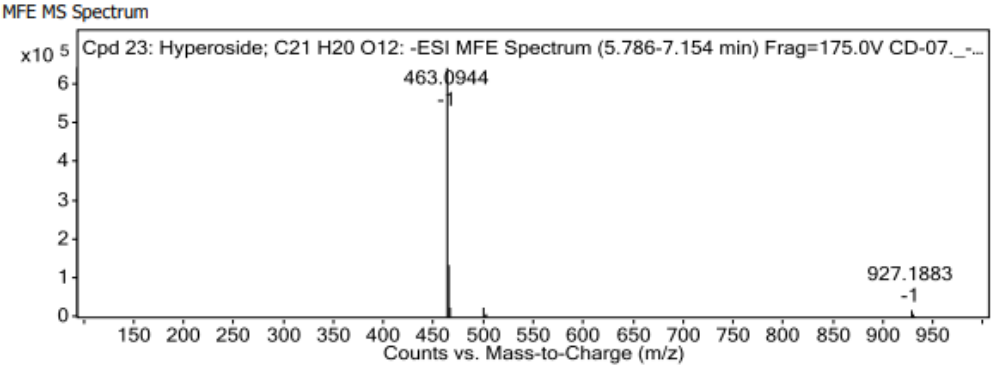
Hyperoside / Quercetin 3-*O*-galactoside (Q3G)

Hyperoside ($C_{21}H_{20}O_{12}$) present in methanolic extract of E. Hirta has the IUPAC name2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxychromen-4-oneis a derivative of quercetin. It appears after

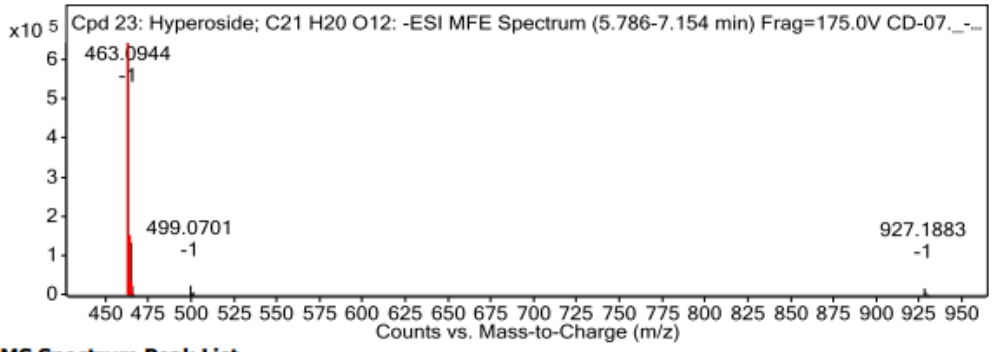
6.51 minutes of sample injection in HR-LCMS on negative ESI mode. Hyperoside is a dietary supplement that has been found to have a number of health benefits. It has potential pharmacological activities including anti-inflammatory, antithrombotic, anti-diabetics, hepatoprotective and antioxidant activity. It has also been found to help reduce cholesterol levels, improve blood circulation, and reduce the risk of cardiovascular disease (Shukla et al., 2019). This data also supports that the E. Hirta extract has medicinal value for the human kind. Quercetin 3-*O*-galactoside (Q3G) has anti-melanogenic effect by reducing melanin production ofα-melanocyte; a melanin stimulating hormone (*α*-MSH) in B16F10 melanoma cells. It inhibits melanogenesis related enzyme and transcription factor MITF (microphthalmia-associated transcription factor) through several pathway, including CREB(The cyclic adenosine monophosphate responsive element binding protein) and GSK3β (Glycogen synthase kinase-3 beta) activation as well as MAPK hyper pigmentation (Karadeniz et al., 2023).

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 23: Hyperoside; C21 H20 O12	**Hyperoside**	463.0944	6.514	Find by Molecular Feature	464.1015

MFE MS Spectrum



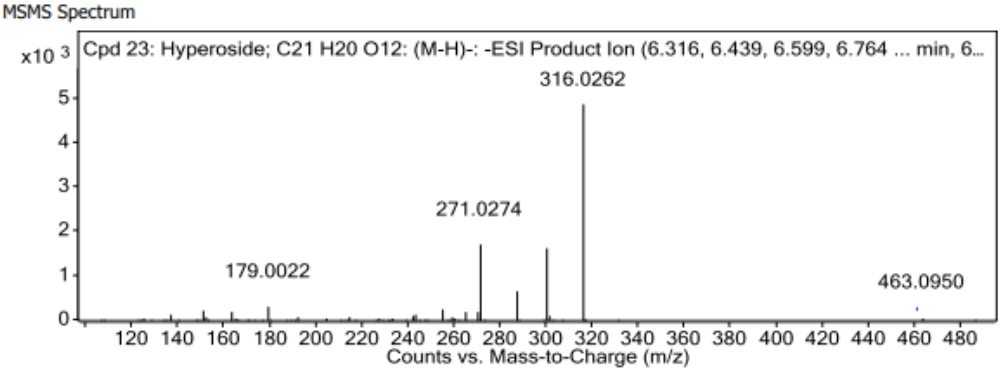
MFE MS Zoomed Spectrum



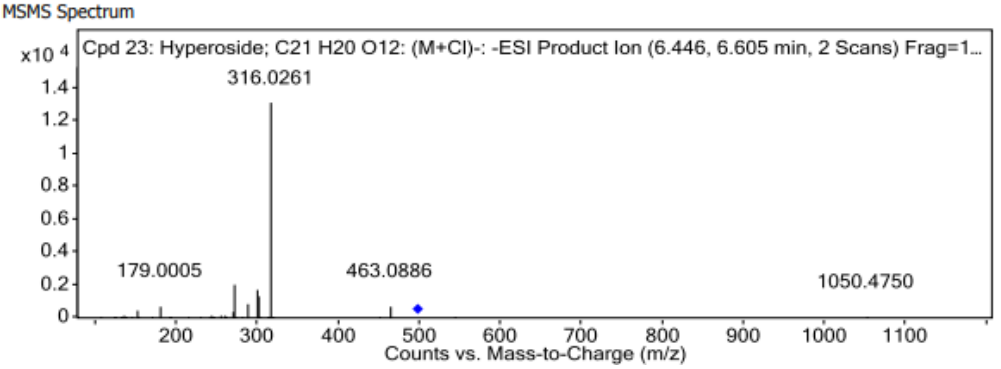
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
463.0944	-1	643711.56	C21 H20 O12	(M-H)-
464.0971	-1	137279.26	C21 H20 O12	(M-H)-
465.0988	-1	26756.31	C21 H20 O12	(M-H)-
466.101	-1	3219.48	C21 H20 O12	(M-H)-
499.0701	-1	25644.41		(M+Cl)-
500.0723	-1	5558.91		(M+Cl)-
501.0682	-1	9085.1		(M+Cl)-
927.1883	-1	20859.54		(2M-H)-
928.1911	-1	9319.27		(2M-H)-
929.1915	-1	3386.64		(2M-H)-

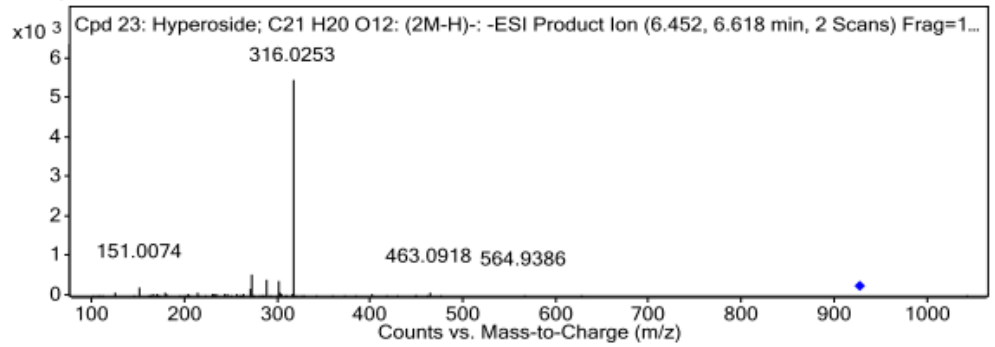
MSMS Spectrum



MSMS Spectrum



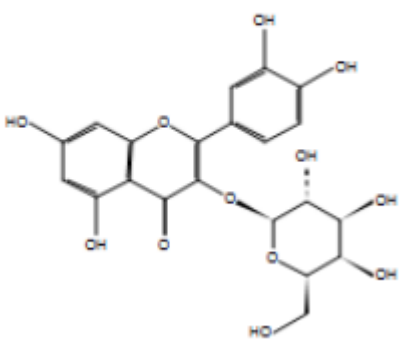
MSMS Spectrum



MS/MS Spectrum Peak List

m/z	z	Abund
151.0073	1	246.12
163.0075	1	195.57
179.0022	1	310.35
255.0338	1	251.11
265.0365	1	195.7
270.0191	1	198.58
271.0274	1	1707.71
287.0233	1	684.53
300.0312	1	1631.84
316.0262	1	4878.73
151.0036	1	447.7
179.0005	1	717.76
255.0276	1	144.6
270.0205	1	375.26
271.0264	1	2011.82
287.0214	1	835
300.0305	1	1737.61
301.0377	1	1348.59
316.0261	1	13118.28
463.0886	1	706.34
151.0074	1	229.52
178.9989	1	108.33
270.0165	1	204.87
271.0266	1	545.26
287.0237	1	431.79
300.0314	1	375.44
301.0373	1	400.11
301.9991	1	105.54
316.0253	1	5480.5
463.0918	1	113.32

Compound Structure



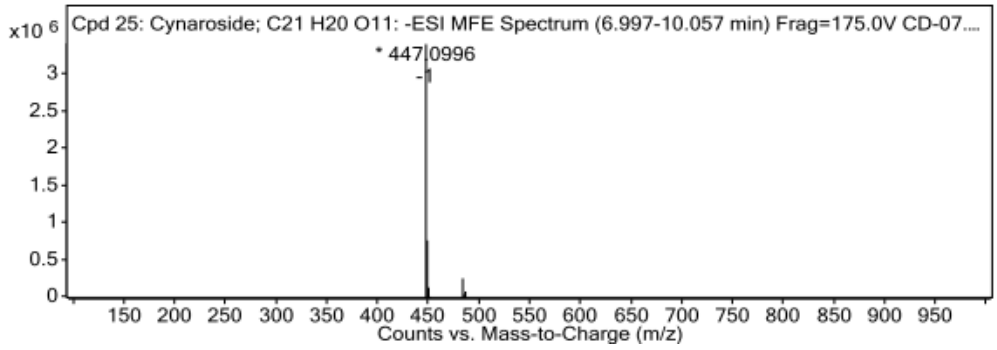
Cynaroside

Cynaroside (Luteolin 7-glucoside) is a flavonoid compound that is present in the methanolic extract of E. Hirta at retention time of 7.263 in HR-LCMS analysis. It exhibits antibacterial, antifungal, anti-inflammatory and anticancer activities. Cynaroside is also a

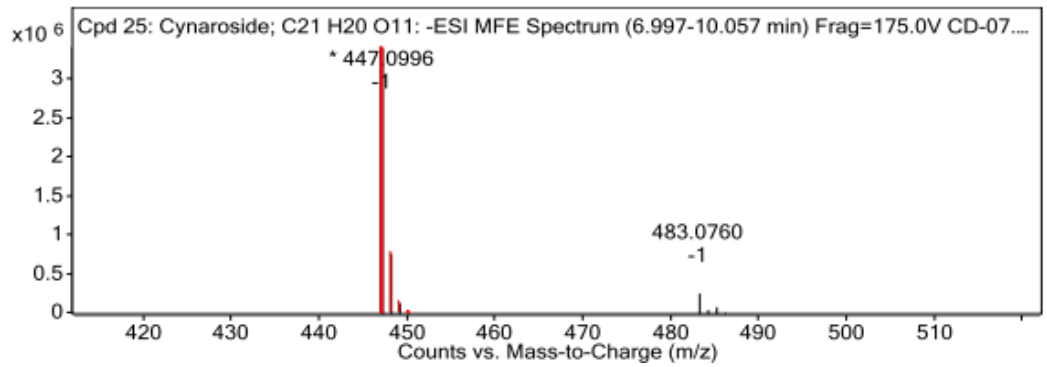
potent influenza RNA-dependent RNA polymerase inhibitor with an **IC**$_{50}$ of 32nM. Cynaroside is also promising inhibitor for H_2O_2-induced apoptosis, has cytoprotection against oxidative stress-induced cardiovascular diseases (Bouyahya et al.,2023)

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 25: Cynaroside; C21 H20 O11	**Cynaroside**	447.0996	7.263	Find by Molecular Feature	448.1069

MFE MS Spectrum



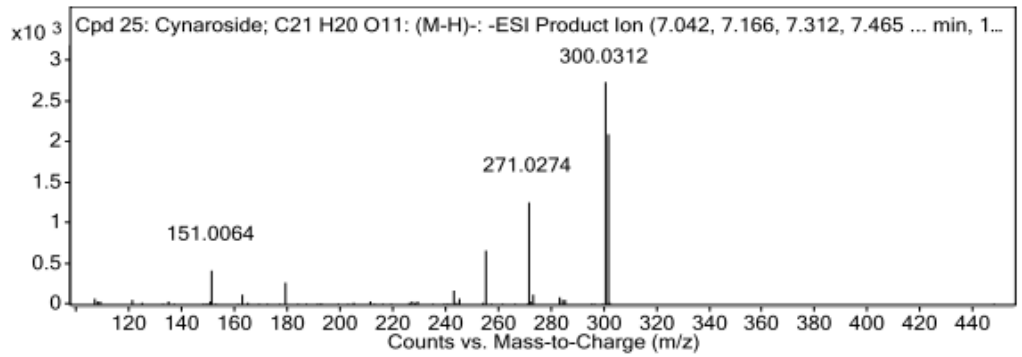
MFE MS Zoomed Spectrum



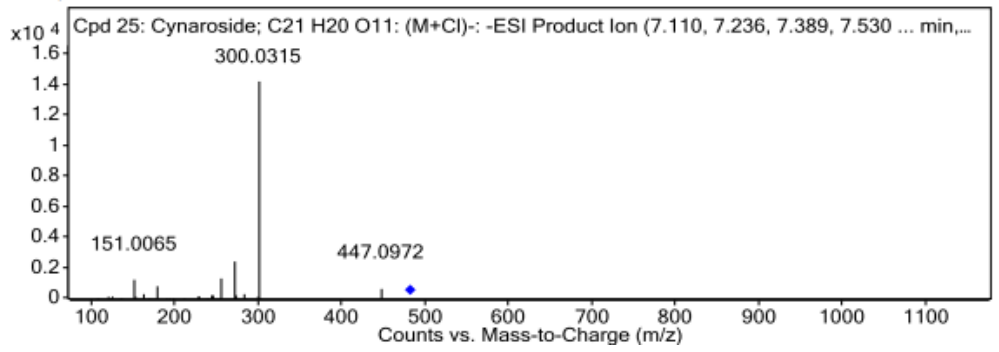
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
447.0996	-1	3404008.75	C21 H20 O11	(M-H)-
448.103	-1	774062.54	C21 H20 O11	(M-H)-
449.1048	-1	140245.05	C21 H20 O11	(M-H)-
450.1066	-1	24106.01	C21 H20 O11	(M-H)-
451.1074	-1	2684.81	C21 H20 O11	(M-H)-
483.076	-1	266044		(M+Cl)-
484.0789	-1	57703.09		(M+Cl)-
485.0737	-1	88479.71		(M+Cl)-
486.0764	-1	18920.03		(M+Cl)-
487.0765	-1	3441.32		(M+Cl)-

MSMS Spectrum



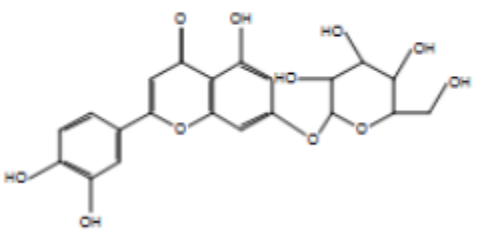
MSMS Spectrum



MS/MS Spectrum Peak List

m/z	z	Abund
151.0064	1	423.96
163.0037	1	129.61
179.0025	1	283.87
243.0331	1	183.86
255.033	1	676.79
271.0274	1	1270.01
273.0439	1	136.53
283.0284	1	96.81
300.0312	1	2749.06
301.0379	1	2096.62
151.0065	1	1250.35
163.0066	1	314.38
179.0014	1	851.04
255.033	1	1350.87
271.0281	1	2483.7
273.043	1	272.54
283.0279	1	368.4
300.0315	1	14251.22
301.0375	1	12305.31
447.0972	1	712.91

Compound Structure



Thus E. Hirta methanolic contains a wide variety of molecules having medicinal importance for human health. The traditional use of this plant as a medicine paid attention

for the scientific investigation. The molecules that are reported from HR- LCMS inspection in positive ESI Modes are listed in table below:

Peak	Tentative Compound	Retention Time (min)	Mass	Molecular Formula	DB Diff ppm	Chemical Class
1	D-1-Aminopropan-2-ol O-phosphate	1.083	155.0335	$C_3 H_{10} N O_4 P$	8.25	Lipid
2	Sorbose	1.129	180.0615	$C_6 H_{12} O_6$	10.31	Carbohydrates
3	6-Deoxyfagomine	1.133	131.0934	$C_6 H_{13} N O_2$	9.36	Alkaloid
4	(R)-Pelletierine	1.136	141.1142	$C_8 H_{15} N O$	8.18	Alkaloids
5	Medicanine	1.162	159.0883	$C_7 H_{13} N O_3$	7.53	amino acid
6	Isoamyl nitrite	1.181	117.0778	$C_5 H_{11} N O_2$	9.84	nitrite ester
7	3beta,6beta-Dihydroxynortropane	1.204	143.0936	$C_7 H_{13} N O_2$	7.44	Alkaloid
8	Lentiginose	1.233	157.1087	$C_8 H_{15} N O_2$	10.01	Alkaloid
9	Retronecine	1.244	155.093	$C_8 H_{13} N O_2$	10.56	Alkaloid
10	2-O-Methyl-L-fucose	1.469	178.0821	$C_7 H_{14} O_5$	11.18	Carbohydrates
11	(R)-2-Ethylmalate	2.783	162.0511	$C_6 H_{10} O_5$	10.42	Enzyme
12	3-Isopropylmalate	4.028	176.0664	$C_7 H_{12} O_5$	11.5	Enzyme
13	Pymetrozine	4.12	217.0933	$C_{10} H_{11} N_5 O$	14.27	Pyridine
14	(2Z,4'Z)-2-(5-Methylthio-4-penten-2-ynylidene)-1,6-dioxaspiro[4.4]non-3-ene	4.956	234.0721	$C_{13} H_{14} O_2 S$	-2.76	Ketal
15	Paramethasone	5.085	392.2001	$C_{22} H_{29} F O_5$	-0.51	Steroid
16	Sonchuionoside C	5.176	386.1924	$C_{19} H_{30} O_8$	4.26	Steroid
17	Flurandrenolide	5.458	436.2262	$C_{24} H_{33} F O_6$	-0.27	Steroid
18	3-O-Caffeoyl-1-O-methylquinic acid	5.633	368.1086	$C_{17} H_{20} O_9$	5.91	Quinic acid
19	Lucidenic acid A	5.814	458.2703	$C_{27} H_{38} O_6$	-7.65	Terpenoid
20	2-Protocatechoylphloroglucinolcarboxylate	5.859	306.0352	$C_{14} H_{10} O_8$	7.66	Phenol
21	3,5,6-Trihydroxy-5-(hydroxymethyl)-2-methoxy-2-cyclohexen-1-on	6.383	204.0615	$C_8 H_{12} O_6$	9.05	Cyclohexanon
22	Isobutyl 2-furanpropionate	6.676	196.108	$C_{11} H_{16} O_3$	10.1	Fatty acid
23	6-C-Galactosylluteolin	7.331	448.0983	$C_{21} H_{20} O_{11}$	5.03	Flavonoid
24	9-Hydroxy-7-megastigmen-3-one glucoside	7.426	372.2125	$C_{19} H_{32} O_7$	6.23	Lipid
25	2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-	7.626	241.0568	$C_{10} H_{11} N O_6$	7.8	Benzoxazino

Peak	Tentative Compound	Retention Time (min)	Mass	Molecular Formula	DB Diff ppm	Chemical Class
26	1-Methylinosine	8.402	282.0957	$C_{11}H_{14}N_4O_5$	2.59	Nucleosides
27	Garcinia lactone dibutyl ester	8.897	302.1345	$C_{14}H_{22}O_7$	6.93	Tricarboxylic acids
28	3'-Ketolactose	9.332	340.1013	$C_{12}H_{20}O_{11}$	-2.12	Carbohydrates
29	4,4-Difluoropregn-5-ene-3,20-dione	10.181	350.2045	$C_{21}H_{28}F_2O_2$	3.63	Steroids
30	2-[Methyl(3-phenylpropanoyl)amino]benzoic acid	11.551/ 11.766	283.1183	$C_{17}H_{17}NO_3$	8.99/8.46	amino benzoic acid
31	13-Hydroxy-9-methoxy-10-oxo-11-octadecenoic acid	12.152	342.2382	$C_{19}H_{34}O_5$	7.2	Fatty acid
32	Valdiate	12.968	310.1757	$C_{17}H_{26}O_5$	7.55	Fatty acid
33	12-oxo-LTB4	13.151	334.2096	$C_{20}H_{30}O_4$	14.3	Fatty acid
34	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid	14.865/ 15.114	228.1341	$C_{12}H_{20}O_4$	8.89/ 8.99	Fatty acid
35	Phthalic acid Mono-2-ethylhexyl Ester	16.712	278.1502	$C_{16}H_{22}O_4$	5.77	Phthalates
36	Epoxysiderol	17.257	362.2415	$C_{22}H_{34}O_4$	11.6	Terpenoids
37	Iriomoteolide 1a	17.441	506.3253	$C_{29}H_{46}O_7$	-1.81	Macrolide
38	Cavipetin D	17.519	418.2732	$C_{25}H_{38}O_5$	-3	Terpenoid
39	PI(18:1(9Z)/18:3(6Z,9Z,12Z))	17.520	858.5292	$C_{45}H_{79}O_{13}P$	-3.95	Lipids
40	Cyclopassifloside II	17.560	682.4246	$C_{37}H_{62}O_{11}$	6.7	Saponins
41	Ginsenoside F3	17.563	770.477	$C_{41}H_{70}O_{13}$	5.99	Saponin
42	Acetylbalchanolide	17.824	292.1652	$C_{17}H_{24}O_4$	7.57	Terpenoids
43	11-Hydroxyandrosterone	18.058	306.2172	$C_{19}H_{30}O_3$	7.49	Steroid
44	Polidocanol	18.121/ 18.39	582.4317	$C_{30}H_{62}O_{10}$	4.47/4.51	Glycerides
45	Methyl (7Z,9Z,9'Z)-6'-apo-y-caroten-6'-oate	18.236/ 18.502	472.3353	$C_{33}H_{44}O_2$	-2.38/-2.39	Terpenoid
46	Glyceryl lactooleate	18.533	428.3091	$C_{24}H_{44}O_6$	10.9	Fatty acid
47	3-Methyl-5-pentyl-2-furannonanoic acid	19.043	308.2328	$C_{19}H_{32}O_3$	7.59	Fatty acid
48	Irinotecan	19.862	586.2787	$C_{33}H_{38}N_4O_6$	0.68	Topoisomerase
49	Pheophorbide a	20.579/20.905	592.2664	$C_{35}H_{36}N_4O_5$	3.61/	Porphyrin
50	Aralionine A	20.65/ 21.004	582.281	$C_{34}H_{38}N_4O_5$	5.59/5.9	Peptide
51	Ganosporelactone A	21.145/21.498	512.2781	$C_{30}H_{40}O_7$	-1.28/	Polyketide

Peak	Tentative Compound	Retention Time (min)	Mass	Molecular Formula	DB Diff ppm	Chemical Class
52	Allosamidine	21.948	622.2762	C_{25} H_{42} N_4 O_{14}	-10.36	Nucleotide
53	Haplophytine	22.052/ 22.333	652.2869	C_{37} H_{40} N_4 O_7	4.25/ 4.73	Alkaloid
54	20-Hydroxy-3,7,11,15,23-pentaoxolanost-8-en-26-oic acid	22.121/ 22.557	528.2744	C_{30} H_{40} O_8	-3.98/ 2.14	Terpenoid
55	Ganoderic acid K	22.576/ 22.907	574.3149	C_{32} H_{46} O_9	-1.3/ 0.89	Steroid
56	Antimycin A1	22.947/ 23.303	548.2758	C_{28} H_{40} N_2 O_9	-5.22/ 2.86	Macrolide

Positive ESI mode of HR-LCMS analysis of the methanolic extract reported 87 compounds. Among them 56 are known and 31 are unknown compounds. Out of which 16 are medicinally active and 7 of adverse health effect. Categorically they are: alkaloids-6, steroids-6, terpenoids-6, saponins-2, flavonoid-1, phenol-1, fatty acids-7, lipids-3, carbohydrates-3, amino acids-2, enzyme-2 and others metabolites-17.

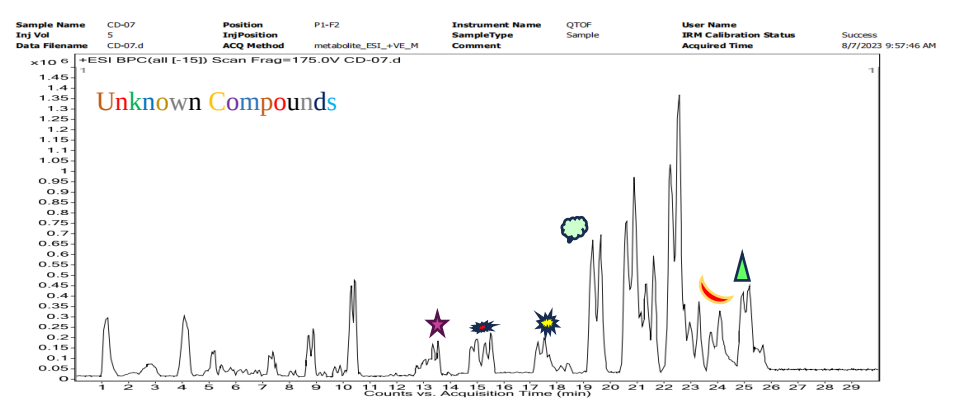


Fig 8 : Base peak chromatogram in positive ESI mode to show unknown compounds

Similarly, labile compounds reported from HR- LCMS inspection in negative ESI Mode were listed in table below as:

Peak	Tentative Compound	Retention Time (min)	Mass	Molecular Formula	DB Diff ppm	Chemical Class
1	5-O-Galloylhamamelofuranose	1.204	332.0787	$C_{13}H_{16}O_{10}$	-13.08	Tannin
2	2-Galloylglucose	1.498	332.0785	$C_{13}H_{16}O_{10}$	-12.36	Phenol
3	Ethyl methanesulfonate	1.717 / 4.146	124.0199	$C_3H_8O_3S$	-3.56/ -3.57	Alkylating agents
4	Mecarbinzid	3.391/ 4.561 /4.814/ 5.553	308.0943	$C_{13}H_{16}N_4O_3S$	0.19/ 0.26/ -0.44/ 0.12	Sulfonamide
5	Vanillin 4-sulfate	4.678/ 5.796	246.0202	$C_9H_{10}O_6S$	-1.46/-3.76	Sulfates
6	N-(Carbomethoxyacetyl)-4-S-chlorotryptophan	4.98	338.0687	$C_{15}H_{15}ClN_2O_5$	-5.16	Complex
7	Punicacortein B	5.006	634.0884	$C_{27}H_{22}O_{18}$	-12.32	Tannin
8	Glyurallin B	5.043	422.1745	$C_{25}H_{26}O_6$	-3.66	Flavonoid
9	1,2,4-Trigalloyl-beta-D-glucopyranose	5.431	636.1024	$C_{27}H_{24}O_{18}$	-9.63	Tannin
10	Benzyl sulfate	5.654	188.0146	$C_7H_8O_4S$	-1.7	Sulfate
11	Syzyginin B	5.957	756.0868	$C_{33}H_{24}O_{21}$	-7.72	Tannin
12	Pibutidine	6.386	356.1893	$C_{19}H_{24}N_4O_3$	-12.59	Antihistamine
13	Hyperoside	6.514	464.1015	$C_{21}H_{20}O_{12}$	-13.01	Flavonoid
14	Cynaroside	7.263	448.1069	$C_{21}H_{20}O_{11}$	-14.06	Flavonoid
15	6-C-Fucosylluteolin	7.938	432.1116	$C_{21}H_{20}O_{10}$	-13.81	Flavonoid
16	5,7,8,3',4'-Pentahydroxyisoflavones	8.966	302.0465	$C_{15}H_{10}O_7$	-12.88	Flavonoid
17	Auxin a	10.055	328.2297	$C_{18}H_{32}O_5$	-14.4	Fatty acid
18	Gnididilatin	14.612	652.3268	$C_{37}H_{48}O_{10}$	-3.19	Terpenoid
19	Geranylfarnesyl diphosphate	16.129/16.461	518.2601	$C_{25}H_{44}O_7P_2$	-7.49/ -7.96	Terpenoid
20	APGPR Enterostatin	18.837	496.2768	$C_{21}H_{36}N_8O_6$	-2.03	Peptide

Peak	Tentative Compound	Retention Time (min)	Mass	Molecular Formula	DB Diff ppm	Chemical Class
21	Ritterazine A	23.962/ 24.284	912.541	C_{54} H_{76} N_2 O_{10}	9.88	Alkaloid
22	3-Hydroxy-b,e-caroten-3'-one	24.177	566.4146	C_{40} H_{54} O_2	-3.87	Carotenoid

30 compounds were reported in Negative ESI mode whereas 22 were known and 8 were unknown according to HR-LCMS data inspection. Out of 22 compounds it was observed that 5 flavonoids, 4 tannins, 2 terpenoids, 1 phenol and 1 alkaloid, one fatty acid and 8 others metabolites.

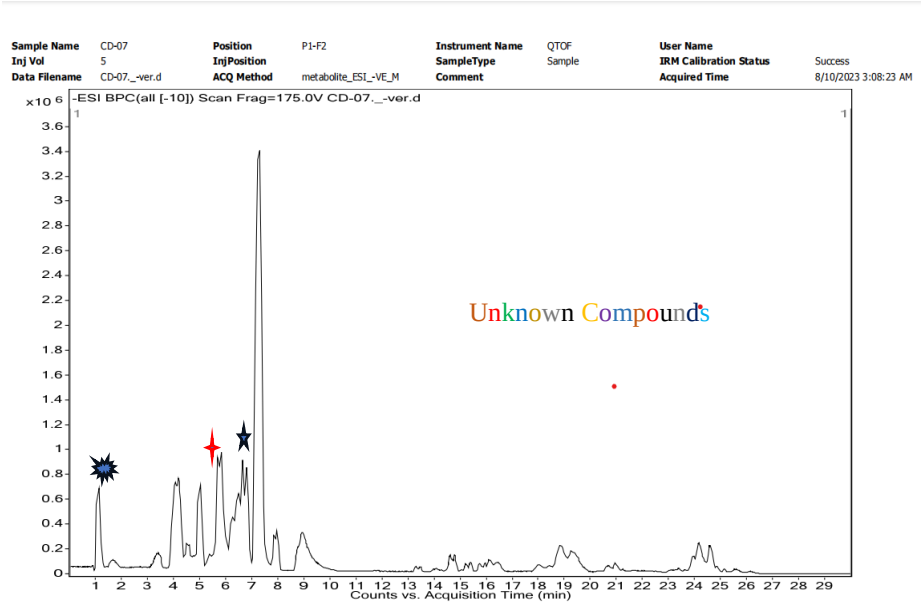


Fig 9: Base peak chromatogram in negative ESI mode to show unknown compounds

Preliminary phytochemical investigation highlights positive results against alkaloids, flavonoids, steroids, terpenoids, carbohydrates and coumarins which reveals the plant sample as goldmine of phytochemical compound.

The quantitative assessments of phytochemical constituents (TPC, TFC and TAA) shows that the plant extract contains substantial quantity of phytochemical constituents. This research result shows TPC 52.22±0.249 mg of GAE/g, TFC 258.04±2.001 mg RE /g and TAA 166.47±0.231 mg AAE/g in methanolic extract. Where as the methanolic plant extract exhibited DPPH IC_{50} as 44.041 µg/ mL. Antibacterial test shows the most effective ZOI value15.33±1.530 mm for *E. coli* and minimum 12.00±0.00 mm with *P. aeruginosa* micro-organism.

The qualitative HRLCMS analysis reveals the presence of substantial numbers of active molecules in the plant, which holds the significant role to cure different diseases. The numbers of molecules present in the extract have wide range of beneficial properties, i.e. active amyl glycosidase inhibitor acts as anti-HIV properties, anti-tumor, immunomodulatory activities, antioxidant, anti-psoriasis drug, sclerosing agent (antipruritic and anastatic) for removal of varicose veins, cytotoxic drug against cancer cell (Topo1).Many of the molecules act as anti-hypertensive agent, immunomodulatory, anti-inflammatory, anti-cancer as well as anti-asthmatic, vasodilator, anti-viral (potential binder against SARS-COV-2) and antifungal. Plant extract can also be used for the medication of cyanide poising also. Besides this, some of the molecules present also used in food and beverage sweetener and preservatives.

The photochemical profiling using HR-LCMS unveiled a total of 117 compounds, comprising 78 identified compounds and 39 hitherto unidentified compounds. Whereas 56 compounds were reported in positive ESI mode out of this 16 were medicinally active. Similarly, 22 compounds are reported in negative ESI mode among them 9 were medicinally active. The relation between the traditional use of E. Hirta and the molecules that are shown in HR-LCMS analysis showed its effective role for the treatment of various kinds of diseases.

5 CHAPTER V: CONCLUSION

5.1 Conclusions

The methanolic extract derived from the leaves of *Euphorbia Hirta* Linn. emerged attention for its significant medicinal uses. The quantitative phytochemical screening of the extract unveiled substantial concentration of total Phenolic (52.22±0.249 mg GAE /g) and total flavonoid (258.04±2.001mg RE /g) contents. Leaves extract also shows anti-bacterial and antioxidant activity (TAA 166.47 ± 0.231mg AAE /g & DPPH IC_{50} as 44.041 µg/ mL). This work uncovers almost all molecules present in the plant and the potential role of E. Hirta to be used as traditional medicine.

HR-LCMS profiling of the sample reveals the existence of 117 compound, comprising 39 unidentified compounds and 78 are identified compounds. These belongs to different classes of molecules such as 8 terpenoids, 8 fatty acids, 7 alkaloids, 6 flavonoids, 6 steroids, 4 tannins, 2 phenols, 2 saponins and 35 others metabolites. Among these molecules, 25 exhibit significant biological activities. These bioactive compounds are reported to have valuable medicinal properties. As a result, this plant appears as promising source for the treatment of broad spectrum of diseases. Furthermore, the molecules present in the extract reported to have efficacy in combating different types of cancers, i.e. epithetical, gastric, colon & rectal cancer in humans. The potential characteristics in combating as hypertension, cyanide poising, lowering of blood cholesterols, removing spider veins, anti-HIV, anti-inflammatory, anti-cancer, anti- bacterial and anti-fungal proved to be a high medicinal value of the plant. Its uses as food and bravage sweetener also signifies its value to use in food products. Report showed that the molecules present in its extract have characteristics of anti- Alzheimer's, antioxidant, cytotoxic, hepatoprotective, vasodilating, and potential binder against SARS-COV-2(anti-viral agent) etc. Thus, the chosen medical plant exhibits potential medicinal importance in ethnobiology.

5.2 Recommendations

The extraction of the molecules from the plant as in its natural form depends on the polarity of solvent used in the process. Therefore, this study recommends extracting the molecules in the solvent of different polarity to get almost all information of the biological molecules. The complete information of the molecules present in the plant and efficacy of the extract has to be further evaluated for

- Bio-pharmacological inspection of extract.
- Toxicity and effective dose of the extract.
- Isolation of pure compounds and their characterization.
- In-silico pharmacokinetic study, molecular docking and their characterizations noble drug
- Unidentified compound appeared in the HR-LCMS should isolate and characterize them.

6 REFERENCE

Abubakar, E. M. (2009). Antibacterial activity of crude extracts of Euphorbia hirta against some bacteria associated with enteric infections. *J Med Plants Res*, *3*(7), 498-505.

Adedapo, A. A., Shabi, O. O., & Adedokun, O. A. (2005). Anthelmintic efficacy of the aqueous crude extract of Euphorbia hirta Linn in Nigerian dogs. *Veterinarskiarhiv*, *75*(1), 39-47.

Ahmad, J., & Ahamad, J. (2020). Bioactive Phytochemicals: Drug Discovery to Product Development. In J. Ahmed (Ed.), Drug Discovery from Plant Sources: Scope, Approach and Challenges. Bentham Science Publishers Pte. Ltd.

Al-Snafi, A. E. (2017). Pharmacology and therapeutic potential of Euphorbia hirta (Syn: Euphorbia pilulifera)-A review. *IOSR Journal of Pharmacy*, *7*(3), 7-20.

Amos Samkumar, R., Premnath, D., & David Paul Raj, R. S. (2019). Strategy for early callus induction and identification of anti-snake venom triterpenoids from plant extracts and suspension culture of Euphorbia hirta L. *3 Biotech*, *9*(7), 266.

Anand, U., Jacobo-Herrera, N., Altemimi, A., &Lakhssassi, N. (2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*, *9*(11), 258. https://doi.org/10.3390/metabo9110258

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., ... &Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology advances*, *33*(8), 1582-1614.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., International Natural Product Sciences Taskforce, &Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature reviews. Drug discovery*, *20*(3), 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Bailly, C. (2019). Irinotecan: 25 years of cancer treatment. *Pharmacological research*, *148*, 104398.

Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life sciences*, *78*(5), 431-441. [PubMed] DOI: 10.1016/j.lfs.2005.09.012

Banez, M. J., Geluz, M. I., Chandra, A., Hamdan, T., Biswas, O. S., Bryan, N. S., & Von Schwarz, E. R. (2020). A systemic review on the antioxidant and anti-inflammatory effects of resveratrol, curcumin, and dietary nitric oxide supplementation on human cardiovascular health. *Nutrition Research*, *78*, 11-26.https://doi.org/10.1016/j.nutres.2020.03.002

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological research*, *196*, 44-68.

Barros, L., Falcão, S., Baptista, P., Freire, C., Vilas-Boas, M., & Ferreira, I. C. (2008). Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays. *Food chemistry*, *111*(1), 61-66.

Basma, A. A., Zakaria, Z., Latha, L. Y., & Sasidharan, S. (2011). Antioxidant activity and phytochemical screening of the methanol extracts of Euphorbia hirta L. *Asian Pacific journal of tropical medicine*, *4*(5), 386-390.https:// doi.org/10.1016/S1995-7645[11]60109-0, PMID 21771682

Basyal, D., Neupane, A., Pandey, D. P., & Pandeya, S. (2021). Phytochemical Screening and In Vitro Antioxidant and Anti-inflammatory Activities of Aerial Parts of Euphorbia hirta L. *Journal of Nepal Chemical Society*, *42*(1), 115-124.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World allergy organization journal*, *5*, 9-19.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature reviews microbiology*, *13*(1), 42-51.

Bouyahya, A., Taha, D., Benali, T., Zengin, G., El Omari, N., El Hachlafi, N., ... & Sahib, N. (2023). Natural sources, biological effects, and pharmacological properties of cynaroside. *Biomedicine & Pharmacotherapy*, *161*, 114337.

Chandran, R., Abrahamse, H., Parimelazhagan, T., & Durai, G. (2017). Syzygiummundagam bark methanol extract restores skin to normal in diabetic wounded rats. *Biomedicine & Pharmacotherapy*, *94*, 781-786.

Chen, L. (1991). Polyphenols from leaves of Euphorbia hirta L. *Zhongguo Zhong yao za zhi= ZhongguoZhongyaoZazhi= China Journal of Chinese Materia Medica*, *16*(1), 38-9.

Ciulei I. Phytochemistry, 2003, 63, 97-104

Coulibaly, A. Y., Hashim, R., Sulaiman, S. F., Sulaiman, O., Ang, L. Z. P., & Ooi, K. L. (2014). Bioprospecting medicinal plants for antioxidant components. *Asian Pacific journal of tropical medicine*, *7*, S553-S559. DOI: 10.1016/S1995-7645(14)60289-3

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.https://doi.org/10.1128/CMR.12.4.564

Dahal, R. H., & Chaudhary, D. K. (2018). Microbial infections and antimicrobial resistance in Nepal: current trends and recommendations. *The open microbiology journal*, *12*, 230.

Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A., &Gharsallah, N. (2019). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. *Arabian Journal of Chemistry*, *12*(8), 3075-3086.

Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A., &Gharsallah, N. (2019). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. *Arabian Journal of Chemistry*, *12*(8), 3075-3086.

Dhami, N. (2008). Ethnomedicinal uses of plants in Western Terai of Nepal: a case study of Dekhatbhuli Vdc of Kanchanpur District. *Medicinal plants in Nepal: an anthology of contemporary research*, 165-177.

Dias, D. A., Urban, S., & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, *2*(2), 303-336.

Dias, T., Gaudêncio, S. P., & Pereira, F. (2018). A computer-driven approach to discover natural product leads for methicillin-resistant Staphylococcus aureus infection therapy. *Marine drugs*, *17*(1), 16.

El-Amier, Y. A., Abbas, M. A., & Dawood, S. H. (2015). Phytotoxic effect of plant extracts from Asteraceae on germination and growth of Echinocloa crus-galli. *International Journal of Development Research*, *5*(7), 4926-4931.

Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, *20*(9), 689-709.

Fu, Y., Chen, J., Li, Y. J., Zheng, Y. F., & Li, P. (2013). Antioxidant and anti-inflammatory activities of six flavonoids separated from licorice. *Food chemistry*, *141*(2), 1063-1071.

Ganesan, A. (2008). The impact of natural products upon modern drug discovery. *Current opinion in chemical biology*, *12*(3), 306-317.https://doi.org/10.1016/j.cbpa.2008.03.016.

Gewali, M. B., & Awale, S. (2008). Aspects of traditional medicine in Nepal. *Japan: Institute of Natural Medicine University of Toyama*, 140-2.

Ghosh, P., Ghosh, C., Das, S., Das, C., Mandal, S., & Chatterjee, S. (2019). Botanical description, phytochemical constituents and pharmacological properties of Euphorbia hirta Linn: a review. *International Journal of Health Sciences and Research*, *9*(3), 273-286.

GoN. (2014). Nepal fifth national report to Convention on Biological Diversity. *Singha Durbar Kathmandu*.

Gu, R., Chang, X., Bai, G., Li, X., Di, Y., Liu, X., ... & Wang, Y. (2021). Effects of household cooking methods on changes of tissue structure, phenolic antioxidant capacity and active component bioaccessibility of quinoa. *Food Chemistry*, *350*, 129138.

Gülçi˙ n, I. L., Beydemi˙ r, Ş., Topal, F., Gagua, N., Bakuridze, A., Bayram, R., &Gepdiremen, A. (2012). Apoptotic, antioxidant and antiradical effects of majdine and

isomajdine from Vinca herbaceaWaldst. and kit. *Journal of enzyme inhibition and medicinal chemistry*, *27*(4), 587-594.

Gülçi˙ n, I. L., Beydemi˙ r, Ş., Topal, F., Gagua, N., Bakuridze, A., Bayram, R., &Gepdiremen, A. (2012). Apoptotic, antioxidant and antiradical effects of majdine and isomajdine from Vinca herbaceaWaldst. and kit. *Journal of enzyme inhibition and medicinal chemistry*, *27*(4), 587-594.

Gülçin, İ., Elmastaş, M., & Aboul-Enein, H. Y. (2012). Antioxidant activity of clove oil–A powerful antioxidant source. *Arabian Journal of chemistry*, *5*(4), 489-499.

Haleshappa, R., Keshamma, E., Girija, C. R., Thanmayi, M., Nagesh, C. G., Lubna Fahmeen, G. H., ... & Patil, S. J. (2020). Phytochemical study and antioxidant properties of ethanolic extracts of Euphorbia milii. *Asian J Biol Sci*, *13*(1), 77-82.

Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

Harvey, A. L. (2008). Natural products in drug discovery. *Drug discovery today*, *13*(19-20), 894-901.

Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature reviews drug discovery*, *14*(2), 111-129.

Hasanuzzaman, M., Bhuyan, M. B., Anee, T. I., Parvin, K., Nahar, K., Mahmud, J. A., & Fujita, M. (2019). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants*, *8*(9), 384.

Heravi, M. M., Zadsirjan, V., Hamidi, H., Daraie, M., & Momeni, T. (2020). Recent applications of the Wittig reaction in alkaloid synthesis. *The Alkaloids: Chemistry and Biology*, *84*, 201-334.

Igwe, K. K., Madubuike, A. J., Akomas, S. C., Otuokere, I. E., &Ukwueze, C. S. (2016). Studies of the medicinal plant Euphorbia hirta methanol leaf extract phytocomponents by GCMS analysis. *International Journal of Scientific and Technical Research in Engineering*, *1*(4), 9-16.

Işık, M., Beydemir, Ş., Yılmaz, A., Naldan, M. E., Aslan, H. E., &Gülçin, İ. (2017). Oxidative stress and mRNA expression of acetylcholinesterase in the leukocytes of ischemic patients. *Biomedicine & pharmacotherapy*, *87*, 561-567.

Johnson, P. B., Abdurahman, E. M., Tiam, E. A., Abdu-Aguye, I., & Hussaini, I. M. (1999). Euphorbia hirta leaf extracts increase urine output and electrolytes in rats. *Journal of Ethnopharmacology*, *65*(1), 63-69.

Joshi, S., Subedi, Y. P., & Paudel, S. K. (2011). Antibacterial and antifungal activity of heartwood of Acacia catechu of Nepal. *J. Nepal. Chem. Soc*, *27*, 94-99.

K.R. Kirtikar, and B.D. Basu., Indian Medicinal Plants, Periodical Experts Books Agency, 2nd edition, Vol. 3, New Delhi 1991.

Karadeniz, F., Oh, J. H., Seo, Y., Yang, J., Lee, H., & Kong, C. S. (2023). Quercetin 3-O-Galactoside Isolated from Limonium tetragonum Inhibits Melanogenesis by Regulating PKA/MITF Signaling and ERK Activation. *International Journal of Molecular Sciences*, *24*(4), 3064.

Karalija, E., Parić, A., Dahija, S., Bešta-Gajević, R., &Ćavar Zeljković, S. (2020). Phenolic compounds and bioactive properties of Verbascum glabratum subsp. bosnense (K. Malý) Murb., an endemic plant species. *Natural product research*, *34*(16), 2407-2411. [CrossRef

Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z. S., Liu, Z., & Kumar, V. (2021). Exploring phytochemicals for combating antibiotic resistance in microbial pathogens. *Frontiers in pharmacology*, *12*, 720726

Kiguchi, M. M., & Dillavou, E. D. (2018). Thermal and nonthermal endovenous ablation options for treatment of superficial venous insufficiency. *Surgical Clinics*, *98*(2), 385-400.

Kling, A., Lukat, P., Almeida, D. V., Bauer, A., Fontaine, E., Sordello, S., ... & Müller, R. (2015). Targeting DnaN for tuberculosis therapy using novel griselimycins. *Science*, *348*(6239), 1106-1112.

Koehn, F. E., & Carter, G. T. (2005). The evolving role of natural products in drug discovery. *Nature reviews Drug discovery*, *4*(3), 206-220.

Krawczyk, H. (2019). The stilbene derivatives, nucleosides, and nucleosides modified by stilbene derivatives. *Bioorganic Chemistry*, *90*, 103073.

Krueger, G. G., O'Reilly, M. A., Weidner, M., Dromgoole, S. H., & Killey, F. P. (1998). Comparative efficacy of once-daily flurandrenolide tape versus twice-daily diflorasone diacetate ointment in the treatment of psoriasis. *Journal of the American Academy of Dermatology*, *38*(2), 186-190.

Kumar, S., Malhotra, R., & Kumar, D. (2010). Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacognosy reviews*, *4*(7), 58.http://www.phcogrev.com/article/2010/4/7/1041030973-78476 5327

Kumari, I., & Pandey, R. K. (2017). Antibacterial Activity of Euphorbia hirta L. In *Applications of biotechnology for sustainable development* (pp. 1-5). Springer Singapore.

Kunwar, R. M., Shrestha, K. P., & Bussmann, R. W. (2010). Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. *Journal of ethnobiology and ethnomedicine*, *6*, 1-18.

Lachance, H., Wetzel, S., Kumar, K., & Waldmann, H. (2012). Charting, navigating, and populating natural product chemical space for drug discovery. *Journal of medicinal chemistry*, *55*(13), 5989-6001.

Lahlou, M. (2007). Screening of natural products for drug discovery. *Expert opinion on drug discovery*, *2*(5), 697-705.

Lešnik, U., Lukežič, T., Podgoršek, A., Horvat, J., Polak, T., Šala, M., ... & Petković, H. (2015). Construction of a new class of tetracycline lead structures with potent antibacterial activity through biosynthetic engineering. *Angewandte Chemie*, *127*(13), 4009-4012.

Li, J. W. H., &Vederas, J. C. (2009). Drug discovery and natural products: end of an era or an endless frontier?. *Science*, *325*(5937), 161-165.

Liu, Y., Murakami, N., Ji, H., Abreu, P., & Zhang, S. (2007). Antimalarial Flavonol Glycosides from Euphorbia hirta. *Pharmaceutical Biology*, *45*(4), 278-281.

López-Alarcón, C., &Denicola, A. (2013). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica chimica acta*, *763*, 1-10.

Mickymaray, S. (2019). Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive Compounds Against Clinically Important Pathogens. Antibiotics, 8(4): 2–57.

Nacoulma, O. G. (1996). Medicinal plants and their traditional uses in Burkina Faso. *Doctoratd'Etat (Ph. D. Thesis), University of Ouagadougou*, *328*.Mamun-Or-Rashid ANM, Shohel M, Mamun-Or-Rashid, A. N. M., Shohel, M., Nayeem, M. T., &Monokesh, K. S. (2013). Compendium ehtnopharmaceutical review on Euphorbia hirta. *Ayurpharm Int J Ayur Alli Sci*, *2*(2), 14-21.

Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of natural products*, *79*(3), 629-661.

Newman, D. J., Cragg, G. M., & Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981− 2002. *Journal of natural products*, *66*(7), 1022-1037.

Ouerghemmi, I., Rebey, I. B., Rahali, F. Z., Bourgou, S., Pistelli, L., Ksouri, R., ... & Tounsi, M. S. (2017). Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Ruta chalepensis. *Journal of food and drug analysis*, *25*(2), 350-359.https://doi.org/https://doi.org/10.1016/j.jfda.2016.04. 001

Parthasarathy, R., & Sathiyabama, M. (2015). Lovastatin-producing endophytic fungus isolated from a medicinal plant Solanum xanthocarpum. *Natural product research*, *29*(24), 2282–2286. https://doi.org/10.1080/14786419.2015.1016938

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, *97*, 55-74.

Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, *209*, 112891. https://doi.org/10.1016/j. ejmech.2020.112891.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*, *2017*.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, *269*(2), 337-341.

Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical interventions in aging*, *2*(2), 219-236.

Ramakrishnan, R., Periyasamy, R., Lakkakula, S., Subramani, P., Arockiam, S. R., & Manikandan, R. (2017). In vitro propagation and conservation of useful endangered medicinal plants with anticancer activity. *J Mol Biol Biotech*, *2*(3), 8.

Rashed, K. (2014). Medicinal Plants with antioxidant Potential: A review. *Hygeia Journal for*.

Rezai, M., Bayrak, C., Taslimi, P., GÜLÇİN, İ., &Menzek, A. (2018). The first synthesis and antioxidant and anticholinergic activities of 1-(4, 5-dihydroxybenzyl) pyrrolidin-2-one derivative bromophenols including natural products. *Turkish journal of chemistry*, *42*(3), 808-825.

Rodríguez-Pérez, C.; Segura-Carretero, A.; Del Mar Contreras, M. Phenolic compounds as natural and multifunctional anti-obesity agents: A review. Crit. Rev. Food Sci. Nutr. 2019, 59(8), 1212–1229. DOI: 10.1080/ 10408398.2017.1399859.

Schippmann, U., Leaman, D. J., & Cunningham, A. B. (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. *Biodiversity and the ecosystem approach in agriculture, forestry and fisheries*.

Shaeer, K. M., Zmarlicka, M. T., Chahine, E. B., Piccicacco, N., & Cho, J. C. (2019). Plazomicin: a next-generation aminoglycoside. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *39*(1), 77-93.

Shahidi, F., &Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods*, *18*, 820-897.https://doi.org/10.1016/j.jff.2015.06.018.

Sharma, K. R. (2020). Phytotoxic, Antioxidant, Antibacterial, And Antileishmanial Activities Of Euphorbia Hirta From Chitwan District Nepal. *Asian J Pharm Clin Res*, *13*(2), 146-149.

Sharma, N. K., & Prasad, R. (2008). Oxidative injury to protein and their protection by phenolic acid antioxidants from Euphorbia hirta leaves. *Journal of Biotechnology*, (136), S720.

Shukla, R., Pandey, V., Vadnere, G. P., & Lodhi, S. (2019). Role of flavonoids in management of inflammatory disorders. In *Bioactive food as dietary interventions for arthritis and related inflammatory diseases* (pp. 293-322). Academic Press.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2020). Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Research International*, *132*, 109114.https://doi. org/10.1016/j.foodres.2020.109114.

Smith, P. A., Koehler, M. F., Girgis, H. S., Yan, D., Chen, Y., Chen, Y., ... & Heise, C. E. (2018). Optimized arylomycins are a new class of Gram-negative antibiotics. *Nature*, *561*(7722), 189-194.

Sorokina, M., & Steinbeck, C. (2020). Review on natural products databases: where to find data in 2020. *Journal of cheminformatics*, *12*(1),20. https://doi.org/10.1186/s13321-020-00424-9

Taslimi, P., &Gulçin, İ. (2018). Antioxidant and anticholinergic properties of olivetol. *Journal of food biochemistry*, *42*(3), e12516.

Thorsen, R. S., & Pouliot, M. (2016). Traditional medicine for the rich and knowledgeable: challenging assumptions about treatment-seeking behaviour in rural and peri-urban Nepal. *Health Policy and Planning*, *31*(3), 314-324.

Tuhin, R. H., Begum, M., Rahman, M. S., Karim, R., Begum, T., Ahmed, S. U., ... & Begum, R. (2017). Wound healing effect of Euphorbia hirtalinn.(Euphorbiaceae) in alloxan induced diabetic rats. *BMC complementary and alternative medicine*, *17*(1), 1-14.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., &Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, *39*(1), 44-84.Williamson EM. Major Herbs of Ayurveda. China: Churchill Livingstone; 2002.

Vijaya, K., Ananthan, S., & Nalini, R. (1995). Antibacterial effect of theaflavin, polyphenon 60 (Camellia sinensis) and Euphorbia hirta on Shigella spp.—a cell culture study. *Journal of ethnopharmacology*, *49*(2), 115-118..

Whayne T. F., Jr (2018). Clinical Use of Digitalis: A State of the Art Review. *American journal of cardiovascular drugs : drugs, devices, and other interventions*, *18*(6), 427–440. https://doi.org/10.1007/s40256-018-0292-1

World Health Organization. (2000). WHO General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, Geneva. Online.

World Health Organization. (2013). *WHO traditional medicine strategy: 2014-2023*. World Health Organization.

Yang, Z. N., Su, B. J., Wang, Y. Q., Liao, H. B., Chen, Z. F., & Liang, D. (2020). Isolation, absolute configuration, and biological activities of chebulic acid and brevifolincarboxylic acid derivatives from Euphorbia hirta. *Journal of natural products*, *83*(4), 985-995.https://doi.org/10.1021/acs.jnatprod.9b00877

Yuet Ping, K., Darah, I., Chen, Y., Sreeramanan, S., & Sasidharan, S. (2013). Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. *BioMed research international*, *2013*., http://dx.doi.org/10.1155/2013/ 182064

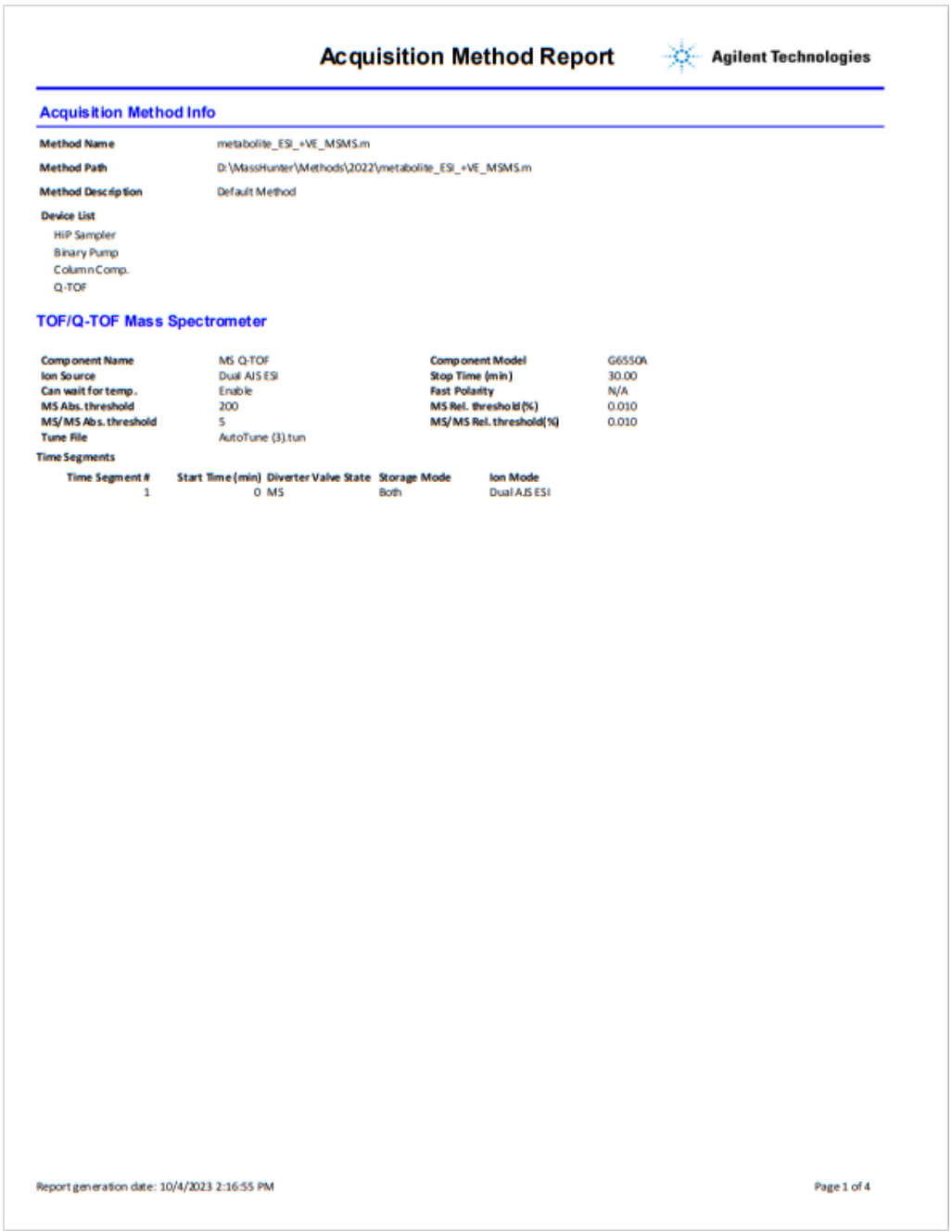
Zafar, S., Sarfraz, I., Rasul, A., Shah, M. A., Hussain, G., Zahoor, M. K., ... & Sarker, S. D. (2021). Osthole: A multifunctional natural compound with potential anticancer, antioxidant and anti-inflammatory Activities. *Mini Reviews in Medicinal Chemistry*, *21*(18), 2747-2763.

Zhang, S. Q., Bi, H. M., & Lisu, C. J. (2007). Extraction of bio-active components from Rhodiola sachalinensis under ultrahigh hydrostatic pressure. *Separation and Purification Technology*, *57*(2), 277-282.

Zuorro, A., & Lavecchia, R. (2012). Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *Journal of Cleaner Production*, *34*, 49-56.

7 APPENDIX – I

HR-LCMS method of analysis



Acquisition Method Report Agilent Technologies

Acquisition Method Info

Method Name	metabolite_ESI_+VE_MSMS.m
Method Path	D:\MassHunter\Methods\2022\metabolite_ESI_+VE_MSMS.m
Method Description	Default Method

Device List

HiP Sampler
Binary Pump
Column Comp.
Q-TOF

TOF/Q-TOF Mass Spectrometer

Component Name	MS Q-TOF	**Component Model**	G6550A
Ion Source	Dual AJS ESI	**Stop Time (min)**	30.00
Can wait for temp.	Enable	**Fast Polarity**	N/A
MS Abs. threshold	200	**MS Rel. threshold(%)**	0.010
MS/MS Abs. threshold	5	**MS/MS Rel. threshold(%)**	0.010
Tune File	AutoTune (3).tun		

Time Segments

Time Segment #	Start Time (min)	Diverter Valve State	Storage Mode	Ion Mode
1	0	MS	Both	Dual AJS ESI

Report generation date: 10/4/2023 2:16:55 PM Page 1 of 4

Time Segment 1

Acquisition Mode AutoMS2

MS Min Range (m/z)	126
MS Max Range (m/z)	1200
MS Scan Rate (spectra/sec)	1.00
MS/MS Scan Rate (spectra/sec)	1.00
Isolation Width MS/MS	Medium (~4 amu)

Ramped Collision Energy

Charge	Slope	Offset
1	8	-2.6
2	6	-2.6
3	4	-2.6

Auto MS/MS Preferred/Exclude Table

Mass	Delta Mass (ppm)	Charge	Type	Retention Time (min)	Delta Ret. Time (min)	Isolation Width	Collision Energy
197.8075	500	1	Exclude	0		Medium (~4 amu)	

Precursor Selection

Max Precursors Per Cycle	10
Threshold (Abs)	10000
Threshold (Rel)(%)	0.010
Precursor abundance based scan speed	Yes
Target (counts/spectrum)	25000.000
Use MS/MS accumulation time limit	Yes
Use dynamic precursor rejection	No
Purity Stringency (%)	100.000
Purity Cutoff (%)	30.000
Isotope Model	Common
Active exclusion enabled	Yes
Active exclusion excluded after (spectra)	1
Active exclusion released after (min)	0.20
Sort precursors	By abundance only

Charge State Preference

Selected Charges
1
2
Unk

Source Parameters

Parameter	Value
Gas Temp (°C)	250
Gas Flow (l/min)	13
Nebulizer (psig)	35
SheathGasTemp	300
SheathGasFlow	11

Scan Segments

Scan Seg #	Ion Polarity	Collision Energy
1	Positive	0

Scan Segment 1

Scan Source Parameters

Parameter	Value
VCap	3500
Nozzle Voltage (V)	1000
Fragmentor	175
Skimmer1	65
OctopoleRFPeak	750

ReferenceMasses

Ref Mass Enabled	Disabled
Ref Nebulizer (psig)	

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Acquisition Method Report

Agilent Technologies

Name: HiP Sampler **Model:** G4226A

Auxiliary	
Draw Speed	100.0 µL/min
Eject Speed	100.0 µL/min
Draw Position Offset	0.0 mm
Wait Time After Drawing	2.0 s
Sample Flush Out Factor	5.0
Vial/Well bottom sensing	Yes
Injection	
Injection Mode	Injection with needle wash
Injection Volume	5.00 µL
Needle Wash	
Needle Wash Location	Flush Port
Wash Time	3.0 s
High throughput	
Automatic Delay Volume Reduction	No
Overlapped Injection	
Enable Overlapped Injection	No
Valve Switching	
Valve Movements	0
Valve Switch Time 1	
Switch Time 1 Enabled	Yes
Switch Time 1	0.01 min
Valve Switch Time 2	
Switch Time 2 Enabled	No
Valve Switch Time 3	
Switch Time 3 Enabled	No
Valve Switch Time 4	
Switch Time 4 Enabled	No
Stop Time	
Stoptime Mode	As pump/No limit
Post Time	
Posttime Mode	Off

Acquisition Method Report

Agilent Technologies

Name: Binary Pump **Model:** G4220B

Flow	0.300 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	1200.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Stroke A	
Automatic Stroke Calculation A	Yes
Stop Time	
Stoptime Mode	Time set
Stoptime	35.00 min
Post Time	
Posttime Mode	Off

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch 2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % Water V.02	0.1% FA in water	100.0 % Water V.02	0.1% FA in water	Ch. 2	Yes	95.00 %
2	B	100.0 % Methanol V.03		100.0 % Acetonitrile V.02		Ch. 2	Yes	5.00 %

Timetable

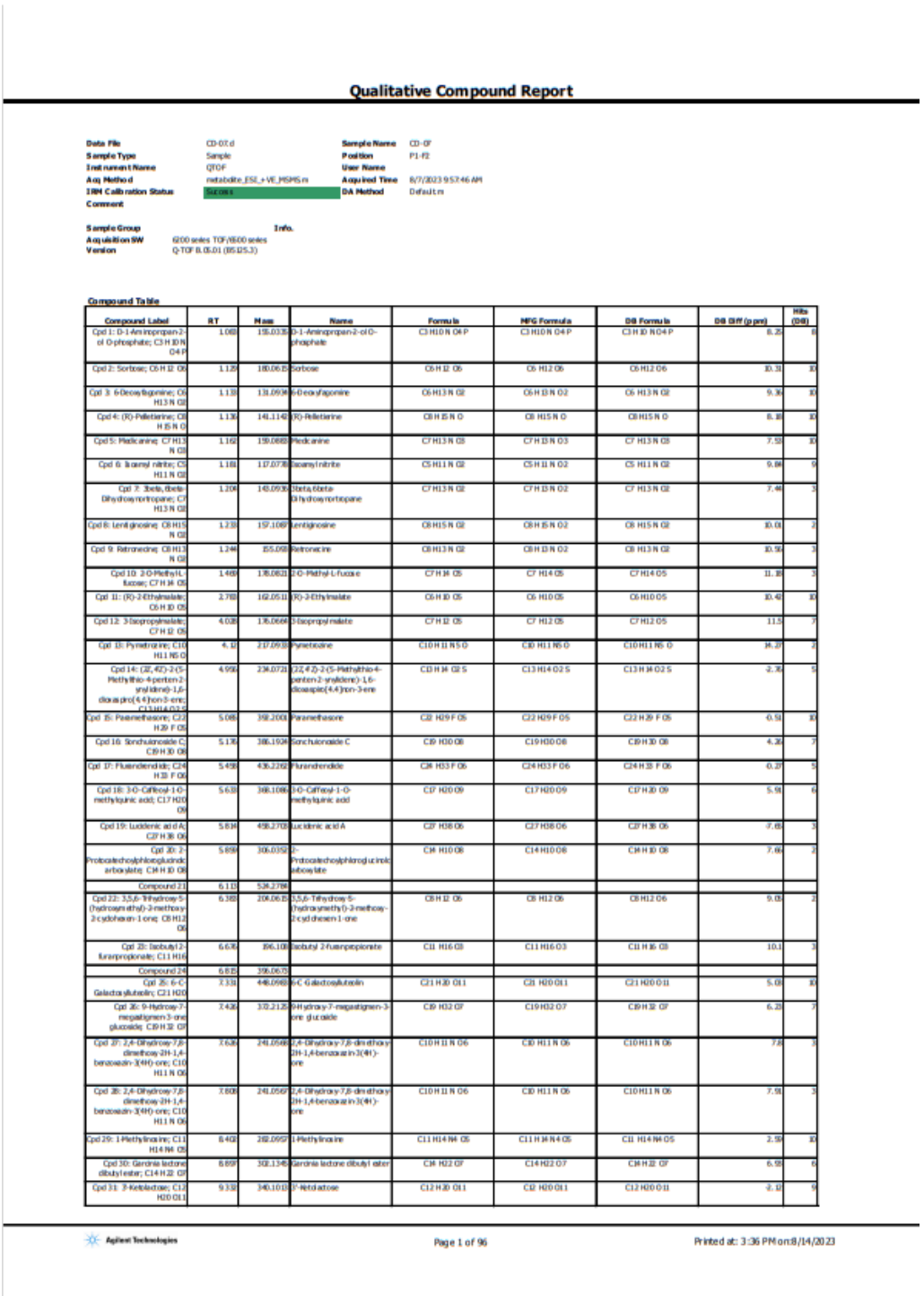
	Time	A	B	Flow	Pressure
1	1.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar
2	25.00 min	0.00 %	100.00 %	0.300 mL/min	1200.00 bar
3	30.00 min	0.00 %	100.00 %	0.300 mL/min	1200.00 bar
4	31.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar
5	35.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar

Name: Column Comp. **Model:** G1316C

Ready when front door open	Yes
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	40.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.80 °C
Right Temperature Control	
Right temperature Control Mode	Temperature Set
Right temperature	40.00 °C
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.80 °C
Stop Time	
Stoptime Mode	As pump/injector
Post Time	
Posttime Mode	Off

8 APPENDIX – II

HR-LCMS Profiling of compound in positive ESI mode



Qualitative Compound Report

Data File	CD-07.d	**Sample Name**	CD-07
Sample Type	Sample	**Position**	P1-F2
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_+VE_HSHS.m	**Acquired Time**	8/7/2023 9:57:46 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)

Compound Table

Compound Label	RT	Mass	Name	Formula	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 1: D-1-Aminopropan-2-ol O-phosphate; C3 H10 N O4 P	1.083	155.0335	D-1-Aminopropan-2-ol O-phosphate	C3 H10 N O4 P	C3 H10 N O4 P	C3 H10 N O4 P	6.25	8
Cpd 2: Sorbose; C6 H12 O6	1.139	180.0635	Sorbose	C6 H12 O6	C6 H12 O6	C6 H12 O6	10.31	10
Cpd 3: 6-Deoxyfagonine; C6 H13 N O2	1.133	131.0934	6-Deoxyfagonine	C6 H13 N O2	C6 H13 N O2	C6 H13 N O2	9.36	10
Cpd 4: (R)-Pelletierine; C8 H15 N O	1.136	141.1143	(R)-Pelletierine	C8 H15 N O	C8 H15 N O	C8 H15 N O	8.38	10
Cpd 5: Medicanine; C7 H13 N O3	1.162	159.0888	Medicanine	C7 H13 N O3	C7 H13 N O3	C7 H13 N O3	7.55	10
Cpd 6: Isoamyl nitrite; C5 H11 N O2	1.181	117.0778	Isoamyl nitrite	C5 H11 N O2	C5 H11 N O2	C5 H11 N O2	9.84	9
Cpd 7: 3beta,6beta-Dihydroxynortropane; C7 H13 N O2	1.204	143.0936	3beta,6beta-Dihydroxynortropane	C7 H13 N O2	C7 H13 N O2	C7 H13 N O2	7.44	3
Cpd 8: Lentiginosine; C8 H15 N O2	1.233	157.1089	Lentiginosine	C8 H15 N O2	C8 H15 N O2	C8 H15 N O2	10.01	2
Cpd 9: Retronecine; C8 H13 N O2	1.244	155.095	Retronecine	C8 H13 N O2	C8 H13 N O2	C8 H13 N O2	10.56	3
Cpd 10: 2-O-Methyl-L-fucose; C7 H14 O5	1.469	178.0821	2-O-Methyl-L-fucose	C7 H14 O5	C7 H14 O5	C7 H14 O5	11.16	3
Cpd 11: (R)-2-Ethylmalate; C6 H10 O5	2.703	162.0511	(R)-2-Ethylmalate	C6 H10 O5	C6 H10 O5	C6 H10 O5	10.43	10
Cpd 12: 3-Isopropylmalate; C7 H12 O5	4.028	176.0668	3-Isopropylmalate	C7 H12 O5	C7 H12 O5	C7 H12 O5	11.3	7
Cpd 13: Pyrrolizine; C10 H11 N5 O	4.12	217.0953	Pyrrolizine	C10 H11 N5 O	C10 H11 N5 O	C10 H11 N5 O	14.27	2
Cpd 14: (2E,4Z)-2-(5-Methylthio-4-pentenylidene)-1,6-dioxaspiro[4.4]non-3-ene; C13 H14 O2 S	4.958	234.0721	(2Z,4Z)-2-(5-Methylthio-4-pentenylidene)-1,6-dioxaspiro[4.4]non-3-ene	C13 H14 O2 S	C13 H14 O2 S	C13 H14 O2 S	-2.36	5
Cpd 15: Paramethasone; C22 H29 F O5	5.085	392.2001	Paramethasone	C22 H29 F O5	C22 H29 F O5	C22 H29 F O5	-0.51	10
Cpd 16: Sinchulonoside C; C19 H30 O8	5.176	386.1934	Sinchulonoside C	C19 H30 O8	C19 H30 O8	C19 H30 O8	4.32	7
Cpd 17: Flurandrenolide; C24 H33 F O6	5.458	436.2292	Flurandrenolide	C24 H33 F O6	C24 H33 F O6	C24 H33 F O6	-0.27	5
Cpd 18: 3-O-Caffeoyl-1-O-methylquinic acid; C17 H20 O9	5.638	368.1086	3-O-Caffeoyl-1-O-methylquinic acid	C17 H20 O9	C17 H20 O9	C17 H20 O9	5.98	6
Cpd 19: Luciidenic acid A; C27 H38 O6	5.814	458.2703	Luciidenic acid A	C27 H38 O6	C27 H38 O6	C27 H38 O6	-7.65	3
Cpd 20: 2-Protocatechoylphlorogluciniol-arboxylate; C14 H10 O8	5.859	306.0352	2-Protocatechoylphlorogluciniol-arboxylate	C14 H10 O8	C14 H10 O8	C14 H10 O8	7.66	2
Compound 21	6.113	536.2788						
Cpd 22: 3,5,6-Trihydroxy-5-(hydroxymethyl)-2-methoxy-2-cyclohexen-1-one; C8 H12 O6	6.388	204.0635	3,5,6-Trihydroxy-5-(hydroxymethyl)-2-methoxy-2-cyclohexen-1-one	C8 H12 O6	C8 H12 O6	C8 H12 O6	9.03	2
Cpd 23: Isobutyl 2-furanpropionate; C11 H16	6.636	166.1089	Isobutyl 2-furanpropionate	C11 H16 O3	C11 H16 O3	C11 H16 O3	10.1	3
Compound 24	6.813	356.0675						
Cpd 25: 6-C-Galactosylluteolin; C21 H20	7.331	448.0968	6-C-Galactosylluteolin	C21 H20 O11	C21 H20 O11	C21 H20 O11	5.08	10
Cpd 26: 9-Hydroxy-7-megastigmen-3-one glucoside; C19 H32 O7	7.436	372.2125	9-Hydroxy-7-megastigmen-3-one glucoside	C19 H32 O7	C19 H32 O7	C19 H32 O7	6.23	7
Cpd 27: 2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one; C10 H11 N O6	7.636	241.0586	2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one	C10 H11 N O6	C10 H11 N O6	C10 H11 N O6	7.8	3
Cpd 28: 2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one; C10 H11 N O6	7.808	241.0587	2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one	C10 H11 N O6	C10 H11 N O6	C10 H11 N O6	7.91	3
Cpd 29: 1-Methylinosine; C11 H14 N4 O5	8.402	282.0957	1-Methylinosine	C11 H14 N4 O5	C11 H14 N4 O5	C11 H14 N4 O5	2.59	10
Cpd 30: Geronlactone dibutylester; C14 H22 O7	8.697	302.1345	Geronlactone dibutylester	C14 H22 O7	C14 H22 O7	C14 H22 O7	6.59	6
Cpd 31: 3-Ketolactose; C12 H20 O11	9.332	340.1013	3-Ketolactose	C12 H20 O11	C12 H20 O11	C12 H20 O11	-2.12	9

Cpd 32: 4,4-Difluoroprogn-5-ene-3,20-dione; C21 H28 F2 O2	10.181	390.2046	4,4-Difluoroprogn-5-ene-3,20-dione	C21 H28 F2 O2	C21 H28 F2 O2	C21 H28 F2 O2	3.68	10
Cpd 33: 2-[(Methyl)(3-phenylpropanoyl)amino]benzoic acid; C17 H17 N O3	11.551	283.1188	2-[(Methyl)(3-phenylpropanoyl)amino]benzoic acid	C17 H17 N O3	C17 H17 N O3	C17 H17 N O3	8.99	7
Cpd 34: 2-[(Methyl)(3-phenylpropanoyl)amino]benzoic acid; C17 H17 N O3	11.700	283.1184	2-[(Methyl)(3-phenylpropanoyl)amino]benzoic acid	C17 H17 N O3	C17 H17 N O3	C17 H17 N O3	8.48	7
Cpd 35: 13-Hydroxy-9-methoxy-10-oxo-11-octadecenoic acid; C19 H34	12.152	342.2382	13-Hydroxy-9-methoxy-10-oxo-11-octadecenoic acid	C19 H34 O5	C19 H34 O5	C19 H34 O5	7.2	6
Cpd 36: Veldiate; C17 H26 O5	12.968	310.1757	Veldiate	C17 H26 O5	C17 H26 O5	C17 H26 O5	7.55	3
Cpd 37: 12-oxo-LTB4; C20 H30 O4	13.152	334.2096	12-oxo-LTB4	C20 H30 O4	C20 H30 O4	C20 H30 O4	14.3	10
Compound 38	13.415	227.2596						
Cpd 39: 5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid; C12 H20 O4	14.883	228.1341	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid	C12 H20 O4	C12 H20 O4	C12 H20 O4	8.86	3
Compound 40	15.000	355.3064						
Cpd 41: 5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid; C12 H20 O4	15.134	228.1341	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid	C12 H20 O4	C12 H20 O4	C12 H20 O4	8.99	3
Compound 42	15.235	288.2059						
Compound 43	15.470	288.2059						
Compound 44	16.499	424.2799						
Cpd 45: Phthalic acid Mono-2-ethylhexyl Ester; C16 H22 O4	16.712	278.1518	Phthalic acid Mono-2-ethylhexyl Ester	C16 H22 O4	C16 H22 O4	C16 H22 O4	5.77	9
Cpd 46: Epoxysiderol; C22 H34 O4	17.257	362.2435	Epoxysiderol	C22 H34 O4	C22 H34 O4	C22 H34 O4	11.6	9
Cpd 47: Isonicotelide 1a; C29 H46 O7	17.441	506.3259	Isonicotelide 1a	C29 H46 O7	C29 H46 O7	C29 H46 O7	-1.61	2
Compound 48	17.493	902.5553						
Compound 49	17.499	462.7698						
Cpd 50: Cavipetin D; C25 H38 O5	17.519	418.2732	Cavipetin D	C25 H38 O5	C25 H38 O5	C25 H38 O5	-3	3
Cpd 51: P[(18:1(9Z)/18:3(6Z,9Z,12Z))]; C45 H79 O13 P	17.52	858.5292	P[(18:1(9Z)/18:3(6Z,9Z,12Z))]	C45 H79 O13 P	C45 H79 O13 P	C45 H79 O13 P	-3.95	10
Compound 52	17.538	814.5038						
Cpd 53: Cyclopassifloside II; C37 H62 O11	17.56	682.4246	Cyclopassifloside II	C37 H62 O11	C37 H62 O11	C37 H62 O11	6.7	1
Cpd 54: Ginsenoside F3; C41 H70 O13	17.562	770.4777	Ginsenoside F3	C41 H70 O13	C41 H70 O13	C41 H70 O13	5.99	4
Cpd 55: Acetylbalchanolide; C17 H24 O4	17.629	292.1682	Acetylbalchanolide	C17 H24 O4	C17 H24 O4	C17 H24 O4	7.57	10
Cpd 56: 11-Hydroxyandrosterone; C19	18.098	306.2172	11-Hydroxyandrosterone	C19 H30 O3	C19 H30 O3	C19 H30 O3	7.46	10
Compound 57	18.098	692.4669						
Compound 58	18.098	648.44						
Cpd 59: Poldocanol; C30 H62 O10	18.121	582.4317	Poldocanol	C30 H62 O10	C30 H62 O10	C30 H62 O10	4.47	2
Compound 60	18.159	540.3836						
Compound 61	18.188	536.3611						
Compound 62	18.200	716.492						
Cpd 63: Plethyl (7Z,9Z,9'Z)-6'-apo-y-caroten-6'-oate; C33 H44 O2	18.230	472.3352	Plethyl (7Z,9Z,9'Z)-6'-apo-y-caroten-6'-oate	C33 H44 O2	C33 H44 O2	C33 H44 O2	-2.38	3
Compound 64	18.333	692.4662						
Compound 65	18.369	648.44						
Cpd 66: Poldocanol; C30 H62 O10	18.39	582.4317	Poldocanol	C30 H62 O10	C30 H62 O10	C30 H62 O10	4.51	2
Compound 67	18.441	540.3837						
Compound 68	18.459	536.3614						
Cpd 69: Plethyl (7Z,9Z,9'Z)-6'-apo-y-caroten-6'-oate; C33 H44 O2	18.502	472.3352	Plethyl (7Z,9Z,9'Z)-6'-apo-y-caroten-6'-oate	C33 H44 O2	C33 H44 O2	C33 H44 O2	-2.38	3
Cpd 70: Glyceryl lactodelate; C24 H44 O6	18.533	428.3091	Glyceryl lactodelate	C24 H44 O6	C24 H44 O6	C24 H44 O6	10.9	2
Cpd 71: 3-Methyl-5-pentyl-2-furannonanoic acid; C19 H32 O3	19.040	308.2328	3-Methyl-5-pentyl-2-furannonanoic acid	C19 H32 O3	C19 H32 O3	C19 H32 O3	7.58	10
Compound 72	19.303	626.2935						
Compound 73	19.620	626.2935						
Cpd 74: Irinotecan; C33 H38 N4 O6	19.862	586.2787	Irinotecan	C33 H38 N4 O6	C33 H38 N4 O6	C33 H38 N4 O6	0.68	5
Compound 75	20.445	636.2708						
Cpd 76: Pheophorbide a; C35 H36 N4 O5	20.579	592.2664	Pheophorbide a	C35 H36 N4 O5	C35 H36 N4 O5	C35 H36 N4 O5	3.61	1
Cpd 77: Aralionine A; C34 H38 N4 O5	20.61	582.281	Aralionine A	C34 H38 N4 O5	C34 H38 N4 O5	C34 H38 N4 O5	5.59	2
Compound 78	20.747	636.2708						
Cpd 79: Pheophorbide a; C35 H36 N4 O5	20.903	592.2664	Pheophorbide a	C35 H36 N4 O5	C35 H36 N4 O5	C35 H36 N4 O5	4.29	1
Cpd 80: Aralionine A; C34 H38 N4 O5	21.006	582.2808	Aralionine A	C34 H38 N4 O5	C34 H38 N4 O5	C34 H38 N4 O5	5.91	2

Qualitative Compound Report

Cpd 81: Ganosporelactone A; C30 H40 O7	21.145	512.2778	Ganosporelactone A	C30 H40 O7	C30 H40 O7	C30 H40 O7	-1.38	10
Compound 82	21.312	638.3078						
Cpd 83: Ganosporelactone A; C30 H40 O7	21.499	512.2782	Ganosporelactone A	C30 H40 O7	C30 H40 O7	C30 H40 O7	-1.62	10
Cpd 84: Allosamidine; C25 H42 N4 O14	21.948	622.2792	Allosamidine	C25 H42 N4 O14	C25 H42 N4 O14	C25 H42 N4 O14	-10.36	2
Cpd 85: Haplophytine; C37 H40 N4 O7	22.052	652.2886	Haplophytine	C37 H40 N4 O7	C37 H40 N4 O7	C37 H40 N4 O7	4.25	2
Cpd 86: 20-Hydroxy-3,7,11,15,23-pentaoxolanost-8-en-26-oic acid; C30 H40 O8	22.121	528.2744	20-Hydroxy-3,7,11,15,23-pentaoxolanost-8-en-26-oic acid	C30 H40 O8	C30 H40 O8	C30 H40 O8	-3.98	5
Compound 87	22.239	606.2823						
Cpd 88: Haplophytine; C37 H40 N4 O7	22.333	652.2886	Haplophytine	C37 H40 N4 O7	C37 H40 N4 O7	C37 H40 N4 O7	4.75	2
Compound 89	22.548	606.2823						
Cpd 90: 20-Hydroxy-3,7,11,15,23-pentaoxolanost-8-en-26-oic acid; C30 H40 O8	22.557	528.2734	20-Hydroxy-3,7,11,15,23-pentaoxolanost-8-en-26-oic acid	C30 H40 O8	C30 H40 O8	C30 H40 O8	-2.14	5
Cpd 91: Ganoderic acid K; C32 H46 O9	22.576	574.3146	Ganoderic acid K	C32 H46 O9	C32 H46 O9	C32 H46 O9	-1.3	8
Cpd 92: Ganoderic acid K; C32 H46 O9	22.907	574.3147	Ganoderic acid K	C32 H46 O9	C32 H46 O9	C32 H46 O9	0.88	8
Cpd 93: Antimycin A1; C28 H40 N2 O9	22.947	548.2758	Antimycin A1	C28 H40 N2 O9	C28 H40 N2 O9	C28 H40 N2 O9	-4.35	1
Cpd 94: Antimycin A1; C28 H40 N2 O9	23.303	548.2762	Antimycin A1	C28 H40 N2 O9	C28 H40 N2 O9	C28 H40 N2 O9	6.22	1
Compound 95	23.412	398.2777						
Compound 96	23.740	337.3331						
Compound 97	23.776	564.2707						
Compound 98	24.080	337.3331						
Compound 99	24.136	564.2707						

9 APPENDIX – III

HR-LCMS profiling of compounds in negative ESI mode

Qualitative Compound Report

Data File	CD-07_-ver.d	**Sample Name**	CD-07
Sample Type	Sample	**Position**	P1-F2
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_-VE_MSMS.m	**Acquired Time**	8/10/2023 3:08:23 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)

Compound Table

Compound Label	RT	Mass	Name	Formula	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
Compound 1	1.112	192.0671						
Cpd 2: 5-O-Galloylhamamelofuranose;	1.204	332.0787	5-O-Galloylhamamelofuranose	C13 H16 O10	C13 H16 O10	C 13 H16 O10	-13.08	9
Cpd 3: 2-Galloylglucose; C13 H16 O10	1.498	332.0785	2-Galloyl glucose	C13 H16 O10	C13 H16 O10	C 13 H16 O10	-12.36	10
Cpd 4: Ethyl methanesulfonate; C3 H8 O3	1.717	124.0199	Ethyl methanesulfonate	C3 H8 O3 S	C3 H8 O3 S	C3 H8 O3 S	-3.56	1
Compound 5	1.725	126.0348						
Cpd 6: Mecarbinzid; C13 H16 N4 O3 S	3.391	308.0943	Mecarbinzid	C13 H16 N4 O3 S	C13 H16 N4 O3 S	C13 H16 N4 O3 S	0.19	10
Cpd 7: Ethyl methanesulfonate; C3 H8 O3	4.146	124.0199	Ethyl methanesulfonate	C3 H8 O3 S	C3 H8 O3 S	C3 H8 O3 S	-3.57	1
Cpd 8: Mecarbinzid; C13 H16 N4 O3 S	4.961	308.0943	Mecarbinzid	C13 H16 N4 O3 S	C13 H16 N4 O3 S	C13 H16 N4 O3 S	0.26	10
Cpd 9: Vanillin 4-sulfate; C9 H10 O6 S	4.978	246.0203	Vanillin 4-sulfate	C9 H10 O6 S	C9 H10 O6 S	C9 H10 O6 S	-1.46	7
Compound 10	4.807	382.0589						
Cpd 11: Mecarbinzid; C13 H16 N4 O3 S	4.814	308.0944	Mecarbinzid	C13 H16 N4 O3 S	C13 H16 N4 O3 S	C13 H16 N4 O3 S	-0.44	9
Cpd 12: N-(Carbomethoxyacetyl)-4-S-chlorotryptophan; C15 H15 Cl N2 O5	4.98	338.0667	N-(Carbomethoxyacetyl)-4-S-chlorotryptophan	C15 H15 Cl N2 O5	C15 H15 Cl N2 O5	C15 H15 Cl N2 O5	-5.16	2
Cpd 13: Punicacortein B; C27 H22 O18	5.006	634.0884	Punicacortein B	C27 H22 O18	C27 H22 O18	C 27 H22 O18	-12.32	4
Cpd 14: Glyurallin B; C25 H26 O6	5.063	422.1745	Glyurallin B	C25 H26 O6	C25 H26 O6	C25 H26 O6	-3.66	2
Cpd 15: 1,2,4-Trigalloyl-beta-D-glucopyranose; C27 H24 O18	5.451	636.1024	1,2,4-Trigalloyl-beta-D-glucopyranose	C27 H24 O18	C27 H24 O18	C 27 H24 O18	-9.63	10
Cpd 16: Mecarbinzid; C13 H16 N4 O3 S	5.953	308.0943	Mecarbinzid	C13 H16 N4 O3 S	C13 H16 N4 O3 S	C13 H16 N4 O3 S	0.12	9
Cpd 17: Benzyl sulfate; C7 H8 O4 S	5.954	188.0146	Benzyl sulfate	C7 H8 O4 S	C7 H8 O4 S	C7 H8 O4 S	-1.7	4
Compound 18	5.991	612.0811						
Cpd 19: Vanillin 4-sulfate; C9 H10 O6 S	5.996	246.0203	Vanillin 4-sulfate	C9 H10 O6 S	C9 H10 O6 S	C9 H10 O6 S	-3.76	3
Cpd 20: Syzygirin B; C33 H24 O21	5.957	756.0868	Syzygirin B	C33 H24 O21	C33 H24 O21	C 33 H24 O21	-7.72	3
Compound 21	6.302	789.117						
Cpd 22: pibutidine; C19 H24 N4 O3	6.386	356.1895	pibutidine	C19 H24 N4 O3	C19 H24 N4 O3	C19 H24 N4 O3	-12.59	4
Cpd 23: Hyperoside; C21 H20 O12	6.514	464.1015	Hyperoside	C21 H20 O12	C21 H20 O12	C 21 H20 O12	-13.01	10
Compound 24	6.745	396.0747						
Cpd 25: Cynaroside; C21 H20 O11	7.263	448.1066	Cynaroside	C21 H20 O11	C21 H20 O11	C 21 H20 O11	-14.06	10
Compound 26	7.271	896.2087						
Cpd 27: 6-C-Fucosylluteolin; C21 H20 O10	7.998	432.1116	6-C-Fucosylluteolin	C21 H20 O10	C21 H20 O10	C 21 H20 O10	-13.81	10
Cpd 28: 5,7,8,3',4'-Pentahydroxyisoflavone; C15 H10 O7	8.966	302.0465	5,7,8,3',4'-Pentahydroxyisoflavone	C15 H10 O7	C15 H10 O7	C15 H10 O7	-12.88	10
Cpd 29: Auxin a; C18 H32 O5	10.055	328.2297	Auxin a	C18 H32 O5	C18 H32 O5	C18 H32 O5	-14.4	4
Cpd 30: Gnidicilatin; C37 H48 O10	14.612	652.3268	Gnidicilatin	C37 H48 O10	C37 H48 O10	C 37 H48 O10	-3.19	2
Compound 31	15.397	506.2263						
Cpd 32: Geranylfarnesyl diphosphate; C25 H44 O7 P2	16.129	518.2601	Geranylfarnesyl diphosphate	C25 H44 O7 P2	C25 H44 O7 P2	C25 H44 O7 P2	-7.65	3
Cpd 33: Geranylfarnesyl diphosphate; C25 H44 O7 P2	16.461	518.2604	Geranylfarnesyl diphosphate	C25 H44 O7 P2	C25 H44 O7 P2	C25 H44 O7 P2	-7.96	3
Cpd 34: APGPR Enterostatin; C21 H36 N8 O6	18.837	496.2768	APGPR Enterostatin	C21 H36 N8 O6	C21 H36 N8 O6	C21 H36 N8 O6	-2.03	6
Cpd 35: Ritterazine A; C54 H76 N2 O10	23.962	912.541	Ritterazine A	C54 H76 N2 O10	C54 H76 N2 O10	C 54 H76 N2 O10	9.86	1
Cpd 36: 3-Hydroxy-b,e-carotene-3'-one; C40 H54 O2	24.177	566.4148	3-Hydroxy-b,e-carotene-3'-one	C40 H54 O2	C40 H54 O2	C40 H54 O2	-3.87	10
Cpd 37: Ritterazine A; C54 H76 N2 O10	24.284	912.5412	Ritterazine A	C54 H76 N2 O10	C54 H76 N2 O10	C 54 H76 N2 O10	9.61	1

10 APPENDIX – IV

PREPARATION OF REAGENTS

Total Phenolic content

Preparation of 20% Sodium Carbonate

20% Sodium carbonate was prepared by dissolving the 20 gram of sodium carbonate in 100 mL of distilled water.

Preparation of Folin-Ciocalteu Reagent

Use brand new FCR no need to any dilution for this case.

Preparation of Standard Gallic Acid Solution for TPC determination

1000 µg/mL of stock solution was prepared by dissolving like 25mg of gallic acid in 25mL of methanol (i.e., 1mg of gallic acid in 1 mL of solvent). The stock solution was diluted to archive final concentration of (1, 2, 4,8 ,16,32) µg/mL (i.e. Then prepared using a two-fold dilution process). It is worth noting that the solution utilize for testing were freshly prepared.

Total Flavonoids content

Preparation of Standard Rutin Solution

Stock solution of Rutin was made in methanol by dissolving 20 mg of rutin in 10 mL methanol (i.e., 2mg/mL). The final concentrations were adjusted in (25,50,75,100,150,200) µg/mL by doing two-fold dilution from the stock solution.

Preparation of plant extracts

The working solution of plant extract i.e., 250 µg/mL were prepared by diluting from the 2.5mg/mL of stock solution.

Preparation of Aluminum 10% Trichloride Hexa-Hydrate & 5% Sodium Nitrate

10% Aluminum trichloride hexahydrate ($AlCl_3.6\ H_2O$) was prepared by dissolving 10 gram of solid mass into the 100 mL of distilled water. Similarly, 2.5 gram of Sodium nitrate ($NaNO_2$) was dissolved in 50 mL of distilled water to get 5% $NaNO_2$ solution

Phosphomolybdenum assay

Preparation of 0.6 M sulfuric acid

330.30 µL of concentrated sulphuric acid was poured into 100 mL of volumetric flask in distilled water to get 0.6 M of dilute Sulphuric acid solution.

Preparation of 28 mM sodium phosphate

0.498 mg of sodium phosphate dry weight was dissolved in distilled water to obtain 28 mM sodium phosphate solution**.**

Preparation of 4 mM ammonium molybdate

4 mM ammonium molybdate solution was prepared by dissolving 0.494 mg of ammonium molybdate solution into the 100 mL of volumetric flask using distilled water as solvent.

At last mix them (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in equal portion to obtained Phosphomolybdenum reagents.

Preparation of Ascorbic acid

Stock solution of Ascorbic acid was made in methanol by dissolving 20 mg of rutin in 10 mL methanol (i.e., 2mg/mL). The final concentrations were adjusted in (60, 80,100,120,140,160) µg/mL by doing two-fold dilution from the stock solution.

Preparation of sample solution

3.3 mg/mL stock solution of sample was prepared to get a final concentration of 0.500 µg/mL solution.

DPPH free radical scavenging assay

Preparation of 0.1 mM DPPH solution

A 0.1mM DPPH solution was mutinously prepared by dissolving 3.9432 mg of DPPH in 100 mL of volumetric flask, which was properly shielded with carbon paper or aluminum foil (i.e., DPPH is light sensitive).

Preparation of Ascorbic Acid solution

Weight out 10 mg of ascorbic acid in the 10 mL of methanol solution to make 1mg/mL of stock solution. Which was further go for two-fold dilution.

Preparation of plant extracts solution

Dissolve 10 mg in10 mL to make 1mg/ml plant extract using vortex mixer. Load them to 10-time higher concentration than desire concentration for 10 mL volumetric flask.

Preparation of Standard Culture (Inoculum)

the microorganism colonies were picked from NA and then subjected into a test tube having 5 mL of Normal saline (NS) solution and turbidity was adjusted similar to the 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL or 10^8 bacteria/mL).

Preparation of Media

B) Nutrient Broth

Nutrient broth was prepared for growing these pathogenic bacteria prepared by agitating 1.3 gm of broth and subsequently diluting it to 100 mL. The broth was subjected through autoclave at 121˚C for a period of 15 minutes. After cooling, 5 mL of nutrient broth was dispersed into a screw cap tube and subjected to secondary sterilization.

C) Muller Hinton Agar

Media was prepared by dissolving 3.42 gm. MHA in 100 mL of distilled water. The solution was heated for 10 minutes further sterilized via autoclaving at 121˚C for a duration of 15 minutes. The medium was allowed to cool to approximately 50˚C and displaced into petri-plates at volume of 20 mL per plates, where it was left undistributed to solidify.

11 APPENDIX – VI

Antimicrobial test,

S N	Susceptible Bacterial Strains	Symbol of PC	(Inhibition Zone Diameter around Circular Well in mm)					Mean ± SD Mm
			PC	NC	A	B	C	
1	*Escherichia coli (-Ve)*	E	25	N	15	17	14	15.33±1.530
2	*Klebsiella pneumoniae (-Ve)*	E	15	N	12	13	13	12.67±0.577
3	*Pseudomonas aeruginosa (-Ve)*	G	22	N	12	12	12	12.00±0.00
4	*Bacillus subtilis (+Ve)*	E	25	N	15	15	15	15.00±0.00
5	*Staphylococcus Aureus (+Ve)*	V	17	N	15	14	14	14.33±0.577

Note: 15µg Erythromycin *(K. pneumonia, and B. subtills, Escherichia coli*),*)*, 10µg Gentamycin (*P. aeruginosa*), 30 µg Vancomycin (*S. aureus)* PC stands Positive controls (*standard drug*), NC stands Negative control (*Solvent used i.e., DMSO*) N= Negative result or No activity. (-Ve) = gram negative bacteria and (+Ve) = gram positive pathogens.

Appendix 3.1: Absorbance of Gallic acid

Concentration	Absorbance			Average absorbance
	A_1	A_2	A_3	
0	0	0	0	0
1	0.067	0.072	0.074	0.071
2	0.154	0.16	0.168	0.161
4	0.329	0.334	0.337	0.333
8	0.544	0.541	0.539	0.541
16	0.926	0.923	0.927	0.925
32	1.429	1.428	1.416	1.824

Appendix 3.2: Absorbance of Rutin

Concentration	Absorbance			Average absorbance
	A_1	A_2	A_3	
0	0	0	0	0
25	0.201	0.198	0.194	0.198
50	0.387	0.381	0.385	0.384
75	0.525	0.531	0.523	0.526
100	0.694	0.7	0.697	0.697
150	0.979	0.983	0.982	0.981
200	1.244	1.241	1.239	1.241

S. N	Concentration (µg/ml)	Absorbance			Total Flavonoid Content			Mean ± SD
		A_1	A_2	A_3	C_1	C_2	C_3	
1	500	0.835	0.839	0.843	253.9	255.19	256.48	255.19±1.291

Appendix 3.2: Absorbance of Ascorbic Acid (TAA)

Concentration	Absorbance			Average absorbance
	A_1	A_2	A_3	
0	0	0	0	0
25	0.201	0.198	0.194	0.198
50	0.387	0.381	0.385	0.384
75	0.525	0.531	0.523	0.526
100	0.694	0.7	0.697	0.697
150	0.979	0.983	0.982	0.981

200	1.244	1.241	1.239	1.241

S. N	Concentration(µg/ml)	Absorbance			Total Antioxidant Assay			Mean ± SD
		A_1	A_2	A_3	C_1	C_2	C_3	
1	500	0.783	0.781	0.783	166.6	166.2	166.6	166.47 ± 0.231

Appendix 3.3: Absorbance and % inhibition of DPPH scavenging assay

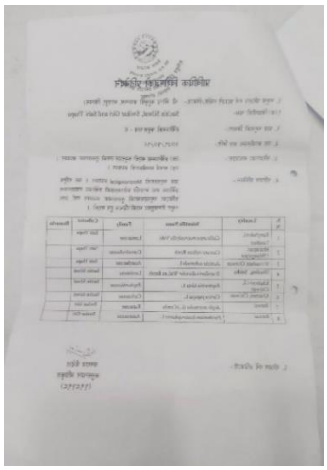
Concentration	Absorbance			Average	% Inhibition
	A_1	A_2	A_3		
0	0	0	0	0	100
8	0.226	0.219	0.221	0.222	44.221
10	0.165	0.172	0.168	0.168	57.705
20	0.112	0.117	0.119	0.116	70.854
40	0.081	0.086	0.084	0.084	78.978
80	0.08	0.77	0.073	0.308	82.697
Control	0.398	0.398	0.398	0.398	

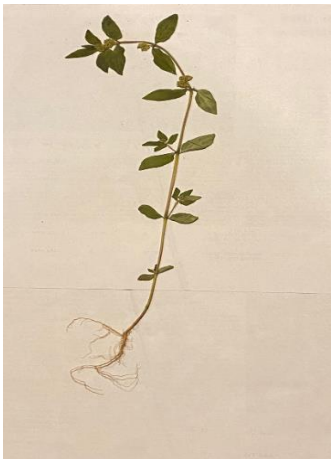
For Sample,

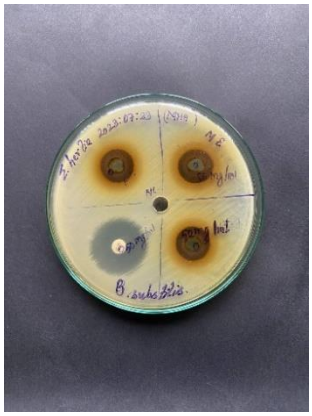
Concentration	Absorbance			Average	% Inhibition
	A_1	A_2	A_3		
0	0	0	0	0	100
8	0.293	0.291	0.289	0.291	26.823
10	0.243	0.243	0.243	0.243	38.893
20	0.186	0.183	0.185	0.185	53.562
40	0.164	0.166	0.16	0.163	58.927
80	0.156	0.159	0.162	0.159	60.017
Control	0.398	0.398	0.397	0.398	

12 APPENDIX – VII

Figure selected plant, it's parts and some lab work.



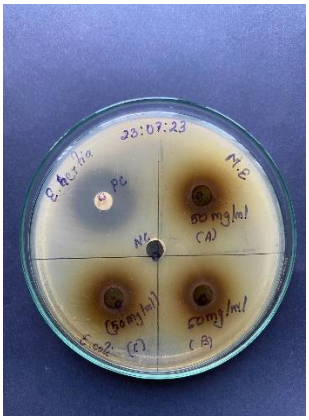




B. subtilis

S. aureus

P. aeruginosa



E. coli

K. pneumonia

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वनस्पति विभाग
राष्ट्रिय हर्वेरियम तथा वनस्पति प्रयोगशाला

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गोदावरी, ललितपुर

पत्र संख्याः ०७९/०८०
च.नं. २६३

गोदावरी, ललितपुर

मिति २०७९/१०/२६

विषयः नमुनाहरु पहिचान सम्वन्धमा।

श्री जीरेन्द्र बहुमुखी क्याम्पस,
भरतपुर, चितवन।

प्रस्तुत विषयमा तहाँको प.सं. ०७९/०८०, च.नं. १३२२, मिति २०७९/१०/१८ को पत्र साथ वनस्पतिका नमुनाहरु प्राप्त भई व्यहोरा अवगत भयो। पत्र मार्फत ल्याइएका नमुनाहरुको पहिचान गरी प्राविधिक विशेषज्ञको प्रतिवेदन (पाना १) का साथै पहिचान गरी पठाइएको व्यहोरा अनुरोध छ।

२०७९/१०/२६

हेम राज पौडेल
अनुसन्धान अधिकृत
(१८२५६१)
नि.कार्यालय प्रमुख

फोन नं.: ५१७४२७७, ५१७४०४७, फ्याक्स: ९७७-०१-५१७४३४९, पोष्ट वक्स नं.: ३७०८
Website: www.kath.gov.np

प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमूना परिक्षण गर्न पठाउने व्यक्ति/निकायः- श्री वीरेन्द्र बहुमुखी क्याम्पस, भरतपुर, चितवन।

१(क) विद्यार्थीको नाम- Sachin Silwal, Swikar Giri and Sabi Thapa

२. प्राप्त नमूनाको विवरणः- हर्बेरियमको नमूना थान - ८

३. यस कार्यालयमा प्राप्त मितिः- २०७९/१०/१८

४. परिक्षणका आधारहरुः- (क) हर्बेरियममा भएको नमूनाहरु संगको तुलनात्मक अध्ययन ।
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

५. पहिचान प्रतिवेदनः- प्राप्त नमूनाहरुको Morphological अध्ययन र यस राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशालाको हर्बेरियम संग्रहालयमा राखिएका नमूनाहरुसंगको तुलनात्मक अध्ययन गर्दा उक्त नमूना निम्नानुसार भएको पहिचान हुन गएको ।

S. N	Locality	Scientific Name	Family	Collector	Remarks
1	Ramjhakots, Tanahun	*Callicarpa macrophylla* Vahl	Lamiaceae	Sabi Thapa	
2	Bharatpur-15Mangalpur	*Cuscuta reflexa* Roxb.	Convolvulaceae	Sabi Thapa	
3	Shivaahats Chitwan	*Justicia adhatoda* L.	Acanthaceae	Sabi Thapa	
4	Dhading, Sakhu	*Scutellaria discolor* Wall.ex Benth.	Lamiaceae	Sachin Silwal	
5	Khaireni-12, Chitwan	*Euphorbia hirta* L.	*Euphorbiaceae*	Sachin Silwal	
6	Khaireni, Chitwan	*Carica papaya* L.	Caricaceae	Sachin Silwal	
7	Butwal	*Aegle marmelos* (L.) Corrêa	Rutaceae	Swikar Giri	
8	Butwal	*Parthenium hysterophorus* L.	Asteraceae	Swikar Giri	

६. परिक्षण गर्ने अधिकारीः-

धनराज कँडेल
अनुसन्धान अधिकृत
(१९८१९८)