NUTRIENT ELEMENTS AND HR-LCMS ANALYSIS OF SECONDARY METABOLITES PRESENT IN METHANOLIC EXTRACTS OF *Colocasia esculenta* (L.) SCHOTT (cocoyam)

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

BY
AJAYA MAHATO
ROLL NO: CHE 2199/077
TU REGISTRATION NO: 5-2-19-577-2011

FEBRUARY, 2025

DECLARATION

I declare that this dissertation entitled "**Nutrient Elements and HR-LCMS Analysis of Secondary Metabolites Present in Methanolic Extracts of (*Colocasia esculenta* (L.) Schott (Cocoyam),**" 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. Ajaya Mahato

Symbol No: CHE 2199/077

T. U. Registration no: 5-2-19-577-2011

RECOMMENDATION

The dissertation entitles "**Nutrient Elements and HR-LCMS Analysis of Secondary Metabolites Present in Methanolic Extracts of (*Colocasia esculenta* (L.) Schott (Cocoyam),**" is submitted by **Mr. Ajaya Mahato** for the completion 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.

Tribhuvan University
BIRENDRA MULTIPLE CAMPUS
Bharatpur, Chitwan

.....

Dr. Ganga Raj Pokhrel
(Supervisor)
Assoc. Professor
Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal

.....

Co- supervisor
Bodh babu Bhattarai (Ph.D.)
(Assistant prof.)
Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal

February, 2025

FOREWORD

The thesis work "**Nutrient Elements and HR-LCMS Analysis of Secondary Metabolites Present in Methanolic Extracts of (*Colocasia esculenta* (L.) Schott (Cocoyam),**" submitted by **Ajaya Mahato** as a part of M.Sc. Course work 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.

Supervisor
Ganga Raj Pokharel, Ph.D.
(Associate professor)
Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal
Date: February, 2025

LETTER OF APPROVAL

Date: 6th February, 2025

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 **Ajaya Mahato** entitled "**Nutrient Elements and HR-LCMS Analysis of Secondary Metabolites Present in Methanolic Extracts of (*Colocasia esculenta* (L.) Schott (Cocoyam)**," is forwarded by Department of Chemistry, Birendra Multiple Campus (BMC) to the office of Dean, IOST, T. U.

Supervisor

Ganga Raj Pokharel, Ph.D.

(Associate professor)

Department of Chemistry

Head of Department of Chemistry

Birendra Multiple Campus,

Bharatpur, Nepal

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, **Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)** for his extraordinary support, guidance, and encouragement throughout the course of my dissertation. His insightful feedback, patience, and unwavering dedication were instrumental in shaping this work. Without his expertise and constant encouragement, this dissertation would not have been possible. I am truly grateful for the opportunity to learn under his mentorship and for the invaluable experience this journey has provided.

Thank you, **Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)**, for your exceptional support and for believing in my abilities even when I doubted myself.

I wish to extend my heartfelt thanks to my co-supervisor, **Bodh Babu Bhattarai, Ph.D. (Asst. Prof.)**, for his extraordinary contribution to my thesis. His deep insights, constructive feedback, and constant encouragement have been invaluable to the development and completion of this work. I am immensely grateful for the time and effort he invested in guiding me, and for his unwavering support throughout this process.

I am very much indebted with Mrs. Pavitra Shrestha Head of Department, Department of Microbiology and Lab incharge Mr. Santa Prasad Dhungana, my seniors Mr. Bipin Khanal, Miss Ranjana Khanal, Miss. Purnima Banjade, in my dissertation. My academic career has been greatly aided by the department's resources, advice, and encouragement. I would like to express my deepest gratitude to Center for Research in Nanotechnology and science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay, 400076, India.

At last but not least, I would like to express my heartfelt gratitude to my parents, my wife and kids for encouraging me throughout this journey.

Ajaya Mahato

Symbol No: CHE 2199/077

T.U. Registration no.: **5-2-19-577-2011**

Email: ajayamahato203@gmail.com

ABSTRACT

Nutrient Elements and HR-LCMS Analysis of Secondary Metabolites Present in Methanolic Extracts of (*Colocasia esculenta* (L.) Schott (Cocoyam) is still not understood clearly. Methanolic extracts of three different parts (tuber, petiole and leaf) of plants from different sampling spots (Palung, Upperdangadhi and Padampur) were utilized to assay antioxidants activities through 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and phosphomolybdate assay (TAA), total phenolic (TPC), total flavonoid (TFC) and total carbohydrate content (TCC) using spectrophotometer (**T80+, PG** Instruments, UK). The antimicrobial susceptibility test of methanolic extracts of plant samples was conducted on seven microorganisms i.e. *E. coli, K. pneumonia, P. aeruginosa, B. subtilis, S. aureus, A. baumannii* and *S. typhi, Pseudomonas and Salmonella species*. Nutrient elements and toxic metals quantification and HR-LCMS analysis of plant tuber, petiole and leaf from Upperdangadhi were carried out at Centre for Research in Nanotechnology and Science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay, 400076, India, using ICP-AES and HR-LCMS spectroscope model G6550A respectively.

IC_{50} value of plant extracts was found in between 240.45-1018.44 µg/mL. TAA by phosphomolybdate assay was found 1.25±0.01 to 28.29±0.01 mg AAE/g DS. TPC was found in between 6.38±0.004 and 19.96±0.01 mg GAE/g DS. TFC was found in the range of 10.46±0.0007 and 89.1±0.006 mg QE/g DS. TCC was found in between 1.78±0.0 and 112±0.0 mg GE/g DS. Concentrations of elements were found in the order of tuber, petiole and leaf as: **Ca** (8910.0, 303.0, 355.0 mg/Kg), **Cr** (0.476, 0.252, 0.427 mg/Kg), **Cu** (1.226, 0.670, 0.727 mg/Kg), **Fe** (15.6, 1.241, 2.41 mg/Kg), **Mg** (96.8, 43.6, 104.0 mg/Kg), **Zn** (128.0, 0.685, 1.942 mg/Kg) and **As** (15.7, 9.8, 65.6 mg/Kg). Concentration of **Pb** was below the detection limit(1ppb).

HR-LCMS analysis of tuber, petiole and leaf revealed that the extract contained 200, 140 unidentified, 60 identified- 11 alkaloids, 4 phenolic, 5 flavonoids, 3 glycosides, 5 steroids, 32 others, 200, 85 unidentified, 115 identified- 12 alkaloids, 15 phenolic, 26 flavonoids, 9 glycosides, 53 others, 197, 106 unidentified, 91 identified- 13 alkaloids, 17 flavonoids, 3 terpenoids, 1 steroid, 12 pharmaceutical drugs, 45 others respectively. Neuraminic acid, Amprenavir, Butin, catechin, cyclopamine,

disopyramide, Funtumine, gentamycin, Jervin, Kaemferol, ketamine, retapamulin, swertiamarin, syringic acid, usambarensine etc. are potentially bioactive.
Key words**:** Cocoyam, Phytochemical screening, Antioxidant activity, Antimicrobial Activity, DPPH, HR-LCMS

सारांश

कोलोकेसिया इस्कुलेन्टा (एल.)स्कट (कर्कलो) को पौष्टिक तत्व र HR-LCMS विश्लेशण अझै स्पष्ट रुपमा बुझ्न सकिएको छैन । पालुङ्ग, उपरदाङगढी र पदमपुरबाट सकंलित कर्कलोको तीन भागहरु (पिडालु, डाँठ र पात) को मिथानोलिक एक्स्ट्रयाक्टबाट एन्टिअक्सिडेनट गतिविधी, जम्मा फिनोलिक मात्रा (टि.पि.सी.) जम्मा फ्लाभोनोइड मात्रा (टि.एफ.सी) जम्मा कार्बोहाइड्रेट मात्रा (टि.सी.सी), स्फेक्ट्रोफोटोमिटर को प्रयोग गरी निकालियो । एन्माइक्रोबियल गतिविधी सात सुक्ष्म जीबहरु (*इ.कोली)* के., न्युमोनि, पि.एरुजिनोसा, बि. सब्टिलिस, एस. आरियस, ए.बैमानी, एस. टाइफी,) को बिरुद्धमा हेरियो । पोशक तत्व र विशाक्त धातुहरुको विश्लेषण एवम् HR-lCMS विश्लेषणको लागी कर्कालोको नमुना सेन्टर फर रिसर्च इन नानोटेक्नोलोजी एण्ड साइन्स, आइ.आइ.टी.बम्बे पठाइएको थियो ।

विरुवाको एक्ट्रयाक्को आइ.सी$_{५०}$ मात्रा २४०.४५-१०१८.४४ माइक्रोग्राम/मि.लि रहेको पाइयो/त्यसैगरी टि.पि.सी, टि.एफ.सी, टि.सी.सी: क्रमशः ६.३८ ± ०.००४ देखी १९.९६ ± ०.०१ मि.ग्रा. जि.ए.इ/ग्रा.डि.एस, १०.४६± ०.०००७ देखी ८९.१± ०.००६ मि.ग्रा क्यु.इ/ग्रा.डि.एस, १.७८± ०.० देखी ११२.० ± ०.० मि.ग्रा.जि.इ/ग्रा डि.एस रहेको पाइयो फोस्फोमोलिब्डेनम एस्सेद्धारा जम्मा एन्टिअक्सिडेन्ट गतिविधी १.२५±०.०१ देखी २८.२९±०.०१ को बिचमा रहेको पाइयो । त्यस्तै पोषक तत्व र विशाक्त तत्वहरुको मात्रा निम्न रहेको पाइयो । क्याल्सियम (०.०७६, ०.२५२, ०.४२७, ३५५ मिग्रा/के. जि, कोमियम (०.४७६, ०.२५२, ०४२७ मि.ग्रा/के.जि) म्याग्नेसियम (९६.८, ४३.६,, १०४.० मि.ग्रा/के.जि) फलाम (१५.६, १.२४१, २.४१ मि.ग्रा/के.जि) जिडक (१२८.०, ०.६८५, १.९४२ मि.ग्रा/के.जि),आर्सेनिक(१५.७,९.८,६५.६मि.ग्रा/के.जि) ।

HR- LCMS विश्लेशण बाट उपरदाङगढीको पिडालुमा जम्मा २०० वटा यौगिकहरु रहेको पाइयो जसमध्ये १४० वटा पहिचान नभएका र ६० वटा पहिचान भएका, पहिचान भएका भएका मध्ये ११ वटा अल्कालोइड, ४ फिनोलिक, ५फ्लाभोनोइड, ३ ग्लाइकोसाइट, ५ स्टेरोइड र ३२ अन्य यौगिकहरु थिए । डाँठमा जम्मा २०० यौगिक मध्ये ८५ पहिचान रहित ११५ पहिचान भएका, पहिचान भएका मध्ये १२ अल्कालोइड, १५ फिनोलिक, २६ फ्लाभोनोट, ९ ग्लाइकोसाइट, ५३ अन्य यौगिक रहेको पइयो । पातको नमुनामा जम्मा १९७ यौगिक जसमध्ये १०६ पहिचानरहित, ९१ पहिचचान भएका, पहिचा भएका मध्ये १३ अल्कालोइड, १६ फ्लाभोनोइड, ३ टर्पिनोइड, १ स्टोरोइट, १२ फर्मासिउटिकल ड्रग्स र ४५ अन्य यौगिक रहेको पाईयो । न्युरामिनिक एसिड, एम्प्रिनाभिर, बुटिन, क्याटेचिन, साइक्लोपामिन, डाइसोपाइरामाइड, फुन्टुमाइन, जेन्टामाइसिन, जर्भिन, क्याम्फेरोल, किटामाइन, स्वेर्टियामरिन, सिरिन्जिक एसिड, उसाम्बरिन्सिन इत्यादि औषधीको रुपमा प्रयोग गरिदै आएको छ ।

मुख्य शव्दहरु: कोकोयाम (कर्कलो), फाइटोकेमिकल स्किनिङ्ग, एन्टिअक्सिडेन्ट गतिविधी एन्टिमाइक्रोवियल गतिविधी, डिपिपिएव,एचआर-एल.सि.एम.एस

LIST OF ACROMYMS AND ABBREVIATIONS

Acetyl CoA	:	Acetyl coenzyme A
ADME	:	Absorption, Distribution, Metabolism and Excretion
Cm	:	Centimeter
DMSO	:	Dimethyl Sulphoxide
DMSO	:	Dimethylsulfoxide
DNA	:	Deoxyribonucleic acid
eGI	:	estimated Glycamic Index
FCR	:	Folin-Ciocalteu Reagent
g/ L	:	Gram per Liter
GAC	:	Gallic acid concentration
GAE	:	Gallic Acid Equivalent
H	:	Hours
H_2SO_4	:	Sulfuric Acid
HCL	:	Hydrochloride Acid
HR-LCMS	:	High Resolution Liquid Chromatography Mass Spectrospectoscopy
HTS	:	High Throughput Screening
Kg	:	kilogram
mg	:	Milligram
MHA	:	Muller Hinton Agar
ml	:	Milliliter
mm		Millimeter
Na_3PO_4	:	Trisodium phosphate
nm	:	Nanometer
NPs	:	Natural products
ppm	:	Parts per million
ROS	:	Reactive Oxygen Species
RTC	:	Root and Tuber Crops
TAA	:	Total antioxidant activity
TFC	:	Total Flavonoid Content

TPC	:	Total Phenolic Content
UV	:	Ultraviolet
wt	:	weight
ZOI	:	Zone of Inhibition

LIST OF SYMBOLS

°C	:	Degree Celsius
%	:	Percentage
&	:	And
λ	:	Lambda
µL	:	Microliter
µg/mL	:	Microgram per milliliter

LIST OF TABLES

Table 1	% yield of samples after drying	20
Table 2	% yield of samples after Soxhlet extraction	20
Table 3	Concentration of various elements through ICP-AES analysis	31
Table 4	Identified compounds in UPT using HR-LCMS	32
Table 5	Identified compounds in UPS using HR-LCMS	34
Table 6	Identified compounds in UPL using HR-LCMS	37

LIST OF FIGURES

Figure 1	Antimicrobial susceptibility test of plant extracts against:	21
Figure 2	Calibration curve of gallic acid to determine total phenolic content	22
Figure 3	Total phenolic contents of samples	23
Figure 4	Calibration curve of Quercetin to evaluate total flavonoid content	24
Figure 5	Total flavonoid contents of samples	25
Figure 6	Calibration curve of glucose dextrose to calculate total carbohydrates content	26
Figure 7	Total carbohydrates contents of samples	26
Figure 8	Calibration curve of ascorbic acid for phosphomolybdate assay	27
Figure 9	TAC of samples	28
Figure 10	Conc vs % inhibition of DPPH by A: BHA, B: IC50 of Samples	29
Figure 11	DPPH free radical scavenging activities of samples (IC50)	30
Figure 12	Chromatogram of UPT in +ve ESI mode using HR-LCMS	38
Figure 13	Chromatogram of UPS in +ve ESI mode	39
Figure 14	Chromatogram of UPL in +ESI mode	39
Figure 15	Chromatogram of UPT in -ve ESI mode	40
Figure 16	Chromatogram of UPS in -ve ESI mode	40
Figure 17	Chromatogram of UPL in -ESI mode	41

TABLE OF CONTENTS

TITLE PAGE i

DECLARATION ii

RECOMMENDATION iii

FOREWORD iv

LETTER OF APPROVAL v

BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL vi

ACKNOWLEDGEMENTS vii

ABSTRACT viii

LIST OF ACROMYMS AND ABBREVIATIONS x

LIST OF SYMBOLS xiii

LIST OF TABLES xiv

LIST OF FIGURES xv

TABLE OF CONTENTS xvi

CHAPTER 1: INTRODUCTION 1

1.1 Natural products 2

1.2 Oxidative stress and antioxidants 3

1.3 Antimicrobial activity 4

1.4 Essential nutrient elements and toxic metals 5

1.5 Research objectives 5

1.6 Rationale 6

CHAPTER 2 : LITERATURE REVIEW 7

CHAPTER 3. MATERIALS AND METHOD 10

3.1 Chemicals and equipment 10

3.2 Collection of plant 10

3.3 Methanolic extraction of phytochemicals. 11

CHAPTER 4 : RESULT AND DISCUSSION 20

4.1. Yield of sample after drying 20

4.2 Yield of samples after Soxhlet extraction. 20

4.3 Antimicrobial susceptibility test analysis 21

4.4 Estimation of total phenolic content 22

4.5 Estimation of total flavonoids content 24

4.6 Estimation of total carbohydrates content 25

4.7 Antioxidant activity 27
4.7.1 Phosphomolybdenum assay 27
4.7.2 DPPH free radical scavenging assay 28
4.8 Quantification of nutrient elements and toxic metals. 30
4.9 HR-LCMS PROFILING 31
CHAPTER 5. CONCLUSIONS and recommednation 56
5.1 Conclusion 56
5.2 Recommendations 57
REFERENCES 58
Appendix I : HR-LCMS METHODS OF ANALYSIS 81
Appendix II : HR-LCMS profiling of compounds in UPT in positive ESI mode. 85
Appendix III : HR-LCMS profiling of compounds in UPS in positive ESI mode. 87
Appendix IV : HR-LCMS profiling of compounds in UPL in positive ESI mode. 89
Appendix V : HR-LCMS profiling of compounds in UPT in negative ESI mode 91
Appendix VI : HR-LCMS profiling of compounds in UPS in negative ESI mode 93
Appendix VII : HR-LCMS profiling of compounds in UPL in negative ESI mode 95
Appendix VIII : PREPARATIONS 97
Appendix IX : SOME PHOTOGRAPHS 100
PLAGARISM REPORT 103

CHAPTER 1: INTRODUCTION

Over 7000 different kinds of edible plants have been used by humans as their food sources. However, to meet the world's food demand researchers has focused on a small number of crops. Just three crops (rice, wheat, and maize) provide more than half of the world's protein and energy demands, and about 35 crop plant species account for 95% of all crop production(Wada et al.., 2019).Cocoyam (*Colocasia esculenta (L.) Schott*), a staple food crop in many tropical and subtropical regions, including parts of Africa, the Caribbean, and Asia, has garnered increasing attention not only for its nutritional value but also as a potential source of chemically valuable compounds. While primarily cultivated for its starchy corms and cormels. various parts of the cocoyam plant, including the leaves, stems, and even the peels, have been traditionally used in folk medicine and have demonstrated potential applications in various industries. This renewed interest stems from the growing recognition of the diverse phytochemical profile of cocoyam, encompassing a range of compounds with potential health benefits and industrial applications. Cocoyam is a significant source of carbohydrates, primarily starch, making it a valuable energy source (Eke et al.., 2024). Beyond carbohydrates, it also contains appreciable amounts of dietary fiber, vitamins (e.g., vitamin C, B vitamins), and minerals (e.g., potassium, calcium, iron) (Chukwu et al., 2022). However, what makes cocoyam particularly interesting from a chemical perspective is its rich content of bioactive compounds. These include polyphenols, flavonoids, alkaloids, and saponins, among others. These phytochemicals have been associated with various biological activities, including antioxidant, anti-inflammatory, antimicrobial, and even anticancer properties ((Awa et al., 2015).

The presence of these diverse chemical constituents suggests that cocoyam could be explored as a source of valuable compounds for various applications. For instance, the high starch content could be utilized in the development of novel biodegradable materials or as a source of modified starches with specific functionalities (e.g., resistant starch). The presence of antioxidant compounds suggests potential applications in the food and pharmaceutical industries as natural preservatives or therapeutic agents. Furthermore, the reported antimicrobial activity of cocoyam

extracts warrants further investigation into the specific compounds responsible and their potential use in developing new antimicrobial agents.

Despite the growing body of research on the nutritional and potential health benefits of cocoyam, a comprehensive understanding of its complex chemical composition, particularly concerning the specific types and concentrations of bioactive compounds present in different parts of the plant and under varying growth conditions, remains limited. Further research is crucial to fully characterize the chemical profile of cocoyam, identify and quantify its valuable constituents, and explore their potential applications in various fields. This thesis aims to address this gap by HR-LCMS analysis of secondary metabolites of methanolic extracts of cocoyam. This study will contribute to a deeper understanding of the chemical composition of cocoyam and its potential for utilization as a valuable resource.

1.1 Natural products

Nature is fundamentally important for human kind supporting life in various aspects for living such as food, shelter, ideal climate based on molecular evolution (Szuba, 2002). Nepal is rich in biodiversity due to its diverse geographical distribution from an altitude ~ 70m from sea level to the tallest canopy, The Mt. Everest (8848m), in south Asia, in between China and India. The biodiversity of Nepal includes 118 varieties of ecosystems harboring over 2% of the flowering plants, 3% of the pteridophytes, 6% of bryophytes, 3.9% mammals, 8.9% of birds and 3.7% of global fauna of butterflies (Poudel et al., 2011).

Biologically active compounds derived from natural sources i.e. plants, animals, microbes etc. are the natural products (NPs). Secondary metabolites are plant NPs produced as end-products, in the forms of biosynthetic intermediate (acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid, 1-deoxyxylulose-5-phosphate), during environmental adaptation or predator defense mechanism beside reproduction and regular growth and development of plant [Newman, 2007]. Natural products escorts with homogenous triumph rate (50%) in the Lipinski and parallel universe due to influence of active transport mechanism [Ganesa, 2008]. Earlier clinical practices of natural products were reported to be originated from North Africa, India and China which revolutionized into new era by the victorious synthesis of quinine, cholesterol,

cortisone, chlorophyll and reserpine by Robert Burns Woodward [Zhang et al., 2020]. New-born male babies of South Californian Indian tribes were faithfully cooked in hot ashes of *Salvia* to nurture as the most robustified member of the tribe and to safeguard from any respiratory maladies throughout the life [Newman, 2007]. The evolution of natural products in modern drug discovery, subsequent to expansion of combinatorial chemistry and High Throughput Screening (HTS) technologies, comprises high affinity and manifold array of biological choices particularly membrane proteins like ion channels, receptors, and tansporters [Irina et al., 2010, Zhang et al., 2020]. The 3D molecular shape, stereochemistry and ring complexity of NPs are highly complicated incorporating far-flung ADME and physiochemical properties as contemplated the drug-like space [Chen et al.,2020]. Many natural products-based drugs like morphine, vinblastine, vincristine, quinine, avermectin, artemisinin, etoposide, teniposide, paclitaxel, and the camptothecin derivatives topotecan irinotecan etc. are being used in treatment of wide range of human diseases including parasitic diseases, Alzheimer's diseases, cardiovascular diseases, cancer, diabetes, neuro-degenerative disorders and many more [Chen et al.,2020, Kinston, 2010, Silva et al.,2014, Bedekar et al., 2010, Franziska et al., 2018,Shaito et al., 2020]. The topmost recognition of pertinence of NPs was 2015 Nobel prize in physiology or medicine for discovery of avermectin and artemisinin [Chen et al., 2020].

1.2 Oxidative stress and antioxidants

Highly reactive chemical species called reactive oxygen species (ROS) are produced in living organisms because of regular cellular mechanisms and external influences which can damage proteins and nucleic acids and change their activities. The physiologically significant ROS are superoxide anion (O_2^-), Hydroxyl radical (OH^-), hydrogen peroxide(H_2O_2) [Birben et al., 2012] alkoxyl and lipid peroxyl radicals, nitric oxide and peroxynitrite [Pisoschia et al.,2015]. The human body, as a defense system against oxidative damage, produces antioxidants. The change in the ratio of oxidants and antioxidants in the favor of oxidants is called oxidative stress [Gupta et al., 2014]. Prolonged manifestation of high levels of pro-oxidant chemicals can cause structural issues with mitochondrial DNA, abnormal gene expression and alterations in enzymatic mechanisms playing a major role in pathogenesis of chronic diseases like diabetes, cancer, heart diseases [Mehdi et al., 2020] neurodegenerative

diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease Amyotrophic Lateral Sclerosis as well as brain and spinal cord damages following stroke and traumatic brain injury [Olufunmilayo et al., 2023] and non-alcoholic steatohepatitis and alcoholic liver disease [Li et al., 2015], hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathy pulmonary fibrosis, chronic obstructive pulmonary disease and asthma [Birben et al., 2012], infertility in both male and female [Bansal et al., 2010] and hepatic parthenogenesis [Ha et al., 2010]. The enzymatic and non-enzymatic antioxidants effectively block ROS in aerobic organisms [Birben et al., 2012]. Xenobiotics induced oxidative stress cause tissue damage persuading carcinogenesis due to gene mutation [Thomson et al., 1998]. Electron paramagnetic resonance only can identify the genuine presence of ROS [Jenkins, 1993]. The compounds, mainly polyphenols, which predominantly initiate the detoxification of ROS are antioxidants [Bjørklund et al., 2017]. Oxygen plays both beneficial as well as toxic role in living body. Oxygen is essential for life while its toxic effect is being used in hyperbaric and radiation therapy. Retinopathy of prematurity (ROP) was reported in new-born infants due to high oxygen concentration in newly launched incubators [Kohen et al., 2004]. The compounds which restrict the production and propagation of ROS are termed as antioxidants. A specific group of compounds (polyphenols, vitamins, trace elements etc.) or a system (enzymatic or non-enzymatic systems) scavenge the ROS inhibiting its ramifications. Polyphenols (flavonoids and phenolic acids) are influential inhibitor of ROS suppressing oxidative stress and lipid peroxidation by chelating effect [Al-Gubory et al., 2010].

This research aims to evaluate the concentration of total polyphenols i.e total phenic acids and total flavonoids present in differently spotted samples of cocoyam.

1.3 Antimicrobial activity

Varying species of microorganisms cause a wide array of health maladies in plants and animals including human beings. People from ancient time used different raw plant extracts to treat such maladies [Khan et al., 2019]. Incompatible varieties of molecules from plants functions as antibiotics, analgesic, antipyretic, anti-inflammatory, antiviral, antitumor etc. [Ramawat et al., 2009]. A revolution in the discovery and development of antibiotics has reached to the fifth generation after discovery of first antibiotic; Penicillin, by Alexender Fleming in August 1928 [Ligon

et al., 2004]. Garlic and clove have antimicrobial, antiseptic and anesthetic properties [Lambert, 2004]. Very low concentration of some non-essential metals (Ag, Hg, Te) has demonstrated their ability to resist/kill bacteria and microbes [Russell et al.,2005]. Isolated phytochemicals (alkaloids, flavonoids, sesquiterpenes, diterpenes, lactones, triterpenes, naphthoquinones) from various medicinal plants are efficient against pathogens [Alanis, 2005]. Some enzyme inhibiting compounds have unpresuming activities against bacteria [Fair et al., 2014]. Escalated use, misuse of antibiotics and the natal aptness of bacteria to resist against have made over 70% of antibiotics ineffective towards at least one or more bacteria [Lu et al., 2011, Odonkor et al., 2011]. It's highly substantial for researchers and pharmaceuticals to seek new and effective compounds to counteract high-resistive bacteria. This research focuses on probing the antimicrobial efficacy of cocoyam.

1.4 Essential nutrient elements and toxic metals

Many biological, chemical, molecular life processes need numerous metal/metalloids like cobalt, iron, manganese, copper, zinc in trace amounts while other metals (arsenic, lead, mercury, cadmium play noxious effect in mammals body including humans [Domingo et al., 2021]. Copper (II) ion is essential in metabolism of neural energy and antioxidants. Remarkable effects of low molecular weight chromium are studied in enhancing efficacy of insulin influencing metabolisms of carbohydrates, lipid and proteins. Zinc is associated with boosting of immune system and reduces sternness of diseases [Pokhrel et al., 2024]. Lead and arsenic have carcinogenic, neurotoxic and hematopoietic effects and affects reproductive system, kidney [Pokhrel et al., 2024, Assi et al., 2016]. The aim of this research is to enumerate the quantities of some nutrient elements as well as the toxic lead and arsenic using Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

1.5 Research objectives

The broad objective of this research is to conduct nutrient elements and HR-LCMS analysis of secondary metabolites present in methanolic extracts of (*Colocasia esculenta* (L.) Schott (Cocoyam).

The specific object of this research are as follows:

- To measure total phenolic (TPC) & total flavonoids content (TFC).
- To evaluate total antioxidant activity (TAA).

- To quantify the carbohydrate contents (TCC).
- To determine the amounts of nutrient elements i.e. iron, zinc, copper, chromium, Calcium and Magnesium.
- To analyze the toxic heavy metals like arsenic and lead.
- To resolve secondary metabolites using HR-LCMS.

1.6 Rationale

Cocoyam, represents a vital, yet often underutilized, staple crop, particularly in developing countries across Africa, Asia, and the Pacific. This research aims to address critical gaps in our understanding of nutrient elements contents and chemical compositions of tuber, petiole and leaf through HR-LCMS and contribute to enhancing its contribution to food security and livelihoods. Cocoyam is a valuable source of carbohydrates, dietary fiber, vitamins (especially Vitamin C and B vitamins), and minerals (including potassium, iron, and calcium) (Okonkwo, 2013). Its low glycemic index makes it a suitable food for managing diabetes (Eleazu et al.., 2016). However, the nutritional composition can vary significantly depending on the variety, growing conditions, and processing methods [Okechukwu, 2017, Adefega,2017, Ndabikunze et al., 2011]. Cocoyam plays a crucial role in food security, particularly in resource-limited communities (FAO, 2019). It is often cultivated as a subsistence crop and serves as a staple food, especially during lean seasons. Furthermore, cocoyam can contribute to income generation through local markets and value-added products [Oyenka, 2014]. Despite its nutritional and economic importance, cocoyam remains underutilized compared to other staple crops. There is a significant potential to expand its cultivation and utilization through improved agronomic practices, development of improved varieties, and diversification of its food applications [Okechukwu, 2017, Adefega,2017, Oyenka, 2014].

Existing research on cocoyam limited in nutrient, toxic element and HR-LCMS analysis of entire plant parts. This research aims to fill this gap by ICP-AES analysis of nutrient elements and HR-LCMS analysis of secondary metabolites present in methanolic extracts of cocoyam. The findings of this study will contribute to process cocoyam in food and pharmaceutical industries.

CHAPTER 2 : LITERATURE REVIEW

Many ethnic groups of people from Nepal uses different locally available plants and their parts as a cure of wide range of health discomforts and diseases. This indicates the prosperity of Nepal in medicinal plant biodiversity [Shrestha et al.,2016]. People from all around world feed mainly in cereals, tuber crops, beans, sugarcane, vegetable plants, fruits. The utility of tuber crops is increasing day by day due to their capability of being grown in varieties of climatic conditions facilitated by genetic diversity [Tay, 2013]. Cocoyam commonly known as taro (English), *karkalo* (Nepali) is one of the mostly used root and tuber crop (RTC) in the world. It is world's six most important root and tuber crop (Boakye et al.., 2018). Mainly two species of cocoyam; *Colocasia esculenta* and *Xanthosoma sagittifolium* are cultivated in more than 65 countries across the world, mostly widespread in tropical regions (Wang, 1983) for their tasty, starchy root (Ce et al.., 2017). Nutrient analysis of reveals that Cocoyam tuber is rich in carbohydrates, protein, fats, fiber [Ukom et al., 2018]. Oxalate content in cocoyam made it as food of under choice because it causes itching sensation in mouth and tongue (Okechukwu, 2017). Phytochemical screening indicates the presence of alkaloids, flavonoids, glycosides, phenols, steroids and tannins [Ogukwe et al., 2017]. Consumption of the parts of cocoyam may lower the risk of colon cancer due to its potential antioxidant effect (Aovi et al., 2018). Rich of antioxidant and nutrient in cocoyam have drawn special attention for the identification of biologically active components to reduce the risk of diseases (Okechukwu, 2017).

Qualitative and quantitative study of phytochemicals and nutrient element analysis were carried out in flowers and stems of two cocoyam varieties: *Xanthosoma sagittifolium* and, *Colocasia esculenta* from Nigeria (Oguke et al., 2017). Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Steroids, Tannins along with carbohydrates, crude proteins, crude fats, fiber arepresent in the organs of cocoyam. Proximate, Mineralsand anti-nutrient contents in Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is also reportedfrom Ethiopia (Ezeonu et al., 2016). Phytochemicals constituents, anti-nutrients along with different elements are also reported in the study. The study reveals that antioxidant potential of cocoyam inflorescence decreases on boiling (Okechukwu, 2017) in Uturu. The nutrient elements Zn, Fe, Cu and vitamins E, B_2 & C is also reported in its mass. Phytochemical investigations and pharmacological screening of *Xanthosoma sagittifoliun* (L.) leaf extract (Aovi et al.,

2018) in Banlgadesh ensured the presence of different phytochemicals, antioxidant activity and mild antimicrobial activity against *Bacillus subtilis, Staphylococcus aureu, Escherichia coli, Salmonella enterica, Vibrio cholera* and *Shigella dysenteriae.* Raw form of Cocoyam contains considerable amount of proximates, phytochemicals, minerals, vitamins, amylose, amylopectin and antioxidants (Awa, 2015). Cocoyam and unripe banana incorporated feed has ameliorating potentials on renal and liver growth of diabetic rats, induced with 55 and 65mg/kg (Eleazu et al., 2013). Significant decrease in estimated glycemic index (eGI) and sugar content with pasting of cocoyam flour was observed. Paste of flour has shown significantly higher α-amylase and α-glucosidase inhibitory activities (Adefega,2017). Cocoyam has significant scope on processing of food products with enhanced nutrition and potential to promote its utilization because of good contents of calcium, magnesium, copper, iron, sodium, zinc, manganese, and potassium (Ndabikunze et al., 2011). The dietetic value of cocoyam flour enriched with cowpea flour was reported to be higher which can be used as dietary supplement to treat malnutrition and chronic diseases caused due to deficiency of protein (Olayiwola et al., 2013). Cocoyam starches exhibited lower water absorption capacity and swelling power, paste clarity and viscosity but higher solubility, gelatinization temperatures and retrogradation tendencies than cassava and corn starches (Mweta et al., 2010). Apical section of Cocoyam tuber was found to rich on protein while distal section rich in ash, fiber and minerals. Potassium was the most abundant mineral found in cocoyam. The content of oxalate reduces with drum drying process (Dedeh, 2002). *Colocasia esculenta* possesses strong antioxidant properties and other compounds that justify its medicinal properties as used in ethno-medicine (Eleazu, 2016). The uses of cocoyam are limited because of high content of anti-nutrients like cyanides, oxalates which varies according to the habitat (Olatunde, 2018). 35 compounds were detected through GC-MS analysis (Rabiu et al., 2024). HPLC-DAD analysis reported 34 phenolic compounds (Ferreres et al., 2012). No any literature about the HRLCMS profiling, nutritional value and antimicrobial activity of Cocoyam grown at different altitudes of Nepal is reported till the date. This research will bridge the gap about the nutritional value of Cocoyam of Nepalese origin and other world.

General overview of *Colocasia esculenta*

Cocoyam (*Colocasia esculenta)* is herbaceous plant grown extensively in tropical regions. It is mainly used as a source of starch and animal feed. Twenty different species of cocoyam are found in Nepal. Cocoyam is found as cultivated as well as wild vegetation in the marginal lands and harsh environment. It is widely cultivated in home garden and fields, along with other plants such as turmeric, ginger (Pandey, 2001). It consists of three parts: leaves, which have a high protein and fiber content; stem, composed of lignocellulose; and a corm, with starch content similar to that of cassava and potatoes (Sebastian et al., 2018). It is herbaceous perennial crops of the family *Araceae* growing to a height of 1.5 to 2 m. The rhizome produces leaves that can grow up to 40 - 25 centimeter in diameter (Ubalua et al.., 2016). Leaves are dark green color upward and light green beneath. The tips of the basal lobes are rounded or sub-rounded, and they have a mucronate, triangular-ovate shape at the apex. The petiole has a height of 0.8–1.2 meters. The spadix has flowering components that can reach a diameter of 8. This rhizome crop can withstand severe weather conditions and a longer time of storage than other root and tuber crops(Ubalua et al., 2016).

Systematic classification of cocoyam

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophytes
Division	:	Magnoliophyta
Class	:	Liliopsida
Subclass	:	Arecidae
Order	:	Arales
Family	:	Araceae
Genus	:	*Colocasia* Schott
Species	:	*esculenta* (L.)
Common name:		Taro
Synonyms:		Alocasia dussil Dammer
		Alocasia illustris W. Bull

Source: United States Department of Agriculture

Natural resources conservation service

https://plants.usda.gov/home/classification/16337

CHAPTER 3. MATERIALS AND METHOD

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.), Sulfuric 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), quercetin (Loba Chemie, Mumbai India), Nutrient Agar (Hi-media Pvt. Ltd, Mumbai India), Hinton Agar (Hi-media, Mumbai India) and other analytical reagents were used without further purification.

Software:

MS Power Point, MS word and MS Excel were used to interpret and analyses all information regarding this research. The software Origin Lab Origin pro was used for the analysis of the data and construction of graphs, curves.

3.2 Collection of plant

Colocasia esculenta (cocoyam) plant samples were gathered at three different elevations in Central Nepal: Padampur (208m), Upperdangadhi (1275m), and Palung (Makawanpur, 2310m). The aboveground plant portions were collected during the blooming season (September 2023) while the underground tubers were taken during the harvest season (January 2024). After being carefully cleaned with tap water and then distilled water, the collected plant pieces were put in a fresh zippered bag.

Identification of plant

The plant was sent to National Herbarium & Plant Laboratories (NHPL), Godawari-5, Lalitpur, for its systematic identification and authentication.

Drying, grinding and storage of plant

The collected plant parts were properly washed under tap water to remove any coarse materials and then with distilled water. The wet weight of sample was taken and recorded. The washed plant parts were chopped into thin slices using a clean stainless-steel knife. The chopped slices of samples were kept under shade drying at room temperature (24-30^{0}C) in a well-ventilated room for about a month. During drying it was made cautious to avoid any fungal growth by continuous reversing in each 2 days until constant weight of samples were obtained. After that the dried sample parts were ground into fine powder using clean stainless-steel bladed grinder. The powdered samples were sieved using 60mm mesh to obtain homogenous powder and kept in a clean polythene zipper bag and kept in a desiccator till phytochemicals extraction to avoid moisture.

3.3 Methanolic extraction of phytochemicals.

Certain weights of the desiccated powder samples were taken using digital balance and packed into 25mm×100mm sized thimble and phytochemicals were extracted by Soxhlet extractor using polar solvent (300ml methanol). The extraction was continued for 10 hours by setting the Soxhlet temperature to the boiling temperature of solvent as per the indication of polarity index table.

Filtration of crude extract

A simple filtration method was carried out to filter the crude extract using Whatman No. 1 (45 μm) to remove unwanted solid particles.

Evaporation of solvent

The solvent from the filtered extract was evaporated in a hot water bath until gummy solid mass was obtained.

Storage of dried samples

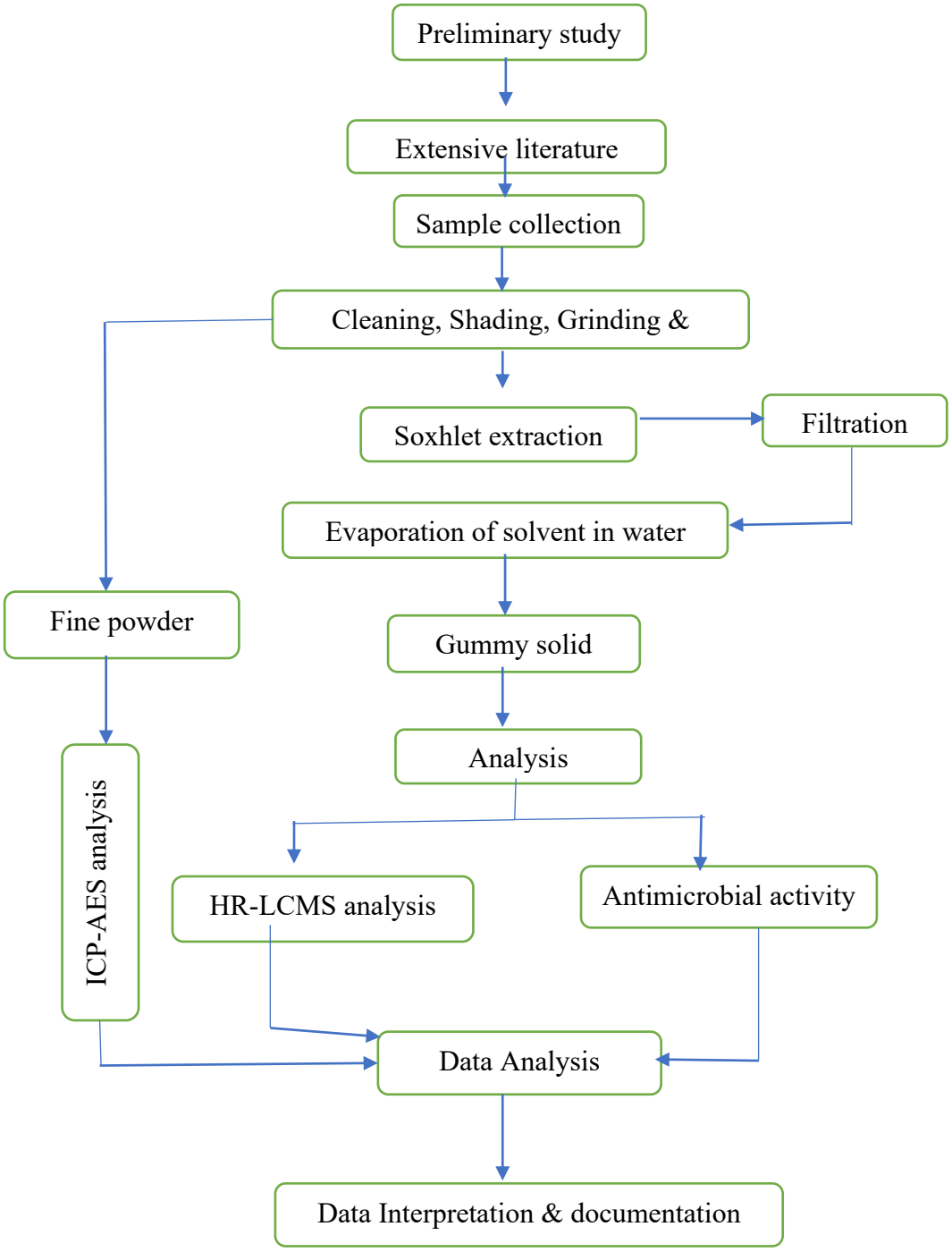
The dried gummy mass of extract was stored in an amber colored glass bottle, air tightly, wrapped with thin parafilm. A portion of samples were sent for HRLCMS profiling to Center for Research in Nanotechnology and science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay, 400076, India.

Percentage yields of methanolic extracts

The % yield of methanolic extract was calculated as per the formula given below.

$$\% \text{ yield} = \frac{weight\ of\ crude\ extract}{weight\ of\ the\ powdered\ sample\ talen} \times 100$$

Schematic diagram showing the methodology of research



[Scheme 1: Flow chart showing entire methodology of research]

Antimicrobial susceptibility test of cocoyam

The antimicrobial susceptibility test of extracts of different parts of cocoyam was carried out by agar well diffusion protocol. The potency of plant extracts against bacterial activity was ascertained zone of inhibition. (Kuper et al., 2012). Clinical

laboratories standard institute 2018 guidelines were followed to conduct the antimicrobial susceptibility test.

Collection of test organisms

The strains of standard pathogenic *bacteria S. aureus, K. pneumoniae, S. typhi, P. aeruginosa, E. coli, A. baumannii* and *B. subtilis* were collected from Bharatpur Hospital, Chitwan, Nepal.

Preparation of plant extract stock.

A stock of 100ppm of each sample was prepared in Dimethyl sulfoxide (DMSO) and stored in a closed vial at -4^0C in a refrigerator for further use.

Mueller Hinton Agar Preparation

45.6g of Mueller Hinton Agar was dissolved completely in 1200ml Distilled water and stirred with autoclaved glass rod. Then the mixture was transferred equally into three 500ml conical flasks. The agar mixture was then autoclaved at 121^0C for 20 minutes. The pressure of autoclave was 15atm. Thus, prepared agar solution was left for few minutes under cooling. The agar plates of 90mm diameter were incubated at 180^0C and left under laminar flow to avoid cross contamination. The agar solution was poured into the agar plates(approx..20ml/plate) and covered with lid. The lid-covered plates were left for 45 minutes to cool. Thus, cooled agar plates were left under refrigeration for 24 hrs. The refrigerated agar plates were left under laminar flow for few minutes. Mueller Hinton Agar (MHA)plates, previously prepared were dried to eliminate excess water on the agar surface. The agar plates were labelled with date of experiment and organisms. The turbid solution of micro-organism was prepared using normal saline purchased from a local pharmacy, in different test-tubes for different micro-organisms. The turbidity of bacterial solution was finalized by comparing with 0.5 McFarland standard. A sterile cotton swab was immersed in the prepared inoculum and any surplus inoculum was removed by gently pressing and rotating the swab against the upper inner wall of test-tube, just above the liquid level. After each swabbing, the plates were rotated at 60-degree and final swabbing was done along the periphery of agar surface. The cross-contamination was avoided by leaving the inoculated plates to dry inside the laminar flow with covered lids. A sterile corks borer having 7mm of diameter was employed to punch the wall on the dried agar plate and marked accurately.80µl plant stock solution was gently loaded into

respective wells with the help of micropipette. The solvent (10&DMSO) was used as negative control while different antibiotics were placed as positive control. The agar plates with controls, samples were covered with lid and left about 45minutes to diffuse the extracts and controls. The plates were uprightly placed in an incubator at 37^0C for 24 hours for appropriate incubation. The clear zone of inhibition around each well in the agar plates were examined and measured using ruler. The average value of zone of inhibition was presented to disclose the strength of plant extract against pathogenic bacteria.

Quantification of total phenolic content

General protocol for estimation of total phenolic content

Estimation of total phenolic content was carried out by Folin-Ciocalteu method with slight modification by Lister and Wilson (2001) (Zahin et al., 2009). Briefly, to 0.5ml of each sample (in triplicate) in graduated glass vial, 2.5ml of 1/10 dilution of Folin-Ciocalteu reagent and 2ml of 7.5% Na_2CO_3 (w/v) were added, shaken vigorously and incubated at 45^0C for 15 minutes under light protected environment in hot water bath. After incubation, the absorbance having λmax at 765nm was determined using UV-VIS spectrophotometer (T80+, PG Instrument, UK). The total phenolic content was expressed in milligrams of gallic acid equivalent (mgGAE/g DS) on the reference of calibration curve achieved with gallic acid as standard.

Measurement of total phenolic content (TPC)

Standard calibration curve of gallic acid was constructed and concentration of total phenolic content was determined by using the equation of straight line. The total phenolic content was calculated using formula;

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

Where, C = Total phenolic content (mg GAE/g DS)

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

v = Volume of extract (mL)

m = weight of plant extract (g)

Standard calibration curve of gallic acid was constructed and total phenolic content was determined from the equation of straight line.

Statistical analysis

The average absorbance from triplicate measurement of each concentration was evaluated which was used to determine linear coefficient and establishing the regression equation;

$y = mx + c$……. (2)

Where, y = absorbance of extract

m = slope of curve

x = concentration extract

c = intercept

The total phenolic content of extract in gm GAE/g, was determined from the regression equation.

Quantification of total flavonoid content

General protocol for estimation of total flavonoid content

The total flavonoid content was determined by aluminium-chloride assay using quercetin as standard (Sagar et al.,2020). Stock solution of quercetin was prepared by dissolving10mg quercetin in 10ml 0f 80% methanol. Varying concentration of quercetin were prepared from stock solution and used for calibration. 1.5ml of 80% methanol was added to standards and samples (0.5ml) followed by addition of 100µl $AlCl_3$, 100µl Potassium acetate and 2.8ml distilled water with vigorous shaking and left for incubation at 30^0C for 30 minutes protected under light free condition. After incubation the absorbance was measured at 410nm using UV-VIS spectrophotometer (T80+, PG Instrument, UK). The total flavonoid content was expressed in milligrams of quercetin equivalent (mgQE/gDS). The measurement of TFC was done in the similar way done for TPC.

Quantification of total carbohydrates content (TCC):

General protocol:

The total carbohydrates of various plant extracts of *Colocasia esculenta* was quantified using modified sulfuric acid-phenol method using glucose dextrose as standard (Masuko et at., 2005). In brief, 500µL of standard and samples were taken in cleaned with distilled water and oven dried test-tubes. 1500µL of conc. H_2SO_4 was added to each test-tubes and shaken up to 30 minutes followed by addition of 300µL 5% phenol. The entire mixture was heated at 90^0C for 5minutes in a static water bath then cooled to room temperature(25^0C) in another water bath for 5 minutes. The absorbance was measured and recorded at 490nm using UV-VIS spectrophotometer. An equation of regression line from standard calibration was implemented to quantify the total carbohydrates (TCC) and expressed as milligrams of glucose equivalent/g of dry sample (mgGE/g DS)

Measurement of total carbohydrates content (TCC):

Standard calibration curve of glucose dextrose was constructed and concentration of total carbohydrates content was determined in the similar way done foe TFC.

The total antioxidant activity (TAA): Phosphomolybdenum assay

General protocol:

The phosphomolybdate method was implemented for estimation of total antioxidant capacity of samples using ascorbic acid as standard (Jie et al.,2011). In brief, 1ml of extract and standard were separately mixed with 4ml of phosphomolybdenum reagent. The phosphomolybdenum reagent was prepared by mixing equal volumes of 0.6M H_2SO_4, 28mM Na_3PO_4 and 4mM ammonium molybdate. The mixture of extract and phosphomolybdenum reagent was the incubated at 95^0C for 90 minutes and cooled to room temperature. After cooling, the absorbance having λmax at 695nm using spectrophotometer (T80+, PG Instrument, UK). The total antioxidant capacity of sample extract was expressed as mg AAE/g DS in comparision to the standard ascorbic acid calibration curve. A blank of 1ml reagent and 4ml methanol was implemented for the estimation of TAA.

Blank = Reagent + Solvent

DPPH free radical scavenging assay:

General protocol

The total antioxidant capacity of different plant extracts of cocoyam was carried out by DPPH free radical scavenging assay (Islam et. at, 2015). 1ml of varying concentrations of ascorbic acid standard and sample extracts were mixed with 2ml of 0.004% of methanolic DPPH and incubated at room temperature for 30 minutes in dark. After incubation, the absorbance of standard and samples were measured at 517nm using UV VIS spectrometer. A blank of 1ml methanol and 2ml 0.004% methanolic DPPH was used as control.

Measurement of DPPH free radical scavenging activity:

The following formula was implemented to estimate the free radical scavenging activity of samples.

$$\text{Percentage scavenging (\%RSA)} = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Solutions of varying concentrations of ascorbic acid as standard was used to evaluate the 50% of radical scavenging capacity **(IC_{50})** and graph was constructed to correlate the %RSA of varying concentrations of samples.

Quantification of nutrient elements and toxic metals

Three plant samples (UPT, UPS and UPL) powder was sent to Center for Research in Nanotechnology and science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay, 400076, India.

0.1g of sample was subjected to microwave digestion (Model: Anton Paar microwave go) using a mixture of 2ml conc. HCl, 1ml conc. HNO_3 and 2ml HF [Pokhrel et al., 2019]. The system was programmed to operate as 190^{o}C for 25 min (15 min ram time) and cooled to room temperature and the volume was made up to 25ml with distilled water. Elements were detected by ICP-AES.

HRLCMS profiling

An Agilent LC instrument column composition (model: G1316C) and a quadrapole time of flight mass spectrometer (QTOF-MS, Agilent technology) with a diode array detector (DAD) (model: G226A) through electro spraying ionization (ESI), was used for chromatographic separation. The chromatographic apparatus was fitted with a binary pump (model: G220B) and hip sampler (model: G4226A). Chromatographic separation was performed in a column compartment that was set at 40 °C. The Hip auto sampler was used with a 5.00µL injection volume, a binary pump with a post time of 35 minutes, a flow rate of 0.3µL/min, a maximum flow gradient of 100mL/min, and a maximum pressure of 1200.00 bar. The mass spectrometer's acquisition method with an MS scan rate (spectra/sec) of 100, was configured for a dual ESI ion source with a range of (m/z) 120–1200. The scan segment was conducted in both positive (+ve) and negative (-ve) modes. The drying gas temperature was set at 250 C with gas flow at 13 L/min, nebulizer gas at 35 psig, capillary voltage at 3500V, fragment voltage at 175V, skimmer voltage at 65V, nozzle voltage at 1000V, and octpole RF peak at 750V. Acetonitrile (B) and water: formic acid (100:0.1) (A) make up the mobile phase. The multistep linear gradient 0-1 min 5% B, 25min 100% B, 30min 100% B, 31min 5% B, and 35min 5% B comprised the chromatographic technique.

The column over temperature was kept at 40°C. Data acquisition and processing were performed using mass hunter workstation software and the mass analyses were done by both positive and negative mode ion modes respectively. The initial scan was done by 190nm to 640nm with m/z 120-1200. The parent ion and source –induced dissociation fragment could provide the accurate mass information. The HR-LC-MS data processing were used for the identification of constituents and ESI chromatogram is obtain to calculate mass to charge ratio (m/z) of the selected peaks

CHAPTER 4 : RESULT AND DISCUSSION

4.1. Yield of sample after drying

The yield of samples of *Colocasia esculenta (L.) Schott.* after shade drying under well ventillated condition is tabulated below.

Table 1 : % yield of samples after drying

SN	SAMPLES CODES	WET WEIGHTS	DRY WEIGHTS	% YIELDS
1.	PDS	106gm	20.22gm	19.07%
2.	PDL	93gm	19.99gm	21.50%
3.	PDT	440gm	91.53gm	20.80%
4.	UPS	100gm	13.00gm	13.00%
5.	UPL	92gm	22.95gm	24.95%
6.	UPT	690gm	184.82gm	26.78%
7.	PS	220gm	37.00gm	16.81%
8.	PL	80gm	19.712 gm	24.64%
9.	PT	795gm	129.85gm	16.33%

The highest yield after drying was obtained from tuber sample from Upperdangadhi (26.78%) and the least yield from leaf sample from same spot (13%).

4.2 Yield of samples after Soxhlet extraction.

The yields of sample extracts obtained from dry weights of samples of *Colocasia esculenta (L.) Schott.* using Soxhlet apparatus and methanol as solvent is tabulated below.

Table 2 : % yield of samples after Soxhlet extraction

SN	SAMPLES	WEIGHT TAKEN(g)	YIELD(g)	% YIELD
1.	PDS	10.024gm	0.948gm	9.45%
2.	PDL	10.002gm	1.127gm	11.27%
3.	PDT	25.030gm	3.511gm	14.02%
4.	UPS	10.022gm	1.186gm	11.83%
5.	UPL	10.016gm	10.016gm	6.29%
6.	UPT	45.010gm	2.420gm	5.37%
7.	PS	15.160gm	4.387gm	28.93%
8.	PL	10.015gm	1.937gm	19.34%
9.	PT	30.024gm	3.051gm	10.16%

The Soxhlet extraction of sample of stem from Palung yielded the highest (28.93%) andthe yield of tuber sample from Upperdangadhi was found to be the least (5.37%).

4.3 Antimicrobial susceptibility test analysis

100ppm (80mL) solutions of methanolic extract of samples in 10% DMSO was used to carry out antimicrobial susceptibility test against both gram +ve and gram -ve bacteria. Same volume of 10% DMSO was used as positive control. The entire antimicrobial activities of samples are shown in the graph below:

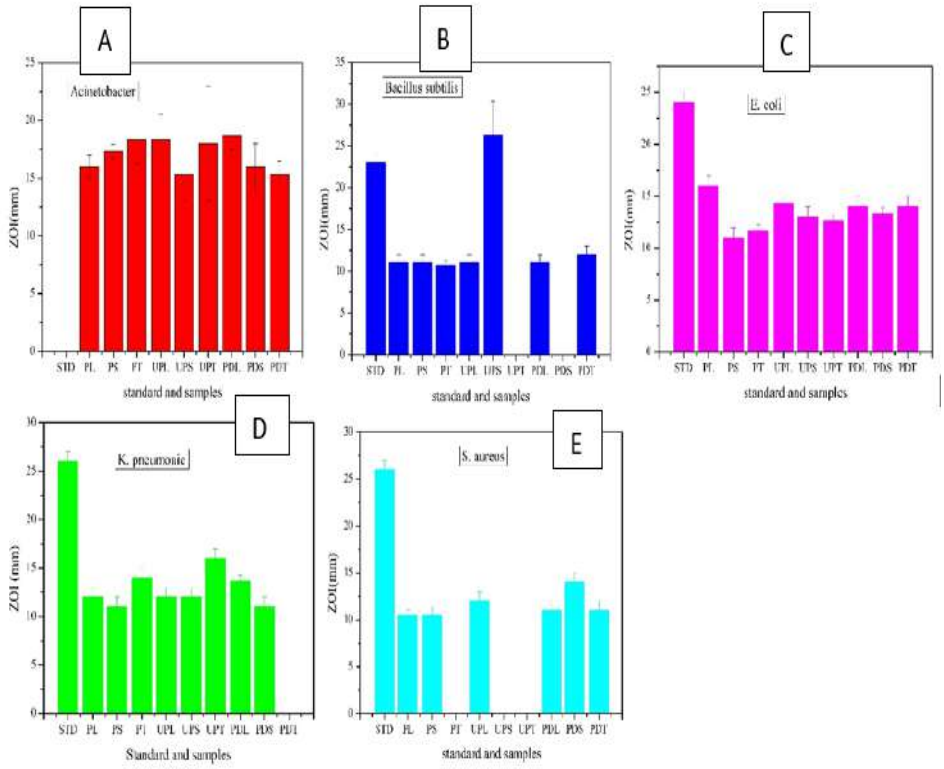


Figure 1 : Antimicrobial susceptibility test of plant extracts against: **A**: Acenitobacter using varieties of antibiotics (Amikacin 30mcg, Ciprofloxacillin 5mcg, Ampicillin 10mcg, Gentamycin 10mcg, Ceftriaxone 30mcg, Ceftazidime 30mcg, Ceftazidime-avibactum 30mcg, Imipenum 10mcg, and Ceftazidime/ clavulanic acid 30/10mcg) as standards, **B**: Bacillus subtilis, Amikacin 30mcg as standard, **C**: Escherichia coli, Amikacin 30mcg as standard, **D**: Klebsiella pneumoniae, Amikacin 30mcg as standard, **E**:S. aureus, Amikacin 30mcg as standard]

The antimicrobial susceptibility test of sample extracts has shown a significant potential to inhibit the examined organisms. All sample extracts are efficient against *A. baumannii* even when a large number of standard antibiotics available in the market show no inhibition of the organism, indicating presence of more potential compound in the plant than the standard resistant *A. baumannii*. The plant extract

samples were seen not to be effective in inhibiting the *Salmonella typhi* and *Pseudomonas aeruginosa.*

4.4 Estimation of total phenolic content

Total phenolic content of sample extracts was estimated using gallic acid standard curve. Varying concentrations of gallic acid (5ppm, 10ppm, 20ppm, 40ppm, 80ppm and 100ppm) were used to calibrate the curve. The calibration curve of instrument was constructed between various concentration of gallic acid and absorbance at 765nm wavelength which is expressed below:

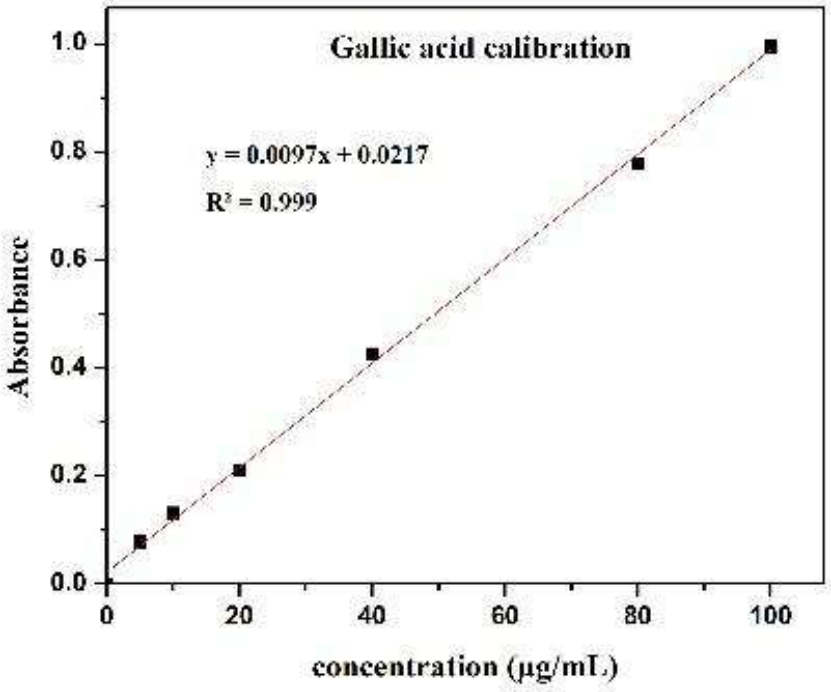


Figure 2 : Calibration curve of gallic acid to determine total phenolic content]

Calculation of total phenolic content of plant samples

The regression equation y = 0.0097x + 0.0217, R^2 = 0.999, obtained from Excel software sheet was employed to calculate the total phenolic contents of sample extracts.

Here, 'x' is concentration of gallic acid in microgram per milliliter (µg/mL)

'y' is absorbance

Slope of the curve (m) = 0.0097 and 'y' intercept (c) = 0.0217

ZA simple equation, TPC = x (V/m) is used to calculate total phenolic contents of plant samples and is expressed in mg GAE/g DS.

From assay for determination of total phenolic content, the leaf extract from Padampur was found to be enriched with maximum phenolic compounds whereas the petiole extract from Padampur was contained with lower concentrations of phenolic compounds.

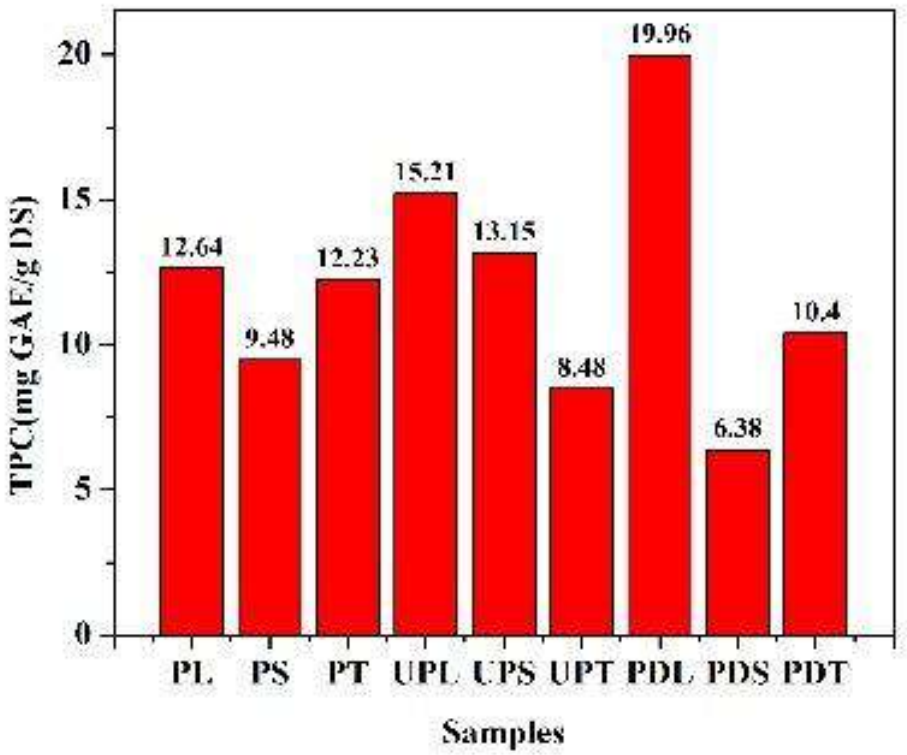


Figure 3 : *Total phenolic contents of samples*

4.5 Estimation of total flavonoids content

Estimation of total flavonoid content of samples was carried out by using Aluminium chloride colorimetric method. Varying concentrations of quercetin (10ppm, 20ppm, 40ppm and 80ppm) were used to calibrate the instrument. A calibration curve obtained from Excel software sheet by plotting concentration Vs absorbance at 410nm wavelength, with regression equation, y = 0.0015x + 0.0048 and R^2 = 0.992 was employed to estimate total flavonoid contents. The TFC was calculated in the similar manner as that for TPC and expressed as mg QE/g DS.

As similar to total phenolic compounds, good contents of flavoinds were found in the leaves of plant samples. The tuber and petiole were poorly enriched with the flavonoids.

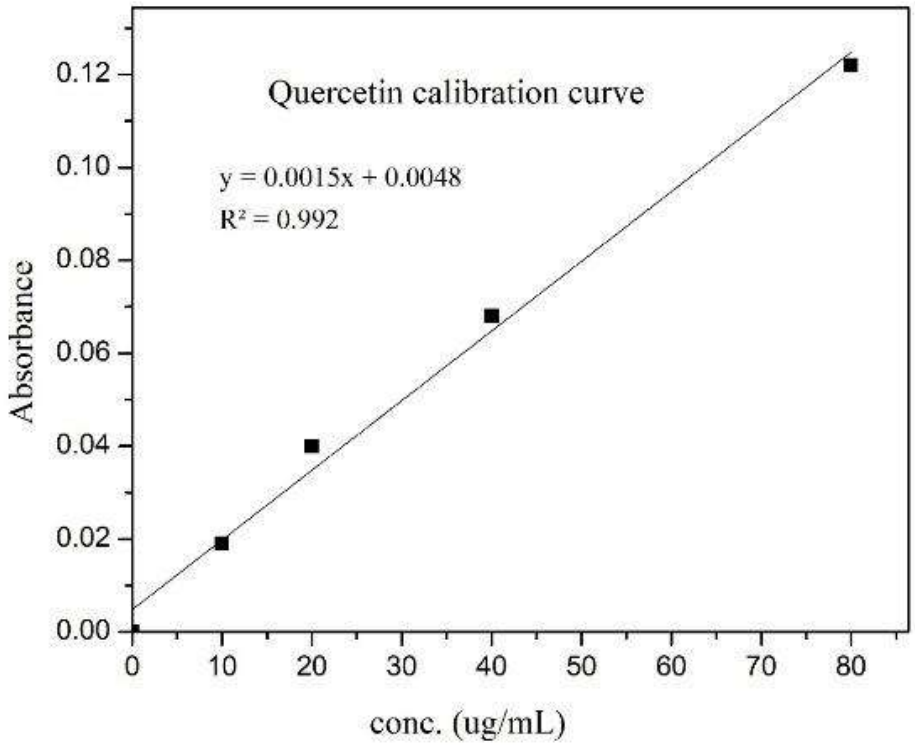


Figure 4 : Calibration curve of Quercetin to evaluate total flavonoid content

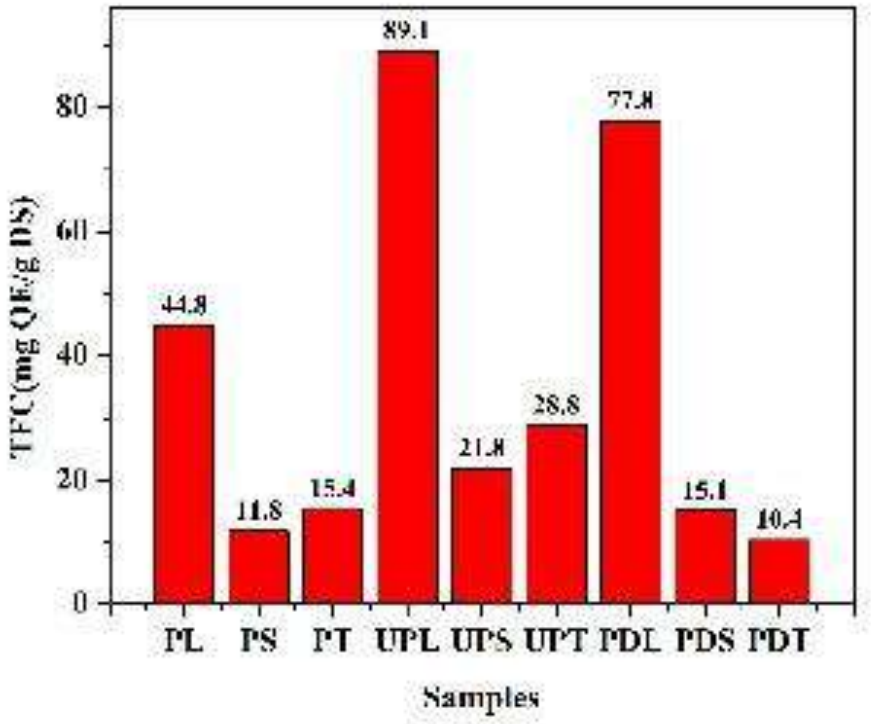


Figure 5 : *Total flavonoid contents of samples*

4.6 Estimation of total carbohydrates content

Modified sulfuric acid-phenol method was implied to quantify total carbohydrates contents (TCC) of samples using glucose dextrose as standard (Masuko et at., 2005). Different concentrations of glucose dextrose (30ppm, 60ppm, 90ppm, 120ppm,150 ppm) ware used to calibrate the spectrophotometer. A calibration curve with regression equation $y = 0.0023x + 0.0149$, $R^2 = 0.986$, obtained from concentration vs absorbance, at 490nm wavelength, plot in Excel software was employed to calculate TCC in mg GE/g DS and the results are shown in table below. The calculation was made in the similar way as for the calculation of TFC.

The tubers of plants contain maximum carbohydrates followed by petioles and the leaves are poorly contained with carbohydrates.

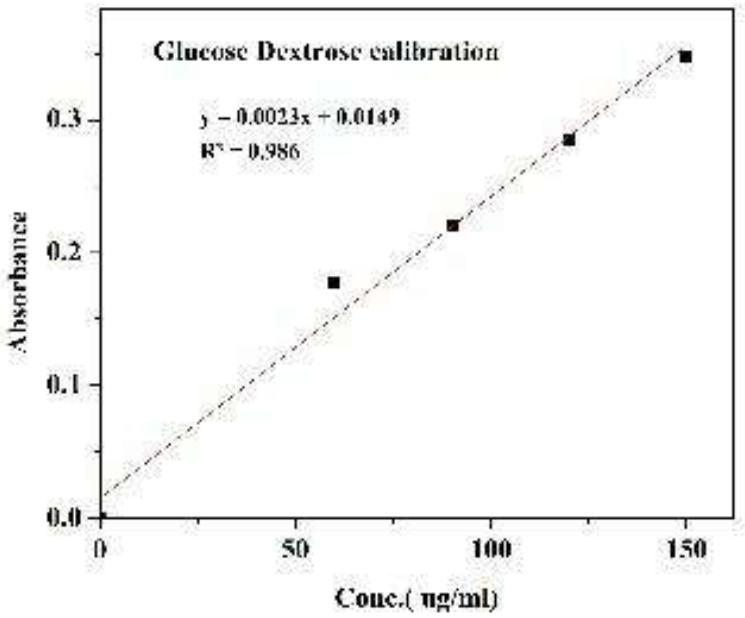


Figure 6 : Calibration curve of glucose dextrose to calculate total carbohydrates content

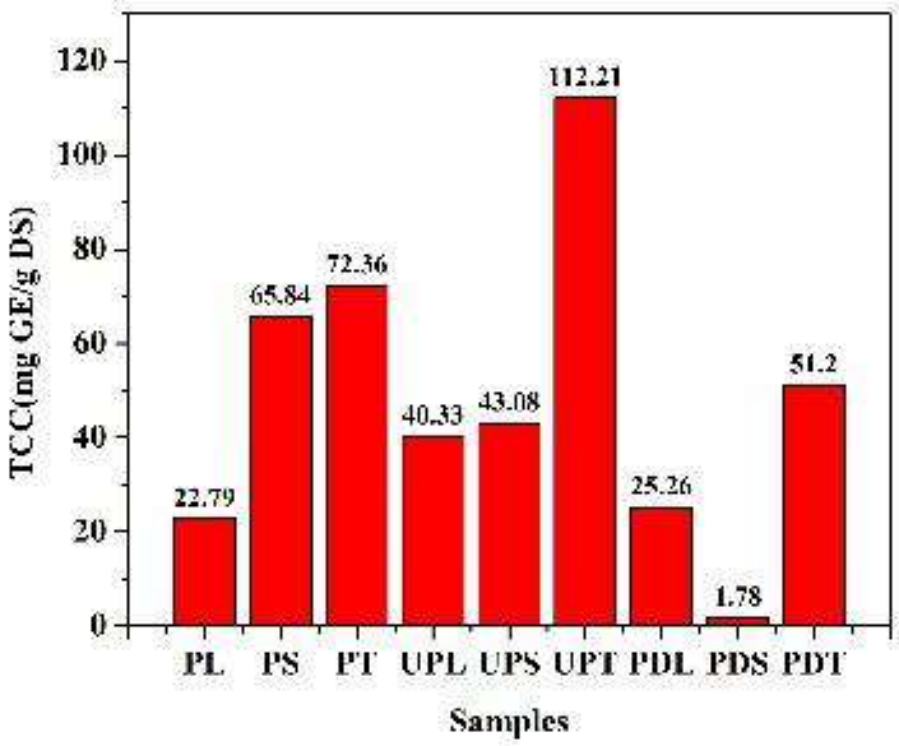


Figure 7 : Total carbohydrates contents of samples

4.7 Antioxidant activity

4.7.1 Phosphomolybdenum assay

The total antioxidant capacity (TAC) of samples was estimated following the protocols of phosphomolybdate assay (Jie et al., 2011) using ascorbic acid as standard. Different concentrations of ascorbic acid (5ppm, 10ppm, 20ppm, 40ppm, 80ppm, 150ppm) were used for calibration at 695nm wavelength. The regression equation, $y = 0.071x + 0.0021$, $R^2 = 0.996$, obtained from a plot between concentrations and absorbance from Excel software sheet was used to calculate total antioxidant capacity in the similar way as that for the calculation of TCC and expressed as mg AAE/g DS which is given in the table below:

Varying antioxidant activities of plant extracts were revealed through phosphomolybdate assay. The highest antioxidant capacity was shown by leaf of plant extracts from Padampur followed by petiole of plant sample from Upperdangadhi. The petiole of plant from Palung have poor antioxidant properties.

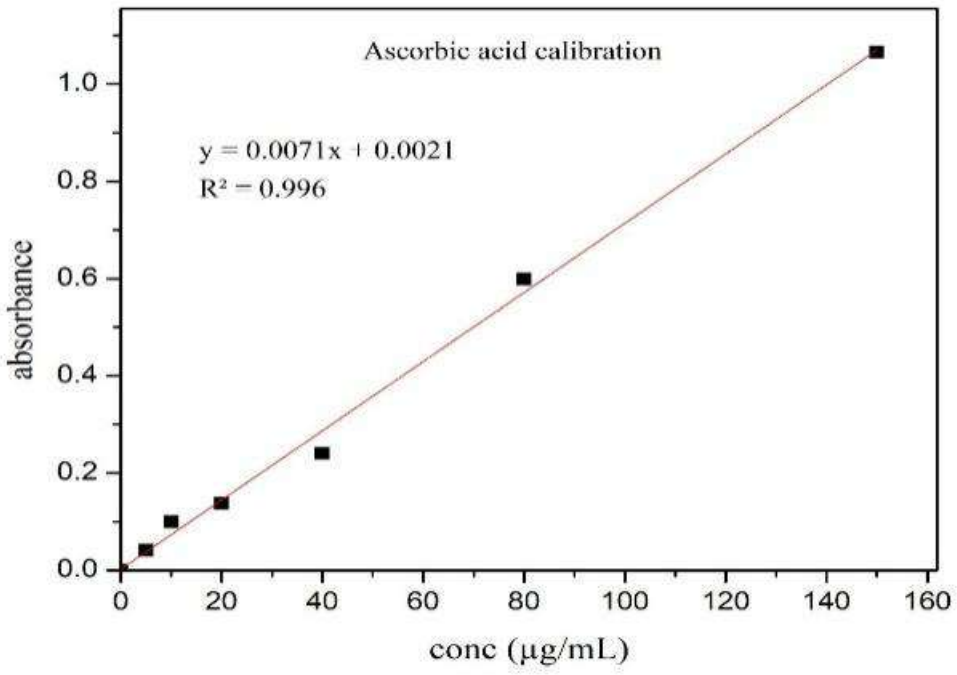


Figure 8 : Calibration curve of ascorbic acid for phosphomolybdate assay

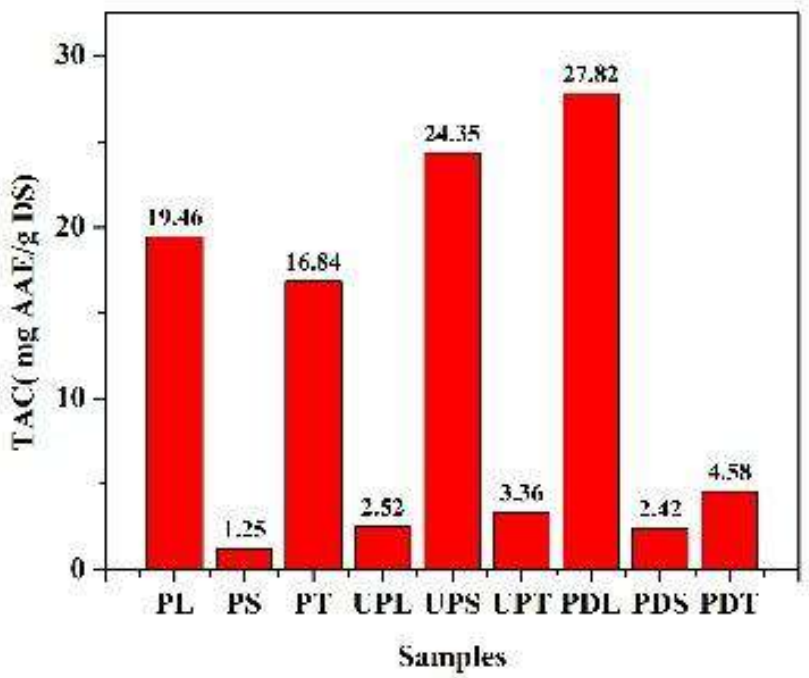
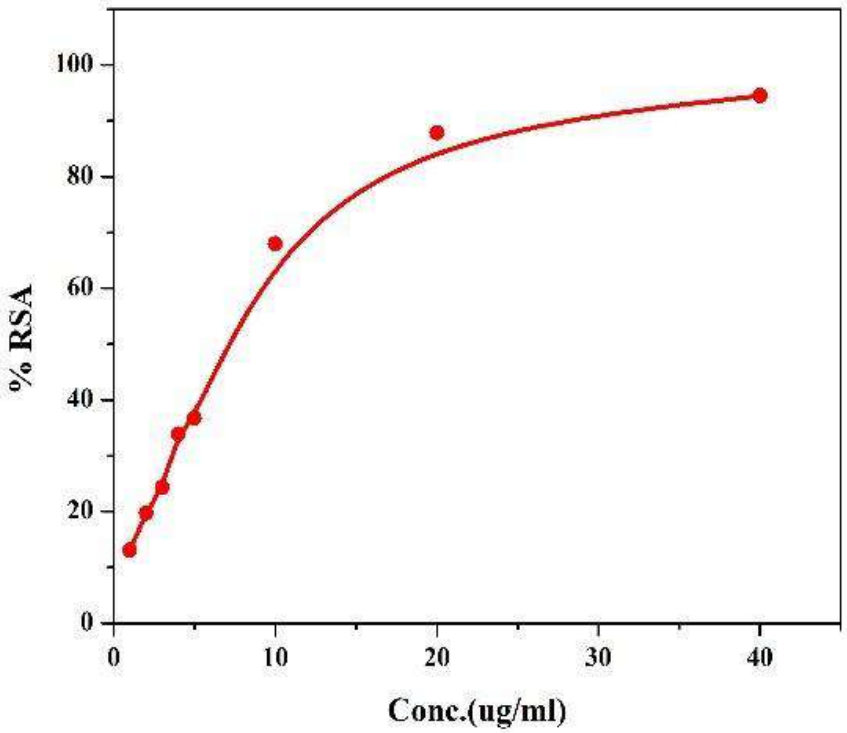


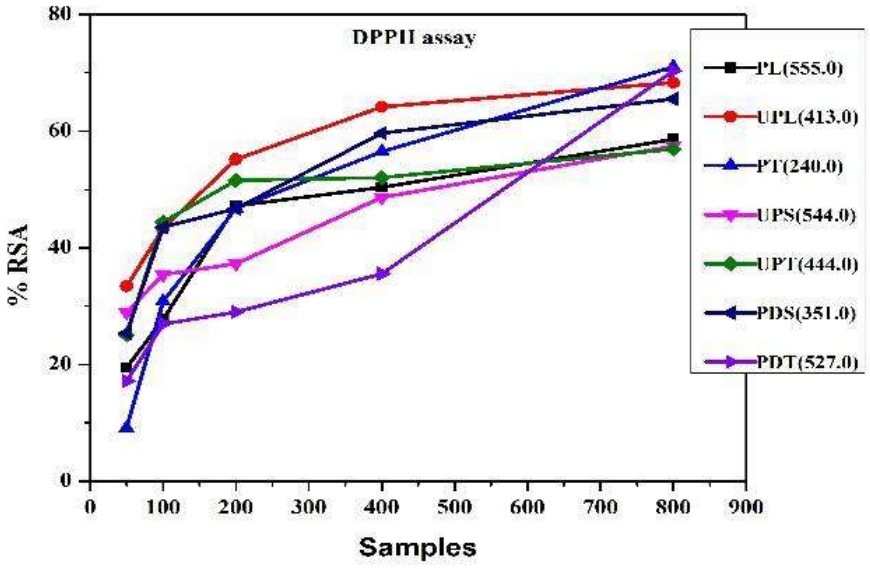
Figure 9 : *TAC of samples*

4.7.2 DPPH free radical scavenging assay

Total antioxidant capacity (TAC) of samples was estimated by DPPH free radical scavenging assay by butylated hydroxy anisole (BHA) as standard (Hasan et. at, 2015). The absorbance was measured at 517nm wavelength. The IC_{50} value of both BHA and samples were calculated from regression equation $y = 6.0779x + 7.2854$, $R^2 = 0.996$, obtained from Excel by a plot between % inhibition and concentration which is shown in the table below:



(A)



(B)

Figure 10 : Conc vs % inhibition of DPPH by **A:** BHA, **B:** IC_{50} of Samples

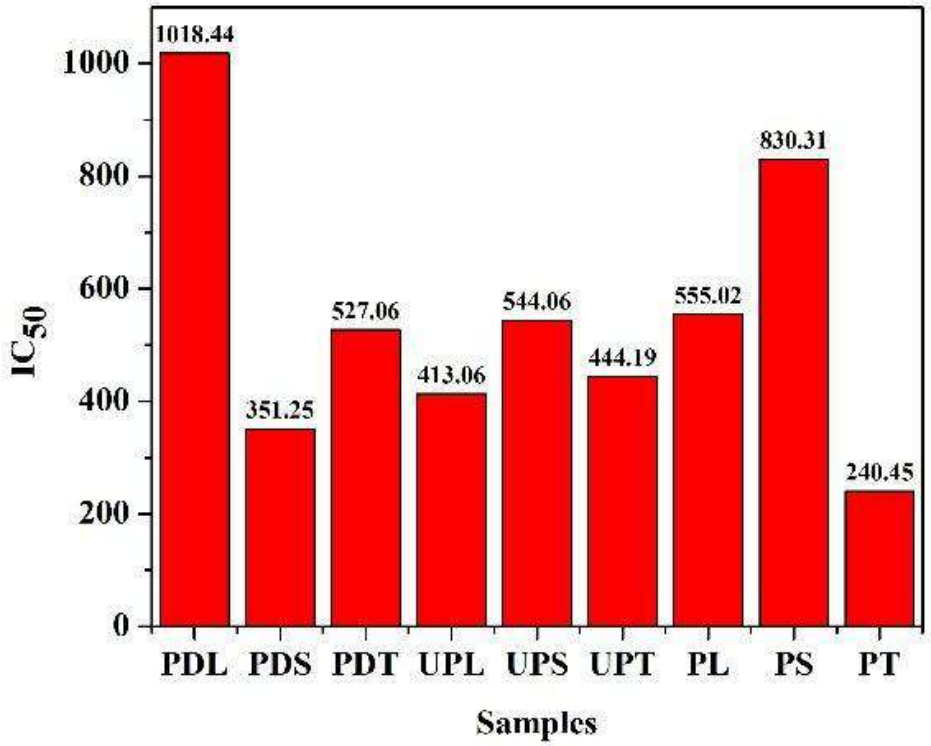


Figure 11 : DPPH free radical scavenging activities of samples (IC50)

The DPPH free radical scavenging assay for estimation of total antioxidant activities of plant extracts using BHA as standard revealed varying potentials of samples to scavenge the free radicals. The minimum IC50 value was for tuber of plant from Palung and the maximum IC50 for leaf of plant extract from Padampur.

4.8 Quantification of nutrient elements and toxic metals.

The quantities of nutrient elements and toxic metals analyzed by ICP-AES at Center for Research in Nanotechnology and science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Bombay, 400076, India, is shown below:

Table 3 : Concentration of various elements through ICP-AES analysis

Name	Ca	Cr	Cu	Fe	Mg	Zn	As
	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg
UPT	8910.0	0.476	1.226	15.600	96.8	128.000	15.700
UPS	303.0	0..252	0.670	1.241	43.6	0.685	9.821
UPL	355.0	0.427	0.727	2.410	104.0	1.942	65.600

4.9 HR-LCMS PROFILING

The methanolic extracts of tuber, petiole and leaf *Colocasia esculents* was subjected to HR-LCMS analysis.

HR-LCMS analysis is conducted in both encompassing both positive and negative modes which revealed total 200 compounds present in UPT, among which 60 are known, 140 are unknown. Among known,11 alkaloids, 4 phenolics, 5 flavonoids, 3 glycosides, 5 steroids and 32 other compounds are present. In the similar way UPS sample contains total 200 compounds, 115 known and 85 are unknown. Out of known, 12 are alkaloids, 15 phenolic, 26 flavonoids, 9 glycosides and 55 other class of compounds. The UPL sample screened total 197 compounds, 89 are known while 116 are unknown. The known compounds include 13 alkaloids, 17 flavonoids, 3 terpenoids, 1 steroid and 57 other compounds.

Table 4 : Identified compounds in UPT using HR-LCMS

S.N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	m/z	Chemical Class	Clinical uses
1	PDT	+ve ESI mode	Diasarone 2	5.139	416.2199	$C_{24}H_{32}O_6$	439.2095	phenolic	Anti dengue viral (Yao et, 2017)
2			Trenbolone	5.839	270.1643	$C_{18}H_{22}O_2$	293.154	steroid	
3			Gentamicin	8.129	477.3206	$C_{21}H_{43}N_5O_7$	478.3281	glyco side	Antibiotics (Chen et al., 2013)
4			Dicyclomine	10.854	309.2627	$C_{19}H_{35}NO_2$	332.252	alkaloid	
5			Jervine	12.243	425.2937	$C_{27}H_{39}NO_3$	448.2831	alkaloid	Anti-tumor, anti-inflammatory (Lei et al., 2020)
6			Glutamyl-lysine	12.515	275.1479	$C_{11}H_{21}N_3O_5$	298.1371	enzyme	
7			funtumine	13.781	317.2675	$C_{21}H_{37}NO$	318.2748	Steroidal alkaloid	Anti-tumor (Badmus et al., 2020)
8			Mangalkanyl glucoside	-6.82	386.2695	$C_{21}H_{38}O_6$	373.2144	glucoside	Antioxidant, anti-inflammatory (Jiang et al., 2022)
9		-ve ESI mode	Vanillic acid	6.435	168.0423	$C_8H_80_4$	167.0349	phenolic	anticancer, antiobesity, antidiabetic, antibacterial, anti-inflammatory, and antioxidant (Kaur et al., 2022)
10			Catechin	7.128	290.0812	$C_{15}H_{14}O_6$	289.0722	flavonoid	Anti-inflammatory, anti-tumor (Musial et al., 2020)

S.N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	m/z	Chemical Class	Clinical uses
11			Usambarensine	11.53	450.2845	$C_{30}H_{34}N_4$	449.2773	alkaloid	anti-amoebic and Anti-plasmodial Activities, anti-tumor (Passemar et al., 2011)
12			Corchorifatty acid F	11.63	328.2259	$C_{18}H_{32}0_5$	327.2185	lipid	antioxidant and anti-inflammatory properties (Yoshikawa et al.,1998)
13			Momordicoside G	11.726	632.429	$C_{37}H_{60}O_8$	677.4275	triterpene	Anticancer ((Du et al., 2019)
14			Macrocarpal B	21.138	472.2839	$C_{28}H_{40}O_6$	471.2767	sesquiterpenoid	Anti-bacterial (Nagata et al.,2006)
15			Diepomuricanin A	18.42	546.4667	$C_{35}H_{62}O_4$	591.465	Acetogenins	antibacterial, anticancer, antidiabetic and anti-inflammatory properties (Al Kazman et al., 2022)
16			Canrenone	23.98	340.2079	$C_{22}H_{28}O_3$	339.201	steroid	Anti-diabeticAntiarrhythmic (Armanini et al., 2014, Dąbrowski et al.,2020)
17			Etidocaine	24.409	276.2223	$C_{17}H_{28}N_2O$	335.2363	Amino acid amide	Anesthetic (Hameroff rt al, 1984)
18			3-keto stearic acid	22.575	298.2518	$C_{18}H_{34}O_3$	297.2446	lipid	anti-inflammatory and anti-cancer (Tiebe et al., 2018)

Table 5 : Identified compounds in UPS using HR-LCMS

S. N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	m/z	Chemical Class	Clinical uses
1	UPS	+ve ESI mode	Neuraminic acid	3.662	267.0818	$C_9H_{17}NO_8$	268.1007	glycoprotein	anti-inflammatory, anti-viral, anti-tumor, anti-hypertensive and skin whitening properties [Mingli et al., 2023].
3			Biperiden	4.548	311.226	$C_{21}H_{29}NO$	334.2154	alkaloid	Parkinson's disease (Kostelnik et al., 2017)
4			Dolasetron	5.075	324.1481	$C_{19}H_{20}N_2O_3$	347.1376	Indole carboxylic acids (alkaloid)	Anti-nausea, anti-vomitting (Meyer et al., 2005)
5			Ketamine	5.291	237.0925	$C_{13}H_{16}ClNO$	238.0998	cyclohexanone	ulcerative cystitis, neurocognitive impairment, deficits inworking and episodic memory [Celia et al., 2011].
6			Mianserin	5.316	264.1645	$C_{18}H_{20}N_2$	287.1539	dibenzoazepine	Antidepressant (Wakeling, 1983)
7			Thienamycin	6.416	272.0836	$C_{11}H_{16}N_2O_4S$	295.0728	Non ribosomal peptides	Antibiotic (Reyes et al., 1985)
8			Sciadopitysin	6.516	580.1313	$C_{33}H_{24}O_{10}$	581.1387	phenolics	anti-inflammatory, anti-oxidant and anti-apoptotic (Ijaz et al.,2023)
9			Naltrindole	15.774	414.1953	$C_{26}H_{26}N_2O_3$	415.2025	alkaloid	anticonvulsant and immunosuppressant properties (Chen et al., 1997)

S. N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	m/z	Chemical Class	Clinical uses
10		-ve ESI mode	Syringic acid	4.056	198.0524	$C_9H_{10}O_5$	257.0668	phenolic	anti-inflammatory, hepatoprotective, cardio protective, neuroprotective, antibacterial, antidiabetic, and antiendotoxic effects [Shimsa et al., 2024] and colorectal cancer [Mihanfar et al., 2020].
11			Caffeic acid	8.336	180.0418	$C_9H_8O_4$	179.0345	polyphenol	antioxidant, anti-inflammatory, and anticancer (Tajner-Czopek, 2020)
12			Chlorogenic acid	9.444	354.0954	$C_{16}H_{18}O_9$	353.088	phenolic	anti-inflammatory, anti-oxidant, anti-bacterial, anti-tumor (Wang et al., 2022)
13			Butin	8.702	272.0692	$C_{15}H_{12}O_5$	317.0679	flavonoid	Antioxidant, anti-tumor (Wu et al., 2022)
14			Sorbose	1.495	180.063	$C_6H_{12}O_6$	179.0557	monosaccharide	Anti-diabetic (Oku et al., 2014)
15			Eudistomin N	1.655	245.9781	$C_{11}H_7BrN_2$	304.9917	alkaloid	Anti-cancer (Yang et al., 2023)
16			Swertiamarin	6.431	374.1189	$C_{16}H_{22}O_{10}$	373.1118	glycoside	hepatoprotective, analgesic, anti-inflammatory, antiarthritis, antidiabetic, antioxidant, neuroprotective and gastroprotective activities (Fadjil et al., 2021)
17			Vanillin	6.987	152.0464	$C_8H_8O_3$	151.0393	phenolic	Antioxidant (Xu et al., 2024)

S. N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	m/z	Chemical Class	Clinical uses
18			Quercetin 3,7-dirhamnoside	8.616	594.161	$C_{27}H_{30}O_{11}$	593.1536	flavonol	anti-inflammatory (He et al., 2023)
19			L-Malic acid	1.49	134.0212	$C_4H_6O_5$	133.0139	Alpha hydroxy acid	antioxidants, disincrustants (Chen et al., 2017)

Table 6 : Identified compounds in UPL using HR-LCMS

S.N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	DB Diff (ppm	m/z	Chemical Class	Clinical uses
1	UPL	+ve ESI mode	Sultamicillin	7.662	594.1467	$C_{25}H_{30}N_4O_9S_2$	-2.2	595.1539	Beta lactamase inhibitor antibiotic	Antibiotic (Friedel et al., 1989)
2			Cyclopamine	7.667	411.3122	$C_{27}H_{41}NO_2$	3.64	434.3013	Alkaloid	anti-cancer (Wilson et al., 2010)
3			Ginkgolide C	8.69	440.1258	$C_{20}H_{24}O_{11}$	13.82	463.1145	Diterpenoid	strong anti-inflammatory and neuroprotective properties (Hebert et al., 2022)
4			Disopyramide	10.669	339.2354	$C_{21}H_{29}N_3O$	-12.82	362.2247	Alkaloid	obstructive hypertrophic cardiomyopathy (Massera et al., 2025)
5			Spiperone	11.149	395.2013	$C_{23}H_{26}FN_3O_2$	-0.96	396.2084	alkaloid	Schizophrenia (Henning et al., 1999)
6			Tiropramide	11.488	467.3169	$C_{28}H_{41}N_3O_3$	-4.59	490.3062	Phenylalanine	Antispasmodic (Takayanagi et al., 1989)
7			Tubulosine	11.983	475.2831	$C_{29}H_{37}N_3O_3$	0.84	476.2904	Alkaloids	Anti breast cancer (Kim et al., 2019)
8			Retapamulin	13.232	517.3292	$C_{30}H_{47}NO_4S$	-12.85	518.3366	Terpenes	Anti-bacterial (Tanus et al., 2014)
9			Somniferine	23.6	608.2513	$C_{36}H_{36}N_2O_7$	1.49	609.2587	Alkaloid	Antiviral (Shree et al., 2022)
10			Pederin	12.716	503.3139	$C_{25}H_{45}NO_9$	-8.79	504.3214	Alkaloid	hemolymph toxin (Kellner and Dettner, 1996)
11			Kaempferol	11.11	286.048	$C_{15}H_{10}O_6$	-1.04	285.0409	Flavonoid	anti-tumorigenic, antiproliferative, and apoptotic effects (Kaur et al., 2024)
12			Luteolin	11.11	286.048	$C_{15}H_{10}O_6$	-1.04	285.0409	flavonoid	anti-inflammatory, anti-proliferative, and antioxidant properties (Fikry et al., 2025)
13			Morindone	12.137	270.0522	$C_{15}H_{10}O_5$	2.16	269.045	phenolic	Anti colorectal cancer (Chee et al., 2024)
14			Anatibant	8.827	710.188	$C_{34}H_{36}Cl_2N_6O_5S$	-4.99	709.1803	Amino acid	traumatic brain injury (Shakur et el, 2009)
15		-ve ESI mode	Tectorigenin	12.416	300.0634	$C_{16}H_{12}O_6$	-0.16	299.0562	Phenolic	hepatoprotective, estrogenic, hypoglycemic and anti-inflammatory activities (Wang et al., 2013)
16			Hexazinone	18.737	252.155	$C_{12}H_{20}N_4O_2$	14.3	297.1535	Triazine heterocyclic compound	Herbicide (Jasemizad and Padhye, 2022)
17			Amprenavir	23.363	505.226	$C_{25}H_{35}N_3O_6S$	-2.65	564.2396	polyphenolic	HIV-1 protease inhibitor (Arvieux and Tribut, 2005)
18			Luteolin-4′-*O*-glucoside	8.742	448.1018	$C_{21}H_{20}O_{11}$	-2.76	447.0943	flavonoid	hyperuricemia and gout (Lin et al., 2018)
19			Ursodeoxycholic acid 3-sulfate	9.154	472.2545	$C_{24}H_{40}O_7S$	-10.56	471.2471	steroid	hepatobiliary diseases (Kobayashi et al., 2000)

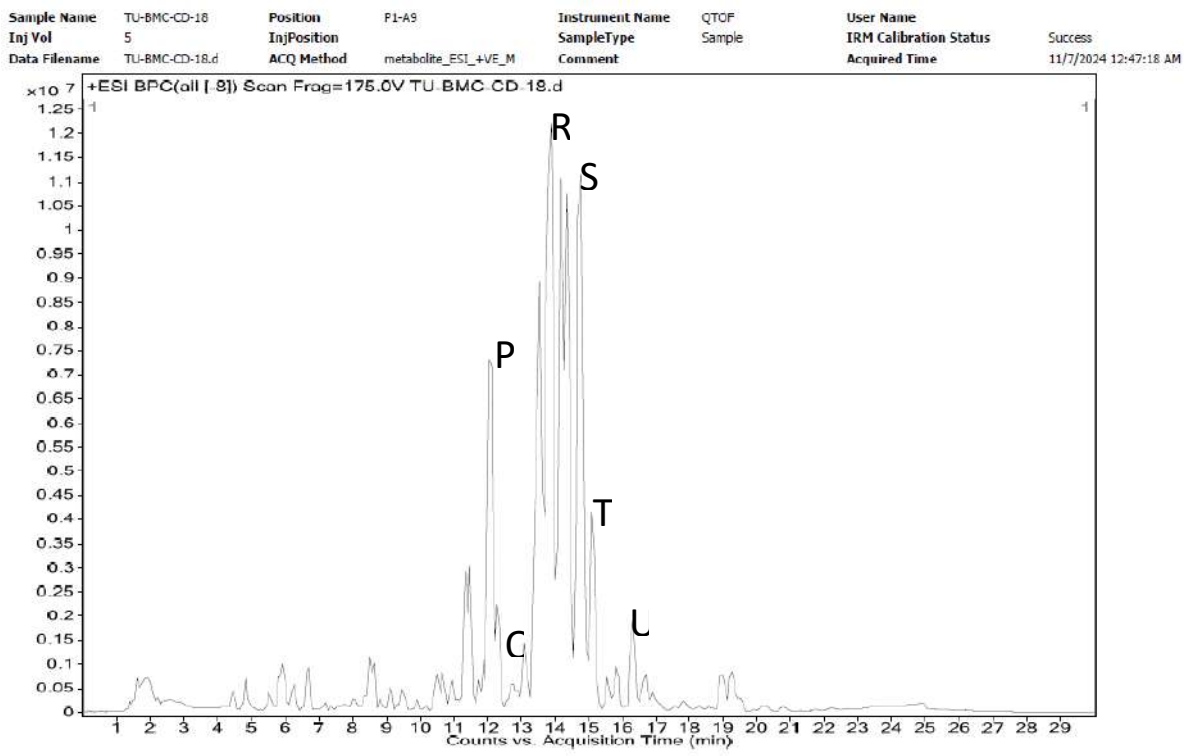


Figure 12 :Chromatogram of UPT in +ve ESI mode using HR-LCMS]

P: Jervine

Q: Funtumine

R: Unknown compound

S: Unknown compound

T: Unknown compound

U: Septentriodine

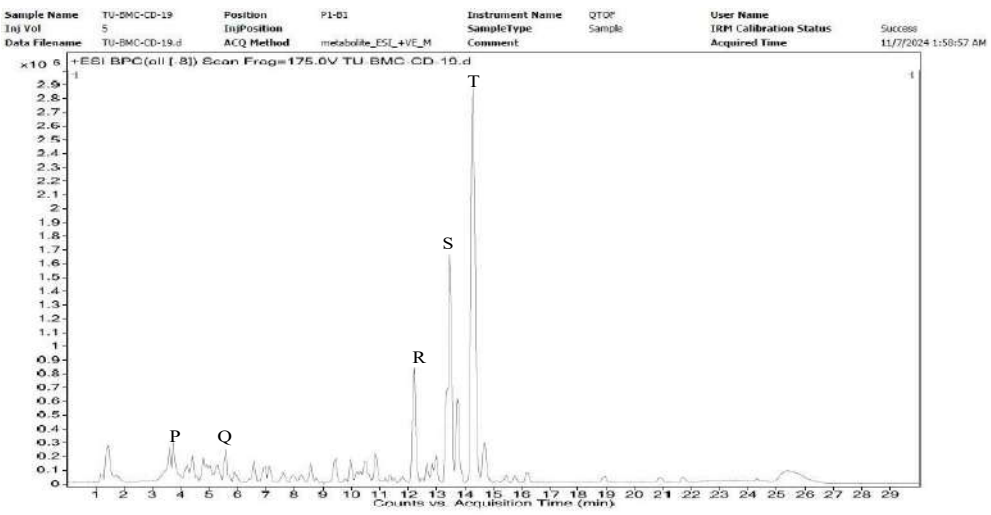


Figure 13 : Chromatogram of UPS in +ve ESI mode]

P: Neuraminic acid

Q: Mianserin

R, S and T: Unknown compounds

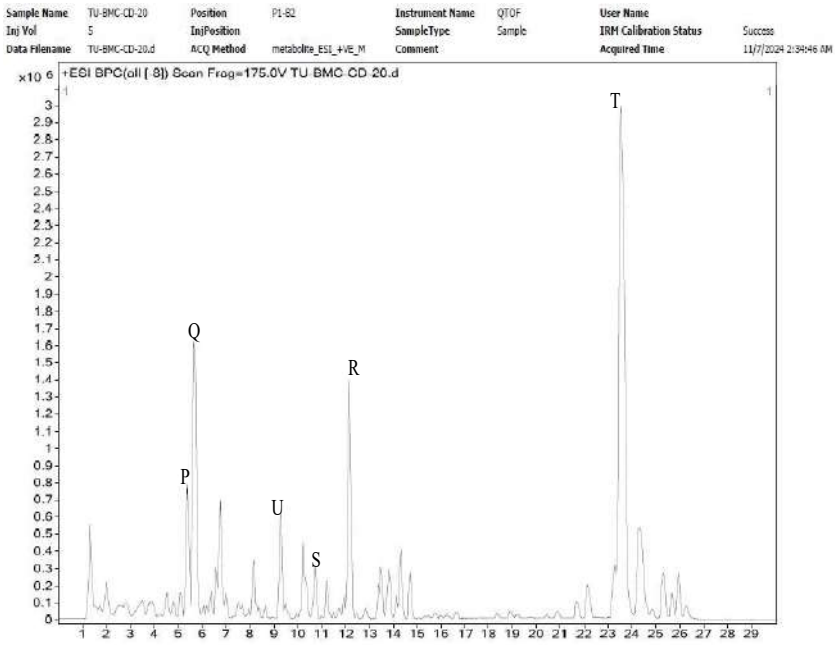


Figure 14 : Chromatogram of UPL in +ESI mode]

P: Abscisic acid glucose ester

Q: 8-pentanoylneosolaniol

R: Unknown compound

S: Unknown compound

T: Jubanine C

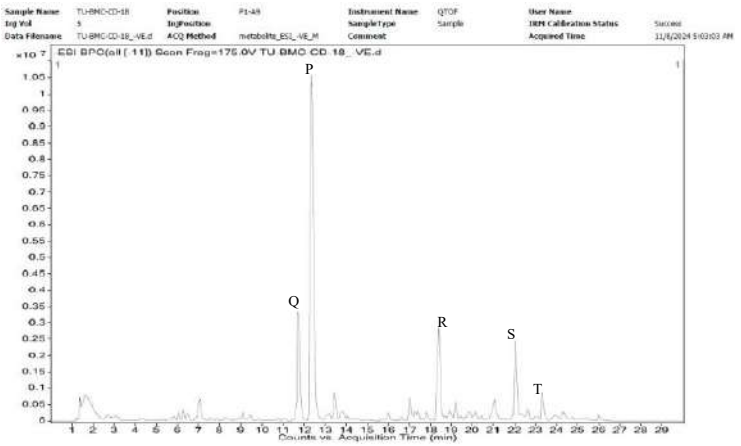


Figure 15 : Chromatogram of UPT in -ve ESI mode]

P: Corchorifatty acid F

Q: 3-Hydroxyquinine

R: Unknown compound

S: Unknown compound

T: Linalyl carprylate

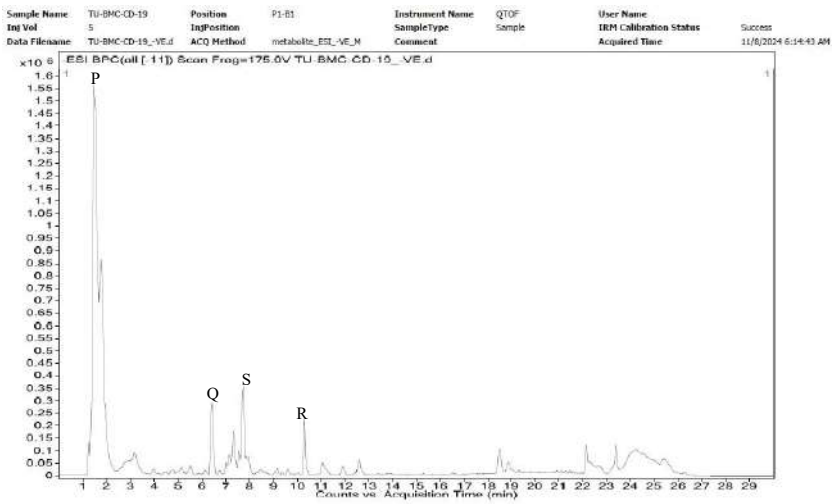


Figure 16 : Chromatogram of UPS in -ve ESI mode

P: Eudistomin N

Q: Swertiamarin

R: Gibberlin A43

S: Luteolin-4'-O-glucoside

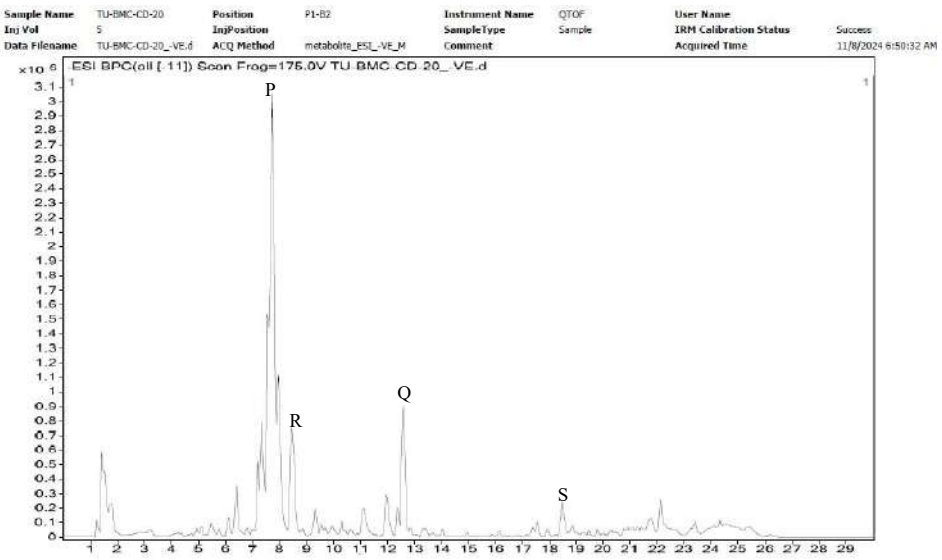


Figure 17 : *Chromatogram of UPL in -ESI mode*

P: Diosmetin 7-O-beta-D-glucuronopyranoside

Q: 9-chloro-10-hydroxy-hexadecanoic acid

R: Quercetin 3,7-dirhamnoside

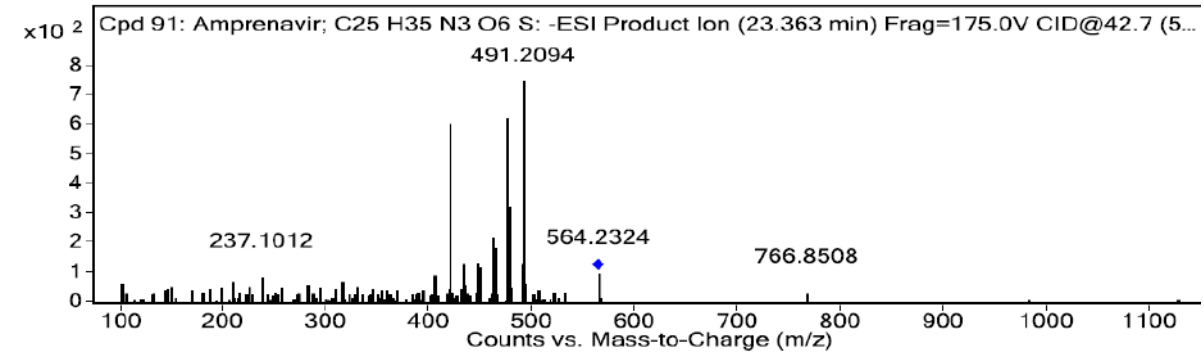
S: Hexazinone

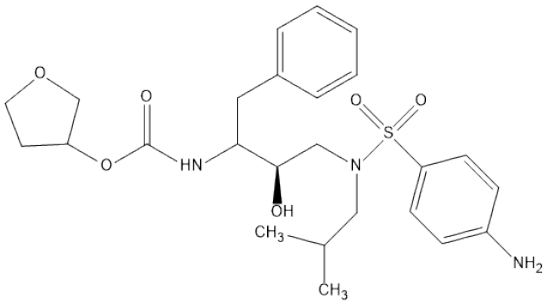
Amprenavir

Amprenavir is a nucleoside reverse transcriptase inhibitors(NRTIs), widely used in amprenavir monotherapy to treat HIV infection which was patented in 1992 and approved by the Food and Drug Administration on April 15, 1999, for clinical use[Stuart et al., 2000].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 91: Amprenavir; C25 H35 N3 O6 S	**Amprenavir**	564.2396	23.363	Auto MS/MS	505.226

MSMS Spectrum





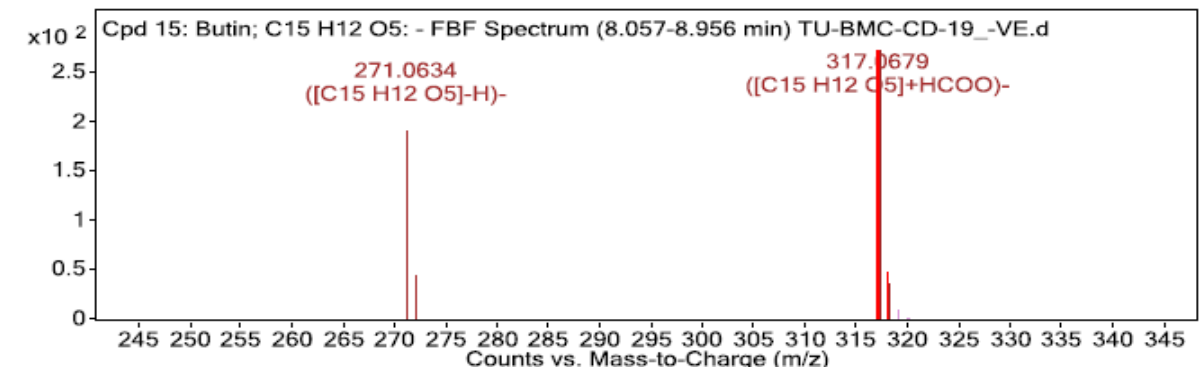
Amprenavir
Chemical Formula: $C_{25}H_{35}N_3O_6S$
Molecular Weight: 505.63

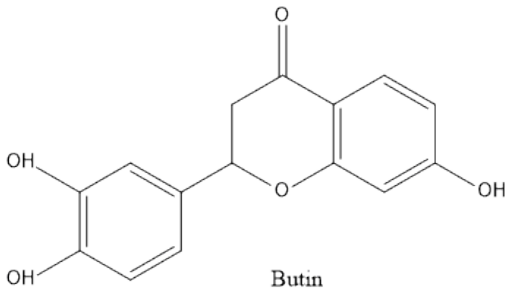
Butin

Butin is a flavonoid that increases intracellular ROS and DPPH radical scavenging capabilities, giving it antioxidant qualities and a cytoprotective impact against oxidative stress. In cells exposed with H2O2, it prevents cellular DNA damage and membrane lipid peroxidation [Zhang et al., 2008]. It can be produced from liquiritigenin through *Bm* TYR-catalyzed hydroxylation [Wu et al., 2022].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 15: Butin; C15 H12 O5	**Butin**	317.0679	8.702	Find By Formula	272.0692

MS Zoomed Spectrum





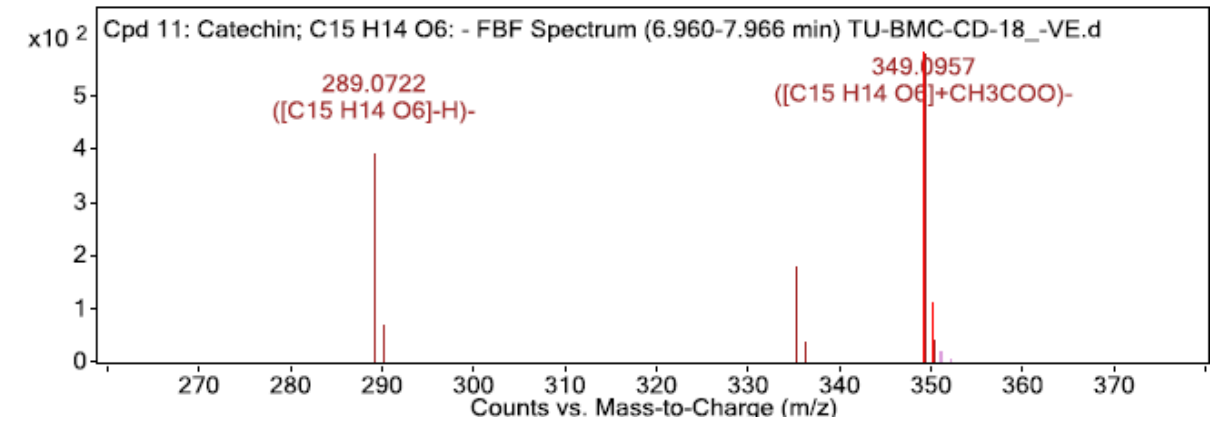
Butin
Chemical Formula: $C_{15}H_{12}O_5$
Molecular Weight: 272.25

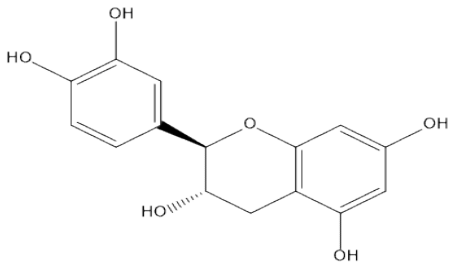
Catechin

T.F.L. Nees von Esenbeck, 1832, successfully isolated crystalline catechin from *Uncaria gambir*. Catechin is well-known for its anti-inflammatory and anti-cancer effects. One powerful characteristic of catechin is its ability to neutralize reactive oxygen and nitrogen species. The prevention of lung, breast, esophagus, stomach, liver, and prostate gland cancers is extremely effective with it [Musial et al., 2020].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 11: Catechin; C15 H14 O6	**Catechin**	289.0722	7.128	Find By Formula	290.0812

MS Zoomed Spectrum





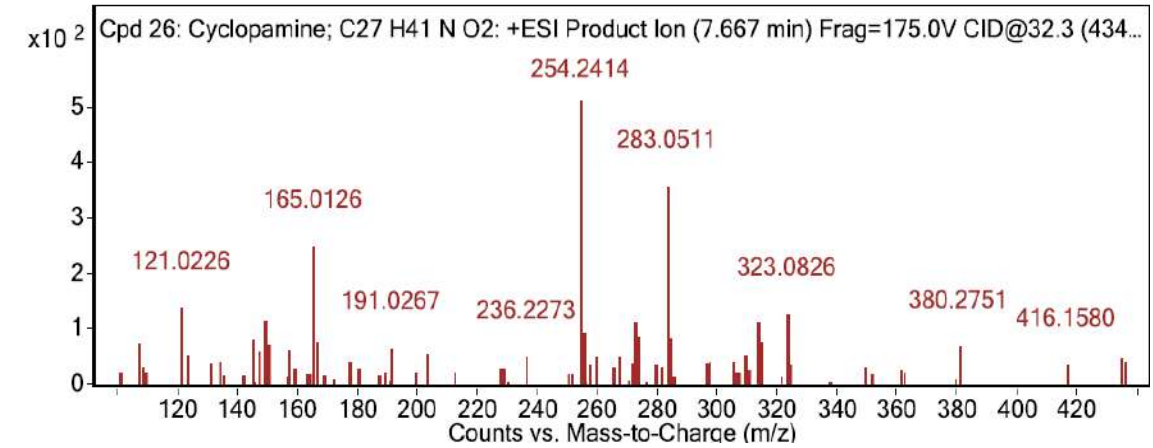
catechin
Chemical Formula: $C_{15}H_{14}O_6$
Molecular Weight: 290.27

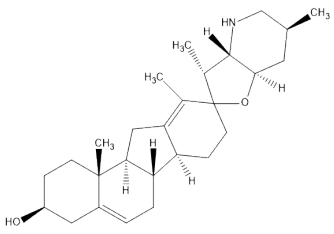
Cyclopamine

The cyclopamine is a terpene alkaloid, isolated from *V. californicum* in 1968. Cyclopamine plays a significant role in treatment of several cancers such as such as basal cell carcinoma, medullablastoma, and rhabdomyosarcoma by inhibiting the Shh signaling pathway for lethal cancers [Lee et al., 2014]

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 26: Cyclopamine; C27 H41 N O2	**Cyclopamine**	434.3013	7.667	Auto MS/MS	411.3122

MSMS Spectrum





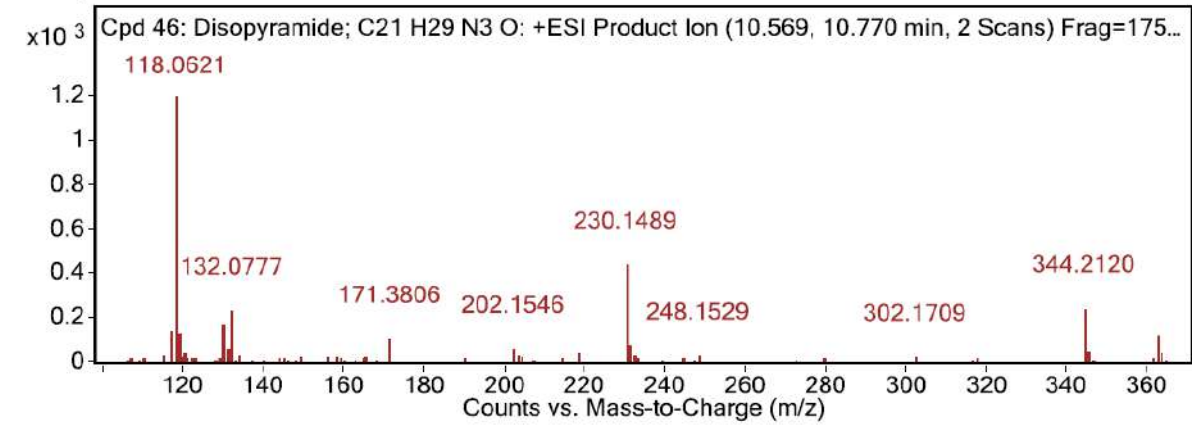
Cyclopamine
Chemical Formula: $C_{27}H_{41}NO_2$
Molecular Weight: 411.62

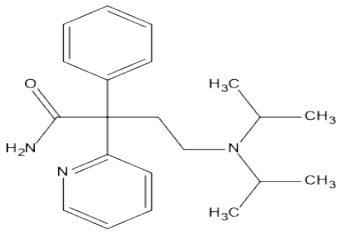
Disopyramide

A sodium channel blocker named disopyramide has an adverse inotropic impact on the ventricular myocardium, which considerably reduces the ability of the heart muscles to contract. It is effective in treating ventricular tachycardia because it inhibits the rise in sodium permeability of cardiac myocyte during Phase 0 of the cardiac action potential with lowering the inward sodium current [Rizos et al., 1987, Kim et al., 1990, Mathur et al., 1972].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 46: Disopyramide; C21 H29 N3 O	**Disopyramide**	362.2247	10.669	Auto MS/MS	339.2354

MSMS Spectrum



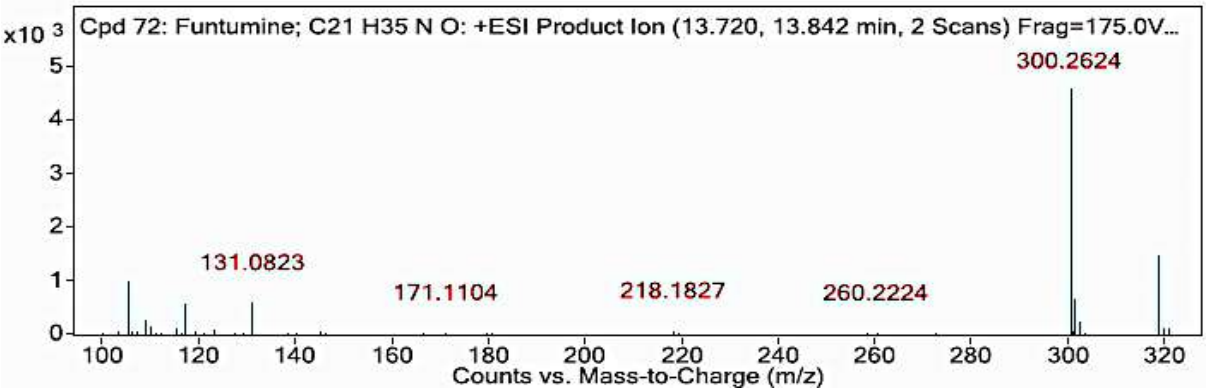


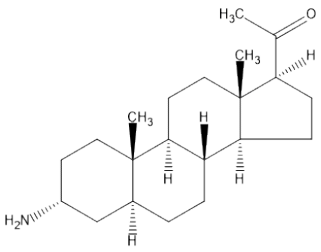
Disopyramide
Chemical Formula: $C_{21}H_{29}N_3O$
Molecular Weight: 339.47

Funtumine

Funtumine is a steroidal alkaloid which has anti-proliferative mechanism of action on cancer cell lines (HT-29, MCF-7 and HeLa) by exploring the mitochondrial depolarization effects, reactive oxygen species (ROS) induction, apoptosis, F-actin perturbation, and inhibition of topoisomerase-I. [Badmus et al., 2020)

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 72: Funtumine; C21 H35 N O	**Funtumine**	318.2748	13.781	Auto MS/MS	317.2675





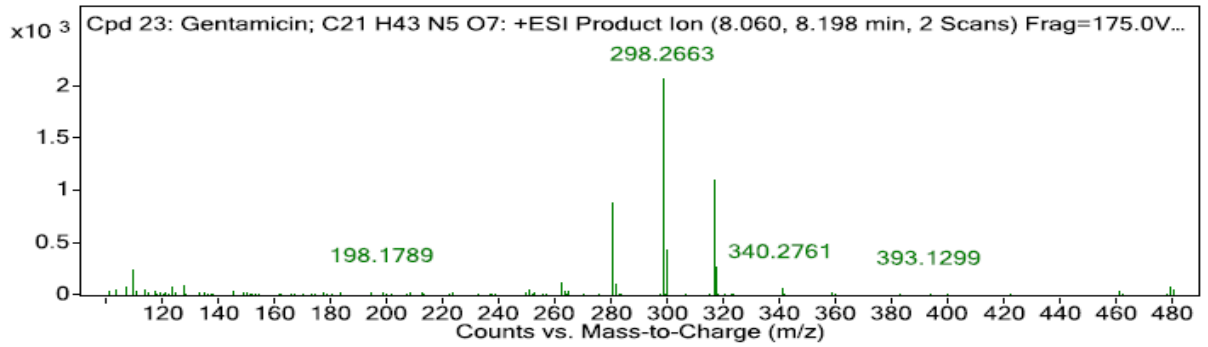
Funtumine
Chemical Formula: $C_{21}H_{35}NO$
Molecular Weight: 317.51

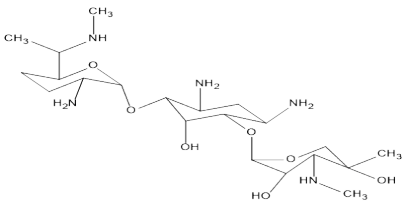
Gentamycin

Weinstein et al., at Schering Corporation in Bloomfield, New Jersey, discovered the aminoglycoside gentamycin from Micromonospora in 1963. It is used to treat a number of infections peritonitis from gastrointestinal tract infections, respiratory tract infections, urinary tract infectious diseases caused by Klebsiella pneumoniae, Escherichia coli, Serratia marcescens, Citrobacter spp., Enterobacteriaceae spp., and Pseudomonas spp. Gentamycin may be used as an adjuvant for febrile neutropenia, female genital infection, uterine infection, postnatal infection, necrotizing enterocolitis in fetuses or newborns, osteomyelitis, pelvic inflammatory disease, plague, gonorrhea, tularemia, prophylaxis of post-cholecystectomy infection,

transrectal prostate biopsy, and postetympanostomy-related infection, malignant otitis externa, and intratympanically or transtympanically for Meniere's disease [Chen et al., 2013].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 23: Gentamicin; C21 H43 N5 O7	**Gentamicin**	478.3281	8.129	Auto MS/MS	477.3206





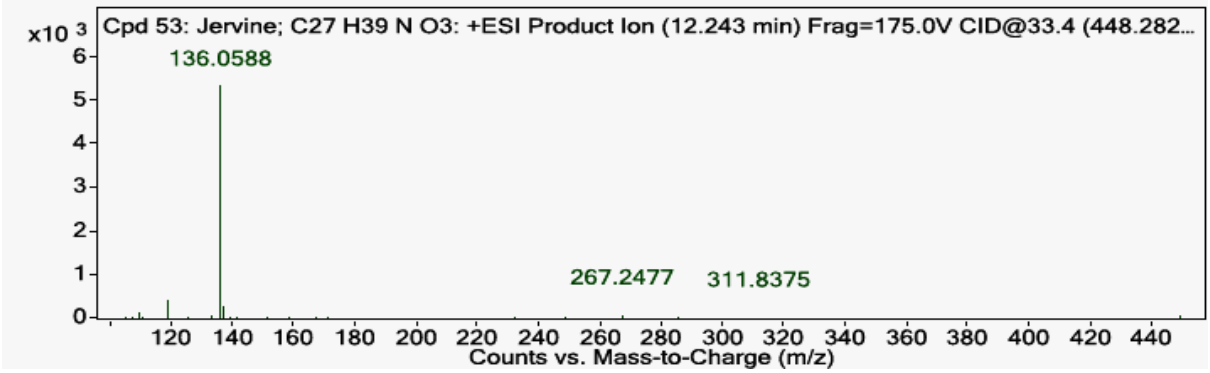
Gentamycin
Chemical Formula: $C_{21}H_{43}N_5O_7$
Molecular Weight: 477.60

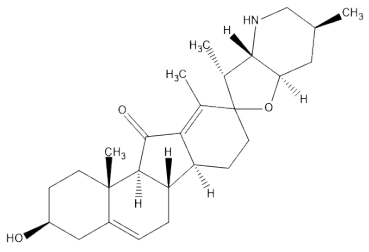
Jervine

Jervin is a steroidal alkaloid identified by Russell Molyneux and James. Jervine is very potent anti-inflammatory agent [Dumlu et al., 2018]. It plays a protective role against radiation-induced gastrointestinal toxicity [Yakan et al., 2019]. Jervine has demonstrated anti-tumor efficacy in non-small cells lung cancer (NSCLC) by reducing proliferation ability of NSCLC cells along with colony formation capacity inhibition [Lei et al., 2020].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 53: Jervine; C27 H39 N O3	**Jervine**	448.2831	12.243	Auto MS/MS	425.2937

MSMS Spectrum





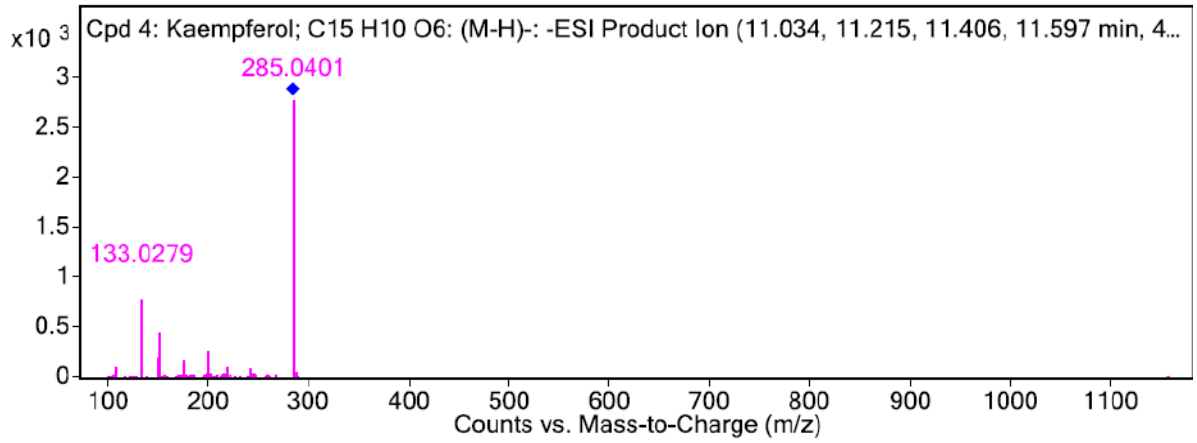
Jervine
Chemical Formula: $C_{27}H_{39}NO_3$
Molecular Weight: 425.60

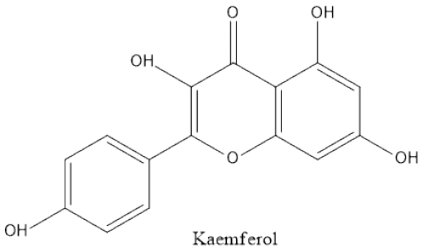
Kaemferol

Kaemferol is a flavon, contains a diphenylpropane structure which make it hydrophobic. Kaemferol is abundantly distributed in plant species of genera Delphinium, Camellia, Berberis, Citrus, Brassica, Allium, Malus, etc. Kaemferol serves as anti-inflammatory agent by modulating pro-inflammatory enzyme activities and gene expression involved in inflammation. It also inhibits transcription factors and is a potent anti-oxidant agent [Devi et al., 2015].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 4: Kaempferol; C15 H10 O6	**Kaempferol**	285.0409	11.11	Find By Formula	286.048

MSMS Spectrum





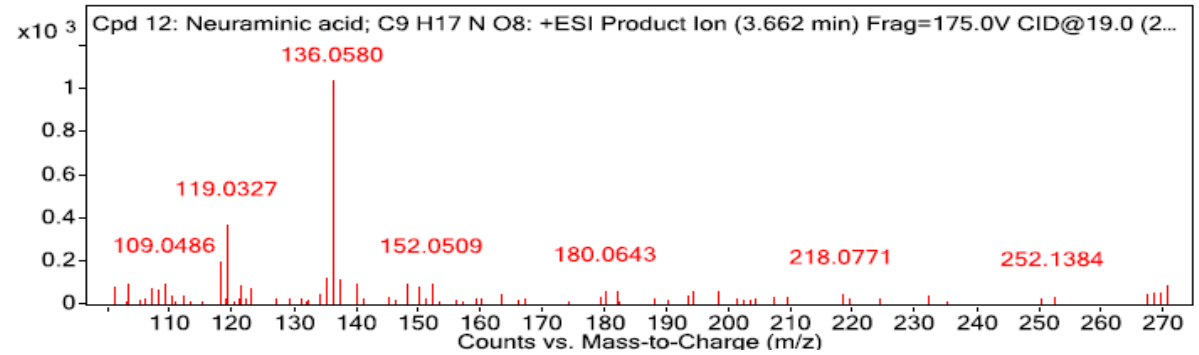
Kaemferol
Chemical Formula: $C_{15}H_{10}O_6$
Molecular Weight: 286.24

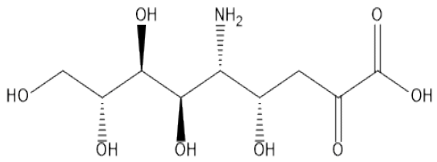
Neuraminic acid

Neuraminic acid is a 9-carbon atom containing monosaccharide which is beneficial in boosting immune, nervous system, gastrointestinal health, brain development. It also has anti-inflammatory, anti-viral, anti-tumor, anti-hypertensive and skin whitening properties [Mingli et al., 2023].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 12: Neuraminic acid; C9 H17 N O8	**Neuraminic acid**	268.1007	3.662	Auto MS/MS	267.0818

MSMS Spectrum





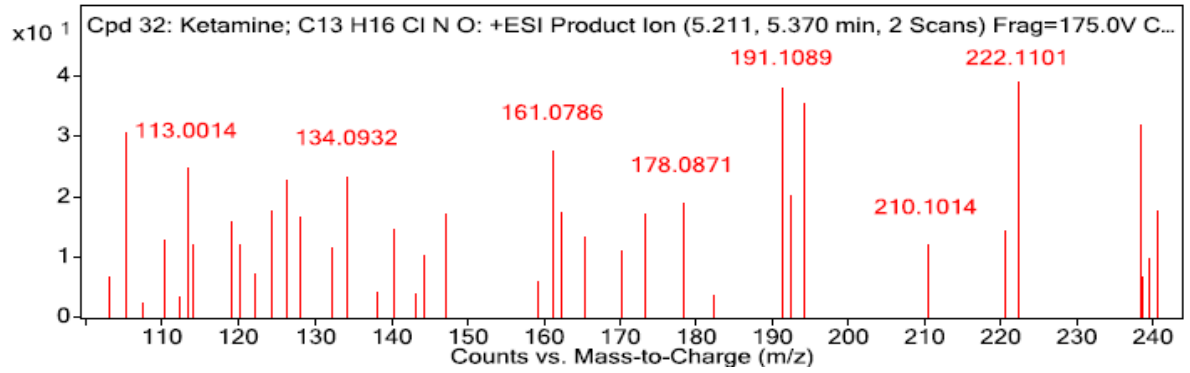
Neuraminic acid
Chemical Formula: $C_9H_{17}NO_8$
Molecular Weight: 267.23

Ketamine

Ketamine, a cyclohexanone, used as anesthesia and pain reliever. The repeated misuse of this molecule has exhibited harmful physical and physiological consequences which includes ulcerative cystitis, neurocognitive impairment, deficits inworking and episodic memory [Celia et al., 2011].

Cpd 32: Ketamine; C13 H16 Cl N O	**Ketamine**	238.0998	5.291	Auto MS/MS	237.0925

MSMS Spectrum

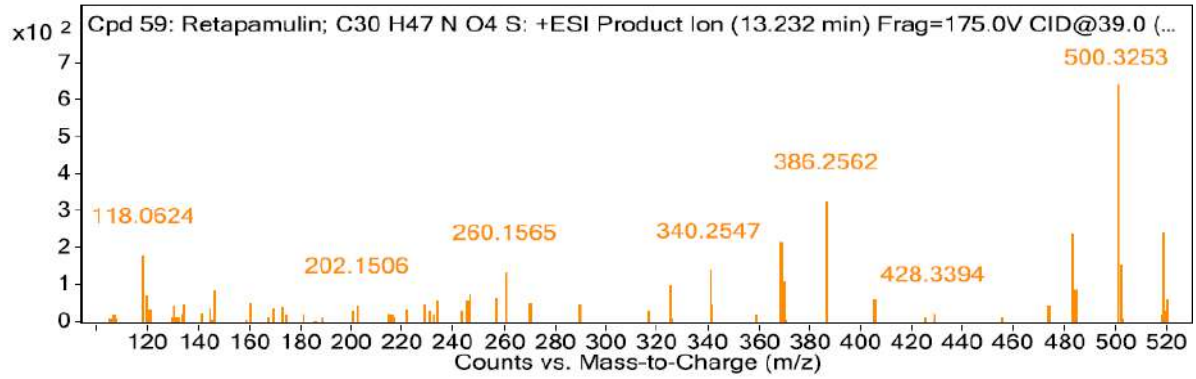


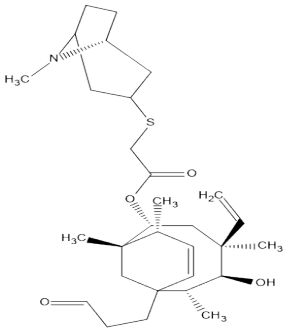
Retapamulin

Retapamulin, a semisynthetic derivative of pleuromutilin, exhibits superior in vitro anti-S. aureus and anti-S. pyogenes action. By attaching to a location on the 50S subunit of the bacterial ribosome, in a manner distinct from that of other ribosomally targeted antibiotics such as macrolides, it specifically prevents the production of proteins by bacteria. In April 2007, the US FDA authorized its topical use for the treatment of bacterial skin infections, such as impetigo, and infected minor cuts, scrapes, or sutured wounds.

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 59: Retapamulin; C30 H47 N O4 S	**Retapamulin**	518.3366	13.232	Auto MS/MS	517.3292

MSMS Spectrum



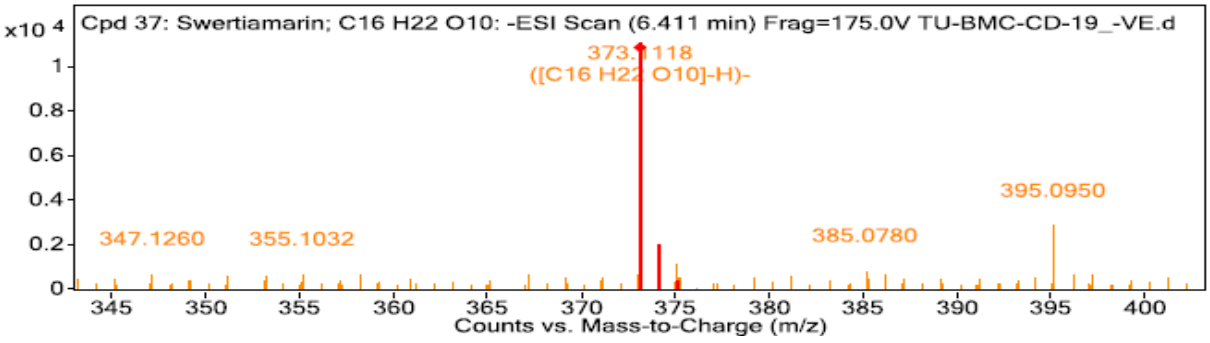


Retapamulin
Chemical Formula: $C_{30}H_{47}NO_4S$
Molecular Weight: 517.76

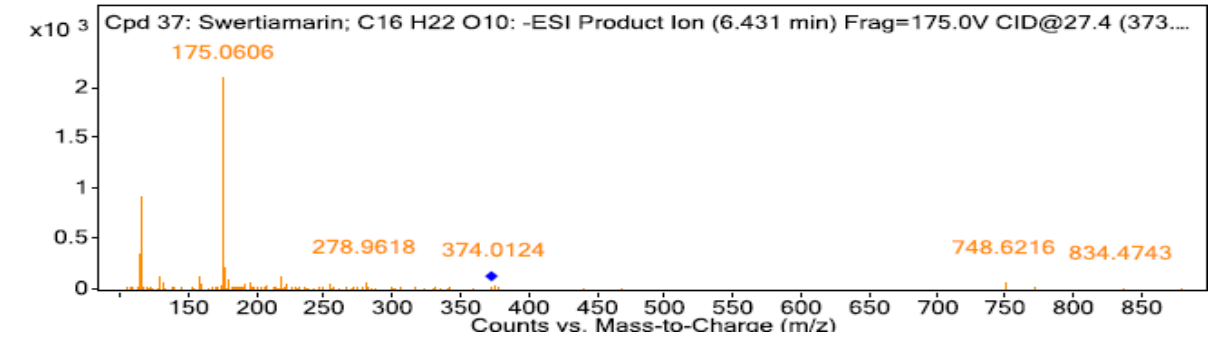
Swertiamarin

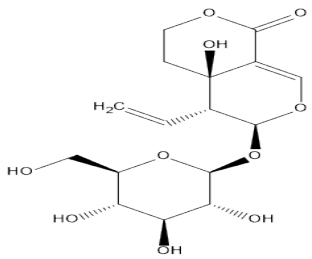
A secoiridoid glycoside, swertiamarin, have a variety of biological properties, including anti-inflammatory, antidiabetic, anti-atherosclerotic, and antioxidant actions. Its impact on a number of signaling pathways linked to cardiac remodeling events, apoptosis, inflammatory and lipid peroxidation markers, and the activation of antioxidant enzymes was the primary cause of its activities [Leong et al., 2016].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 37: Swertiamarin; C16 H22 O10	**Swertiamarin**	373.1118	6.431	Auto MS/MS	374.1189



MSMS Spectrum





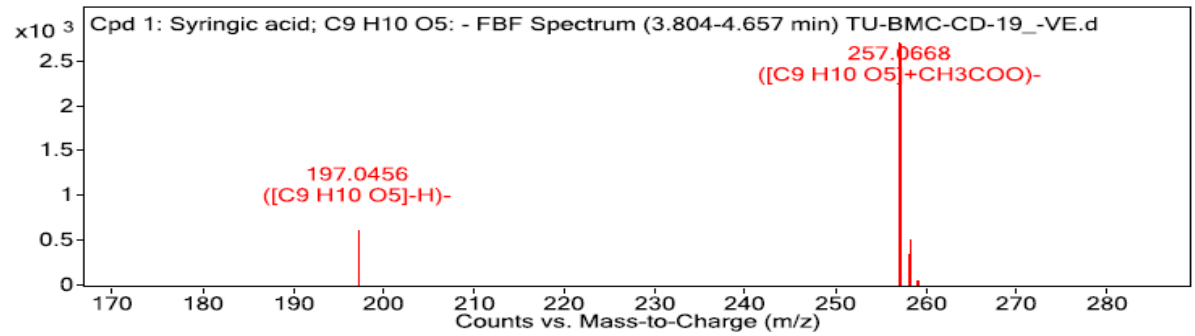
Swartiamarin
Chemical Formula: $C_{16}H_{22}O_{10}$
Molecular Weight: 374.34

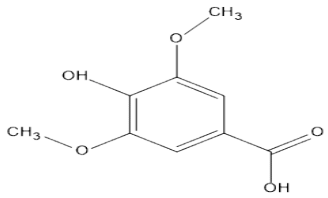
Syringic acid

Syringic acid is one of the most prevalent phenolic acids, member of the hydroxybenzoic acid. Olives, dates, grapes, walnuts, radishes, and pumpkins are the main foods that contain it. The chemical structure of syringic acid exhibits a benzene ring with a hydroxyl (-OH), one carboxylic acid (-COOH), and two methoxy (-OCH3) groups linked to the ring.The medicinal qualities of syringic acid are attributed due to the presence of methoxy groups on the aromatic ringat positions 3 and 5. Wide variety of pharmacological characteristics are exhibited by syringic acid, such as anti-inflammatory, hepatoprotective, cardioprotective, neuroprotective, antibacterial, antidiabetic, and antiendotoxic effects [Shimsa et al., 2024] and colorectal cancer [Mihanfar et al., 2020].

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 1: Syringic acid; C9 H10 O5	**Syringic acid**	257.0668	4.056	Find By Formula	198.0524

MS Zoomed Spectrum





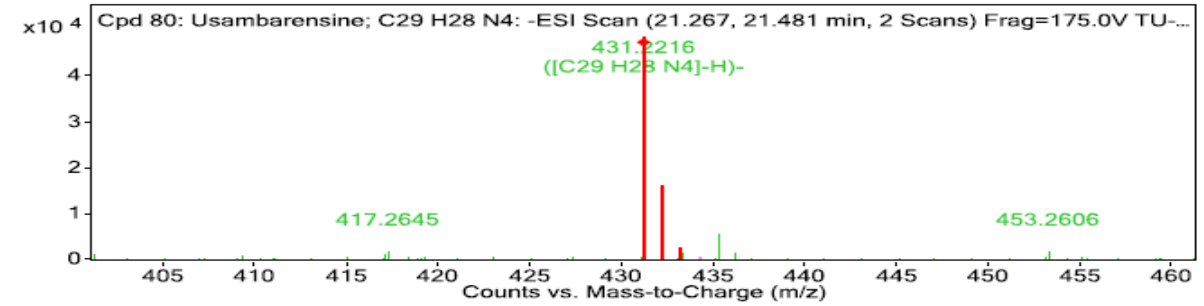
Syringic acid
Chemical Formula: $C_9H_{10}O_5$
Molecular Weight: 198.17

Usambarensine

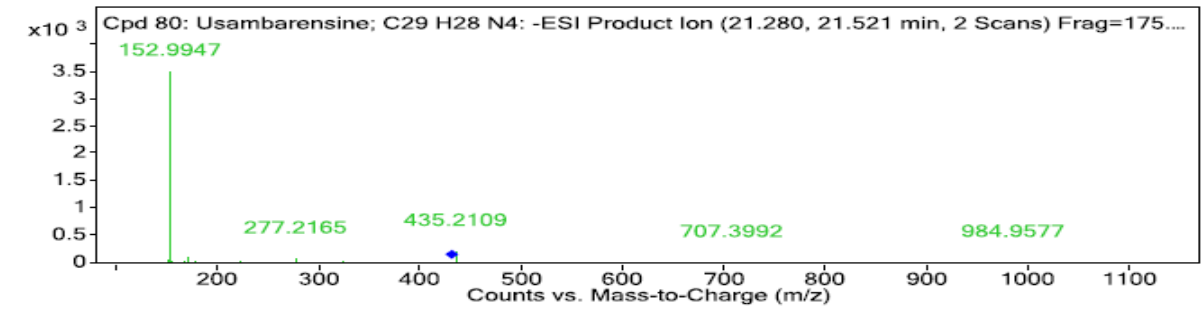
Usambarensine is a tertiary amine alkaloid. Usambarensine has atropine-like and spasmolytic properties, anti-amoebic and Anti-plasmodial Activities [Wright et al., 1991], as antineoplastic drugs for cancer chemotherapy. [Isah,2016] and antiplasmodial activity [Passemar et al., 2011].

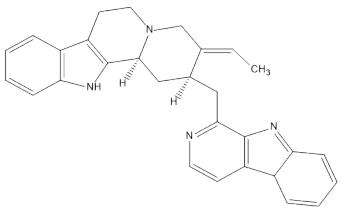
Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 80: Usambarensine; C29 H28 N4	**Usambarensine**	431.2216	21.401	Auto MS/MS	432.2289

MS Zoomed Spectrum



MSMS Spectrum





Usambarensine
Chemical Formula: $C_{29}H_{28}N_4$
Molecular Weight: 432.56

HR-LCMS profiling of plant samples from Upperdangadhi, screened a large variety of compounds bearing anti-bacterial, anti-viral, anti-fungal, anti-tumor, antioxidant, anti-inflammatory, anti-malarial, anti-helmintic, anti-hypertensive, anti-vomiting, anti-depression and anxiety, anti-atoptosis, purgative properties. Some compounds are potential to treat Parkinson's disease and to enhance mitochondrial normal function.

From designed tests and their results, it is obvious that cocoyam can be the best alternative of cereal crops in nutrient supplement and a good source of pharmaceutical products along with other known medicinally rich bios.

CHAPTER 5. CONCLUSIONS AND RECOMMEDNATION

5.1 Conclusion

The research presented in this thesis has provided valuable insights into the chemical composition of cocoyam (*Colocasia esculenta*) and its potential as a source of valuable chemical compounds. Through the application of UV-VIS spectrophotometer, HR-LCMS and ICP-AES, a comprehensive analysis of tuber, petiole and leaf revealed the presence and quantification of a range of phytochemicals. TPC and TFC were more in leaf than petiole and tuber 19.96±0.01 mg GAE/g DS, 89.1±0.006 mg QE/g DS). Highest antioxidant activity in Padampur leaf (27.82±0.01mg AAE/g DS and palung tuber (IC_{50} =240.45 µg/ml). Maximum TCC reported in Upperdangadhi tuber (112±0.0 mg GE/g DS). Significant amounts of essential elements (Ca, Cu, Fe, Mg, Zn) and Trace levels of toxic metals like arsenic (As, Cr) were recorded via ICP-AES.

The HR-LCMS analysis of UPT in both positive and negative ESI mode revealed total 200 compounds, among which 60 are known, 140 are unknown. Among known,11 alkaloids, 4 phenolics, 5 flavonoids, 3 glycosides, 5 steroids and 32 other compounds are present. In the similar way UPS sample contains total 200 compounds, 115 known and 85 are unknown. Out of known, 12 are alkaloids, 15 phenolic, 26 flavonoids, 9 glycosides and 55 other class of compounds. The UPL sample screened total 197 compounds, 89 are known while 116 are unknown. The known compounds include 13 alkaloids, 17 flavonoids, 3 terpenoids, 1 steroid and 57 other compounds. These compounds have demonstrated Anti-tumor, anti-inflammatory, Antioxidant anti-obesity, antidiabetic, antibacterial, anti-amoebic, Anti-plasmodial, Anesthetic anti-hypertensive, skin whitening properties, Anti-nausea, anti-vomitting, Antidepressant properties and also effective in Parkinson's disease.

The findings of this research contribute to the existing body of knowledge on the chemical profile of cocoyam and support its traditional uses in various applications which suggest that cocoyam could be a valuable source of natural antioxidants for the food and pharmaceutical industries. In conclusion, this research has demonstrated the rich chemical diversity of cocoyam and its potential as a source of valuable compounds with applications in various fields such as food, pharmaceuticals, cosmetics.

5.2 Recommendations

Based on the findings presented in this thesis on the chemical composition of cocoyam (*Colocasia esculenta*), the following recommendations are put forth for future research and development:

1. Isolation of pure compounds and their characterization.
2. In-silico pharmacokinetic study, molecular docking and their characterization as to noble drug
3. Unidentified compound appeared in the HR-LCMS should be isolated and characterized.

REFERENCES

Adefegha, S. A. (2018). Impact of pasting on starch composition, estimated glycemic index, phenolic constituents, antioxidant activities and antidiabetic properties of flour produced from cocoyam (*Colocasia esculenta*) corm. *Journal of Food Biochemistry*, *42*(4), e12514. https://doi.org/10.1111/jfbc.12514

Ahmad Khan, M. S., & Ahmad, I. (2019). Herbal Medicine. In *New Look to Phytomedicine* (pp. 3–13). Elsevier. https://doi.org/10.1016/B978-0-12-814619-4.00001-X

Ainaz Mihanfar, Saber Ghazizadeh Darband, Shirin Sadighparvar b, Mojtaba Kaviani c, & Mohammad Mirza-Aghazadeh-Attari d,e, Bahman Yousefi f, Maryam Majidinia. (2020). *In vitro and in vivo anticancer effects of syringic acid on colorectal cancer: Possible mechanistic view*. https://doi.org/10.1016/j.cbi.2020.109337

Al Kazman, B. S. M., Harnett, J. E., & Hanrahan, J. R. (2022). Traditional Uses, Phytochemistry and Pharmacological Activities of Annonacae. *Molecules*, *27*(11), 3462. https://doi.org/10.3390/molecules27113462

Alanis, A. J. (2005). Resistance to Antibiotics: Are We in the Post-Antibiotic Era? *Archives of Medical Research*, *36*(6), 697–705. https://doi.org/10.1016/j.arcmed.2005.06.009

Al-Gubory, K. H., Fowler, P. A., & Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *The International Journal of Biochemistry & Cell Biology*, *42*(10), 1634–1650. https://doi.org/10.1016/j.biocel.2010.06.001

Armanini, D., Sabbadin, C., Donà, G., Clari, G., & Bordin, L. (2014). Aldosterone receptor blockers spironolactone and canrenone: Two multivalent drugs. *Expert Opinion on Pharmacotherapy*, *15*(7), 909–912. https://doi.org/10.1517/14656566.2014.896901

Arroyo, G., Bustos, J. A., Lescano, A. G., Gonzales, I., Saavedra, H., Rodriguez, S., Pretell, E. J., Bonato, P. S., Lanchote, V. L., Takayanagui, O. M., Horton, J., Gonzalez, A. E., Gilman, R. H., Garcia, H. H., Cysticercosis Working Group in Peru, Tsang, V. C. W., O'Neal, S., Martinez, M., Zimic, M., … Friedland, J. (2019). Albendazole Sulfoxide Plasma Levels and Efficacy of Antiparasitic Treatment in Patients With Parenchymal

Neurocysticercosis. *Clinical Infectious Diseases*, *69*(11), 1996–2002. https://doi.org/10.1093/cid/ciz085

Arvieux, C., & Tribut, O. (2005). Amprenavir or Fosamprenavir plus Ritonavir in HIV Infection: Pharmacology, Efficacy and Tolerability Profile. *Drugs*, *65*(5), 633–659. https://doi.org/10.2165/00003495-200565050-00005

Assi, M. A., Hezmee, M. N. M., Haron, A. W., Sabri, M. Y., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Veterinary World*, *9*(6), 660–671. https://doi.org/10.14202/vetworld.2016.660-671

Awa, E., & Eleazu, C. (2015). Bioactive constituents and antioxidant activities of raw and processed cocoyam (Colocasia esculenta): Chemical composition of raw and processed cocoyam. *Nutrafoods*, *14*(3), 133–140. https://doi.org/10.1007/s13749-015-0033-x

Badmus, J. A., Ekpo, O. E., Sharma, J. R., Sibuyi, N. R. S., Meyer, M., Hussein, A. A., & Hiss, D. C. (2020). An Insight into the Mechanism of Holamine- and Funtumine-Induced Cell Death in Cancer Cells. *Molecules*, *25*(23), 5716. https://doi.org/10.3390/molecules25235716

Baek, J., & Lee, M.-G. (2016). Oxidative stress and antioxidant strategies in dermatology. *Redox Report*, *21*(4), 164–169. https://doi.org/10.1179/1351000215Y.0000000015

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bansal, A. K., & Bilaspuri, G. S. (2011). Impacts of Oxidative Stress and Antioxidants on Semen Functions. *Veterinary Medicine International*, *2011*, 1–7. https://doi.org/10.4061/2011/686137

Barbara Holmes and Eugene M. Sorkin. (1986). Indoramin A Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy in Hypertension and Related Vascular, Cardiovascular and Airway Diseases. *ADIS Drug Information Services*, *Drugs 31 : 467-499 (1986)*.

Bedekar, A., Shah, K., & Koffas, M. (2010). Natural Products for Type II Diabetes Treatment. In *Advances in Applied Microbiology* (Vol. 71, pp. 21–73). Elsevier. https://doi.org/10.1016/S0065-2164(10)71002-9

Bernardini, S., Tiezzi, A., Laghezza Masci, V., & Ovidi, E. (2018). Natural products for human health: An historical overview of the drug discovery approaches. *Natural Product Research*, *32*(16), 1926–1950. https://doi.org/10.1080/14786419.2017.1356838

Beutler, J. A. (2009). Natural Products as a Foundation for Drug Discovery. *Current Protocols in Pharmacology*, *46*(1). https://doi.org/10.1002/0471141755.ph0911s46

Bjørklund, G., & Chirumbolo, S. (2017). Role of oxidative stress and antioxidants in daily nutrition and human health. *Nutrition*, *33*, 311–321. https://doi.org/10.1016/j.nut.2016.07.018

Boakye, A. A., Wireko-Manu, F. D., Oduro, I., Ellis, W. O., Gudjónsdóttir, M., & Chronakis, I. S. (2018). Utilizing cocoyam (*Xanthosoma sagittifolium*) for food and nutrition security: A review. *Food Science & Nutrition*, *6*(4), 703–713. https://doi.org/10.1002/fsn3.602

Ce, O., Pc, A., & Ck, E. (2017). Studies on the Flowers and Stems of Two Cocoyam Varieties: Xanthosoma sagittifolium and Colocasia esculenta. *Natural Products Chemistry & Research*, *05*(06). https://doi.org/10.4172/2329-6836.1000263

Chang, Chia-Chi, Yang, Ming-Hua, Wen, Hwei-Mei, & Chern, Jiing-Chuan. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*(3), 178–182.

Chee, C. W., Mohd Hashim, N., & Nor Rashid, N. (2024). Morindone as a potential therapeutic compound targeting TP53 and KRAS mutations in colorectal cancer cells. *Chemico-Biological Interactions*, *392*, 110928. https://doi.org/10.1016/j.cbi.2024.110928

Chen, C., Chen, Y., Wu, P., & Chen, B. (2014). Update on new medicinal applications of gentamicin: Evidence-based review. *Journal of the Formosan Medical Association*,

113(2), 72–82. https://doi.org/10.1016/j.jfma.2013.10.002

Chen, X., Wang, Y., Dong, X., Hu, G., & Liu, L. (2017). Engineering rTCA pathway and C4-dicarboxylate transporter for l-malic acid production. *Applied Microbiology and Biotechnology*, *101*(10), 4041–4052. https://doi.org/10.1007/s00253-017-8141-8

Chen, Y., & Kirchmair, J. (2020). Cheminformatics in Natural Product-based Drug Discovery. *Molecular Informatics*, *39*(12), 2000171. https://doi.org/10.1002/minf.202000171

Chen, Y. L., Law, P. Y., & Loh, H. H. (2004). Inhibition of Akt/Protein Kinase B Signaling by Naltrindole in Small Cell Lung Cancer Cells. *Cancer Research*, *64*(23), 8723–8730. https://doi.org/10.1158/0008-5472.CAN-03-3091

Cheuka, P., Mayoka, G., Mutai, P., & Chibale, K. (2016). The Role of Natural Products in Drug Discovery and Development against Neglected Tropical Diseases. *Molecules*, *22*(1), 58. https://doi.org/10.3390/molecules22010058

Dąbrowski, R., Syska, P., Mączyńska, J., Farkowski, M., Sawicki, S., Kubaszek-Kornatowska, A., Michałek, P., Kowalik, I., Szwed, H., & Hryniewiecki, T. (2020). Clinical efficacy of potassium canreonate-canrenone in sinus rhythm restoration among patients with atrial fibrillation—A protocol of a pilot, randomized, double - blind, placebo-controlled study (CANREN-AF trial). *Trials*, *21*(1), 397. https://doi.org/10.1186/s13063-020-04277-3

Daum, R. S., Kar, S., & Kirkpatrick, P. (2007). Retapamulin. *Nature Reviews Drug Discovery*, *6*(11), 865–866. https://doi.org/10.1038/nrd2442

David-Chukwu, N., Amadi, A., Onuabuchi, I., & Chukwu, M. (2022). Influence of storage life and variety on the micro-nutrient compositions of stored cocoyam-based products. *Food Therapy and Health Care*, *4*(2), 9. https://doi.org/10.53388/FTHC20220501009

Dias, D. A., Urban, S., & Roessner, U. (2012). A Historical Overview of Natural Products in Drug Discovery. *Metabolites*, *2*(2), 303–336. https://doi.org/10.3390/metabo2020303

Domingo, J. L., & Marquès, M. (2021). The effects of some essential and toxic

metals/metalloids in COVID-19: A review. *Food and Chemical Toxicology*, *152*, 112161. https://doi.org/10.1016/j.fct.2021.112161

Du, Z., Zhang, S., Lin, Y., Zhou, L., Wang, Y., Yan, G., Zhang, M., Wang, M., Li, J., Tong, Q., Duan, Y., & Du, G. (2019). Momordicoside G Regulates Macrophage Phenotypes to Stimulate Efficient Repair of Lung Injury and Prevent Urethane-Induced Lung Carcinoma Lesions. *Frontiers in Pharmacology*, *10*, 321. https://doi.org/10.3389/fphar.2019.00321

Dumlu, F. A., Aydin, T., Odabasoglu, F., Berktas, O. A., Kutlu, Z., Erol, H. S., Halici, M. B., Cadirci, E., & Cakir, A. (2019). Anti-inflammatory and antioxidant properties of jervine, a sterodial alkaloid from rhizomes of Veratrum album. *Phytomedicine*, *55*, 191–199. https://doi.org/10.1016/j.phymed.2018.06.035

Eleazu, C. O. (2016). Characterization of the natural products in cocoyam (*Colocasia esculenta*) using GC–MS. *Pharmaceutical Biology*, *54*(12), 2880–2885. https://doi.org/10.1080/13880209.2016.1190383

Elif Acar Arslan, MD,* Erhan Arslan, MD,† Anıl Kılınç, MD,‡ and Özkan Göksu, PhD§. (2017). Effect of Biperiden Treatment in Acute Orofacial and Extremity Dyskinesia With Methylphenidate Therapy. *Pediatric Emergency Care*, *Volume 00*(Number 00). www.pec-online.com

Esra Birben PhD,1 Umit Murat Sahiner MD,1 Cansin Sackesen MD,1 & Serpil Erzurum MD,2 and Omer Kalayci, MD1. (2012). Oxidative Stress and Antioxidant Defense. *WAO Journal*.

Ezeonu, C. S., & Ejikeme, C. M. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science*, *2016*, 1–9. https://doi.org/10.1155/2016/5601327

Fair, R. J., & Tor, Y. (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, *6*, PMC.S14459. https://doi.org/10.4137/PMC.S14459

Ferreres, F., Gonçalves, R. F., Gil-Izquierdo, A., Valentão, P., Silva, A. M. S., Silva, J. B.,

Santos, D., & Andrade, P. B. (2012). Further Knowledge on the Phenolic Profile of *Colocasia esculenta* (L.) Shott. *Journal of Agricultural and Food Chemistry*, *60*(28), 7005–7015. https://doi.org/10.1021/jf301739q

Fikry, H., Saleh, L. A., Sadek, D. R., & Alkhalek, H. A. A. (2025). The possible protective effect of luteolin on cardiovascular and hepatic changes in metabolic syndrome rat model. *Cell and Tissue Research*, *399*(1), 27–60. https://doi.org/10.1007/s00441-024-03927-1

Foti, M. C. (2015). Use and Abuse of the DPPH• Radical. *Journal of Agricultural and Food Chemistry*, *63*(40), 8765–8776. https://doi.org/10.1021/acs.jafc.5b03839

Foyer, C. H., & Shigeoka, S. (2011). Understanding Oxidative Stress and Antioxidant Functions to Enhance Photosynthesis. *Plant Physiology*, *155*(1), 93–100. https://doi.org/10.1104/pp.110.166181

Frederick M. Kahan, Helmut Kropp, Jon G. Sundelof and Jerome Birnbaum. (1983). Thienamycin: Development of imipenem-cilastatin. *Journal of Antimicrobial Chemotherapy*, *12*, 1–35.

Friedel, H. A., Campoli-Richards, D. M., & Goa, K. L. (1989). Sultamicillin: A Review of its Antibacterial Activity, Pharmacokinetic Properties and Therapeutic Use. *Drugs*, *37*(4), 491–522. https://doi.org/10.2165/00003495-198937040-00005

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

Granot, E., & Kohen, R. (2004). Oxidative stress in childhood—In health and disease states. *Clinical Nutrition*, *23*(1), 3–11. https://doi.org/10.1016/S0261-5614(03)00097-9

Gupta, R. K., Patel, A. K., Shah, N., Choudhary, A. K., Jha, U. K., Yadav, U. C., Gupta, P. K., & Pakuwal, U. (2014). Oxidative Stress and Antioxidants in Disease and Cancer: A Review. *Asian Pacific Journal of Cancer Prevention*, *15*(11), 4405–4409. https://doi.org/10.7314/APJCP.2014.15.11.4405

Ha, H.-L. (2010). Oxidative stress and antioxidants in hepatic pathogenesis. *World Journal of Gastroenterology*, *16*(48), 6035. https://doi.org/10.3748/wjg.v16.i48.6035

Halliwell, B. (2014). Cell culture, oxidative stress, and antioxidants: Avoiding pitfalls. *Biomedical Journal*, *0*(0), 0. https://doi.org/10.4103/2319-4170.128725

Harvey, A. (2008). Natural products in drug discovery. *Drug Discovery Today*, *13*(19–20), 894–901. https://doi.org/10.1016/j.drudis.2008.07.004

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. https://doi.org/10.1038/nrd4510

Hassan, A., Mekhael, MagedK. G., Hanna, A., Simon, A., Tóth, G., & Duddeck, H. (2019). Phytochemical investigation of Corchorus olitorius and Corchorus capsularis (Family Tiliaceae) that grow in Egypt. *Egyptian Pharmaceutical Journal*, *0*(0), 0. https://doi.org/10.4103/epj.epj_51_18

Hauser, R. A., Isaacson, S., & Clinch, T. (2014). Randomized, placebo-controlled trial of trimethobenzamide to control nausea and vomiting during initiation and continued treatment with subcutaneous apomorphine injection. *Parkinsonism & Related Disorders*, *20*(11), 1171–1176. https://doi.org/10.1016/j.parkreldis.2014.08.010

He, X., Sun, Y., Lu, X., Yang, F., Li, T., Deng, C., Song, J., & Huang, X. (2023). Assessment of the anti-inflammatory mechanism of quercetin 3,7-dirhamnoside using an integrated pharmacology strategy. *Chemical Biology & Drug Design*, *102*(6), 1534–1552. https://doi.org/10.1111/cbdd.14346

Hébert, M., Bellavance, G., & Barriault, L. (2022). Total Synthesis of Ginkgolide C and Formal Syntheses of Ginkgolides A and B. *Journal of the American Chemical Society*, *144*(39), 17792–17796. https://doi.org/10.1021/jacs.2c08351

Henning, U., Krieger, K., & Klimke, A. (1999). Specific binding of 3h-spiperone to peripheral blood cells: Relevance for the interpretation of binding studies in psychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *23*(2), 225–241. https://doi.org/10.1016/S0278-5846(98)00104-3

Igbinoba, S., Onyeji, C., & Akanmu, M. (2016). Modulation of cytochrome P450 3A4 mediated quinine metabolism in healthy volunteers by two honey samples from different floral and geographical sources. *International Journal of Basic and Clinical Pharmacology*, 823–828. https://doi.org/10.18203/2319-2003.ijbcp20161528

Ijaz, M. U., Qamer, M., Hamza, A., Ahmed, H., Afsar, T., Abulmeaty, M., Ayub, A., & Razak, S. (2023). RETRACTED ARTICLE: Sciadopitysin mitigates spermatological and testicular damage instigated by paraquat administration in male albino rats. *Scientific Reports*, *13*(1), 19753. https://doi.org/10.1038/s41598-023-46898-z

Isah, T. (2016). Anticancer alkaloids from trees: Development into drugs. *Pharmacognosy Reviews*, *10*(20), 90. https://doi.org/10.4103/0973-7847.194047

James Q. Del Rosso, DO; Joseph Bikowski, MD. (2008). Trolamine-Containing Topical Emulsion: Clinical Applications in Dermatology. *Drug Therapy Topics*, *81*, 209–214.

Jasemizad, T., & Padhye, L. P. (2022). Photodegradation and adsorption of hexazinone in aqueous solutions: Removal efficiencies, kinetics, and mechanisms. *Environmental Science and Pollution Research*, *29*(32), 48330–48339. https://doi.org/10.1007/s11356-022-19205-y

Jenkins, R. R. (1993). Exercise, Oxidative Stress, and Antioxidants: A Review. *International Journal of Sport Nutrition*, *3*(4), 356–375. https://doi.org/10.1123/ijsn.3.4.356

Jensen, S. R., Li, H.-Q., Albach, D. C., & Gotfredsen, C. H. (2008). Phytochemistry and molecular systematics of Triaenophora rupestris and Oreosolen wattii (Scrophulariaceae). *Phytochemistry*, *69*(11), 2162–2166. https://doi.org/10.1016/j.phytochem.2008.05.010

Jiang, L., Liu, L., Chen, H., Zhang, W., He, L., & Zeng, X. (2022). Effects of autochthonous starter cultures on bacterial communities and metabolites during fermentation of Yu jiangsuan, a Chinese traditional fermented condiment. *LWT*, *168*, 113874. https://doi.org/10.1016/j.lwt.2022.113874

Jie Bi, Qingli Yang1*, Jie Sun, Jian Chen and Juan Zhang. (2011). Study on Ultrasonic Extraction Technology and Oxidation Resistance of Total Flavonoids from Peanut

Hull. *Food Sci. Technol. Res.*, *17*(3), 187–198.

JOS~E E. LEYSEN, W. GOMMEREN and PIERRE M. LADURON. (1978). SPIPERONE: A LIGAND OF CHOICE RECEPTORS 1. KINETICS AND CHARACTERISTICS OF FOR NEUROLEPTIC IN VITRO BINDING. *BmchemlcaPlh Armacology*, *21*, 307~ 316.

Joseph Onyeka. (2014). Status of Cocoyam (Colocasia esculenta and Xanthosoma spp) in West and Central Africa: Production, Household Importance and the Threat from Leaf Blight. *CGIAR Research Program on Roots, Tubers and Bananas (RTB)*.

Joshi, A., Sharma, V., Singh, J., & Kaushik, V. (2022). Chemi-Informatic Approach to Investigate Putative PharmacoactiveAgents of Plant Origin to Eradicate COVID-19. *Coronaviruses*, *3*(3), e170322188687. https://doi.org/10.2174/2666796701999201203210036

Julia A. Balfour and Karen L. Goa. (1997). Dolasetron A Review of its Pharmacology and Therapeutic Potential in the Management of Nausea and Vomiting Induced by Chemotherapy, Radiotherapy or Surgery. *ADIS DRUG EVALUATION*, *Drug s 1997 Aug; 54 (2); 273-298*.

Kasi Pandima Devia, Dicson Sheeja Malara, Seyed Fazel Nabavib, Antoni Suredac, & Jianbo Xiaod,e, Seyed Mohammad Nabavib,∗∗,1, Maria Dagliaf,∗,1. (2015). Kaempferol and inflammation: From chemistry to medicine. *Pharmacological Research*.

Kaur, J., Gulati, M., Singh, S. K., Kuppusamy, G., Kapoor, B., Mishra, V., Gupta, S., Arshad, M. F., Porwal, O., Jha, N. K., Chaitanya, M. V. N. L., Chellappan, D. K., Gupta, G., Gupta, P. K., Dua, K., Khursheed, R., Awasthi, A., & Corrie, L. (2022). Discovering multifaceted role of vanillic acid beyond flavours: Nutraceutical and therapeutic potential. *Trends in Food Science & Technology*, *122*, 187–200. https://doi.org/10.1016/j.tifs.2022.02.023

Kaur, S., Mendonca, P., & Soliman, K. F. A. (2024). The Anticancer Effects and Therapeutic Potential of Kaempferol in Triple-Negative Breast Cancer. *Nutrients*, *16*(15), 2392. https://doi.org/10.3390/nu16152392

Kellner, R. L. L., & Dettner, K. (1996). Differential efficacy of toxic pederin in deterring potential arthropod predators of Paederus (Coleoptera: Staphylinidae) offspring. *Oecologia*, *107*(3), 293–300. https://doi.org/10.1007/BF00328445

Kim, B.-H., Yi, E. H., Li, Y.-C., Park, I.-C., Park, J. Y., & Ye, S.-K. (2019). Anticancer Activity of Tubulosine through Suppression of Interleukin-6-Induced Janus Kinase 2/Signal Transducer and Activation of Transcription 3 Signaling. *Journal of Breast Cancer*, *22*(3), 362. https://doi.org/10.4048/jbc.2019.22.e34

Kim, S. Y., & Benowitz, N. L. (1990). Poisoning Due to Class IA Antiarrhythmic Drugs Quinidine, Procainamide and Disopyramide: *Drug Safety*, *5*(6), 393–420. https://doi.org/10.2165/00002018-199005060-00002

Kingston, D. G. I. (2011). Modern Natural Products Drug Discovery and Its Relevance to Biodiversity Conservation. *Journal of Natural Products*, *74*(3), 496–511. https://doi.org/10.1021/np100550t

Kirby, W. M. M., G. M. Yoshihara, K. S. Sundsted, and J. H. Warren. (1957). Clinical usefulness of a single disc method for antibiotic sensitivity testing. *Antibiotics Annu.*, *892*, 1956–1957.

Knight, V., Sanglier, J.-J., DiTullio, D., Braccili, S., Bonner, P., Waters, J., Hughes, D., & Zhang, L. (2003). Diversifying microbial natural products for drug discovery. *Applied Microbiology and Biotechnology*, *62*(5–6), 446–458. https://doi.org/10.1007/s00253-003-1381-9

Kobayashi, N., Katsumata, H., Katayama, H., Oiwa, H., Goto, J., & Takeuchi, Y. (2000). A monoclonal antibody-based enzyme-linked immunosorbent assay of ursodeoxycholic acid 3-sulfates in human urine. *The Journal of Steroid Biochemistry and Molecular Biology*, *72*(5), 265–272. https://doi.org/10.1016/S0960-0760(00)00032-7

Koehn, F. E., & Carter, G. T. (2005). The evolving role of natural products in drug discovery. *Nature Reviews Drug Discovery*, *4*(3), 206–220. https://doi.org/10.1038/nrd1657

Kohen, R., & Nyska, A. (2002). Invited Review: Oxidation of Biological Systems: Oxidative Stress Phenomena, Antioxidants, Redox Reactions, and Methods for Their

Quantification. *Toxicologic Pathology*, *30*(6), 620–650. https://doi.org/10.1080/01926230290166724

Kostelnik, A., Cegan, A., & Pohanka, M. (2017). Anti-Parkinson Drug Biperiden Inhibits Enzyme Acetylcholinesterase. *BioMed Research International*, *2017*, 1–5. https://doi.org/10.1155/2017/2532764

Kuper, K. M., Boles, D. M., Mohr, J. F., & Wanger, A. (2009). Antimicrobial Susceptibility Testing: A Primer for Clinicians. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *29*(11), 1326–1343. https://doi.org/10.1592/phco.29.11.1326

Lambert, P. (2005). Bacterial resistance to antibiotics: Modified target sites. *Advanced Drug Delivery Reviews*, *57*(10), 1471–1485. https://doi.org/10.1016/j.addr.2005.04.003

Lee, S. T., Welch, K. D., Panter, K. E., Gardner, D. R., Garrossian, M., & Chang, C.-W. T. (2014). Cyclopamine: From Cyclops Lambs to Cancer Treatment. *Journal of Agricultural and Food Chemistry*, *62*(30), 7355–7362. https://doi.org/10.1021/jf5005622

Leong, X. Y., Thanikachalam, P. V., Pandey, M., & Ramamurthy, S. (2016). A systematic review of the protective role of swertiamarin in cardiac and metabolic diseases. *Biomedicine & Pharmacotherapy*, *84*, 1051–1060. https://doi.org/10.1016/j.biopha.2016.10.044

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. https://doi.org/10.1126/science.1168243

Li, S., Tan, H.-Y., Wang, N., Zhang, Z.-J., Lao, L., Wong, C.-W., & Feng, Y. (2015). The Role of Oxidative Stress and Antioxidants in Liver Diseases. *International Journal of Molecular Sciences*, *16*(11), 26087–26124. https://doi.org/10.3390/ijms161125942

Ligon, B. L. (2004). Penicillin: Its discovery and early development. *Seminars in Pediatric Infectious Diseases*, *15*(1), 52–57. https://doi.org/10.1053/j.spid.2004.02.001

Lin, Y., Liu, P.-G., Liang, W.-Q., Hu, Y.-J., Xu, P., Zhou, J., Pu, J.-B., & Zhang, H.-J. (2018). Luteolin-4′- O -glucoside and its aglycone, two major flavones of Gnaphalium affine D. Don, resist hyperuricemia and acute gouty arthritis activity in animal models. *Phytomedicine*, *41*, 54–61. https://doi.org/10.1016/j.phymed.2018.02.002

MARYAM ZAHIN1, , FARRUKH AQIL2, & AND IQBAL AHMAD*. (2009). THE IN VITRO ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF FOUR INDIAN MEDICINAL PLANTS. *International Journal of Pharmacy and Pharmaceutical Sciences*, *Vol. 1*.

Massera, D., Sherrid, M. V., Adlestein, E., Bokhari, N., Alvarez, I. C., Wu, W. Y., Reuter, M. C., Maron, M. S., Maron, B. J., & Rowin, E. J. (2025). Disopyramide Revisited for Treatment of Symptomatic Obstructive Hypertrophic Cardiomyopathy: Efficacy and Safety in Patients Treated for at Least 5 Years. *Journal of the American Heart Association*, *14*(2), e037639. https://doi.org/10.1161/JAHA.124.037639

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Analytical Biochemistry*, *339*(1), 69–72. https://doi.org/10.1016/j.ab.2004.12.001

Mathur, P. P. (1972). Cardiovascular effects of a newer antiarrhythmic agent, disopyramide phosphate. *American Heart Journal*, *84*(6), 764–770. https://doi.org/10.1016/0002-8703(72)90069-5

Meyer, T. A., Roberson, C. R., Rajab, M. H., Davis, J., & McLeskey, C. H. (2005). Dolasetron Versus Ondansetron for the Treatment of Postoperative Nausea and Vomiting: *Anesthesia & Analgesia*, *100*(2), 373–377. https://doi.org/10.1213/01.ANE.0000144421.96275.D1

Mishra, B. B., & Tiwari, V. K. (2011). Natural products: An evolving role in future drug discovery. *European Journal of Medicinal Chemistry*, *46*(10), 4769–4807. https://doi.org/10.1016/j.ejmech.2011.07.057

Morgan, C. J. A., Curran, H. V., & the Independent Scientific Committee on Drugs (ISCD). (2012). Ketamine use: A review. *Addiction*, *107*(1), 27–38. https://doi.org/10.1111/j.1360-0443.2011.03576.x

Muhamad Fadzil, N. S., Sekar, M., Gan, S. H., Bonam, S. R., Wu, Y. S., Vaijanathappa, J., Ravi, S., Lum, P. T., & Dhadde, S. B. (2021). Chemistry, Pharmacology and Therapeutic Potential of Swertiamarin – A Promising Natural Lead for New Drug Discovery and Development. *Drug Design, Development and Therapy*, *Volume 15*, 2721–2746. https://doi.org/10.2147/DDDT.S299753

Musial, C., Kuban-Jankowska, A., & Gorska-Ponikowska, M. (2020). Beneficial Properties of Green Tea Catechins. *International Journal of Molecular Sciences*, *21*(5), 1744. https://doi.org/10.3390/ijms21051744

Mweta, D. E., Labuschagne, M. T., Bonnet, S., Swarts, J., & Saka, J. D. K. (2010). Isolation and physicochemical characterisation of starch from cocoyam (Colocasia esculenta) grown in Malawi. *Journal of the Science of Food and Agriculture*, n/a-n/a. https://doi.org/10.1002/jsfa.4029

Nagata, H., Inagaki, Y., Yamamoto, Y., Maeda, K., Kataoka, K., Osawa, K., & Shizukuishi, S. (2006). Inhibitory effects of macrocarpals on the biological activity of *Porphyromonas gingivalis* and other periodontopathic bacteria. *Oral Microbiology and Immunology*, *21*(3), 159–163. https://doi.org/10.1111/j.1399-302X.2006.00269.x

Newman, D. J. (2008). Natural Products as Leads to Potential Drugs: An Old Process or the New Hope for Drug Discovery? *Journal of Medicinal Chemistry*, *51*(9), 2589–2599. https://doi.org/10.1021/jm0704090

Niki, E. (2016). Oxidative stress and antioxidants: Distress or eustress ? *Archives of Biochemistry and Biophysics*, *595*, 19–24. https://doi.org/10.1016/j.abb.2015.11.017

Ogut, E., Armagan, K., & Gül, Z. (2022). The role of syringic acid as a neuroprotective agent for neurodegenerative disorders and future expectations. *Metabolic Brain Disease*, *37*(4), 859–880. https://doi.org/10.1007/s11011-022-00960-3

Oku, T., Murata-Takenoshita, Y., Yamazaki, Y., Shimura, F., & Nakamura, S. (2014). D-Sorbose inhibits disaccharidase activity and demonstrates suppressive action on postprandial blood levels of glucose and insulin in the rat. *Nutrition Research*, *34*(11), 961–967. https://doi.org/10.1016/j.nutres.2014.09.009

Olatunde, K., Adebayo, K., Muhumuza, J., & Bada, B. (2018). Assessment of variability in proximate/anti-nutritive composition of cocoyam within Nigeria and Uganda. *Journal of Applied Sciences and Environmental Management*, *22*(5), 737. https://doi.org/10.4314/jasem.v22i5.20

Olayiwola, I., Folaranmi, F., Adebowale, A. A., Oluseye, O., Ajoke, S., & Wasiu, A. (2013). Chemical, mineral composition, and sensory acceptability of cocoyam-based recipes enriched with cowpea flour. *Food Science & Nutrition*, *1*(3), 228–234. https://doi.org/10.1002/fsn3.30

Olufunmilayo, E. O., Gerke-Duncan, M. B., & Holsinger, R. M. D. (2023). Oxidative Stress and Antioxidants in Neurodegenerative Disorders. *Antioxidants*, *12*(2), 517. https://doi.org/10.3390/antiox12020517

Otekunrin, O. A., Sawicka, B., Adeyonu, A. G., Otekunrin, O. A., & Rachoń, L. (2021). Cocoyam [Colocasia esculenta (L.) Schott]: Exploring the Production, Health and Trade Potentials in Sub-Saharan Africa. *Sustainability*, *13*(8), 4483. https://doi.org/10.3390/su13084483

Owusu-Darko, P. G., Paterson, A., & Omenyo, E. L. (2014). Cocoyam (corms and cormels)—An underexploited food and feed resource. *Journal of Agricultural Chemistry and Environment*, *03*(01), 22–29. https://doi.org/10.4236/jacen.2014.31004

Passemar, C., Saléry, M., Soh, P. N., Linas, M.-D., Ahond, A., Poupat, C., & Benoit-Vical, F. (2011). Indole and aminoimidazole moieties appear as key structural units in antiplasmodial molecules. *Phytomedicine*, *18*(13), 1118–1125. https://doi.org/10.1016/j.phymed.2011.03.010

Patricia G. Owusu-Darko, Alistair Paterson, Emmanuel L. Omenyo. (2014). Cocoyam (corms and cormels)—an underexploited food and feed resource. *Journal of Agricultural Chemistry and Environment*, *03*. http://dx.doi.org/10.4236/jacen.2014.31004

Paudel, P. K., Bhattarai, B. P., & Kindlmann, P. (2012). An Overview of the Biodiversity in Nepal. In P. Kindlmann (Ed.), *Himalayan Biodiversity in the Changing World* (pp. 1–40). Springer Netherlands. https://doi.org/10.1007/978-94-007-1802-9_1

Paurakh Magar, Galaxy Pokhrel, Sachin Silwal, , Sabi Thapa, , Ajaya Mahato, , Bishisha Adhikara, , Shiva Bagale, , Pabitra shrestha, , & Bodhbabu Bhattaraia*, Ganga Raj Pokhrel a*. (2024). Antioxidant Activity and HR-LCMS Analysis of Phytochemicals Present in the Methanolic Extract of the Rhizomes of Paris Polyphylla (Satuwa). *Api Journal of Science*, *1*(1), 8–17.

Perrone, S., Negro, S., Tataranno, M. L., & Buonocore, G. (2010). Oxidative stress and antioxidant strategies in newborns. *The Journal of Maternal-Fetal & Neonatal Medicine*, *23*(sup3), 63–65. https://doi.org/10.3109/14767058.2010.509940

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. https://doi.org/10.1016/j.ejmech.2015.04.040

Pohl, F., & Kong Thoo Lin, P. (2018). The Potential Use of Plant Natural Products and Plant Extracts with Antioxidant Properties for the Prevention/Treatment of Neurodegenerative Diseases: In Vitro, In Vivo and Clinical Trials. *Molecules*, *23*(12), 3283. https://doi.org/10.3390/molecules23123283

Pokhrel, G., Silwal, S., Thapa, S., Giri, S., Mahato, A., Adhikari, B., Bagale, S., Bhattarai, B., & Pokhrel, G. R. (2024). Assessment of Micronutrients and Toxic Elements in Rice Grains: Implications for Food Safety and Nutrition. *BMC Journal of Scientific Research*, *7*(1), 37–49. https://doi.org/10.3126/bmcjsr.v7i1.72942

Poole, K., Russell, A., & Lambert, P. (2005). Mechanisms of antimicrobial resistance: Opportunities for new targeted therapies. *Advanced Drug Delivery Reviews*, *57*(10), 1443–1445. https://doi.org/10.1016/j.addr.2005.05.001

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. https://doi.org/10.1006/abio.1999.4019

Rabiu, I., Yusha'u, M., Isah Abdullahi, J., & Muhammed, A. (2024). Phytochemical analysis, Thin Layer Chromatography and Gas Chromatography Mass Spectroscopy profile of colocasia esculenta and manihot esculanta extracts and their potential for drug discovery. *Drug Discovery*, *18*(41), 1–20. https://doi.org/10.54905/disssi.v18i41.e10dd1975

Ramawat, K. G. (Ed.). (2009). *Herbal Drugs: Ethnomedicine to Modern Medicine*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-79116-4

Rao, A. V., & Balachandran, B. (2002). Role of Oxidative Stress and Antioxidants in Neurodegenerative Diseases. *Nutritional Neuroscience*, *5*(5), 291–309. https://doi.org/10.1080/1028415021000033767

Reyes, S., Abdelraouf, K., & Nicolau, D. P. (2020). In vivo activity of human-simulated regimens of imipenem alone and in combination with relebactam against Pseudomonas aeruginosa in the murine thigh infection model. *Journal of Antimicrobial Chemotherapy*, dkaa145. https://doi.org/10.1093/jac/dkaa145

Rizos, I., Brachmann, J., Lengfelder, W., Schmitt, C., Olshausen, K. V., Kübler, W., & Senges, J. (1987). Effects of intravenous disopyramide and quinidine on normal myocardium and on the characteristics of arrhythmias: Intraindividual comparison in patients with sustained ventricular tachycardia. *European Heart Journal*, *8*(2), 154–163. https://doi.org/10.1093/oxfordjournals.eurheartj.a062243

Rodrigues, T., Reker, D., Schneider, P., & Schneider, G. (2016). Counting on natural products for drug design. *Nature Chemistry*, *8*(6), 531–541. https://doi.org/10.1038/nchem.2479

Sagar, N. A., Pareek, S., & Gonzalez-Aguilar, G. A. (2020). Quantification of flavonoids, total phenols and antioxidant properties of onion skin: A comparative study of fifteen Indian cultivars. *Journal of Food Science and Technology*, *57*(7), 2423–2432. https://doi.org/10.1007/s13197-020-04277-w

Sahari Shimsa, Sukanta Mondal , Saraswathy Mini. (2024). Syringic acid: A promising phenolic phytochemical with extensive therapeutic applications. *R&D of Functional Food Products*, *1*(5), 1–14.

Saugstad, O. D. (2003). Bronchopulmonary dysplasia—Oxidative stress and antioxidants. *Seminars in Neonatology*, *8*(1), 39–49. https://doi.org/10.1016/S1084-2756(02)00194-X

Sefa-Dedeh, S., & Agyir-Sackey, E. K. (2004). Chemical composition and the effect of processing on oxalate content of cocoyam Xanthosoma sagittifolium and Colocasia esculenta cormels. *Food Chemistry*, *85*(4), 479–487. https://doi.org/10.1016/S0308-8146(02)00244-3

Senyilmaz-Tiebe, D., Pfaff, D. H., Virtue, S., Schwarz, K. V., Fleming, T., Altamura, S., Muckenthaler, M. U., Okun, J. G., Vidal-Puig, A., Nawroth, P., & Teleman, A. A. (2018). Dietary stearic acid regulates mitochondria in vivo in humans. *Nature Communications*, *9*(1), 3129. https://doi.org/10.1038/s41467-018-05614-6

Serna-Loaiza, S., Carmona-Garcia, E., & Cardona, C. A. (2018). Potential raw materials for biorefineries to ensure food security: The Cocoyam case. *Industrial Crops and Products*, *126*, 92–102. https://doi.org/10.1016/j.indcrop.2018.10.005

Shady, N. H., Mokhtar, F. A., Abdullah, H. S., Abdel-Aziz, S. A., Mohamad, S. A., Imam, M. S., El Afify, S. R., & Abdelmohsen, U. R. (2024). In Vitro and Randomized Controlled Clinical Study of Natural Constituents' Anti-HPV Potential for Treatment of Plantar Warts Supported with In Silico Studies and Network Analysis. *Pharmaceuticals*, *17*(6), 759. https://doi.org/10.3390/ph17060759

Shaito, A., Thuan, D. T. B., Phu, H. T., Nguyen, T. H. D., Hasan, H., Halabi, S., Abdelhady, S., Nasrallah, G. K., Eid, A. H., & Pintus, G. (2020). Herbal Medicine for

Cardiovascular Diseases: Efficacy, Mechanisms, and Safety. *Frontiers in Pharmacology*, *11*, 422. https://doi.org/10.3389/fphar.2020.00422

Shakur, H., Andrews, P., Asser, T., Balica, L., Boeriu, C., Quintero, J. D. C., Dewan, Y., Druwé, P., Fletcher, O., Frost, C., Hartzenberg, B., Mantilla, J. M., Murillo-Cabezas, F., Pachl, J., Ravi, R. R., Rätsep, I., Sampaio, C., Singh, M., Svoboda, P., & Roberts, I. (2009). The BRAIN TRIAL: A randomised, placebo controlled trial of a Bradykinin B2 receptor antagonist (Anatibant) in patients with traumatic brain injury. *Trials*, *10*(1), 109. https://doi.org/10.1186/1745-6215-10-109

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., … Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in Physiology*, *11*, 694. https://doi.org/10.3389/fphys.2020.00694

Shree, P., Mishra, P., Selvaraj, C., Singh, S. K., Chaube, R., Garg, N., & Tripathi, Y. B. (2022). Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants – *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Giloy) and *Ocimum sanctum* (Tulsi) – a molecular docking study. *Journal of Biomolecular Structure and Dynamics*, *40*(1), 190–203. https://doi.org/10.1080/07391102.2020.1810778

Shrestha, N., Shrestha, S., Koju, L., Shrestha, K. K., & Wang, Z. (2016). Medicinal plant diversity and traditional healing practices in eastern Nepal. *Journal of Ethnopharmacology*, *192*, 292–301. https://doi.org/10.1016/j.jep.2016.07.067

Silva, T., Reis, J., Teixeira, J., & Borges, F. (2014). Alzheimer's disease, enzyme targets and drug discovery struggles: From natural products to drug prototypes. *Ageing Research Reviews*, *15*, 116–145. https://doi.org/10.1016/j.arr.2014.03.008

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu

reagent. In *Methods in Enzymology* (Vol. 299, pp. 152–178). Elsevier. https://doi.org/10.1016/S0076-6879(99)99017-1

Sleda, M. A., Li, Z.-H., Behera, R., Baierna, B., Li, C., Jumpathong, J., Malwal, S. R., Kawamukai, M., Oldfield, E., & Moreno, S. N. J. (2022). The Heptaprenyl Diphosphate Synthase (Coq1) Is the Target of a Lipophilic Bisphosphonate That Protects Mice against Toxoplasma gondii Infection. *mBio*, *13*(5), e01966-22. https://doi.org/10.1128/mbio.01966-22

Stephen T Odonkor a*,Kennedy K Addo. (2011). Bacteria Resistance to Antibiotics: Recent Trends and Challenges. *International Journal of Biological & Medical Research*, *(4): 1204-1210*(2), 1204–1210.

Stephen T Odonkor ,Kennedy K Addo. (2011). Bacteria Resistance to Antibiotics: Recent Trends and Challenges. *International Journal of Biological & Medical Research*, *2*(4), 1204–1210.

Stuart Noble and Karen L. Goa. (n.d.). Amprenavir A Review of its Clinical Potential in Patients with HIV Infection. *ADIS DRUG EVALUATION*, *Drugs 2000 Dec; 60 (6): 1383-1410*.

Stuart R. Hameroff, MD, Juan Carlos Lerman, PhD and Casey D. Blitt, MD. (1984). Etidocaine Aerosol for Laryngotracheal Anesthesia Clinical Evaluation Using Exploratory Data Analysis. *BMJ Journals*, *9*(4). https://doi.org/10.1136/rapm-00115550-198409040-00006

Szuba, T. (2002). Was There Collective Intelligence Before Life on Earth? Considerations on the Formal Foundations of Intelligence, Life, and Evolution. *World Futures*, *58*(1), 61–80. https://doi.org/10.1080/02604020210404

Tajner-Czopek, A., Gertchen, M., Rytel, E., Kita, A., Kucharska, A. Z., & Sokół-Łętowska, A. (2020). Study of Antioxidant Activity of Some Medicinal Plants Having High Content of Caffeic Acid Derivatives. *Antioxidants*, *9*(5), 412. https://doi.org/10.3390/antiox9050412

Takayanagi, I., Hisayama, T., Iwase, M., Sakuma, N., & Nagai, H. (1989). Pharmacological properties of tiropramide, an antispasmodic drug. *General Pharmacology: The Vascular System*, *20*(3), 335–339. https://doi.org/10.1016/0306-3623(89)90269-3

Tanus, T., Scangarella-Oman, N. E., Dalessandro, M., Li, G., Breton, J. J., & Tomayko, J. F. (2014). A Randomized, Double-blind, Comparative Study to Assess the Safety and Efficacy of Topical Retapamulin Ointment 1% Versus Oral Linezolid in the Treatment of Secondarily Infected Traumatic Lesions and Impetigo Due to Methicillin-Resistant Staphylococcus aureus. *Advances in Skin & Wound Care*, *27*(12), 548–559. https://doi.org/10.1097/01.ASW.0000456631.20389.ae

Tay, D. (2013). Tropical and Subtropical Root and Tuber Crops. In M. N. Normah, H. F. Chin, & B. M. Reed (Eds.), *Conservation of Tropical Plant Species* (pp. 249–292). Springer New York. https://doi.org/10.1007/978-1-4614-3776-5_12

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, K. (2018). Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. *International Journal of Molecular Sciences*, *19*(6), 1578. https://doi.org/10.3390/ijms19061578

Thomson, A., Hemphill, D., & Jeejeebhoy, K. N. (1998). Oxidative Stress and Antioxidants in Intestinal Disease. *Digestive Diseases*, *16*(3), 152–158. https://doi.org/10.1159/000016859

Tohru Dairi, Kazuo Yamaguchi, and Mamoru Hasegawa. (1992). N-Formimidoyl fortimicin A synthase, a unique oxidase involved in fortimicin A biosynthesis: Purification, characterization and gene cloning. *Mol Gen Genet (1992) 236:49-59*.

Tufikul Islam, Mohammad Raquibul Hasan, Aumit Roy, Md. Shafiqul Islam, Md. Afaz Uddin, Md. Ariful Islam, Md. Nuruzzaman Neon, Md. Sohel Rana. (2015). SCREENING OF IN-VITRO ANTIOXIDANT, BRINE SHRIMP LETHALITY BIOASSAY AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF BRIDELIA RETUSA (L.) SPRENG. FRUIT. *International Journal of Pharmacy*, *5*(4), 1058–1067.

Vetter, I., Davis, J. L., Rash, L. D., Anangi, R., Mobli, M., Alewood, P. F., Lewis, R. J., & King, G. F. (2011). Venomics: A new paradigm for natural products-based drug discovery. *Amino Acids*, *40*(1), 15–28. https://doi.org/10.1007/s00726-010-0516-4

Wada, E., Feyissa, T., & Tesfaye, K. (2019). Proximate, Mineral and Antinutrient Contents of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Ethiopia. *International Journal of Food Science*, *2019*, 1–7. https://doi.org/10.1155/2019/8965476

Wakeling, A. (1983). Efficacy and side effects of mianserin, a tetracyclic antidepressant. *Postgraduate Medical Journal*, *59*(690), 229–231. https://doi.org/10.1136/pgmj.59.690.229

Wang, J.-K., & Higa, S. (Eds.). (1983). *Taro, a review of colocasia esculenta and its potentials*. University of Hawaii Press.

Wang, L., Pan, X., Jiang, L., Chu, Y., Gao, S., Jiang, X., Zhang, Y., Chen, Y., Luo, S., & Peng, C. (2022). The Biological Activity Mechanism of Chlorogenic Acid and Its Applications in Food Industry: A Review. *Frontiers in Nutrition*, *9*, 943911. https://doi.org/10.3389/fnut.2022.943911

Wang, S., Gong, T., Lu, J., Kano, Y., & Yuan, D. (2013). Simultaneous determination of tectorigenin and its metabolites in rat plasma by ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry. *Journal of Chromatography B*, *933*, 50–58. https://doi.org/10.1016/j.jchromb.2013.06.009

Wei Lei , Zhenyun Huo, W. L. a, Zhenyun Huo. (2020). Jervine inhibits non-small cell lung cancer (NSCLC) progression by suppressing Hedgehog and AKT signaling via triggering autophagyregulated apoptosis. *Biochemical and Biophysical Research Communications*.

Wilson, S. R., Strand, M. F., Krapp, A., Rise, F., Petersen, D., & Krauss, S. (2010). Hedgehog antagonist cyclopamine isomerizes to less potent forms when acidified. *Journal of Pharmaceutical and Biomedical Analysis*, *52*(5), 707–713. https://doi.org/10.1016/j.jpba.2010.02.017

Wirz-Justice, A., & Lichtsteiner, M. (1976). Sex specific differences in noradrenaline uptake and its inhibition by maprotiline. *The Journal of Pharmacy and Pharmacology*, *28*(2), 172–175. https://doi.org/10.1111/j.2042-7158.1976.tb04124.x

Wright, C., Bray, D., O'Neill, M., Warhurst, D., Phillipson, J., Quetin-Leclercq, J., & Angenot, L. (1991). Antiamœbic and Antiplasmodial Activities of Alkaloids Isolated from *Strychnos usambarensis*. *Planta Medica*, *57*(04), 337–340. https://doi.org/10.1055/s-2006-960112

Wu, J.-Y., Ding, H.-Y., Wang, T.-Y., Cai, C.-Z., & Chang, T.-S. (2022). Application of Biotransformation-Guided Purification in Chinese Medicine: An Example to Produce Butin from Licorice. *Catalysts*, *12*(7), 718. https://doi.org/10.3390/catal12070718

W.-W. MA, J.E. ANDERSON, A.T. MCKENZIE, S.R. BYRN, J.L. MCLAUGHLIN. (1990). TUBULOSINE: AN ANTITUMOR CONSTITUENT OF POGONOPUS SPECIOSUS. *Journal of Natural Prodwts*, *53*(4), 1009–1014.

Xu, M., Zhang, L., Zeng, Y., Zhou, Z., & Han, Y. (2025). Preparation and characterization of Levan composite film incorporating vanillin for use as a potential edible coating for peony seed oil. *International Journal of Biological Macromolecules*, *288*, 138732. https://doi.org/10.1016/j.ijbiomac.2024.138732

Yakan, S., Aydin, T., Gulmez, C., Ozden, O., Eren Erdogan, K., Daglioglu, Y. K., Andic, F., Atakisi, O., & Cakir, A. (2019). The protective role of jervine against radiation-induced gastrointestinal toxicity. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *34*(1), 789–798. https://doi.org/10.1080/14756366.2019.1586681

Yang, G., Xie, H., Wang, C., Zhang, C., Yu, L., Zhang, L., Liu, X., Xu, R., Song, Z., Liu, R., & Ueda, M. (2023). Design, synthesis, and discovery of Eudistomin Y derivatives as lysosome-targeted antiproliferation agents. *European Journal of Medicinal Chemistry*, *250*, 115193. https://doi.org/10.1016/j.ejmech.2023.115193

Yao, X., Ling, Y., Guo, S., He, S., Wang, J., Zhang, Q., Wu, W., Zou, M., Zhang, T., Nandakumar, K. S., Chen, X., & Liu, S. (2018). Inhibition of dengue viral infection by diasarone-I is associated with 2'O methyltransferase of NS5. *European Journal of Pharmacology*, *821*, 11–20. https://doi.org/10.1016/j.ejphar.2017.12.029

Yoshikawa, M., Murakami, T., Shimada, H., Yoshizumi, S., Saka, M., Yamahara, J., & Matsuda, H. (1998). Medicinal Foodstuffs. XIV. On the Bioactive Constituents of Moroheiya. (2): New Fatty Acids, Corchorifatty Acids A, B, C, D, E, and F, from the Leaves of Corchorus olitorius L. (Tiliaceae): Structures and Inhibitory Effect on NO Production in Mouse Peritoneal Macrophages. *Chemical and Pharmaceutical Bulletin*, *46*(6), 1008–1014. https://doi.org/10.1248/cpb.46.1008

Zhang, L., Song, J., Kong, L., Yuan, T., Li, W., Zhang, W., Hou, B., Lu, Y., & Du, G. (2020). The strategies and techniques of drug discovery from natural products. *Pharmacology & Therapeutics*, *216*, 107686. https://doi.org/10.1016/j.pharmthera.2020.107686

Zhang, R., Chae, S., Kang, K. A., Piao, M. J., Ko, D. O., Wang, Z. H., Park, D. B., Park, J. W., You, H. J., & Hyun, J. W. (2008). Protective effect of butin against hydrogen peroxide-induced apoptosis by scavenging reactive oxygen species and activating antioxidant enzymes. *Molecular and Cellular Biochemistry*, *318*(1–2), 33–42. https://doi.org/10.1007/s11010-008-9854-x

Zhao, M., Zhu, Y., Wang, H., Zhang, W., & Mu, W. (2023). Recent advances on N-acetylneuraminic acid: Physiological roles, applications, and biosynthesis. *Synthetic and Systems Biotechnology*, *8*(3), 509–519. https://doi.org/10.1016/j.synbio.2023.06.009

APPENDIX I

HR-LCMS METHODS OF ANALYSIS

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	Ch2 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 % Acetonitrile 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

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	Ch2 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 % Acetonitrile 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

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

Time Segment 1

Acquisition Mode AutoMS2

MS Min Range (m/z)	120
MS Max Range (m/z)	1300
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 (%)	50.000
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)	55
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

Report generation date: 11/7/2024 3:57:40 PM

Acquisition Method Report Agilent Technologies

Acquisition Method Info

Method Name	metabolite_ESI_+VE_MSMS.m
Method Path	D:\MassHunter\Methods\2024\metabolite_ESI_+VE_MSMS.m
Method Description	Default Method

Device List

- HiP Sampler
- Binary Pump
- Column Comp.
- DAD
- 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.tun		

Time Segments

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

APPENDIX II

HR-LCMS profiling of compounds in UPT in positive ESI mode.

Acquisition Method Report Agilent Technologies

Name: DAD Model: G4212B

Peakwidth	>0.10 min (2.0 s response time) (2.5 Hz)
UV Lamp Required	Yes
Analog Output 1	
Analog 1 Zero Offset	5 %
Analog 1 Attenuation	1000 mAU
Signals	
Prepare Mode	
Margin for negative Absorbance	100 mAU
Autobalance	
Autobalance Prerun	Yes
Autobalance Postrun	No
Spectrum	
Spectrum Range WL from	190.0 nm
Spectrum Range WL to	640.0 nm
Spectrum Step	2.0 nm
Spectrum Store	All
Stoptime	
Stoptime Mode	As pump/injector
Posttime	
Posttime Mode	Off
Signals	
Signal table	

	Use Sig.	Signal	Wavelength	Bandwidth	Use Ref.	Ref Wavel.	Ref Bandw.
1	Yes	Signal A	280.0 nm	4.0 nm	Yes	360.0 nm	100.0 nm
2	Yes	Signal B	300.0 nm	4.0 nm	Yes	360.0 nm	100.0 nm
3	Yes	Signal C	245.0 nm	4.0 nm	Yes	360.0 nm	100.0 nm
4	Yes	Signal D	254.0 nm	4.0 nm	Yes	360.0 nm	100.0 nm
5	No	Signal E					
6	No	Signal F					
7	No	Signal G					
8	No	Signal H					

Data File	TU-BMC-CI-18.d	**Sample Name**	TU-BMC-CI-18
Sample Type	Sample	**Position**	P1-A9
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_+VE_HSRS.m	**Acquired Time**	11/7/2024 12:47:18 AM
DA Method	Default.m	**DA Method**	Default.m
Comment			

Sample Group Info.
Acquisition SW 6200 series TOF/6500 series
Version Q-TOF B.05.01 (B0325.J)

Compound Table

Compound Label	RT	Mass	Absnd	Name	Formula	MFE Formula	DB Formula	DB DBE (ppm)	DBE (DB)
Compound 1	4.468		13841						
Cpd 2: O-Ureidohomoserine; C5 H11 N3 O4	4.746	177.0749	63440	O-Ureidohomoserine	C5 H11 N3 O4	C5 H11 N3 O4	C5 H11 N3 O4	0.16	4
Cpd 3: Benzoxinuline; C20 H19 N O2	4.865	305.1431	10461	Benzoxinuline	C20 H19 N O2	C20 H19 N O2	C20 H19 N O2	-1.06	1
Cpd 4: Diosmone 2; C24 H22 O6	5.139	418.2394		Diosmone 2	C24 H22 O6	C24 H22 O6	C24 H22 O6	-0.05	15
Cpd 5: Abscisic acid glucose ester; C21 H30 O9	5.54	426.1924		Abscisic acid glucose ester	C21 H30 O9	C21 H30 O9	C21 H30 O9	-7.9	4
Compound 6	5.609								
Cpd 7: 7-keto-D-Glucopyranosyloxybutylbenzophthalide; C18 H22 O6	5.615	366.1346		7-keto-D-Glucopyranosyloxybutylbenzophthalide	C18 H22 O6	C18 H22 O6	C18 H22 O6	-6.67	2
Cpd 8: Abscisic acid glucose ester; C21 H30 O9	5.812	426.1923		Abscisic acid glucose ester	C21 H30 O9	C21 H30 O9	C21 H30 O9	-6.19	3
Cpd 9: 6-Pentacosynoxoadanol; C24	5.839	466.2225	901.16	6-Pentacosynoxoadanol	C24 H34 O6	C24 H34 O6	C24 H34 O6	-4.66	2
Cpd 10: Trewbolone; C18 H22 O2	5.839	270.1643		Trewbolone	C18 H22 O2	C18 H22 O2	C18 H22 O2	-4.77	6
Compound 11	5.96		32900						
Cpd 12: Abscisic acid glucose ester; C21 H30 O9	6.13	426.1913		Abscisic acid glucose ester	C21 H30 O9	C21 H30 O9	C21 H30 O9	-5.21	4
Compound 13	6.167		27966						
Cpd 14: 6-Pentacosynoxoadanol; C24	6.244	466.2234		6-Pentacosynoxoadanol	C24 H34 O6	C24 H34 O6	C24 H34 O6	-4.75	2
Cpd 15: Istamycin A3; C18 H35 N6 O5	6.324	418.2683		Istamycin A3	C18 H35 N6 O5	C18 H35 N6 O5	C18 H35 N6 O5	-11.45	2
Cpd 16: Mangiferyl glucoside; C21 H28 O9	6.638	398.2945	34805	Mangiferyl glucoside	C21 H28 O9	C21 H28 O9	C21 H28 O9	-6.82	4
Cpd 17: Cincecetol C; C20 H28 O7	7.081	380.1884	31562	Cincecetol C	C20 H28 O7	C20 H28 O7	C20 H28 O7	-4	6
Compound 18	7.561		129236						
Compound 19	7.724		144238						
Cpd 20: Lagerstroemine; C26 H31 N O5	7.793	437.2229	122265	Lagerstroemine	C26 H31 N O5	C26 H31 N O5	C26 H31 N O5	-6.63	2
Cpd 21: Alpha-Tilsacharide; C28 H37 N O14	7.806	515.2314		Alpha-Tilsacharide	C28 H37 N O14	C28 H37 N O14	C28 H37 N O14	-19.42	1
Compound 22	8.123		47920						
Cpd 23: Gentamicin; C21 H43 N5 O7	8.126	477.3206		Gentamicin	C21 H43 N5 O7	C21 H43 N5 O7	C21 H43 N5 O7	-6.18	1
Cpd 24: Geranyl anabinopyranosyl-glucoside; C21 H36 O10	8.287	448.2246		Geranyl anabinopyranosyl-glucoside	C21 H36 O10	C21 H36 O10	C21 H36 O10	13.84	3
Compound 25	8.374								
Compound 26	8.44								
Compound 27	8.45		351265						
Compound 28	8.453								
Compound 29	8.546								
Cpd 30: Geranyl anabinopyranosyl-glucoside; C21 H36 O10	8.574	448.2256	92936	Geranyl anabinopyranosyl-glucoside	C21 H36 O10	C21 H36 O10	C21 H36 O10	11.43	3
Cpd 31: 6beta-Fluoro-5alpha-hydroxypregnane-3,20-dione; C21 H31 F O3	8.738	350.2251		6beta-Fluoro-5alpha-hydroxypregnane-3,20-dione	C21 H31 F O3	C21 H31 F O3	C21 H31 F O3	1.71	4
Compound 32	9.189								
Compound 33	9.335		114035						
Compound 34	9.408								
Cpd 35: (20S)-23,25-dihdroxy-24-oxocholestane D3 23-(beta-glucuronide); C33 H50 O10	10.346	606.3344		(20S)-23,25-dihdroxy-24-oxocholestane D3 23-(beta-glucuronide)	C33 H50 O10	C33 H50 O10	C33 H50 O10	9.85	1
Cpd 36: Dicyclanine; C19 H25 N O2	10.854	299.2827		Dicyclanine	C19 H25 N O2	C19 H25 N O2	C19 H25 N O2	13.16	1
Compound 37	11.056								
Cpd 38: Dicyclanine; C19 H25 N O2	11.124	299.2824	176567	Dicyclanine	C19 H25 N O2	C19 H25 N O2	C19 H25 N O2	14.25	1
Compound 39	11.187		116182						
Cpd 40: Cryptophorine; C17 H27 N O	11.236	261.2093		Cryptophorine	C17 H27 N O	C17 H27 N O	C17 H27 N O	15.15	1
Compound 41	11.324								
Compound 42	11.404								
Cpd 43: 16-Oxo-palmitate; C16 H30 O3	11.434	270.2156	189031	16-Oxo-palmitate	C16 H30 O3	C16 H30 O3	C16 H30 O3	13.6	2
Compound 44	11.531								
Compound 45	11.767		51865						
Cpd 46: Dicyclanine; C19 H25 N O2	11.797	299.2823		Dicyclanine	C19 H25 N O2	C19 H25 N O2	C19 H25 N O2	14.81	1
Compound 47	11.835		56813						
Compound 48	12.027								
Cpd 49: Cyclopaxilfoside X; C37 H62 O12	12.166	698.4163		Cyclopaxilfoside X	C37 H62 O12	C37 H62 O12	C37 H62 O12	11.31	3

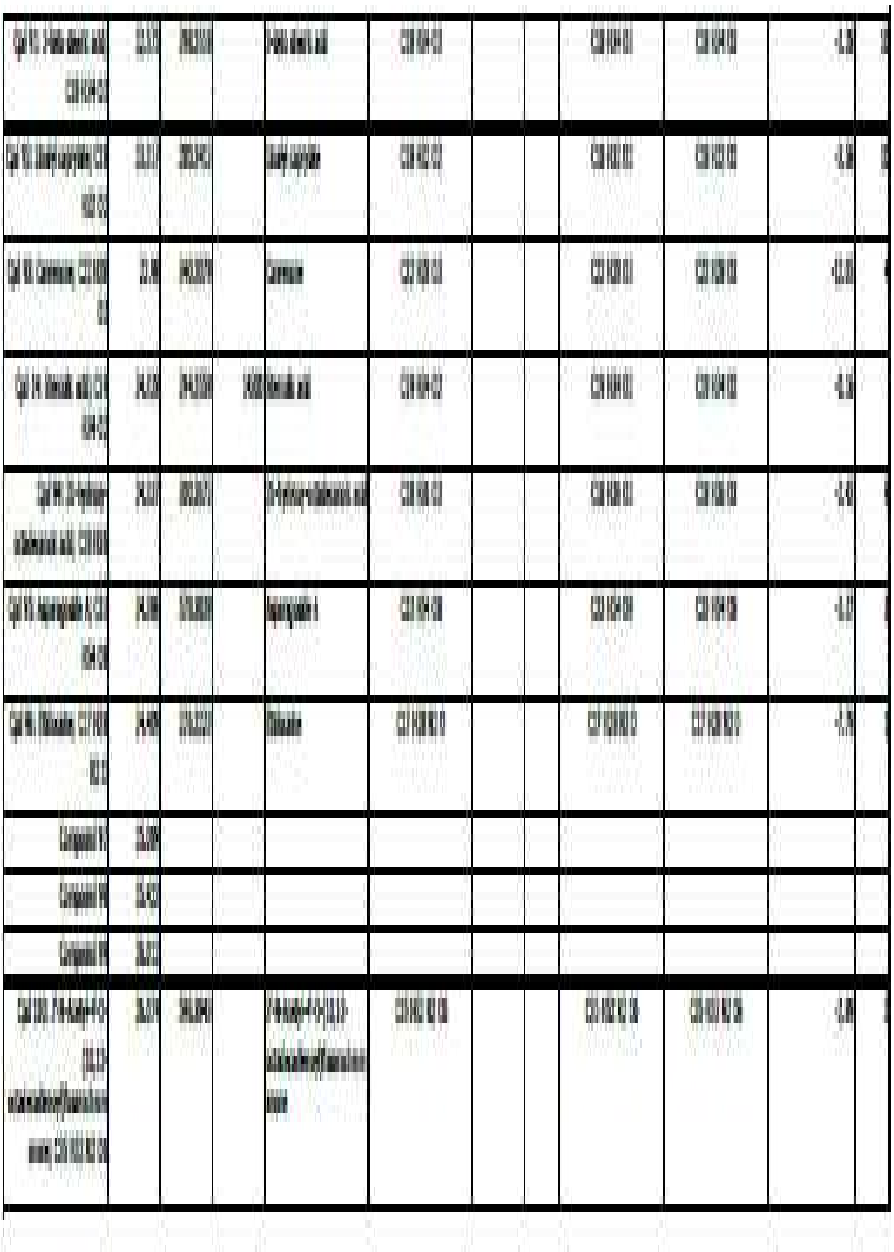
APPENDIX III

HR-LCMS profiling of compounds in UPS in positive ESI mode.

Cpd 50: DG(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:4(6Z,9Z,12Z,15Z)/0:0); C43 H64 O5	12.113	660.4844		DG(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:4(6Z,9Z,12Z,15Z)/0:0)	C43 H64 O5	C43 H64 O5	C43 H64 O5	0.71	1
Compound 51	12.138								
Compound 52	12.210								
Cpd 53: Jervine; C27 H39 N O3	12.240	425.2987		Jervine	C27 H39 N O3	C27 H39 N O3	C27 H39 N O3	-1.70	1
Compound 54	12.286								
Cpd 55: 2-Hexadecenoyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol; C28 H38 O4	12.466	578.4233		2-Hexadecenoyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol	C28 H38 O4	C28 H38 O4	C28 H38 O4	17.82	1
Compound 56	12.510								
Cpd 57: Glutamyl-lysine; C11 H21 N3 O5	12.522	275.1479		Glutamyl-lysine	C11 H21 N3 O5	C11 H21 N3 O5	C11 H21 N3 O5	0.00	4
Compound 58	12.563		602132						
Compound 59	12.726								
Compound 60	12.807		1908668						
Compound 61	13.106		908678						
Compound 62	13.151								
Compound 63	13.151								
Compound 64	13.202								
Compound 65	13.291								
Compound 66	13.401								
Compound 67	13.478								
Compound 68	13.514								
Compound 69	13.722		1982705						
Compound 70	13.771								
Compound 71	13.791								
Cpd 72: Pumiliotine; C21 H25 N O	13.791	307.1929		Pumiliotine	C21 H25 N O	C21 H25 N O	C21 H25 N O	13.82	1
Compound 73	13.792								
Compound 74	13.798								
Compound 75	13.846								
Compound 76	13.943								
Compound 77	14.107								
Compound 78	14.107								
Cpd 79: N-Hexadecanoylpyrrolidine; C20	14.126	309.3083		N-Hexadecanoylpyrrolidine	C20 H39 N O	C20 H39 N O	C20 H39 N O	9.26	1
Compound 80	14.138								
Compound 81	14.21								
Compound 82	14.3								
Compound 83	14.317								
Compound 84	14.372								
Compound 85	14.373								
Compound 86	14.726								
Compound 87	14.723								
Compound 88	14.727								
Compound 89	14.806								
Compound 90	14.807								
Compound 91	14.976								
Compound 92	15.197								
Compound 93	15.754								
Compound 94	16.240								
Cpd 95: Septemtriodine; C17 H32 N2 O11	16.864	768.3543		Septemtriodine	C17 H32 N2 O11	C17 H32 N2 O11	C17 H32 N2 O11	4.69	1
Compound 96	20.047								
Compound 97	20.403								
Cpd 98: DG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(4Z,7Z,10Z,13Z,16Z)/0:0); C47 H72 O5	22.192	716.5377	722231	DG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(4Z,7Z,10Z,13Z,16Z)/0:0)	C47 H72 O5	C47 H72 O5	C47 H72 O5	14.28	4
Cpd 99: Lisuride; C22 H33 N4 O4	22.564	375.2382	321991	Lisuride	C22 H33 N4 O4	C22 H33 N4 O4	C22 H33 N4 O4	13.33	1
Compound 100	23.211		539731						

APPENDIX IV

HR-LCMS profiling of compounds in UPL in positive ESI mode.



Data File	TU-BME-CD-19.d	**Sample Name**	TU-BME-CD-19
Sample Type	Sample	**Position**	P1-B1
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_+HS_HSMS.m	**Acquired Time**	11/7/2024 1:36:57 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	Abund	Name	Formula	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
Compound 1	1.247								
Compound 2	1.408								
Compound 3	1.458		90801						
Compound 4	1.468								
Compound 5	1.508								
Compound 6	1.805								
Cpd 7: 3''-Hydroxy-geranylhydroquinone; C16 H22 O2	3.196	262.1577	18617	3''-Hydroxy-geranylhydroquinone	C16 H22 O2	C16 H22 O2	C16 H22 O2	-1.23	1
Compound 8	3.596		19620						
Compound 9	3.641		70567						
Compound 10	3.648		11434						
Cpd 11: Minwan; C33 H21 O3 N2	3.83	248.1403	46036	Minwan	C33 H21 O3 N2	C33 H21 O3 N2	C33 H21 O3 N2	-0.27	1
Cpd 12: Neuraminic acid; C9 H17 N O8	3.682	267.0959		Neuraminic acid	C9 H17 N O8	C9 H17 N O8	C9 H17 N O8	30.86	7
Cpd 13: 2-Methylpiperidine; C6 H13 N	3.782	99.1047	155510	2-Methylpiperidine	C6 H13 N	C6 H13 N	C6 H13 N	1.48	2
Cpd 14: 2-Naphthalenol 2-aminobenzoate; C17 H13 N O2	3.794	263.0954	19189	2-Naphthalenol 2-aminobenzoate	C17 H13 N O2	C17 H13 N O2	C17 H13 N O2	4.6	4
Cpd 15: N-(3-Methyl-3-oxidooxy)alkaline; C10 H19 N O3	3.798	201.1339	23513	N-(3-Methyl-3-oxidooxy)alkaline	C10 H19 N O3	C10 H19 N O3	C10 H19 N O3	14.48	2
Cpd 16: L-prolyl-L-glycine; C7 H12 N2 O3	3.863	172.0851		L-prolyl-L-glycine	C7 H12 N2 O3	C7 H12 N2 O3	C7 H12 N2 O3	9.62	2
Compound 17	3.941		39172						
Cpd 18: 2-Methylpiperidine; C6 H13 N	4.037	99.1044		2-Methylpiperidine	C6 H13 N	C6 H13 N	C6 H13 N	4.48	2
Cpd 19: Albendazole sulfone; C12 H15 N3 O4 S	4.111	297.1761		Albendazole sulfone	C12 H15 N3 O4 S	C12 H15 N3 O4 S	C12 H15 N3 O4 S	7.01	2
Compound 20	4.186		58968						
Cpd 21: Trolamine; C6 H15 N O3	4.251	149.1043	140139	Trolamine	C6 H15 N O3	C6 H15 N O3	C6 H15 N O3	6.15	1
Compound 22	4.307		12280						
Cpd 23: (S)-N-[3-(3,4-Methylenedioxyphenyl)-2-(mercaptomethyl)-1-oxopropyl]glycine; C15 H18 N2 O6 S	4.533	354.0982	28725	(S)-N-[3-(3,4-Methylenedioxyphenyl)-2-(mercaptomethyl)-1-oxopropyl]glycine	C15 H18 N2 O6 S	C15 H18 N2 O6 S	C15 H18 N2 O6 S	6.84	1
Cpd 24: Bipentiav; C21 H29 N O	4.548	311.224	21170	Bipentiav	C21 H29 N O	C21 H29 N O	C21 H29 N O	-2.47	2
Cpd 25: Lisdine; C20 H17 N O7	4.582	383.1064		Lisdine	C20 H17 N O7	C20 H17 N O7	C20 H17 N O7	0.18	2
Cpd 26: 9-[3,4-Dimethoxyphenyl]-3-methoxy-1H-phenalen-1-one; C22 H18 O4	4.605	346.119		9-(3,4-Dimethoxyphenyl)-3-methoxy-1H-phenalen-1-one	C22 H18 O4	C22 H18 O4	C22 H18 O4	4.26	1
Cpd 27: Mebendizole; C16 H13 N3 O3	4.631	295.0965		Mebendizole	C16 H13 N3 O3	C16 H13 N3 O3	C16 H13 N3 O3	-3.88	1
Cpd 28: N-Chloroacetyl-2,6-diethylaniline; C12 H16 Cl N O	4.851	225.0934	76898	N-Chloroacetyl-2,6-diethylaniline	C12 H16 Cl N O	C12 H16 Cl N O	C12 H16 Cl N O	-5.9	1
Cpd 29: Macubein; C14 H16 N2 O3	4.872	260.1177	23562	Macubein	C14 H16 N2 O3	C14 H16 N2 O3	C14 H16 N2 O3	-6.08	1
Cpd 30: 3-O-Caffeoyl-4-O-methylquinic acid; C17 H20 O9	5.074	368.1109	26040	3-O-Caffeoyl-4-O-methylquinic acid	C17 H20 O9	C17 H20 O9	C17 H20 O9	-2.16	10
Cpd 31: Dolasetron; C19 H20 N2 O3	5.075	324.1481		Dolasetron	C19 H20 N2 O3	C19 H20 N2 O3	C19 H20 N2 O3	-3.32	9
Cpd 32: Ketamine; C13 H16 Cl N O	5.241	237.0925	68008	Ketamine	C13 H16 Cl N O	C13 H16 Cl N O	C13 H16 Cl N O	-1.76	1
Compound 33	5.296		29635						
Cpd 34: Manswirin; C18 H20 N2	5.316	264.1645	40630	Manswirin	C18 H20 N2	C18 H20 N2	C18 H20 N2	-6.99	2
Compound 35	5.370								
Cpd 36: Stiliamidine; C16 H16 N4	5.462	264.1451	12776	Stiliamidine	C16 H16 N4	C16 H16 N4	C16 H16 N4	-14.07	1
Compound 37	5.979		13491						
Compound 38	6.025		17615						
Compound 39	6.265		18796						
Compound 40	6.275								
Compound 41	6.358		13425						
Cpd 42: Thiexanycin; C11 H16 N2 O4 S	6.456	272.0839	31460	Thiexanycin	C11 H16 N2 O4 S	C11 H16 N2 O4 S	C11 H16 N2 O4 S	-1.96	9
Cpd 43: Scleodipsyn; C33 H24 O10	6.536	580.1313		Scleodipsyn	C33 H24 O10	C33 H24 O10	C33 H24 O10	9.73	1
Cpd 44: Nicofexanate; C20 H16 N2 O	6.542	302.1467	13867	Nicofexanate	C20 H16 N2 O	C20 H16 N2 O	C20 H16 N2 O	4.14	2
Cpd 45: Norfthiexamycin; C10 H14 N2 O4 S	6.546	258.0667	14402	Norfthiexamycin	C10 H14 N2 O4 S	C10 H14 N2 O4 S	C10 H14 N2 O4 S	2.65	6
Compound 46	6.680		41502						
Cpd 47: 3,3'-Bisergosrufone; C28 H22 O4	6.841	342.1553		3,3'-Bisergosrufone	C28 H22 O4	C28 H22 O4	C28 H22 O4	-6.34	1

APPENDIX V

HR-LCMS profiling of compounds in UPT in negative ESI mode

Compound 49	6.933		11354						
Compound 49	7.080		10588						
Compound 50	7.54		10240						
Compound 51	7.376		17215						
Compound 52	7.431		10795						
Cpd 53: 7,7-Dihydroxy-4'-methoxy-8-prenylflavan; C21 H24 O4	7.576	340.1669		7,7-Dihydroxy-4'-methoxy-8-prenylflavan	C21 H24 O4	C21 H24 O4	C21 H24 O4	-4.26	1
Compound 54	7.657		21405						
Compound 55	7.845		17996						
Compound 56	7.91								
Cpd 57: Gentamicin; C21 H43 N5 O7	8.039	477.3199		Gentamicin	C21 H43 N5 O7	C21 H43 N5 O7	C21 H43 N5 O7	-7.42	1
Compound 58	8.219		18066						
Compound 59	8.327		11569						
Compound 60	8.536		37720						
Cpd 61: 4-O-alpha-D-Galactopyranosylcalystegine B2; C13 H23 N O9	8.757	337.1361	11179	4-O-alpha-D-Galactopyranosylcalystegine B2	C13 H23 N O9	C13 H23 N O9	C13 H23 N O9	2.46	8
Compound 62	8.852		11716						
Compound 63	8.958		10999						
Cpd 64: 2-Undecyl-4(1H)-quinolinone; C20 H29 N O	9.39	299.221		2-Undecyl-4(1H)-quinolinone	C20 H29 N O	C20 H29 N O	C20 H29 N O	13.02	1
Cpd 65: N7-Acetylgentamicin; C19 H36 N4 O11	9.471	496.2457		N7-Acetylgentamicin	C19 H36 N4 O11	C19 H36 N4 O11	C19 H36 N4 O11	-7.24	3
Cpd 66: Julanine C; C29 H47 N5 O5	9.712	665.356		Julanine C	C29 H47 N5 O5	C29 H47 N5 O5	C29 H47 N5 O5	-12.4	2
Compound 67	9.809		34722						
Compound 68	10.012								
Cpd 69: Julanine A; C40 H49 N5 O6	10.424	695.376		Julanine A	C40 H49 N5 O6	C40 H49 N5 O6	C40 H49 N5 O6	-11.08	1
Compound 70	10.908								
Cpd 71: CB-108; C37 H57 N O14	11.139	739.3928		CB-108	C37 H57 N O14	C37 H57 N O14	C37 H57 N O14	-8.63	2
Cpd 72: Kuwaiguine; C20 H21 N O3	11.261	323.1557	11585	Kuwaiguine	C20 H21 N O3	C20 H21 N O3	C20 H21 N O3	-11.11	4
Compound 73	11.322		17604						
Compound 74	11.445		19905						
Compound 75	11.501		22416						
Cpd 76: Kuwaiguine; C20 H21 N O3	11.894	323.1554	17762	Kuwaiguine	C20 H21 N O3	C20 H21 N O3	C20 H21 N O3	-10.19	4
Compound 77	11.964		15435						
Compound 78	12.221								
Compound 79	12.376								
Compound 80	12.467								
Compound 81	12.644								
Compound 82	14.08								
Compound 83	14.377								
Compound 84	14.617								
Compound 85	15.216		11872						
Cpd 86: 1-Octen-3-yl primeveroside; C19 H34 O10	15.65	422.2109		1-Octen-3-yl primeveroside	C19 H34 O10	C19 H34 O10	C19 H34 O10	2.02	7
Compound 87	15.749								
Cpd 88: Nalbinolide; C26 H26 N2 O3	15.774	414.1953		Nalbinolide	C26 H26 N2 O3	C26 H26 N2 O3	C26 H26 N2 O3	-2.27	3
Cpd 89: ORG 26599; C25 H40 Cl N O3	15.793	437.2764		ORG 26599	C25 H40 Cl N O3	C25 H40 Cl N O3	C25 H40 Cl N O3	-1.7	1
Cpd 90: Octanoylglucuronide; C14 H24 O8	16.239	320.1451	32519	Octanoylglucuronide	C14 H24 O8	C14 H24 O8	C14 H24 O8	6.16	2
Cpd 91: LysoPE(24:0/0:0); C29 H60 N O7 P	16.33	565.4089		LysoPE(24:0/0:0)	C29 H60 N O7 P	C29 H60 N O7 P	C29 H60 N O7 P	2.21	3
Cpd 92: 2,6-Di-tert-butyl-4-ethylphenol; C16 H26 O	16.58	234.1944		2,6-Di-tert-butyl-4-ethylphenol	C16 H26 O	C16 H26 O	C16 H26 O	14.88	1
Compound 93	16.798								
Cpd 94: N-Desmethylclomiphen; C25	20.9	270.1474		N-Desmethylclomiphen	C25 H29 NO	C25 H29 NO	C25 H29 NO	1.29	2
Compound 95	21.736								
Compound 96	23.575								
Compound 97	24.148								
Cpd 98: Dipperamide A; C34 H38 N2 O6	24.276	570.2752	20276	Dipperamide A	C34 H38 N2 O6	C34 H38 N2 O6	C34 H38 N2 O6	-2.88	4
Compound 99	24.53								
Compound 100	24.817								

Data File	TU-BMC-CD-19_VE.d	**Sample Name**	TU-BMC-CD-19
Sample Type	Sample	**Position**	P2-B1
Instrument Name	QTOF	**User Name**	
Acq Method	Metabolite_ESI_-VE_MSMS.m	**Acquired Time**	11/8/2024 6:14:43 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

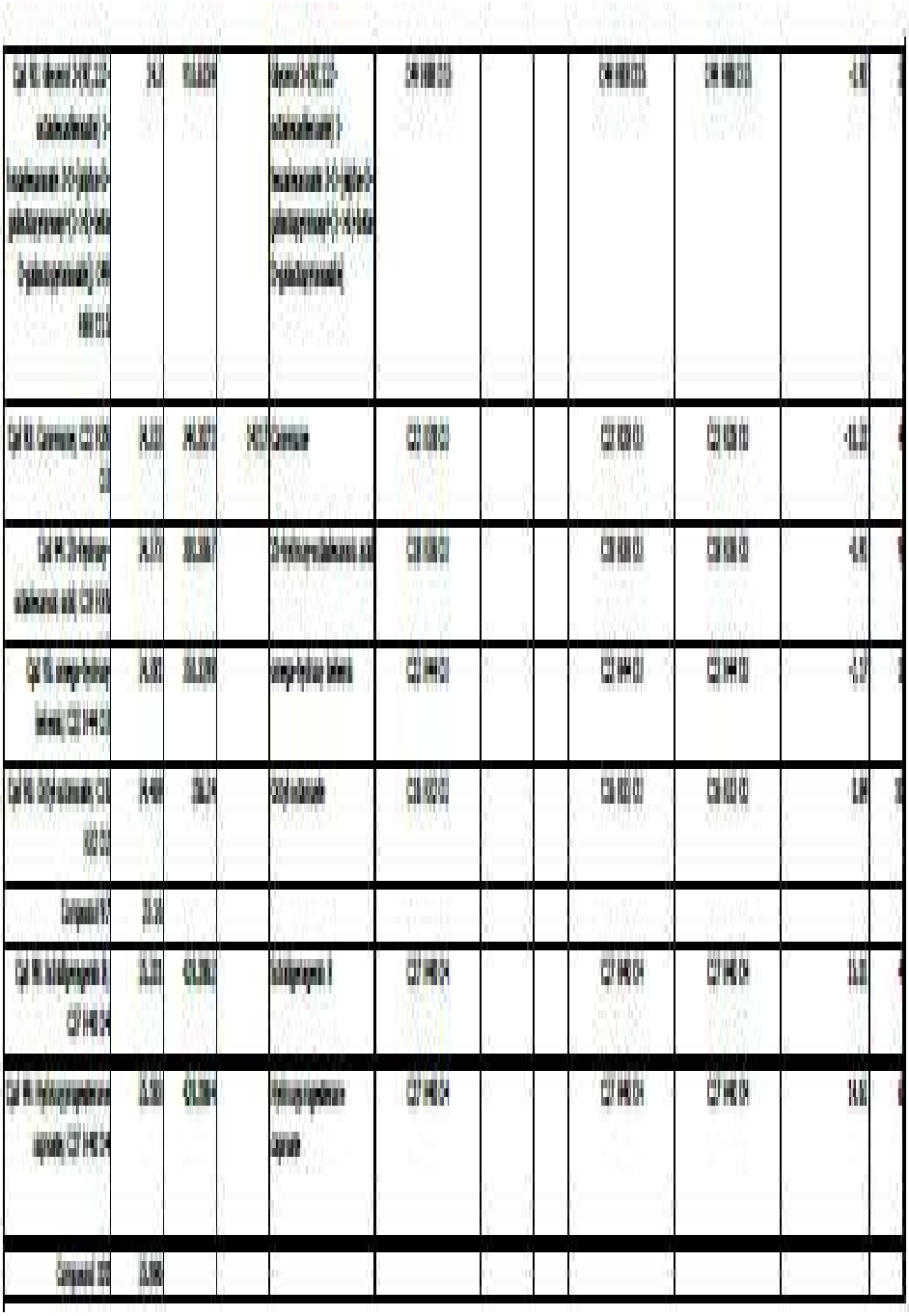
Sample Group	Info.
Acquisition SW	6200 series TOF/6530 series
Version	Q-TOF B.03.01 (B0119.2)

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula	DB Diff (ppm)	MSn (DB)
Cpd 16: 3-O-Feruloylquinic acid;	1.334	384.069		3-O-Feruloylquinic acid	C16 H18 O11			C16 H18 O11	C16 H18 O11	2.21	
Cpd 17: 2-Ethyl-O-(3-methyl-4-nitrophenyl)-methylphosphonothioate; C12 H21 N1 O4 P1 S	1.414	322.0967		O-Ethyl-O-(3-methyl-4-nitrophenyl) methylphosphonothioate	C13 H21 N1 O4 P1 S			C13 H21 N2 O4 P1 S	C13 H21 N2 O4 P1 S	-2.09	
Cpd 18: Vicianose; C11 H18 O10	1.442	310.1064		Vicianose	C11 H20 O10			C11 H20 O10	C11 H20 O10	-1.93	
Cpd 19: 3-Feruloylcatalpol; C25 H30 O13	1.446	538.1761		3-Feruloylcatalpol	C25 H30 O13			C25 H30 O13	C25 H30 O13	-14.6	
Cpd 14: Malic acid; C4 H6 O5	1.463	134.0212	699286	Malic acid	C4 H6 O5			C4 H6 O5	C4 H6 O5	2.98	
Cpd 20: L-Malic acid; C4 H6 O5	1.49	134.0212		L-Malic acid	C4 H6 O5			C4 H6 O5	C4 H6 O5	2.99	
Cpd 21: Sorbose; C6 H12 O6	1.493	180.063		Sorbose	C6 H12 O6			C6 H12 O6	C6 H12 O6	2.13	
Cpd 22: 2-Deoxy-3-keto-D-gluconic acid; C6 H10 O6	1.502	178.0474		2-Deoxy-3-keto-D-gluconic acid	C6 H10 O6			C6 H10 O6	C6 H10 O6	2.11	
Cpd 23: Dihydroferulic acid 4-sulfate; C10 H12 O7 S	1.582	276.029		Dihydroferulic acid 4-sulfate	C10 H12 O7 S			C10 H12 O7 S	C10 H12 O7 S	5.13	
Cpd 24: Sulpiride; C15 H23 N3 O4 S	1.582	322.031		Sulpiride	C15 H23 O2 P S3			C15 H23 O2 P S3	C15 H23 O2 P S3	-7.71	
Cpd 25: 3'-O-Methyl-(-)-epicatechin-5-O-sulphate; C16 H16 O7 S	1.646	352.0618		3'-O-Methyl-(-)-epicatechin-5-O-sulphate	C16 H16 O7 S			C16 H16 O7 S	C16 H16 O7 S	-8.34	
Cpd 26: Eudesmin B; C11 H7 Br N2	1.653	245.9781		Eudesmin B	C11 H7 Br N2			C11 H7 Br N2	C11 H7 Br N2	4.83	
Cpd 27: L-Malic acid; C4 H6 O5	1.77	134.0212		L-Malic acid	C4 H6 O5			C4 H6 O5	C4 H6 O5	1.95	
Cpd 28: Oxyhellebrin; C8 H19 O2 P S3	1.775	280.0257		Oxyhellebrin	C8 H19 O2 P S3			C8 H19 O2 P S3	C8 H19 O2 P S3	-8.21	
Cpd 29: Eudesmin B; C11 H7 Br N2	1.863	245.9766		Eudesmin B	C11 H7 Br N2			C11 H7 Br N2	C11 H7 Br N2	10.87	
Cpd 30: Dihydroferulic acid 4-sulfate; C10 H12 O7 S	1.889	276.0291	32066	Dihydroferulic acid 4-sulfate	C10 H12 O7 S			C10 H12 O7 S	C10 H12 O7 S	3.94	
Cpd 1: Syringic acid; C9 H10 O5	4.834	198.0524	3754	Syringic acid	C9 H10 O5			C9 H10 O5	C9 H10 O5	1.45	
Cpd 11: C9 H10 O5	4.836	198.0525	3754		C9 H10 O5	198.0528	-1.61	C9 H10 O5	C9 H10 O5		
Cpd 11: 3-Hydroxyferulate; C10 H12 O5	4.967	212.0529	11472	3-Hydroxyferulate	C10 H12 O5			C10 H12 O5	C10 H12 O5	0.94	
Cpd 12: Isovitexinoxoside; C24 H27 N5 O8	5.259	513.1976	16532	Isovitexinoxoside	C24 H27 N5 O8			C24 H27 N5 O8	C24 H27 N5 O8	0.39	
Cpd 13: Methyl 2,3-dihydro-2,5-dihydroxy-2-oxo-3-tolubenzoic acid; C11 H11 N O5	5.397	237.0639	16713	Methyl 2,3-dihydro-2,5-dihydroxy-2-oxo-3-tolubenzoic acid	C11 H11 N O5			C11 H11 N O5	C11 H11 N O5	-8.13	
Cpd 14: 2,5-dihydroxybenzoic acid; C7 H6 O4	5.521	154.0256	17131	2,5-dihydroxybenzoic acid	C7 H6 O4			C7 H6 O4	C7 H6 O4	5.71	
Cpd 15: Pinoline; C13 H20 N2 O3	5.719	240.1469	13082	Pinoline	C13 H20 N2 O3			C13 H20 N2 O3	C13 H20 N2 O3	2.14	
Cpd 16: (a)-Glycerol 1,3-diacetate; C7 H12 O5	5.835	176.0679	46279	(a)-Glycerol 1,3-diacetate	C7 H12 O5			C7 H12 O5	C7 H12 O5	3.52	
Cpd 17: Sweroside; C16 H22 O10	6.431	374.1189		Sweroside	C16 H22 O10			C16 H22 O10	C16 H22 O10	6.46	
Cpd 18: Monomethyl phthalate; C9 H8 O4	6.717	180.0419	33680	Monomethyl phthalate	C9 H8 O4			C9 H8 O4	C9 H8 O4	2.27	
Cpd 19: Quercetin 3-rhamnoside-7-glucoside; C27 H30 O16	6.803	610.1551	10276	Quercetin 3-rhamnoside-7-glucoside	C27 H30 O16			C27 H30 O16	C27 H30 O16	-2.76	
Cpd 45: Vanillin; C8 H8 O3	6.967	152.0464	13079	Vanillin	C8 H8 O3			C8 H8 O3	C8 H8 O3	6.12	
Cpd 41: Quercetin 3,7-dirhamnoside; C27 H30 O15	7.214	594.1585		Quercetin 3,7-dirhamnoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-1.62	
Cpd 42: Kaempferol 3-rhamnoside-7-rhamnoglucoside; C33 H40 O19	7.23	740.2111		Kaempferol 3-rhamnoside-7-rhamnoglucoside	C33 H40 O19			C33 H40 O19	C33 H40 O19	0.27	
Cpd 43: Kaempferol 3-rhamnoside-7-glucoside; C27 H30 O15	7.23	580.1456		Kaempferol 3-rhamnoside-7-glucoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-4.83	
Cpd 44: Isoquercitrin; C21 H20 O12	7.271	464.0955		Isoquercitrin	C21 H20 O12			C21 H20 O12	C21 H20 O12	1.29	
Cpd 45: Epiafzelechin-(4a->8)-epiafzelechin; C30 H26 O10	7.271	546.1526		Epiafzelechin-(4a->8)-epiafzelechin	C30 H26 O10			C30 H26 O10	C30 H26 O10	4.93	
Cpd 46: Kaempferol 3-rhamnoside-7-glucoside; C27 H30 O15	7.271	594.1585		Kaempferol 3-rhamnoside-7-glucoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-1.62	
Cpd 47: Quercetin 3-rhamnoside-7-glucoside; C27 H30 O16	7.271	610.1551		Quercetin 3-rhamnoside-7-glucoside	C27 H30 O16			C27 H30 O16	C27 H30 O16	-2.76	
Cpd 48: Quercetin 3,7-dirhamnoside; C27 H30 O15	7.271	594.1585		Quercetin 3,7-dirhamnoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-1.62	
Cpd 49: Luteolin 4'-O-glucoside; C21 H20 O11	7.302	448.1021		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H20 O11	C21 H20 O11	-1.38	
Cpd 50: Kaempferol 3-rhamnoside-7-xyloside; C26 H28 O14	7.486	564.1481		Kaempferol 3-rhamnoside-7-xyloside	C26 H28 O14			C26 H28 O14	C26 H28 O14	-3.9	
Cpd 51: 3-Acetyl-6-methoxybenzaldehyde; C10 H10 O3	8.29	178.0633	30193	3-Acetyl-6-methoxybenzaldehyde	C10 H10 O3			C10 H10 O3	C10 H10 O3	6.29	
Cpd 9: Caffeic acid; C9 H8 O4	8.336	180.0419	2935	Caffeic acid	C9 H8 O4			C9 H8 O4	C9 H8 O4	2.71	
Cpd 52: Genkwanin 8-C-glucoside; C21 H20 O10	8.443	432.1063	12122	Genkwanin 8-C-glucoside	C21 H20 O10			C21 H20 O10	C21 H20 O10	-2.11	

APPENDIX VI

HR-LCMS profiling of compounds in UPS in negative ESI mode



Data File	TU-EMC-CD-20.d	**Sample Name**	TU-EMC-CD-20
Sample Type	Sample	**Position**	P1-B2
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_+vE_HSMS.m	**Acquired Time**	11/7/2024 2:34:46 AM
ISH 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	Abund	Name	Formula	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
Compound 1:	1.39								
Compound 2:	1.401								
Compound 3:	1.51								
Compound 4:	1.754		85529						
Compound 5:	1.887		56763						
Compound 6:	2.115								
Cpd 7: L-prolyl-L-glycine; C7 H12 N2 O3	2.462	172.0831	43997	L-prolyl-L-glycine	C7 H12 N2 O3	C7 H12 N2 O3	C7 H12 N2 O3	9.60	1
Cpd 8: L-prolyl-L-glycine; C7 H12 N2 O3	2.767	172.0835	54088	L-prolyl-L-glycine	C7 H12 N2 O3	C7 H12 N2 O3	C7 H12 N2 O3	7.46	1
Cpd 9: Dolworon; C19 H20 N2 O3	4.754	324.1476	25898	Dolworon	C19 H20 N2 O3	C19 H20 N2 O3	C19 H20 N2 O3	-0.7	1
Cpd 10: Mitoxantrin; C38 H32 N2	5.101	284.1947	79634	Mitoxantrin	C38 H32 N2	C38 H32 N2	C38 H32 N2	-7.92	1
Cpd 11: Abscisic acid glucose ester; C21 H30 O9	5.241	426.1915		Abscisic acid glucose ester	C21 H30 O9	C21 H30 O9	C21 H30 O9	-5.82	1
Cpd 12: Stilbamidine; C18 H16 N4	5.48	284.1488	20918	Stilbamidine	C18 H16 N4	C18 H16 N4	C18 H16 N4	-12.34	1
Compound 13:	5.737								
Cpd 14: 9-Pentacosyleicosanol; C24	5.832	468.2222		9-Pentacosyleicosanol	C24 H24 O9	C24 H24 O9	C24 H24 O9	-4.16	1
Cpd 15: Stilbamidine; C18 H16 N4	6.071	284.1484	23448	Stilbamidine	C18 H16 N4	C18 H16 N4	C18 H16 N4	-12.95	1
Cpd 16: Sclerophytin; C23 H24 O10	6.227	460.1368		Sclerophytin	C23 H24 O10	C23 H24 O10	C23 H24 O10	6.95	1
Cpd 17: Sultamicillin; C25 H30 N4 O9 S2	6.338	594.1467		Sultamicillin	C25 H30 N4 O9 S2	C25 H30 N4 O9 S2	C25 H30 N4 O9 S2	-2.13	10
Cpd 18: Sclerophytin; C23 H24 O10	6.45	460.1374		Sclerophytin	C23 H24 O10	C23 H24 O10	C23 H24 O10	7.33	1
Cpd 19: 3,7-Eleangonifone; C28 H22 O4	6.548	342.1571		3,7-Eleangonifone	C28 H22 O4	C28 H22 O4	C28 H22 O4	-4.8	1
Compound 20:	6.677								
Compound 21:	6.948								
Cpd 22: 3,7-Eleangonifone; C28 H22 O4	7.024	342.1556		3,7-Eleangonifone	C28 H22 O4	C28 H22 O4	C28 H22 O4	-7.02	1
Compound 23:	7.037								
Cpd 24: 7a-Hydroxy-O-carbamoyl-deacetylcephalosporin C; C15	7.525	432.0971	51176	7a-Hydroxy-O-carbamoyl-deacetylcephalosporin C	C15 H20 N4 O9 S	C15 H26 N4 O9 S	C15 H20 N4 O9 S	-4.34	1
Cpd 25: Sultamicillin; C25 H30 N4 O9 S2	7.642	594.1467		Sultamicillin	C25 H30 N4 O9 S2	C25 H30 N4 O9 S2	C25 H30 N4 O9 S2	-2.2	1
Cpd 26: Cyclopamine; C27 H41 N O2	7.667	411.3122	34252	Cyclopamine	C27 H41 N O2	C27 H41 N O2	C27 H41 N O2	3.64	1
Cpd 27: Murrayacinine; C23 H23 N O2	7.732	345.1759		Murrayacinine	C23 H23 N O2	C23 H23 N O2	C23 H23 N O2	2.77	2
Cpd 28: 4''-Methyl-4''-(3,4-dihydroxy-5-cinnamoyl)isoorientin; C31	7.884	624.1572		4''-Methyl-4''-(3,4-dihydroxy-5-cinnamoyl)isoorientin	C31 H28 O14	C31 H28 O14	C31 H28 O14	-14.86	1
Compound 29:	7.948		42133						
Cpd 30: 11,12,14-Trihydroxy-7-methoxy-6,11,13-stemarene-20,6-olide; C21 H28 O6	8.053	376.1913	15412	11,12,14-Trihydroxy-7-methoxy-6,11,13-stemarene-20,6-olide	C21 H28 O6	C21 H28 O6	C21 H28 O6	-7.29	1
Cpd 31: 2,4,6-Trimethyl-1,3,5-trioxane; C9 H18 O3	8.173	174.1259	53108	2,4,6-Trimethyl-1,3,5-trioxane	C9 H18 O3	C9 H18 O3	C9 H18 O3	9.77	1
Cpd 32: N-[[(Ethoxycarbonyl)methyl]-pyrrolidine-3-carboxamide; C15 H27 N O3	8.189	269.1951		N-[[(Ethoxycarbonyl)methyl]-pyrrolidine-3-carboxamide	C15 H27 N O3	C15 H27 N O3	C15 H27 N O3	15.01	1
Compound 33:	8.402		33009						
Compound 34:	8.464								
Compound 35:	8.589		30834						
Cpd 36: Ginkgolide C; C20 H24 O11	8.681	440.1258		Ginkgolide C	C20 H24 O11	C20 H24 O11	C20 H24 O11	13.82	1
Cpd 37: 1-Carbazol-9-yl-3-(3,5-dimethylpyrazol-1-yl)-propan-2-ol; C20 H21 N3 O	8.868	319.1786	10548	1-Carbazol-9-yl-3-(3,5-dimethylpyrazol-1-yl)-propan-2-ol	C20 H21 N3 O	C20 H21 N3 O	C20 H21 N3 O	-6.86	1
Cpd 38: Indusamin; C22 H25 N3 O	8.994	347.2004	13472	Indusamin	C22 H25 N3 O	C22 H25 N3 O	C22 H25 N3 O	-6.18	1
Cpd 39: N7-Acetylgeranamide; C19 H35 N4 O11	9.284	496.243		N7-Acetylgeranamide	C19 H35 N4 O11	C19 H36 N4 O11	C19 H36 N4 O11	-10.05	1
Cpd 40: Julanine C; C39 H47 N5 O5	9.603	665.3589		Julanine C	C39 H47 N5 O5	C39 H47 N5 O5	C39 H47 N5 O5	-12.33	2
Cpd 41: Indusamin; C22 H25 N3 O	9.959	347.2018	40448	Indusamin	C22 H25 N3 O	C22 H25 N3 O	C22 H25 N3 O	-5.62	1
Compound 42:	10.197		9264						
Cpd 43: Julanine A; C40 H49 N5 O6	10.232	695.379		Julanine A	C40 H49 N5 O6	C40 H49 N5 O6	C40 H49 N5 O6	-13.94	1
Compound 44:	10.448								
Compound 45:	10.582		11562						

APPENDIX VII

HR-LCMS profiling of compounds in UPL in negative ESI mode

Data File	TU-EMC-CD-20_-VE.d	**Sample Name**	TU-EMC-CD-20
Sample Type	Sample	**Position**	P1-B2
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ES(-)_-VE_HSMS.m	**Acquired Time**	11/8/2024 6:30:32 AM
DA Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.10.01 (B10125.2)

Compound Table

Compound Label	RT	Mass	Absnd	Name	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 10: Malic acid; C4 H6 O5	1.476	134.0221	276358	Malic acid	C4 H6 O5			C4 H6 O5	C4 H6 O5	2.21	
Cpd 11: 7-Hydroxy-6-methyl-8-tidyl lotaxine; C12 H16 N4 O7	1.511	328.1018		7-Hydroxy-6-methyl-8-tidyl lotaxine	C12 H16 N4 O7			C12 H16 N4 O7	C12 H16 N4 O7	0.43	
Cpd 1: C7 H6 O2	5.212	121.0284	826		C7 H6 O2	122.0368	-1.86	C7 H6 O2	C7 H6 O2		
Cpd 9: C7 H6 O2	5.212	121.0284	826		C7 H6 O2	122.0368	-1.86	C7 H6 O2	C7 H6 O2		
Cpd 12: C9 H16 O4	5.33	189.1041	8259		C9 H16 O4	188.1049	-0.93	C9 H16 O4	C9 H16 O4		
Cpd 14: Yunnanpseudolone; C28 H31 N9 O10 S2	5.711	737.1717		Yunnanpseudolone	C28 H31 N9 O10 S2			C28 H31 N9 O10 S2	C28 H31 N9 O10 S2	9.1	
Compound 15	5.746										
Cpd 16: Quercetin 3-rhamnoside-7-glucoside; C27 H30 O16	6.734	609.1512		Quercetin 3-rhamnoside-7-glucoside	C27 H30 O16			C27 H30 O16	C27 H30 O16	-2.96	
Cpd 17: Kudzusaide; C26 H28 O13	7.074	548.146		Kudzusaide	C26 H28 O13			C26 H28 O13	C26 H28 O13	-0.3	
Cpd 18: Quercetin 3,7-dirhamnoside; C27 H30 O15	7.139	594.1616		Quercetin 3,7-dirhamnoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-5.29	
Cpd 19: Congo red; C32 H24 N6 O6 S2	7.221	652.1189		Congo red	C32 H24 N6 O6 S2			C32 H24 N6 O6 S2	C32 H24 N6 O6 S2	1.2	
Cpd 20: Ephedrannin A; C30 H20 O11	7.264	556.0988		Ephedrannin A	C30 H20 O11			C30 H20 O11	C30 H20 O11	3.22	
Cpd 21: Chesnutin; C27 H30 O18	7.271	626.1317		Chesnutin	C27 H30 O18			C27 H30 O18	C27 H30 O18	15.97	
Cpd 23: Diosmetin 7-O-beta-D-glucuronopyranoside; C22 H20 O12	7.376	476.0969		Diosmetin 7-O-beta-D-glucuronopyranoside	C22 H20 O12			C22 H20 O12	C22 H20 O12	-0.96	
Compound 23	7.374										
Cpd 24: Kudzusaide; C26 H28 O13	7.442	548.146		Kudzusaide	C26 H28 O13			C26 H28 O13	C26 H28 O13	-5.49	
Cpd 25: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	7.444	564.151		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H28 O14	C26 H28 O14	-5.41	
Cpd 26: Ephedrannin A; C30 H20 O11	7.531	556.0988		Ephedrannin A	C30 H20 O11			C30 H20 O11	C30 H20 O11	3.51	
Cpd 27: Genistein 4',7-O-diglucuronide; C27 H26 O17	7.619	622.1073		Genistein 4',7-O-diglucuronide	C27 H26 O17			C27 H26 O17	C27 H26 O17	15.23	
Compound 28	7.636										
Compound 29	7.67										
Compound 30	7.676										
Compound 31	7.693										
Compound 32	7.703										
Cpd 33: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	7.731	564.151		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H28 O14	C26 H28 O14	-5.41	
Cpd 34: Quercetin 3,7-dirhamnoside; C27 H30 O15	7.842	594.1616		Quercetin 3,7-dirhamnoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-5.37	
Compound 35	7.874										
Cpd 36: Luteolin 4'-O-glucoside; C21 H20 O11	7.891	448.1028		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H20 O11	C21 H20 O11	-5.09	
Compound 37	7.92										
Cpd 38: Genistein 4',7-O-diglucuronide; C27 H26 O17	7.943	622.1074		Genistein 4',7-O-diglucuronide	C27 H26 O17			C27 H26 O17	C27 H26 O17	15.47	
Cpd 39: Diosmetin 7-O-beta-D-glucuronopyranoside; C22 H20 O12	7.957	476.0971		Diosmetin 7-O-beta-D-glucuronopyranoside	C22 H20 O12			C22 H20 O12	C22 H20 O12	-0.77	
Cpd 40: Quercetin 3,7-dirhamnoside; C27 H30 O15	8.288	594.1616		Quercetin 3,7-dirhamnoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-5.37	
Cpd 41: Quercetin 3-rhamnoside-7-glucoside; C27 H30 O16	8.293	610.1565		Quercetin 3-rhamnoside-7-glucoside	C27 H30 O16			C27 H30 O16	C27 H30 O16	-5.99	
Cpd 42: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	8.296	564.151		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H28 O14	C26 H28 O14	-5.42	
Cpd 43: Isoquercitrin; C27 H30 O16	8.303	578.1669		Isoquercitrin	C27 H30 O16			C27 H30 O16	C27 H30 O16	5.43	
Cpd 44: Luteolin 4'-O-glucoside; C21 H20 O11	8.221	448.1027		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H20 O11	C21 H20 O11	-5.63	
Cpd 45: Genistein 8-C-glucoside; C21 H20 O10	8.435	432.1077		Genistein 8-C-glucoside	C21 H20 O10			C21 H20 O10	C21 H20 O10	-4.85	
Cpd 46: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	8.437	564.1467		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H28 O14	C26 H28 O14	-3.19	
Cpd 47: Quercetin 3,7-dirhamnoside; C27 H30 O15	8.544	594.1613		Quercetin 3,7-dirhamnoside	C27 H30 O15			C27 H30 O15	C27 H30 O15	-4.94	
Cpd 48: Daidzein 4',7-diglucoside; C27 H30 O14	8.554	578.1638		Daidzein 4',7-diglucoside	C27 H30 O14			C27 H30 O14	C27 H30 O14	-0.48	
Cpd 49: CMP-9-glycolylneuraminate; C20 H31 N4 O17 P	8.571	630.1362		CMP-9-glycolylneuraminate	C20 H31 N4 O17 P			C20 H31 N4 O17 P	C20 H31 N4 O17 P	9.53	
Cpd 50: Genistein 8-C-glucoside; C21 H20 O10	8.661	432.1063	46048	Genistein 8-C-glucoside	C21 H20 O10			C21 H20 O10	C21 H20 O10	-2.59	
Cpd 51: Luteolin 4'-O-glucoside; C21 H20 O11	8.742	448.1028		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H20 O11	C21 H20 O11	-3.76	
Cpd 52: Isoorientin; C21 H20 O11	8.79	462.1182	53082	Isoorientin	C21 H20 O11			C21 H20 O11	C21 H20 O11	-6.92	

APPENDIX VIII
PREPARATIONS

Total Phenolic content

Preparation of 10% Sodium Carbonate

10% Sodium carbonate was prepared by dissolving the 10 gram of sodium carbonate in

100 ml of distilled water.

Preparation of 10% Folin-Ciocalteu Reagent

10ml FCR was diluted in 80ml of distilled water.

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 (5, 10, 20, 40, 80, 100)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 Quercetin Solution

Stock solution of Quercetin was made in methanol by dissolving 10 mg of rutin in 10 ml

methanol (i.e., 1mg/ml). The final concentrations were adjusted in

(10, 20, 40,80) µg/mLby 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 & 1M potassium acetate

10% Aluminum trichloride hexahydrate (AlCl3.6 H2O) was prepared by dissolving 10

gram of solid mass into the 100 ml of distilled water. Similarly, 1M potassium acetate was prepared by dissolving 9.815g pot. Acetate into 100ml distilled water.

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 sulfuric 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 10 mg of ascorbic acid in 10ml methanol (i.e., 1mg/ml). The final concentrations were adjusted in (5, 10, 20, 40, 80, 100) µg/mL by doing two-fold dilution from the stock solution

DPPH free radical scavenging assay

Preparation of 0.004% DPPH solution

0.004% DPPH solution was mutinously prepared by dissolving 20mg of DPPH in 500 ml of volumetric flask, which was properly shielded with aluminum foil (i.e., DPPH is light sensitive).

Preparation of Butylated Hydroxy Anisole (BHA) solution

Weight out 10 mg of BHA in the 10 ml of methanol solution to make 1mg/ml of stock solution. Which was further diluted to prepare (1, 2, 3, 4, 5, 10, 20, 40, 80) µg/mL working solutions.

Preparation of plant extracts solution

Dissolve 25mg in 25ml 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 Media

45.6g of Mueller Hinton Agar was dissolved completely in 1200ml Distilled water and stirred with autoclaved glass rod. Then the mixture was transferred equally into three 500ml conical flasks. The agar mixture was then autoclaved at 121^0C for 20 minutes. The pressure of autoclave was 15atm. Thus, prepared agar solution was left for few minutes under cooling.

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वनस्पति विभाग

राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशाला

गोदावरी, ललितपुर

पत्र संख्या: ०८०।०८९
च न १९२४

मिति २०८०/०६/२६

विषय: नमूना पहिचान सम्वन्धमा।

श्री बीरेन्द्र बहुमुखी क्याम्पस,
भरतपुर, चितवन।

प्रस्तुत विषयमा तहाँ क्यापसको प.सं. ०८०/०८१, च.नं. ६४४, मिति २०८०/०६/२१ को पत्र साथ वनस्पतिको नमूना प्राप्त भई व्यहोरा अवगत भयो। पत्र मार्फत ल्याइएको नमूनाको पहिचान गरी प्राविधिक विशेषज्ञको प्रतिवेदन (पाना १) यसै पत्रसाथ संलग्न गरी पठाइएको व्यहोरा अनुरोध छ।

२०८०।६।२६

सुभाष खत्री
वरिष्ठ अनुसन्धान अधिकृत
(१६३६८१)
कार्यालय प्रमुख

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

प्राविधिक विश्लेषणको प्रतिवेदन

१. नमूना परिक्षण गर्न पठाउने व्यक्ति/निकायः- श्रीमती ब्रह्ममुखी क्याम्पस, भरतपुर, चितवन।

१(क) विद्यार्थीहरुको नामः- श्री अजय महतो (त्रि.वि. दर्ता नं. ५-२-१९-५७७-२०११)

२. प्राप्त नमूनाको विवरणः- वनस्पतिको नमूना थान-१

३. यस कार्यालयमा प्राप्त मितिः- २०८०/०६/२५

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

५. पहिचान प्रतिवेदनः- प्राप्त नमूनाको Morphological अध्ययन तथा यस राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशालाको हर्बेरियममा राखिएका नमूनाहरु संगको तुलनात्मक अध्ययन गर्दा उक्त नमूनाहरु निम्नानुसार भएको पहिचान हुन गएको।

S.N	Scientific Name	Family	Remarks
1	*Colocasia esculenta* (L.) Schott	Araceae	

६. परिक्षण गर्ने अधिकारीः-

रिता क्षेत्री
अनुसन्धान अधिकृत
(१९८२००)

PLAGARISM REPORT

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY

☎ : 056 { 493253 / 493689 / 493159

वीरेन्द्र बहुमुखी क्याम्पस

BIRENDRA MULTIPLE CAMPUS

भरतपुर, चितवन
Bharatpur, Chitwan

पत्रसंख्या :
च. नं.(Ref.) :

मिति : २०८१।१०।२०
Date :

जो जस सँग सम्वन्धित छ ।

तपाई AJAYA MAHATO ले यस पुस्तकालयमा प्लेजारिजम परीक्षण गर्नका लागि हार्डकपी र सफ्टकपीको विषय वस्तुमा कुनै फरक छैन भनी स्वघोषणा गरी पेनड्राइभ/ईमेल मार्फत विभागमा पेश गर्नुभएकोले विभागबाट इमेल मार्फत प्राप्त सफ्टकपी NUTRIENT ELEMENTS AND HR-LCMS ANALYSIS OF SECONDARY METABOLITES PRESENT IN METHANOLIC EXTRACTS OF COCOYAM *Colocasia esculenta (l.) schott)* शिर्षकको **M.Sc. in CHEMISTRY** तहको उपाधिका लागि तयार गरिएको Dissertation / Thesis मा प्लेजारिजम परीक्षण पछिको समानता सूची (Similarity Index) १०(दश) प्रतिशत रहेको व्यहोरा प्रमाणित गरिन्छ ।

.....
महेन्द्रप्रसाद अधिकारी
पुस्तकालय प्रमुख

Library LP_4

NUTRIENT ELEMENTS AND HR-LCMS ANALYSIS OF SECON...

By: AJAYA MAHATO

As of: Feb 2, 2025 4:17:58 PM
9,033 words - 70 matches - 55 sources

Similarity Index
10%

Mode: Summary Report

sources:

58 words / 1% - Internet from 05-Jan-2023 12:00AM
www.researchgate.net

52 words / 1% - Internet from 08-Feb-2023 12:00AM
www.researchgate.net

48 words / 1% - Internet from 21-May-2022 12:00AM
www.researchgate.net

73 words / 1% - Internet from 15-Mar-2023 12:00AM
physc.sgtuniversity.ac.in

14 words / < 1% match - Internet from 03-Feb-2023 12:00AM
www.researchgate.net

11 words / < 1% match - Internet from 30-Jan-2023 12:00AM
www.researchgate.net

9 words / < 1% match - Internet from 28-Jan-2023 12:00AM
www.researchgate.net

38 words / < 1% match - Internet from 18-Feb-2024 12:00AM
elibrary.tucl.edu.np

33 words / < 1% match - Internet from 18-Jan-2024 12:00AM
elibrary.tucl.edu.np

9 words / < 1% match - Internet from 02-Feb-2024 12:00AM
elibrary.tucl.edu.np

30 words / < 1% match - Internet from 03-Dec-2021 12:00AM
www.pubfacts.com