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

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

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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.

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.....

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

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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 organs, i.e. tuber, petiole and leaves of plants were utilized for antioxidants activities using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and phosphomolybdate assay (TAA). Total phenolic (TPC), total flavonoid (TFC) and total carbohydrate contents (TCC) were analyzed using spectrophotometer. The antimicrobial susceptibility test was conducted on seven microorganisms i.e. *E. coli, K. pneumonia, P. aeruginosa, B. subtilis, S. aureus, A. baumannii* and *S. typhispecies*. The ZOI was evaluated as:*A. baumannii*(18.60 ±1.15 mm), *B. subtilis*(26.30±4.04 mm), *E. coli* (14.33 ±0.57 mm), *S. aureus*(14.00±1.00 mm), *K. pneumonia*(16.00±1.00 mm) respectively.Nutrient elements and toxic metallic element quantificationand secondary metabolites analysis of the extract of underground and above ground organs (i.e. tuber, petiole and leaves) were carried out using ICPAES and HR-LCMS respectively.

IC_{50} value of extracts lied between 240.45-1018.44 µg/mL.The amount of TAA using phosphomolybdate assay in the samples were found1.25±0.01 to 28.29±0.01mg AAE/g DS.The analysis of TPC in different sampleswere evaluated between 6.38±0.00 and 19.96±0.01 mg GAE/g DS.The total amount of TFC was in the range of 10.46±0.00 and 89.10±0.00 mg QE/g DS. The overall amount of TCC in the samples remainedin between 1.78±0.00 and 112.00±0.00mg GE/g DS. Concentrations of nutrient elements in tuber, petiole and leaves were foundin the order as:**Ca** (8910.00, 303.00, 355.00 mg/Kg), **Cr** (0.47, 0.25, 0.42 mg/Kg), **Cu** (1.22, 0.67, 0.72 mg/Kg), **Fe** (15.60, 1.24, 2.41 mg/Kg), **Mg** (96.80, 43.60, 104.00 mg/Kg), **Zn** (128.00, 0.68, 1.94mg/Kg) andtoxic elements**As**(15.70, 9.80, 65.60 mg/Kg) whereas the concentration of **Pb** was below the detection limit(1ppb).

HR-LCMS analysis of the methanolic extracts of tuber, petiole and leaf of *Colocasia esculenta* revealed large number of phytochemical constituents.The tuber extract contained 200 different molecules out of which 140 remained unidentified, while 60 were found listed in the HR-LCMS library. The identified compounds comprised 11 alkaloids, 4 phenolic compounds, 5 flavonoids, 3 glycosides, 5 steroids, and 32 other miscellaneous compounds.

Similarly, the extract of petiole also contained 200 different plant secondary metabolites, including 85 unidentified and 115 identified constituents. Among the identified compounds, 12 were alkaloids, 15 phenolic compounds, 26 flavonoids, 9 glycosides, and 53 other compounds.

Likewise, in the leaf extract, a total of 197 compounds were detected, among them 106 were unidentified and 91 were identified. The identified constituents contained 13 alkaloids, 17 flavonoids, 3 terpenoids, 1 steroid, 12 pharmaceutical drugs, and 45 other compounds.

Notably, several potentially bioactive compounds were detected across the extracts, including neuraminic acid, amprenavir, butin, catechin, cyclopamine, disopyramide, funtumine, gentamycin, jervine, kaempferol, ketamine, retapamulin, swertiamarin, syringic acid, and usambarensine, among others. These compounds are known to possess diverse pharmacological activities, highlighting the therapeutic potential of *Colocasia esculenta*.

***Key words*:** Cocoyam, Phytochemical screening, Antioxidant activity, Antimicrobial Activity, DPPH, HR-LCMS

सारांश

कोलोकेसिया इस्कुलेन्टा (एल.)स्कट (कर्कलो) को पौष्टिक तत्व र HR-LCMSविश्लेशण अझै स्पष्ट रुपमा बुझ्न सकिएको छैन । पालुङ्ग, उपरदाङगढी र पदमपुरबाट सर्कलित 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- LCMSविश्लेशण 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 शव्दहरुः कोकोयाम (कर्कलो), फाइटोकेमिकल स्किनिङ्ग,एन्टिअक्सिडेन्ट गतिविधी एन्टिमाइक्रोवियलगतिविधी,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

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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.Cocoyamis 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

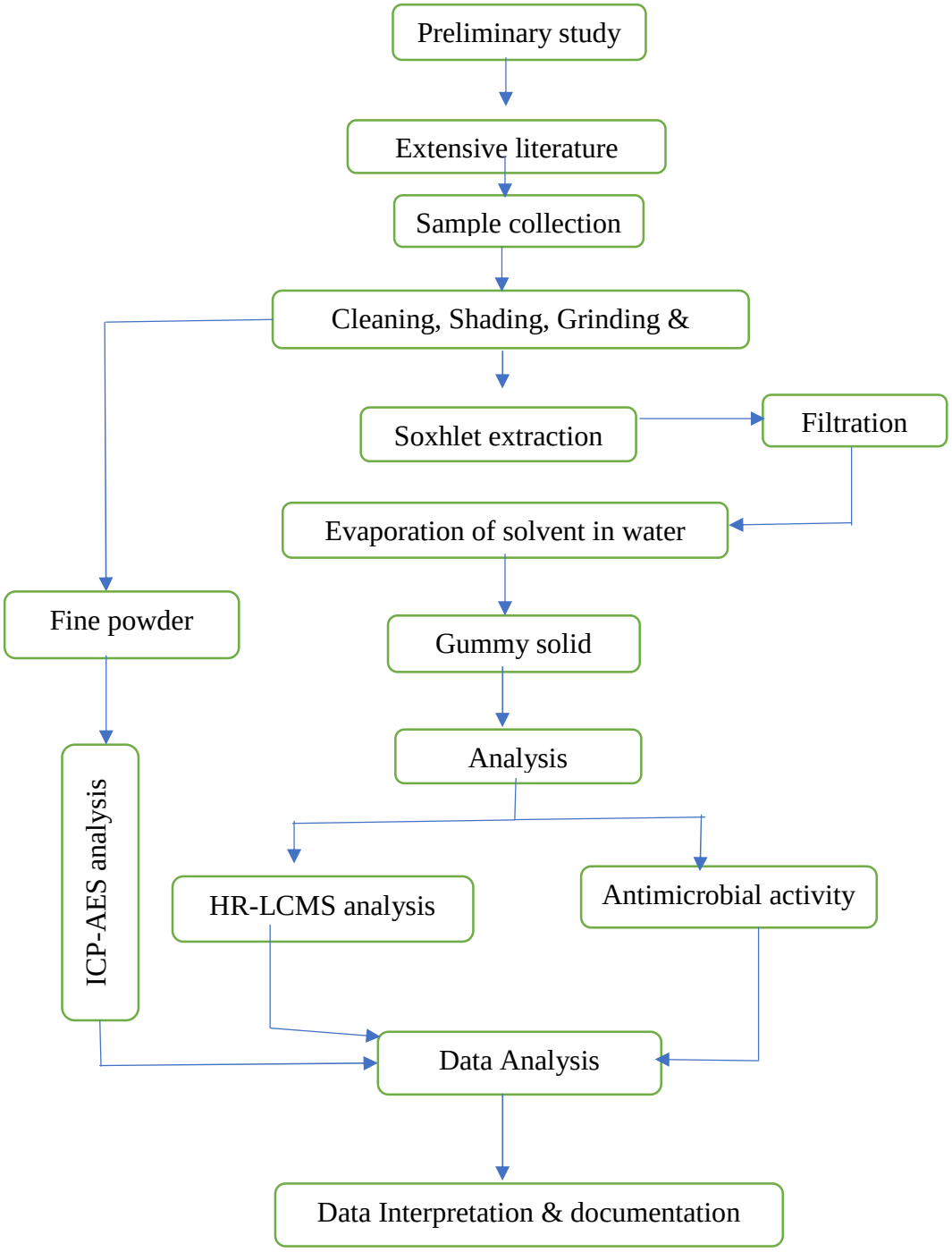
The dried gummy mass of extract was stored in an amber colored glass bottle, air tightly, wrapped with thin parafilm. A portion of samples were sent for HRLCMS profiling to Center for Research in Nanotechnology and science (CRNTS), Sophisticated Analytical Instrument Facility (SAIF), Indian Institute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

Percentage yields of methanolic extracts

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

$$\% \text{ yield} = \frac{w \quad ht\,o \quad c \quad e}{w \quad ht\,o \quad the\,p \quad sa \quad t} \times 100$$

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 wascarriedout by agar well diffusion protocol. The potency of plant extracts against bacterialactivity was ascertained zone of inhibition. (Kuper et al., 2012). Clinical

laboratories standardinstitute 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 flowto 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 minutesto cool. Thus, cooled agar plates were left under refrigeration for 24 hrs. The refrigeratedagar 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 andorganisms. The turbid solution of micro-organism was prepared using normal salinepurchased from a local pharmacy, in different test-tubes for different micro-organisms.The turbidity of bacterial solution was finalized by comparing with 0.5 McFarlandstandard. A sterile cotton swab was immersed in the prepared inoculum and any surplusinoculum was removed by gently pressing and rotating the swab against the upper innerwall of test-tube, just above the liquid level. After each swabbing, the plates were rotatedat 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 flowwith covered lids. A sterile corks borer having 7mm of diameter was employed to punchthe wall on the dried agar plate and marked accurately.80µl plant stock solution wasgently loaded into respective wells

with the help of micropipette.The solvent (10&DMSO) was used as negative control while different antibiotics were placed as positivecontrol.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 anincubator at 37^0C for 24 hours for appropriate incubation. The clear zone of inhibitionaround each well in the agar plates were examined and measured using ruler.The averagevalue of zone of inhibition was presented to disclose the strength of plant extract againstpathogenic 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 withslight modification by Lister and Wilson (2001) (Zahin etal., 2009). Briefly, to 0.5ml ofeach 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 andincubated 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-VISspectrophotometer (T80+, PG Instrument, UK). The total phenolic content was expressedin milligrams of gallic acid equivalent (mgGAE/g DS) on the reference of calibrationcurve 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 phenoliccontent was calculated using formula;

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

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 wasdetermined from the equation of straight line.

Statistical analysis

The average absorbance from triplicate measurement of each concentration was evaluatedwhich 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 regressionequation.

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 werepreprared from stock solution and used for calibration. 1.5ml of 80% methanol was addedto standards and samples (0.5ml) followed by addition of 100µl $AlCl_3$, 100µl Potassiumacetate and 2.8ml distilled water with vigorous shaking and left for incubation at 30^0C for30 minutes protected under light free condition. After incubation the absorbance wasmeasured at 410nm using UV-VIS spectrophotometer (T80+, PG Instrument, UK). Thetotal 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 quantifiedusing modified sulfuric acid-phenol method using glucose dextrose as standard (Masukoet at., 2005). In brief, 500µL of standard and samples were taken in cleaned with distilledwater and oven dried test-tubes. 1500µL of conc. H_2SO_4 was added to each test-tubes andshaken up to 30 minutes followed by addition of 300µL 5% phenol. The entire mixturewas 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 recordedat 490nm using UV-VIS spectrophotometer. An equation of regression line from standardcalibration was implemented to quantify the total carbohydrates (TCC) and expressed asmilligrams 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 oftotalcarbohydrates 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 antioxidantcapacity of samples using ascorbic acid as standard (Jie et al.,2011). In brief, 1ml ofextract and standard were separately mixed with 4ml of phosphomolybdenum reagent.The phosphomolybdenum reagent was prepared by mixing equal volumes of 0.6MH_2SO_4, 28mM Na_3PO_4 and 4mM ammonium molybdate. The mixture of extract andphosphomolybdenum reagent was the incubated at 95^0C for 90 minutes and cooled toroom temperature. After cooling, the absorbance having λmax at 695nm usingspectrophotometer (T80+, PG Instrument, UK). The total antioxidant capacity of sampleextract 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 theestimation 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 byDPPH free radical scavenging assay (Islam et. at, 2015). 1ml of varying concentrationsof ascorbic acid standard and sample extracts were mixed with 2ml of 0.004% ofmethanolic DPPH and incubated at room temperature for 30 minutes in dark. Afterincubation, the absorbance of standard and samples were measured at 517nm using UVVIS spectrometer. A blank of 1ml methanol and 2ml 0.004% methanolic DPPH was usedas control.

Measurement of DPPH free radical scavenging activity:

The following formula was implemented to estimate the free radical scavengingactivityof samples.

$$\text{Percentage scavenging (\%RSA)} = \frac{A \quad o \; c_{\cdot} \quad -a \quad o \; sa \quad b_{\cdot}}{A \quad o \; c_{\cdot}} \times 100$$

Solutions of varying concentrations of ascorbic acid as standard was used to evaluate the50% 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 Paarmicrowave go) using a mixture of 2ml conc. HCl, 1ml conc. HNO_3 and2ml 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

Theyield of samples of *Colocasia esculenta(L.) Schott.* after shade drying under wellventillated condition is tabulated below.

Table 1 : % yield of samples after drying

SN	SAMPLES CODES	WET WEIGHTS	DRY WEIGHTS	% YIELDS
1.	PDS	106.00gm	20.22gm	19.07%
2.	PDL	93.00gm	19.99gm	21.50%
3.	PDT	440.00gm	91.53gm	20.80%
4.	UPS	100.00gm	13.00gm	13.00%
5.	UPL	92.00gm	22.95gm	24.95%
6.	UPT	690.00gm	184.82gm	26.78%
7.	PS	220.00gm	37.00gm	16.81%
8.	PL	80.00gm	19.71 gm	24.64%
9.	PT	795.00gm	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 *Colocasiaesculenta (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	10.02gm	0.94gm	9.45%
2.	PDL	10.00gm	1.12gm	11.27%
3.	PDT	25.03gm	3.51gm	14.02%
4.	UPS	10.02gm	1.18gm	11.83%
5.	UPL	10.01gm	10.01gm	6.29%
6.	UPT	45.01gm	2.42gm	5.37%
7.	PS	15.16gm	4.38gm	28.93%
8.	PL	10.01gm	1.93gm	19.34%
9.	PT	30.02gm	3.05gm	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:

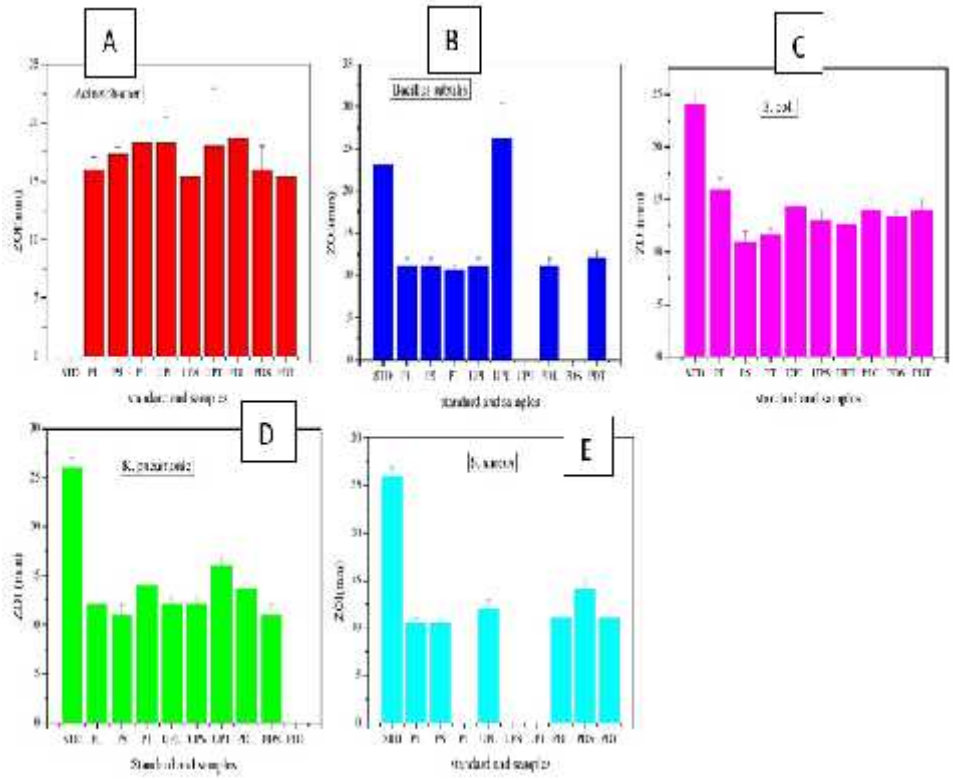


Figure 1 : Antimicrobial susceptibility test of plant extracts against: **A**: Acenitobacter using varieties of antibiotics (Amikacin 30mcg, Ciprofloxacillin 5mcg, Ampicillin 10mcg, Gentamycin 10mcg, Ceftriaxone 30mcg, Ceftazidime 30mcg, Ceftazidime-avibactum 30mcg, Imipenum 10mcg, and Ceftazidime/ clavulanic 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:

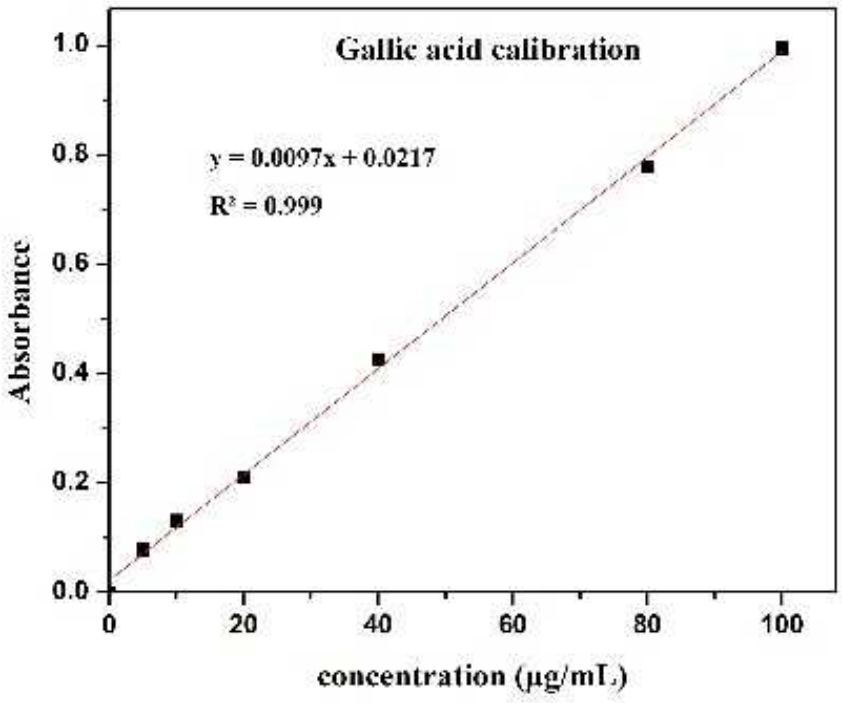


Figure 2 : Calibration curve of gallic acid to determine total phenolic content]

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 compoundswhereas the petiole extract fromPadampurwas contained with lowerconcentrations of phenolic compounds.

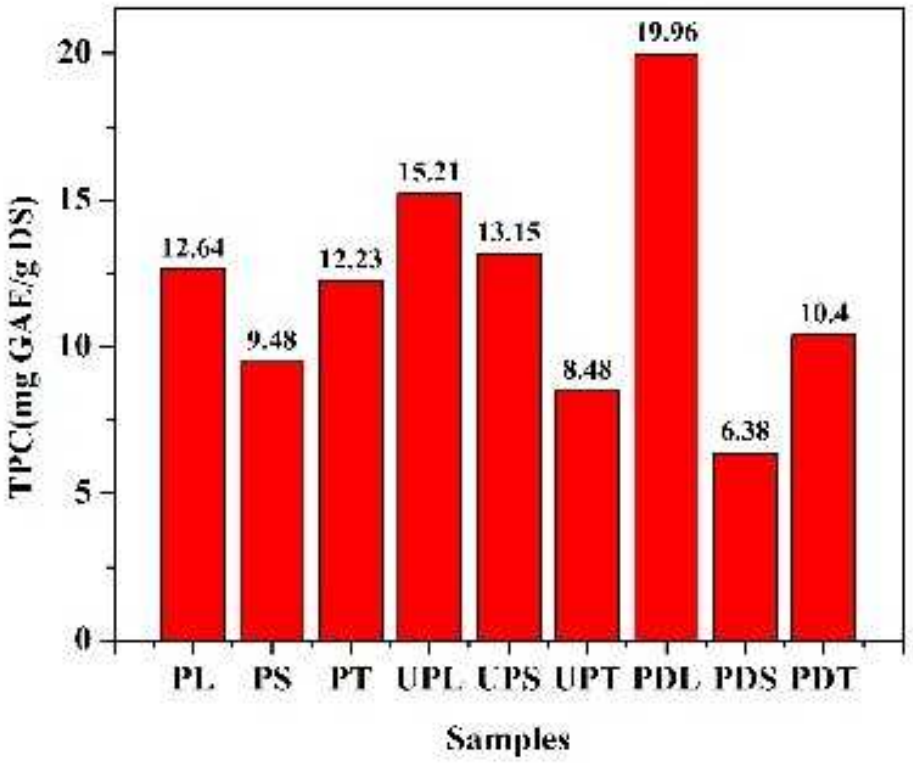


Figure 3 :*Total phenolic contents of samples*

4.5Estimation 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.

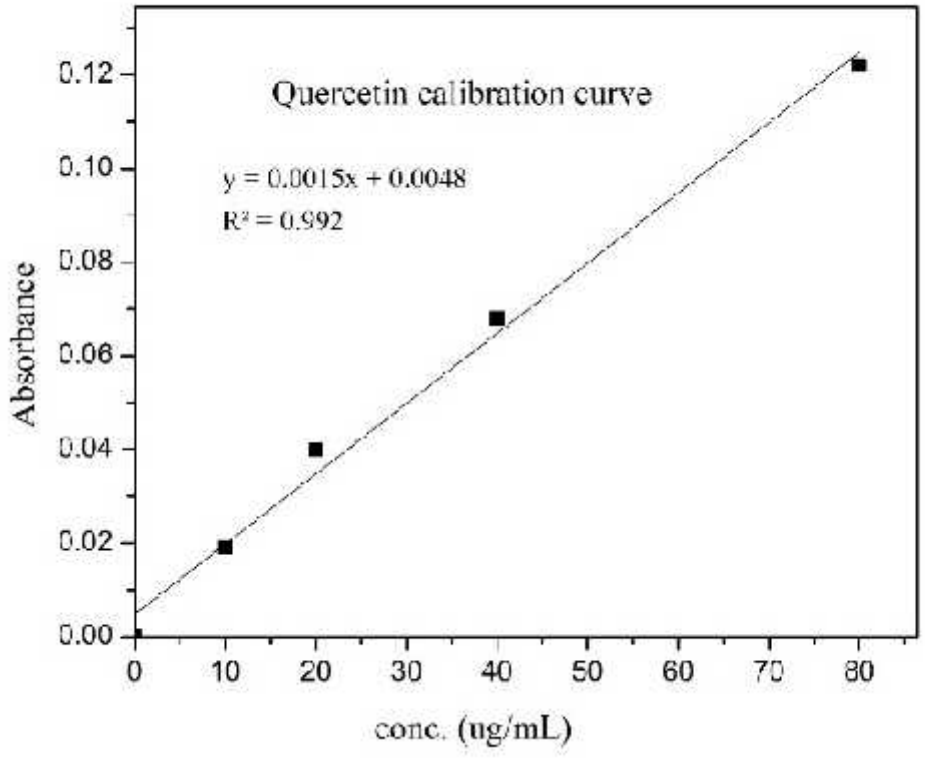


Figure 4 : Calibration curve of Quercetin to evaluate total flavonoid content

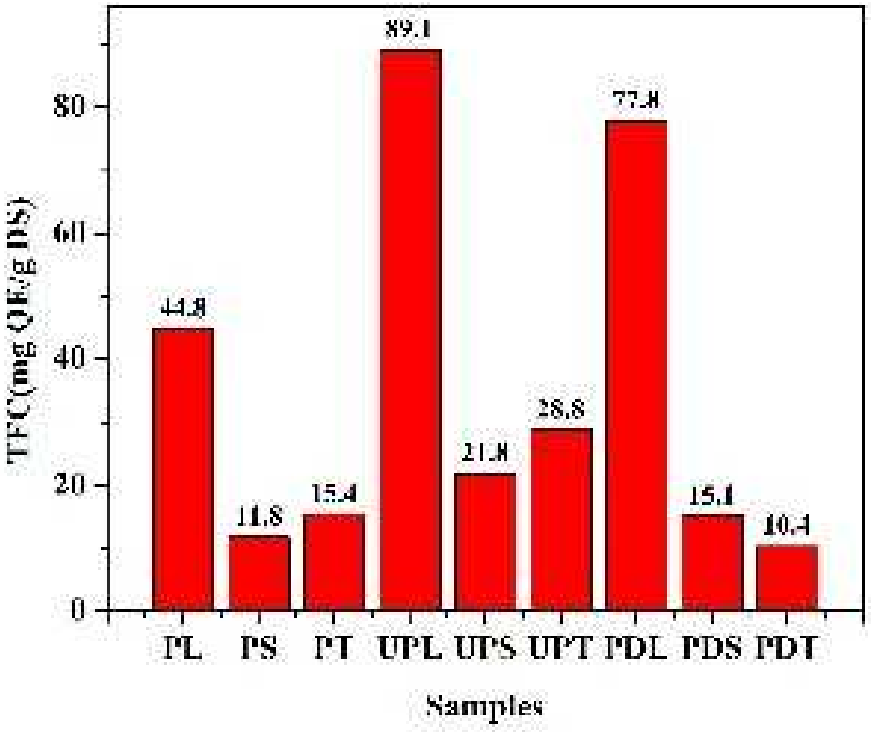


Figure 5 :*Total flavonoid contents of samples*

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 (Masukoetat., 2005). Different concentrations of glucose dextrose (30ppm,60ppm,90ppm,120ppm,150ppm) 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.

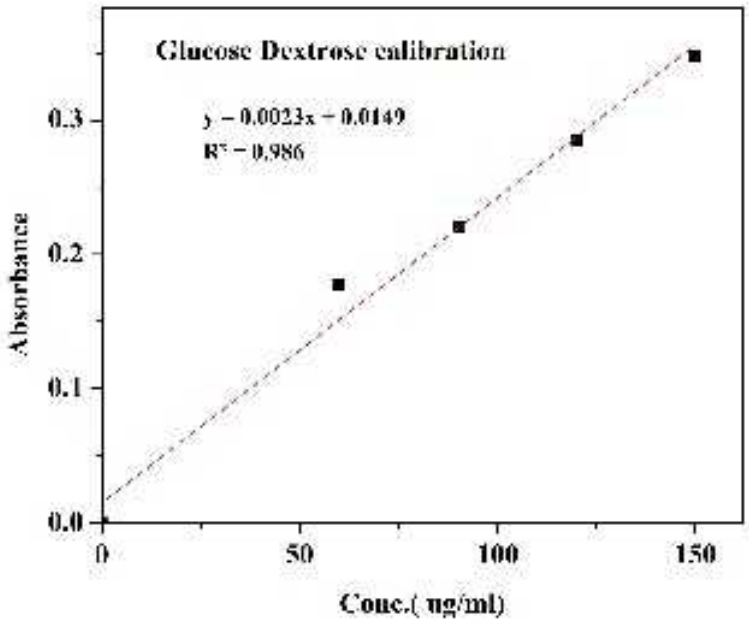


Figure 6 : Calibration curve of glucose dextrose to calculate total carbohydrates content

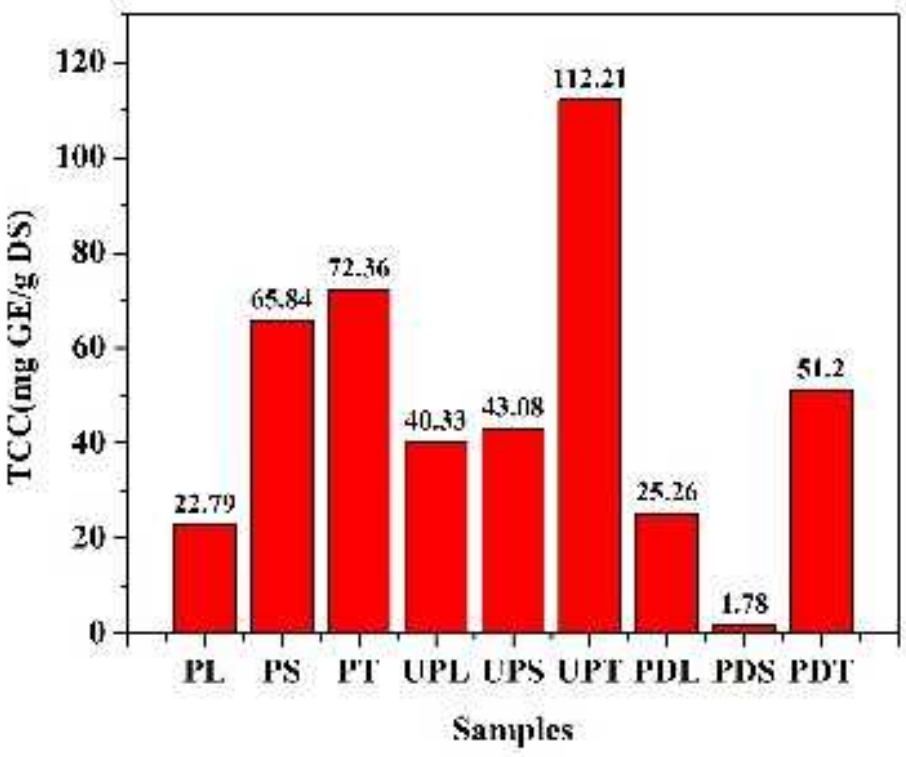


Figure 7 : Total carbohydrates contents of samples

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.

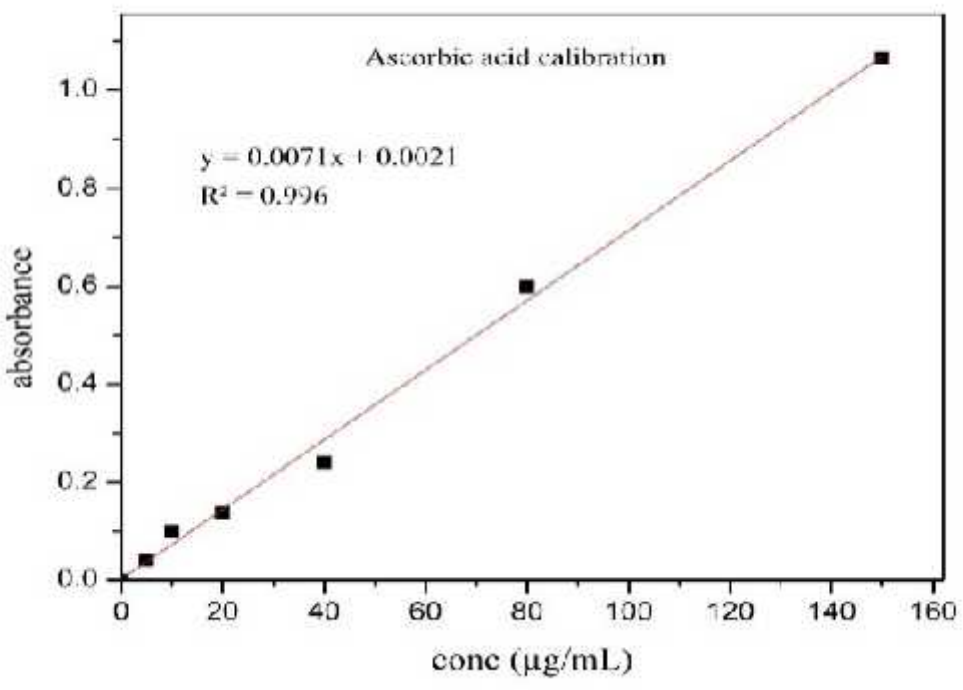


Figure 8 : Calibration curve of ascorbic acid for phosphomolybdate assay

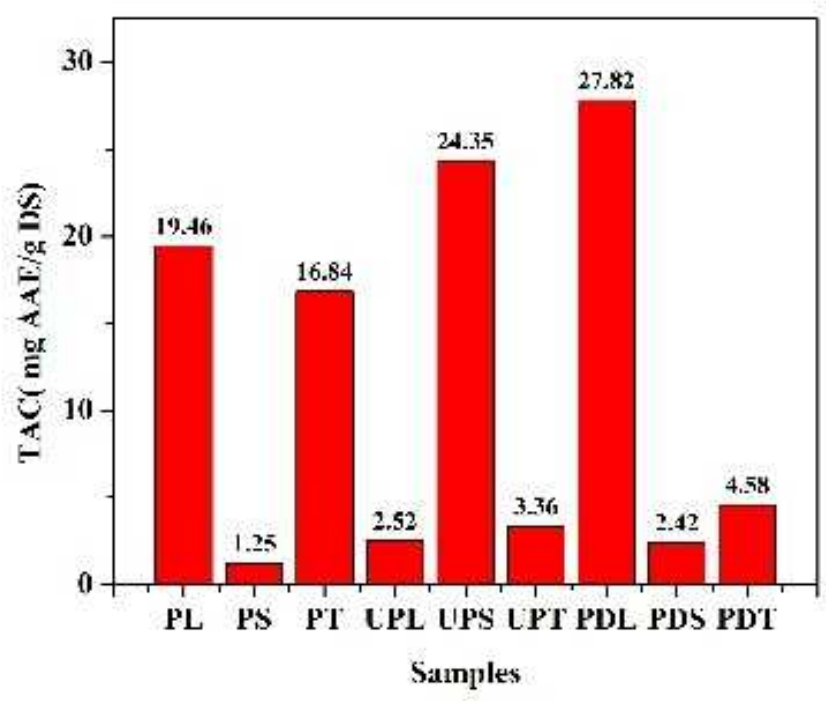
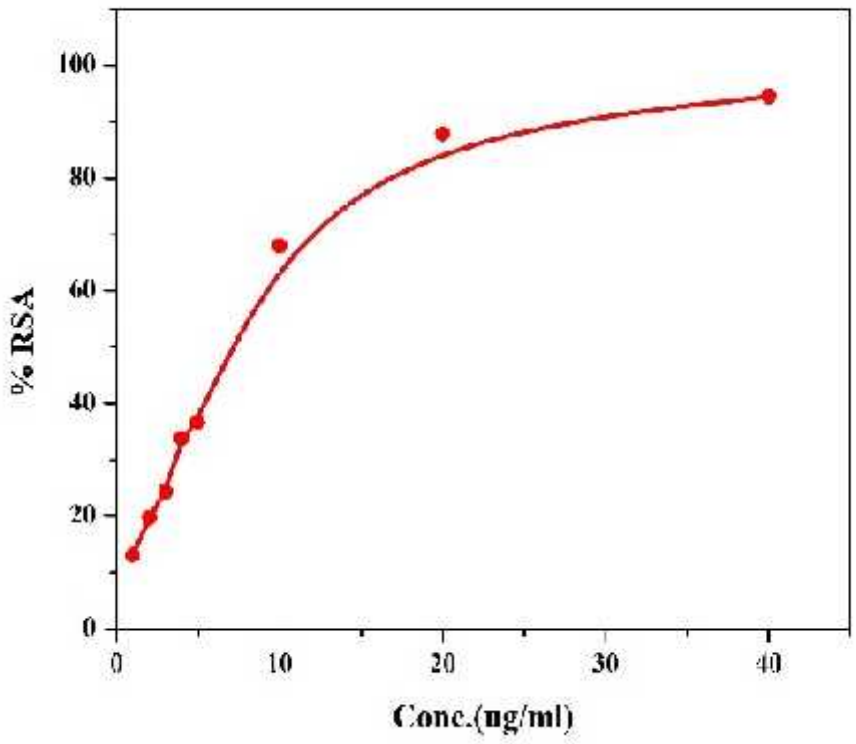


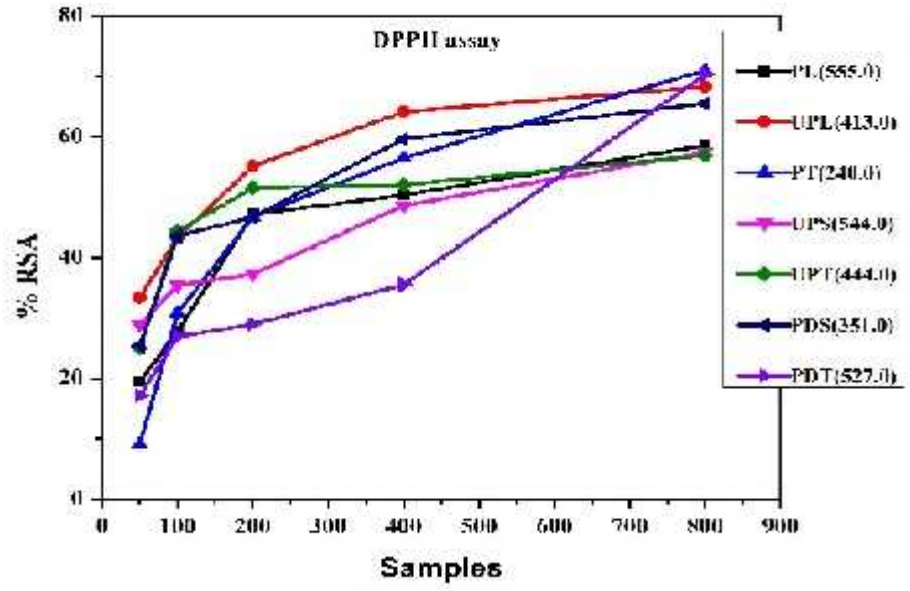
Figure 9 :*TAC of samples*

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

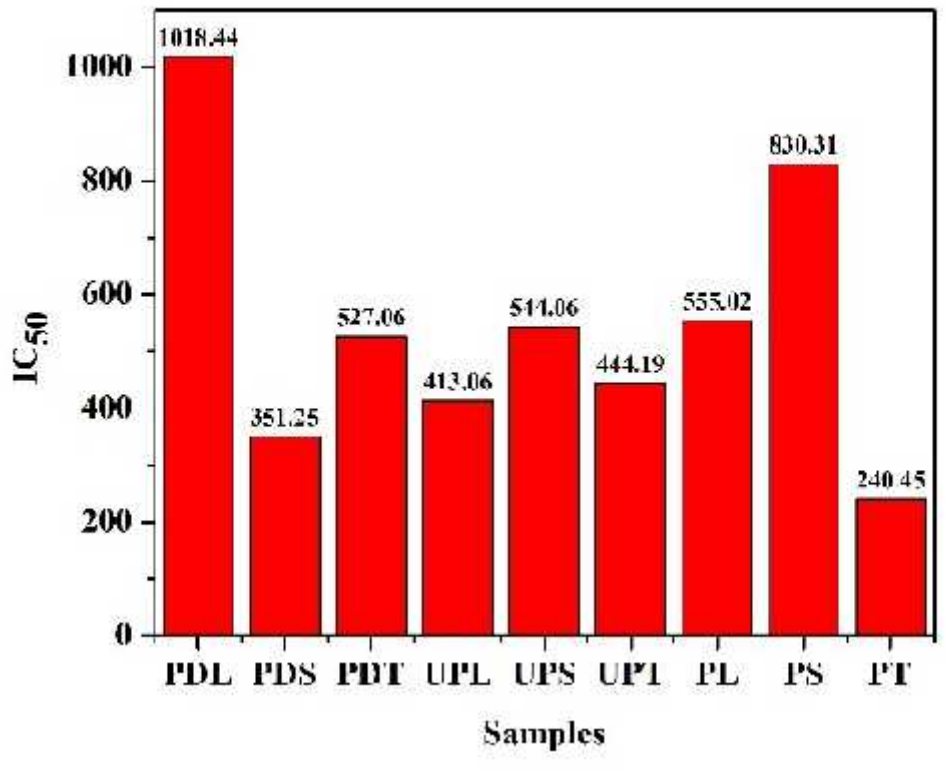


Figure 11 :DPPH free radical scavenging activities of samples (IC50)

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	8910.00	0.47	1.22	15.60	96.80	128.00	15.70
UPS	303.00	0.25	0.67	1.24	43.60	0.68	9.82
UPL	355.00	0.42	0.72	2.41	104.00	1.94	65.60

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 (Reyeset 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 (Chenet al., 1997)

S. N	Sample	Mode	Putative compounds	Retention Time(Min)	Mass	Molecular Formula	m/z	Chemical Class	Clinical uses
10			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		-ve ESI mode	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		-ve ESI mode	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			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)

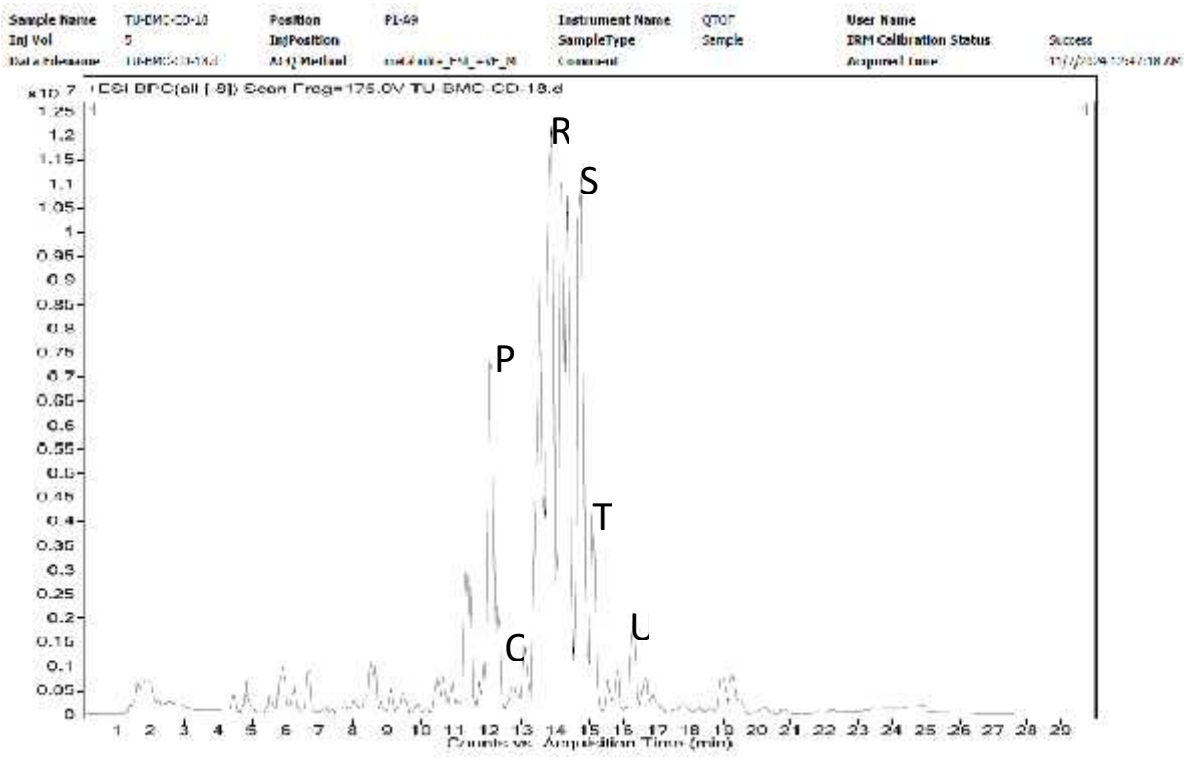


Figure 12 :Chromatogram of UPT in +ve ESI mode using HR-LCMS]

P: Jervine

Q: Funtumine

R: Unknown compound

S: Unknown compound

T: Unknown compound

U: Septentriodine

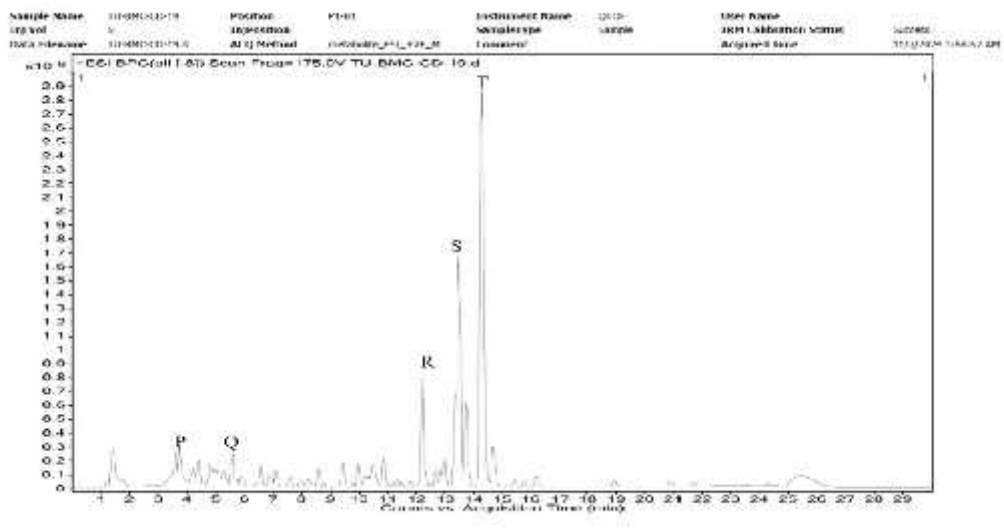


Figure 13 : Chromatogram of UPS in +ve ESI mode]

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

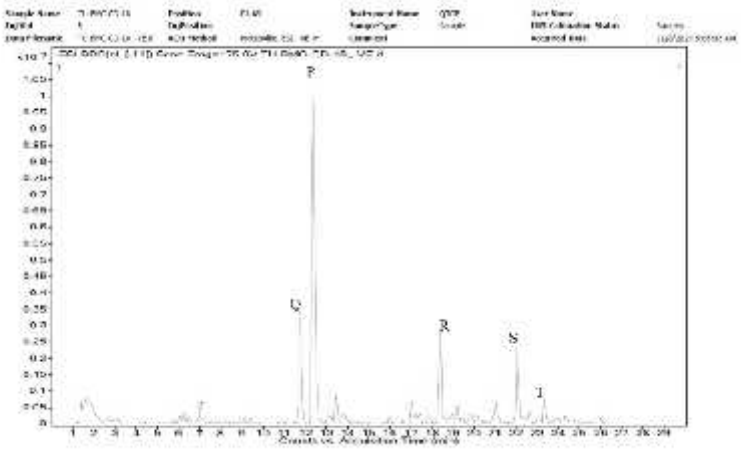


Figure 15 : Chromatogram of UPT in -ve ESI mode]

P: Corchorifatty acid F

Q: 3-Hydroxyquinine

R: Unknown compound

S: Unknown compound

T: Linalyl carprylate

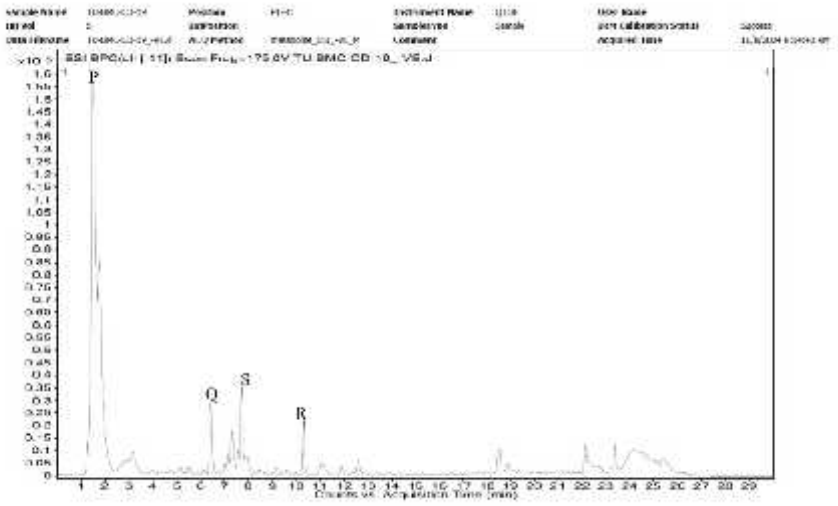


Figure 16 : Chromatogram of UPS in -ve ESI mode

P: Eudistomin N

Q: Swertiamarin

R: Gibberlin A43

S: Luteolin-4′-O-glucoside

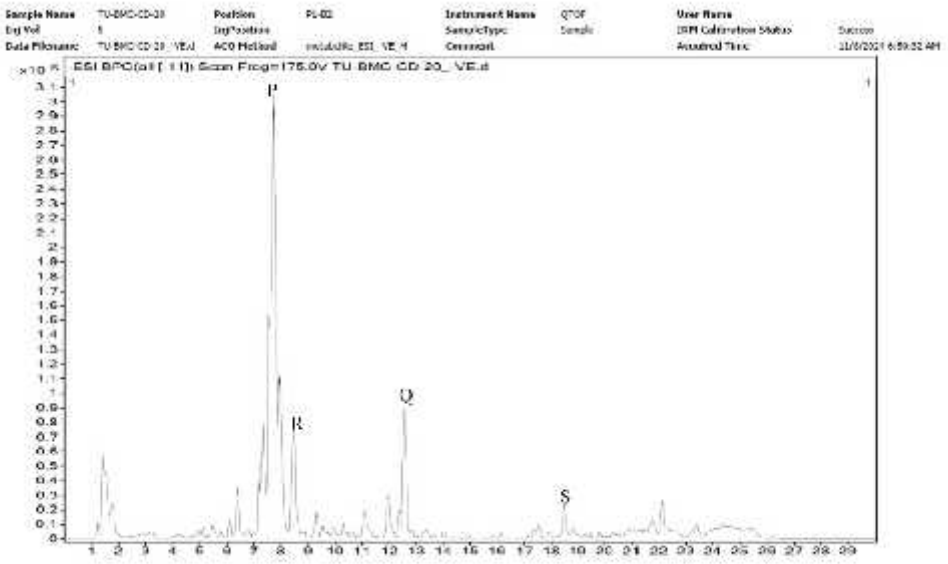


Figure 17 :*Chromatogram of UPL in -ESI mode*

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

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

R: Quercetin 3,7-dirhamnoside

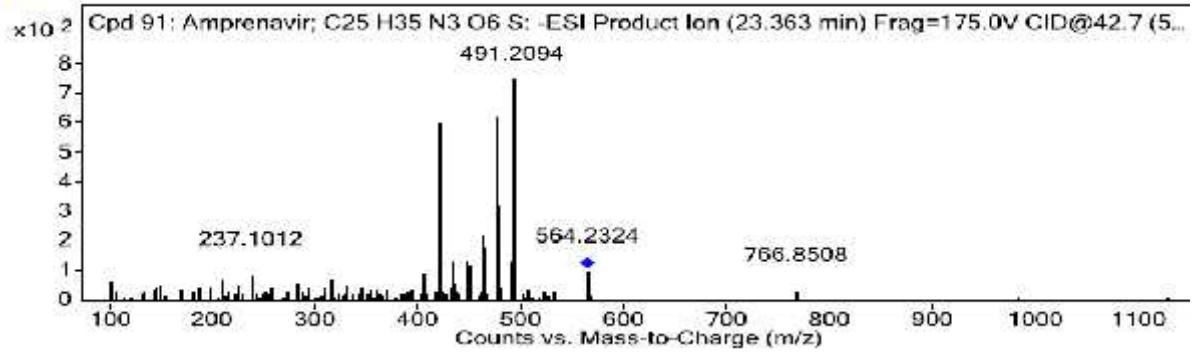
S: Hexazinone

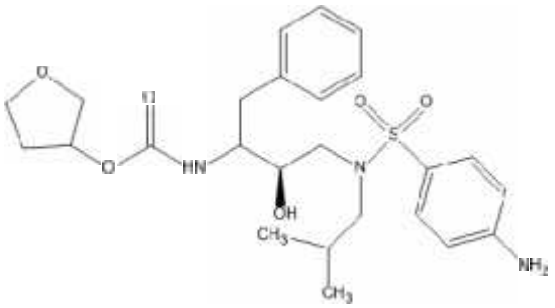
Amprenavir

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



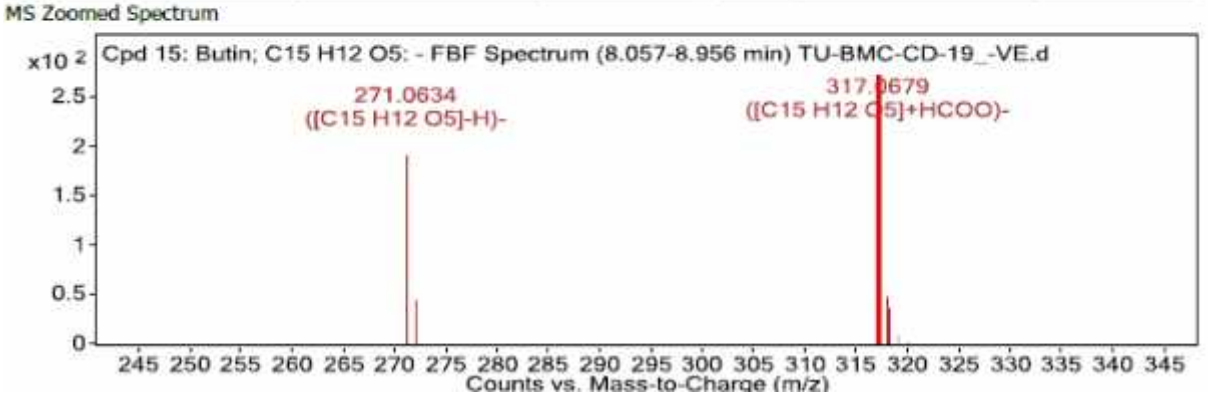


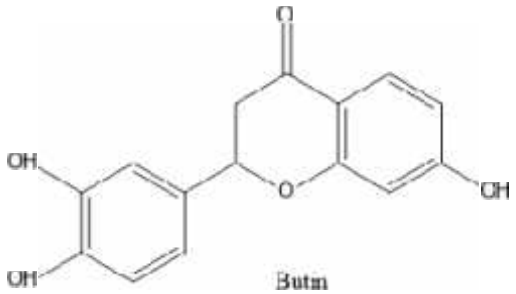
Amprenavu
Chemical Formula: $C_{25}H_{35}N_3O_6S$
Molecular Weight: 505.63

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





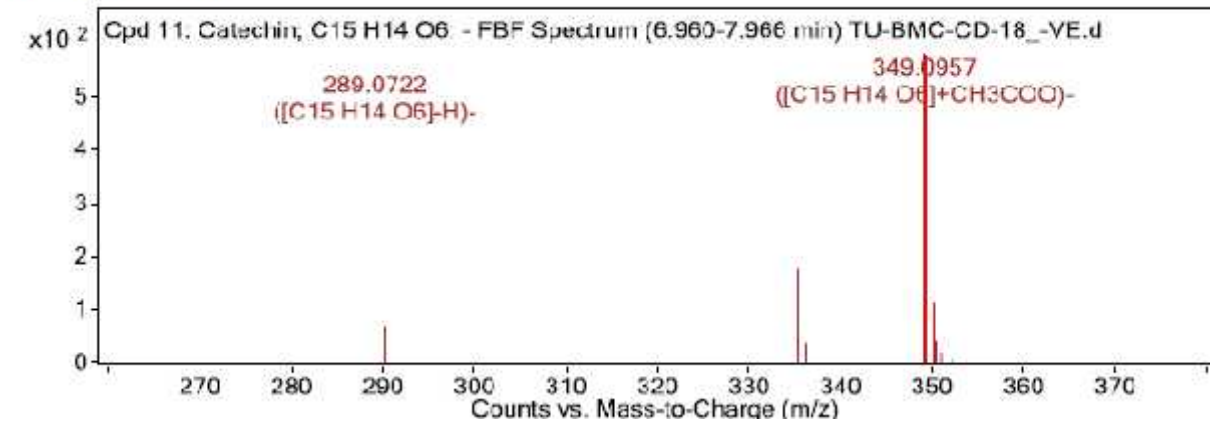
Butin
Chemical Formula: $C_{15}H_{12}O_5$
Molecular Weight: 272.25

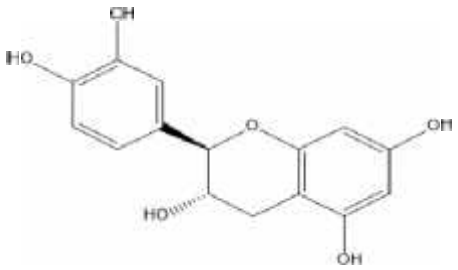
Catechin

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



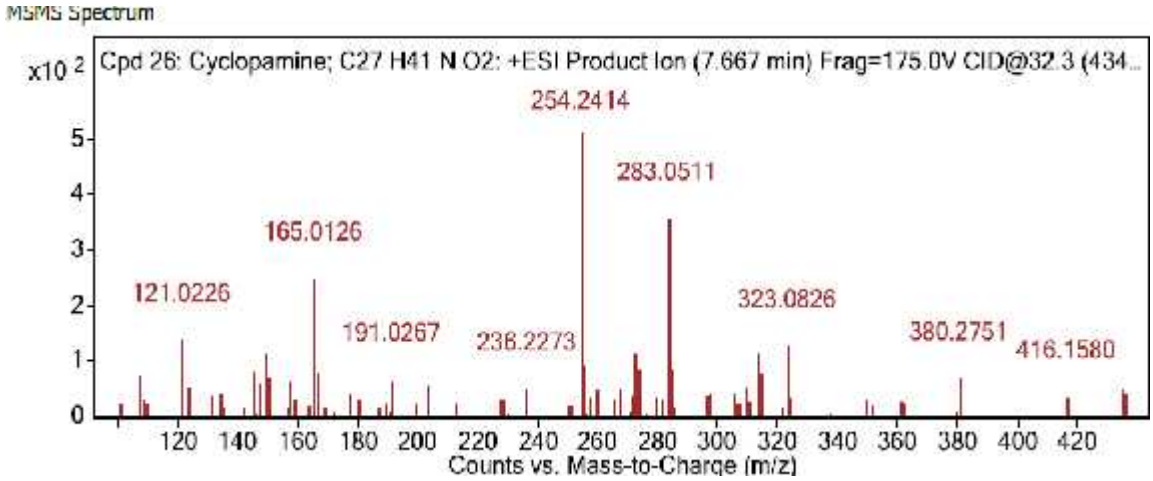


catechin
Chemical Formula: $C_{15}H_{14}O_6$
Molecular Weight: 290.27

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

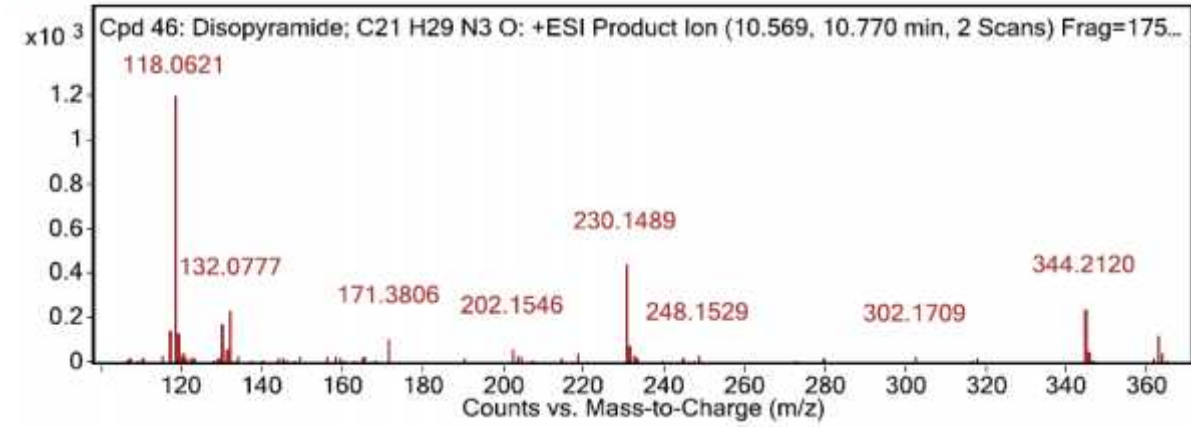


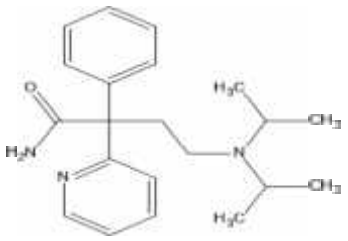
Disopyramide

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



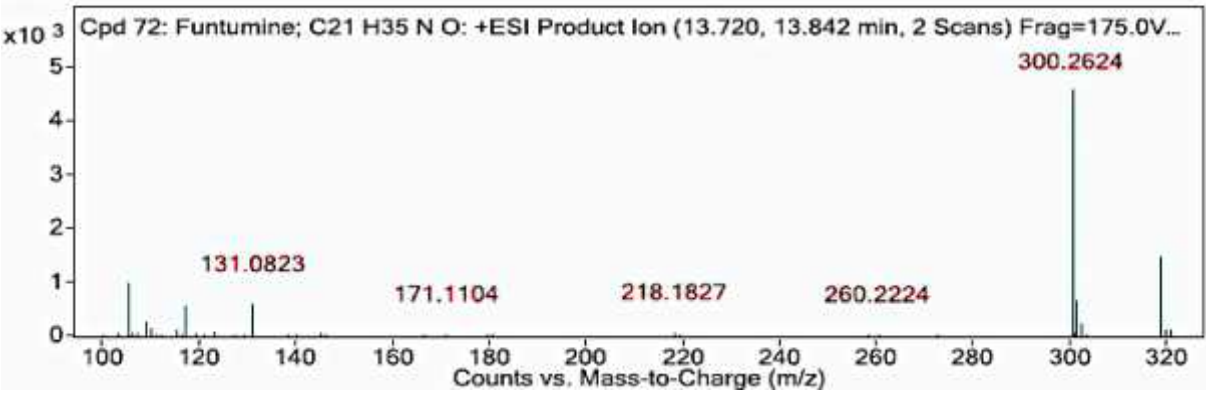


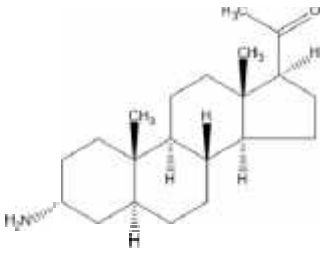
Disopyramide
Chemical Formula: $C_{21}H_{29}N_3O$
Molecular Weight: 339.47

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





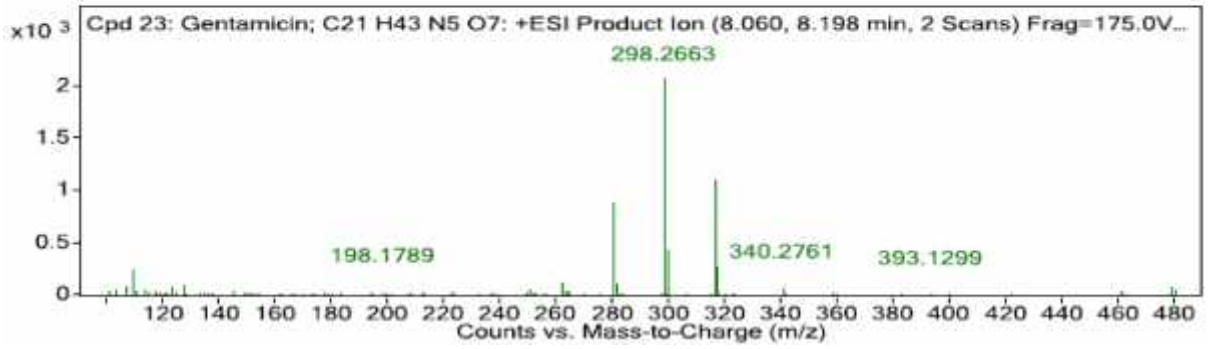
Funtumine
Chemical Formula: $C_{28}H_{35}NO$
Molecular Weight: 317.51

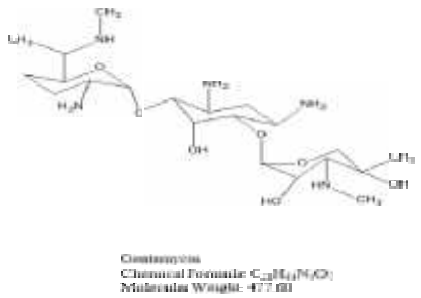
Gentamycin

Weinstein etal., 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





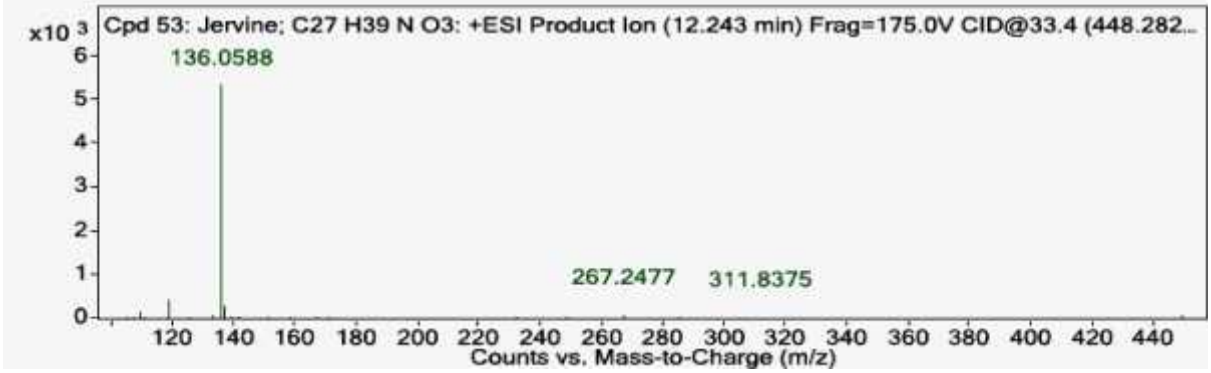
Gentamycin
Chemical Formula: $C_{21}H_{43}N_5O_7$
Molecular Weight: 477.60

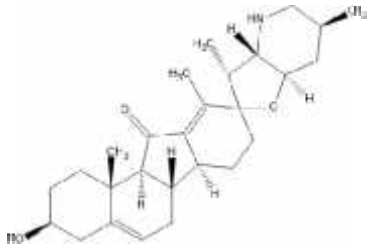
Jervine

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





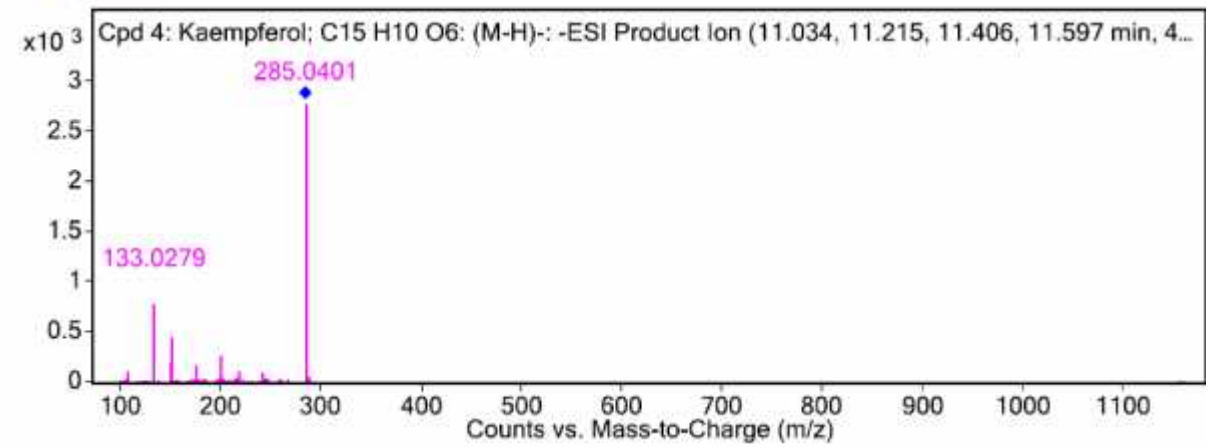
Jervine
Chemical Formula: $C_{27}H_{39}NO_3$
Molecular Weight: 425.60

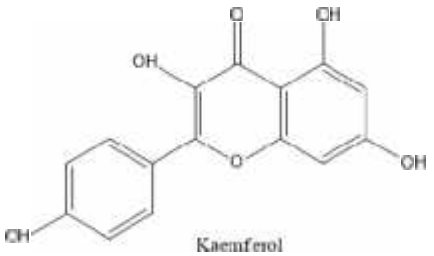
Kaemferol

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





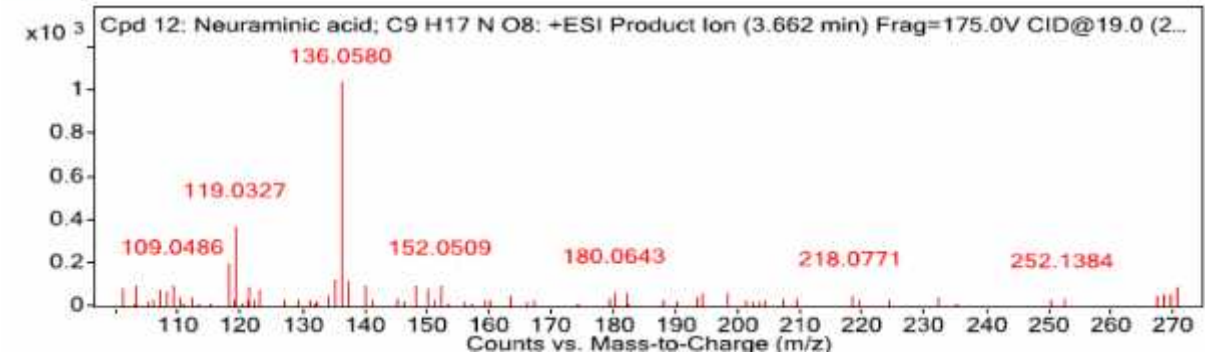
Kaempferol
Chemical Formula: $C_{15}H_{10}O_6$
Molecular Weight: 286.24

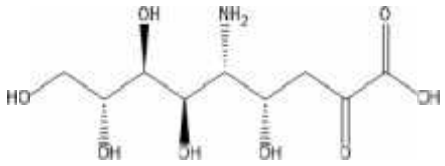
Neuraminic acid

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





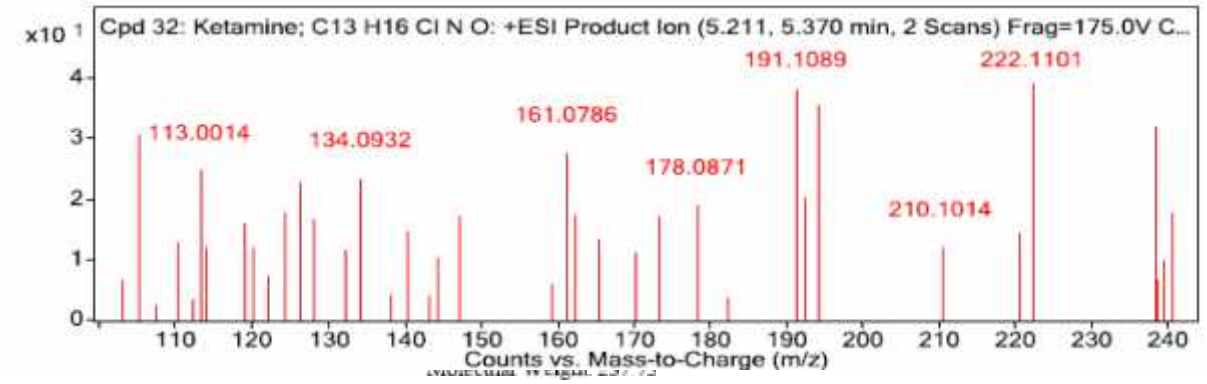
Neuraminic acid
Chemical Formula: $C_9H_{17}NO_8$
Molecular Weight: 267.23

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].

Compound Label	Name	m/z	RT	Algorithm	Mass
Cpd 32: Ketamine; C13 H16 Cl N O	Ketamine	238.0998	5.291	Auto MS/MS	237.0925

MSMS Spectrum

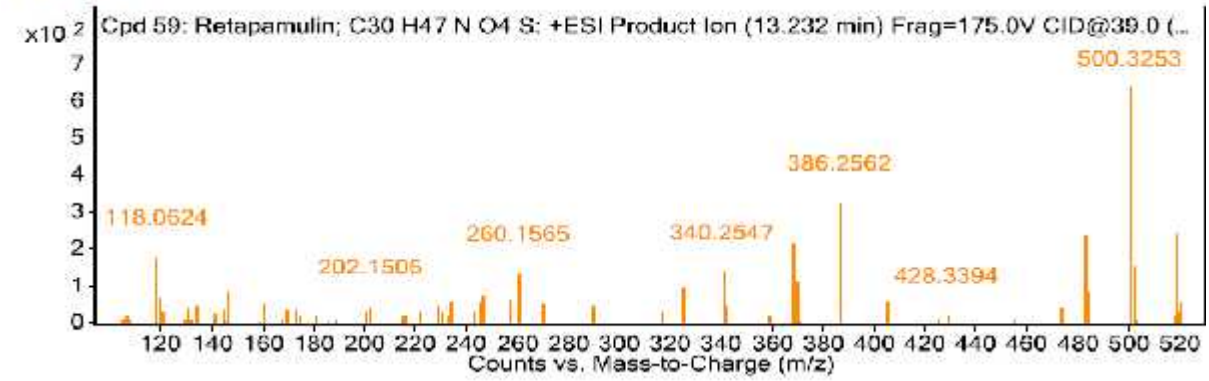


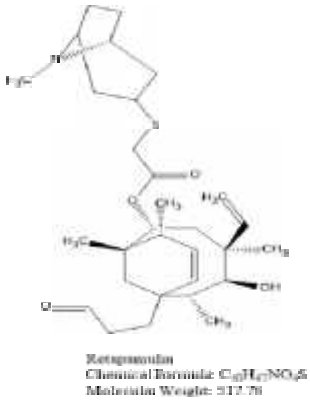
Retapamulin

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



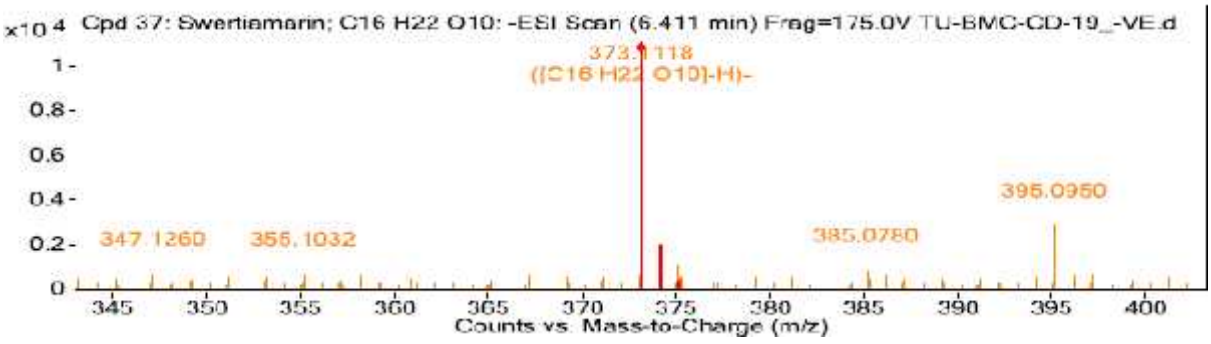


Ketopumilin
Chemical Formula: $C_{16}H_{22}NO_6$
Molecular Weight: 317.78

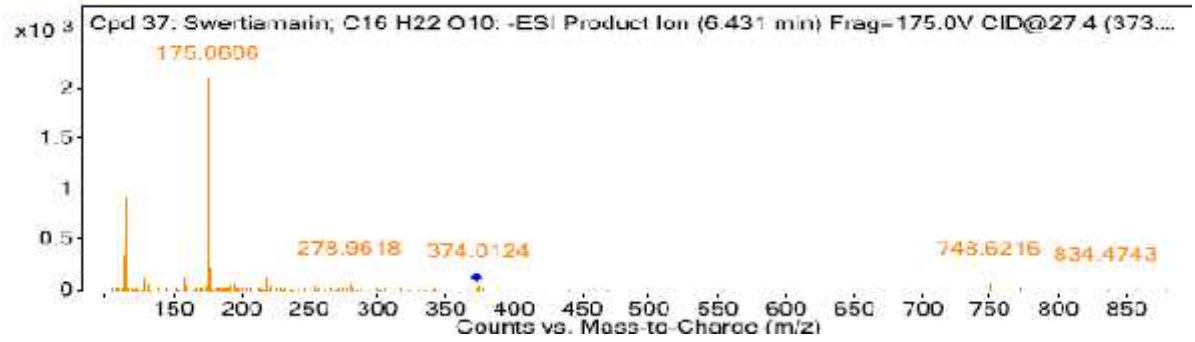
Swertiamarin

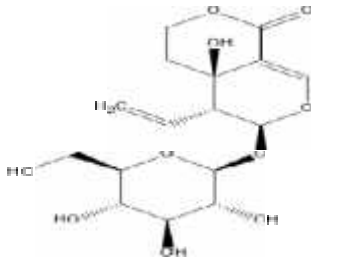
A secoiridoid glycoside, swertiamarin, have a variety of biological properties, including anti-inflammatory, antidiabetic, anti-atherosclerotic, and antioxidant actions. Its impact on a number of signaling pathways linked to cardiac remodeling events, apoptosis, inflammatory and lipid peroxidation markers, 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



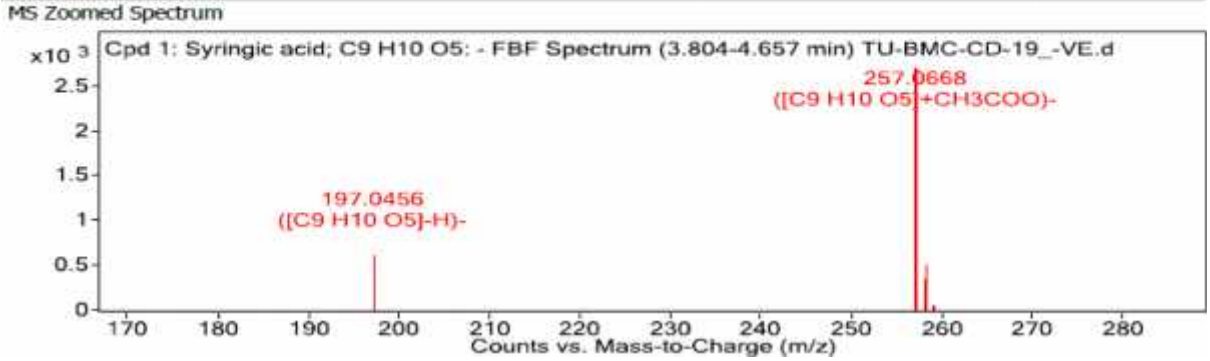


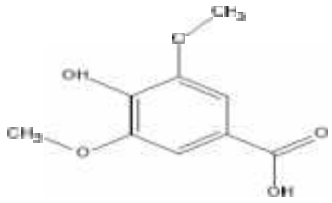
Syringic acid

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. Widevarietyofpharmacologicalcharacteristicsareexhibitedbysyringicacid,suchasanti-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





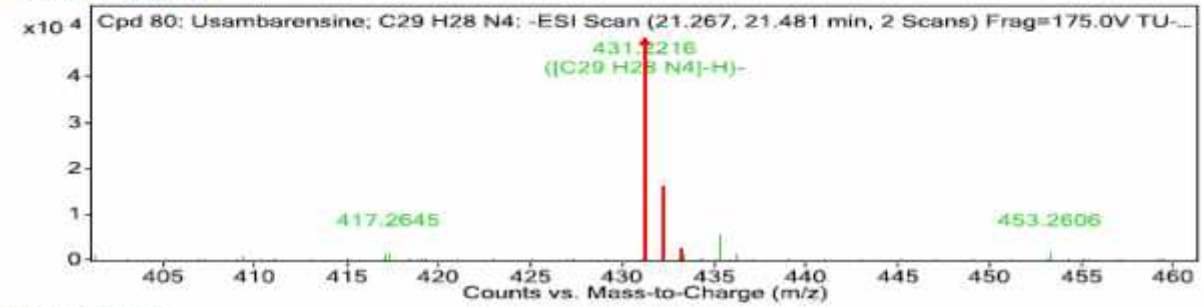
Syringic acid
Chemical Formula: $C_9H_{10}O_5$
Molecular Weight: 198.17

Usambarensine

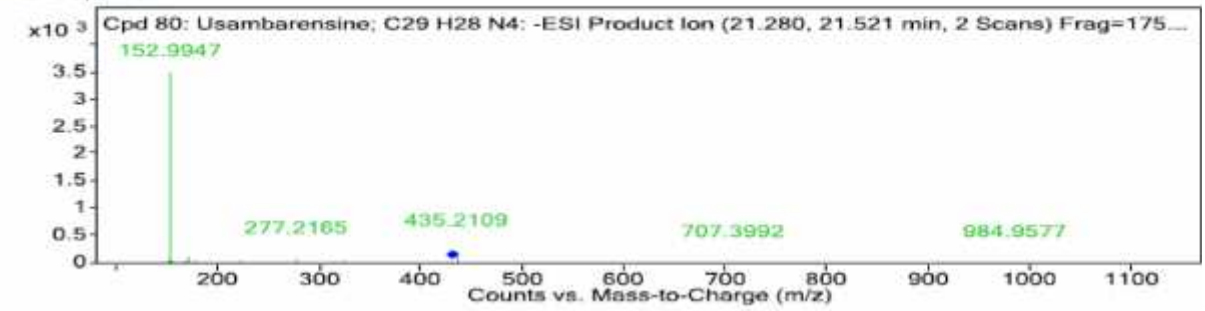
Usambarensine is a tertiary amine alkaloid. Usambarensine has atropine-like and spasmolytic properties, anti-amoebic and Anti-plasmodial Activities [Wright et al., 1991], as antineoplastic drugs for cancer chemotherapy. [Isah,2016] and antiplasmodial activity [Passemar et al., 2011].

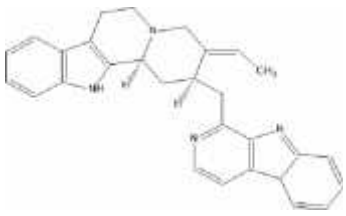
Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 80: Usambarensine; C29 H28 N4	**Usambarensine**	431.2216	21.401	Auto MS/MS	432.2289

MS Zoomed Spectrum



MSMS Spectrum





Usambarensine
Chemical Formula: $C_{29}H_{28}N_4$
Molecular Weight: 432.58

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

Cocoyam (*Colocasia esculenta*) is potential source of secondary metabolites and nutrient elements beneficial for human health. The chemical constituents present inthe extracts proves that it may become the super food for the oppressed society having low economic status.The application of UV-VIS spectrophotometer, HR-LCMS and ICP-AES, a comprehensive analysis of tuber, petiole and leaves revealed the presence varieties 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 DSand 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, out of which 115 known and 85 are unknown. Out of known compounds, 12 are alkaloids, 15 phenolic, 26 flavonoids, 9 glycosides and 55 other class of compounds. The UPL sample screened total 197 compounds, among them, 89 are known while 116 are unknown. In the core of 89 known compounds: 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 in the chemical profile of cocoyam and it might become a valuable source of natural antioxidants.To sum up, Cocoyam is the rich source of varieties of chemical constituents and nutrient elements. Hence it may be potential for the use in processed food, pharmaceuticals, cosmetics etc.

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 asto noble drug
3. Unidentified compound appeared in the HR-LCMS should be isolated andcharacterized.

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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 V02	0.1% FA in water	Ch. 2	Yes	95.00 %
2	B	100.0 % Acetonitrile V.03		100.0 % Acetonitrile V02		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

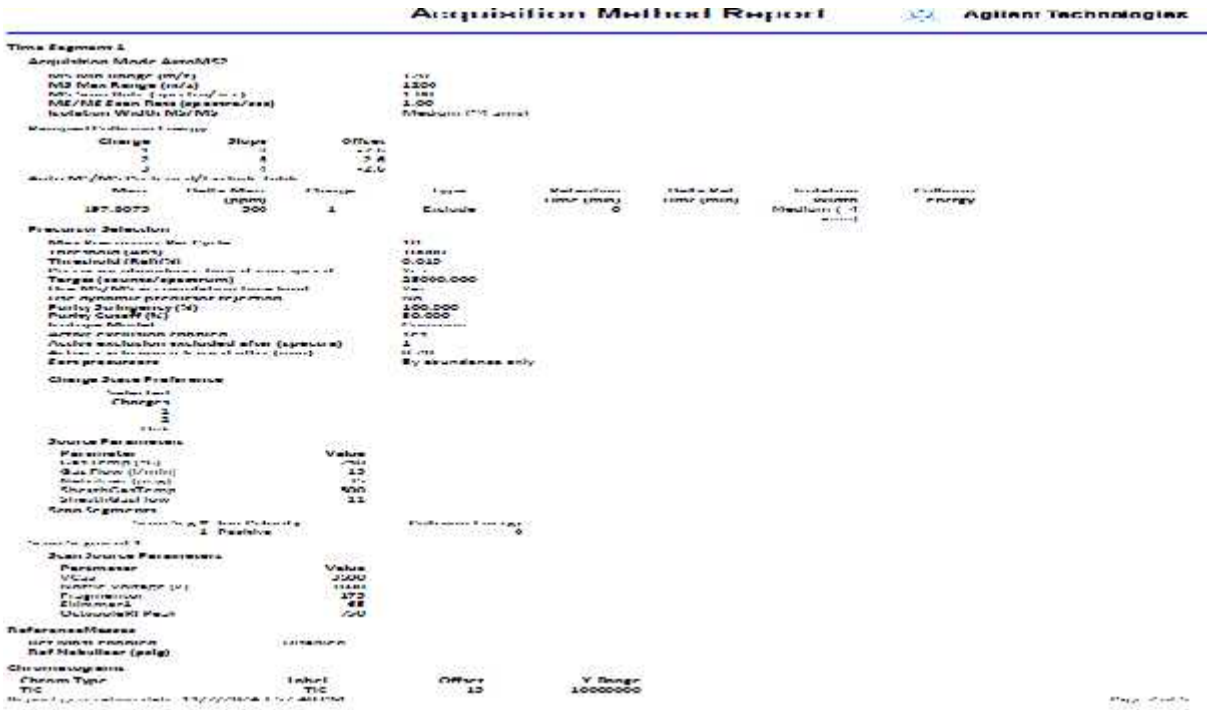
Ready when front door open	Yes
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	40.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.80 °C
Right Temperature Control	
Right temperature Control Mode	Temperature 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



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 : 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	260.0 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					

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	HFG Formula	BB Formula	BB Diff (ppm)	Tag (BB)
Compound 1	4.46								
Cpd 2: [illegible]	4.74	277.0744	92443	[illegible]ranoside	C12 H14 N6 O4	C12 H14 N6 O4	C12 H14 N6 O4	0.68	1
Cpd 3: [illegible]	4.80	332.1453	32463	[illegible]	C16 H19 N O5	C16 H19 N O5	C16 H19 N O5	-0.08	1
Cpd 4: Thiamine [illegible]	5.16	434.1188		Thiamine 3	C14 H20 N4 O6	C14 H20 N4 O6	C14 H20 N4 O6	-0.09	1
Cpd 5: Abscisic acid glucose ester	5.34	426.1924		Abscisic acid glucose ester	C21 H30 O9	C21 H30 O9	C21 H30 O9	-0.6	1
Compound 6	5.606								
Cpd 7: 7-beta-D-Glucopyranosyloxy-4-ethylbenzopyranone	5.64	446.1316		7-beta-D-Glucopyranosyloxy-4-ethylbenzopyranone	C19 H26 O9	C19 H26 O9	C19 H26 O9	0.07	1
Cpd 8: [illegible]	5.81	426.1971		[illegible]	C19 H30 O9	C19 H30 O9	C19 H30 O9	-0.75	1
Cpd 9: 6-Formylpyranosidenol	5.84	166.2201	99618	6-Formylpyranosidenol	C21 H24 O9	C21 H24 O9	C21 H24 O9	1.66	1
Cpd 10: Hirtiosone	5.89	270.1342		Hirtiosone	C18 H22 O2	C18 H22 O2	C18 H22 O2	-0.77	1
Compound 11	6.06		209600						
Cpd 12: Abscisic acid glucose ester	6.48	426.1762		Abscisic acid glucose ester	C21 H30 O9	C21 H30 O9	C21 H30 O9	0.24	1
Compound 13	6.537		27668						
[illegible]	6.738	464.1716		[illegible]	C19 H28 N6 O8	C19 H28 N6 O8	C19 H28 N6 O8	-2.15	1
Cpd 15: Salicylic Ac [illegible]	6.821	116.2862		Salicylic Ac	C20 H26 N6 O5	C20 H26 N6 O5	C20 H26 N6 O5	0.57	1
Cpd 16: Phenylalanyl [illegible]	6.838	280.2961	24870	Phenylalanyl [illegible]	C21 H28 O5	C21 H28 O5	C21 H28 O5	-0.82	1
Cpd 17: Gonosterol G [illegible]	7.061	386.3363	31000	Gonosterol G	C29 H38 O7	C29 H38 O7	C29 H38 O7	0	1
Compound 18	7.261		23520						
Compound 19	7.724		34608						
Cpd 20: Lagrimicrolino [illegible]	7.756	197.2327	32009	Lagrimicrolino	C25 H31 N O5	C25 H31 N O5	C25 H31 N O5	0.49	1
Cpd 21: AbrierTriacetamide [illegible]	7.809	513.2314		AbrierTriacetamide	C29 H37 N O14	C29 H37 N O14	C29 H37 N O14	-1.9	1
Compound 22	8.121		47900						
Cpd 23: Cantharidin [illegible]	8.126	177.2361		Cantharidin	C21 H16 N6 O7	C21 H16 N6 O7	C21 H16 N6 O7	0.48	1
Cpd 24: Geranyl anthocyanosyl glucoside [illegible]	8.247	448.2348		Geranyl anthocyanosyl glucoside	C21 H36 O10	C21 H36 O10	C21 H36 O10	0.84	1
Compound 25	8.354								
Compound 26	8.45								
Compound 27	8.45		31000						
Compound 28	8.477								
Compound 29	8.545								
Cpd 30: Geranyl anthocyanosyl glucoside	8.734	448.3762	37000	Geranyl anthocyanosyl glucoside	C21 H36 O10	C21 H36 O10	C21 H36 O10	0.43	1
Cpd 31: [illegible]	8.776	190.1751		[illegible]	C13 H21 N O2	C13 H21 N O2	C13 H21 N O2	1.37	1
Compound 32	9.188								
Compound 33	9.232		124000						
Compound 34	9.456								
Cpd 35: [illegible]	9.726	356.1924		[illegible]	C19 H28 O7	C19 H28 O7	C19 H28 O7	0.56	1
Cpd 36: [illegible]	10.062	192.1671		[illegible]	C13 H20 N4 O	C13 H20 N4 O	C13 H20 N4 O	0.5	1
Compound 37	10.289								
Cpd 38: Dipalmitoleoyl [illegible]	11.124	335.2824	126000	Dipalmitoleoyl	C19 H33 N O2	C19 H33 N O2	C19 H33 N O2	0.23	1
Compound 39	11.182		116000						
Cpd 40: Cryptophorbolide [illegible]	11.218	332.2663		Cryptophorbolide	C17 H37 N O	C17 H37 N O	C17 H37 N O	0.5	1
Compound 41	11.324								
Compound 42	11.424								
Cpd 43: 12-Oxo palmitoleoyl [illegible]	11.531	276.2456	95000	12-Oxo palmitoleoyl	C16 H32 O2	C16 H32 O2	C16 H32 O2	0.6	1
Compound 44	11.631								
Compound 45	11.737		53400						
Cpd 46: Dipalmitoleoyl [illegible]	11.787	335.2823		Dipalmitoleoyl	C19 H33 N O2	C19 H33 N O2	C19 H33 N O2	0.23	1
Compound 47	11.838		35400						
Compound 48	12.027								
Cpd 49: Cholestadienol 1 [illegible]	12.208	386.4167		Cholestadienol 1	C27 H46 O	C27 H46 O	C27 H46 O	0.13	1

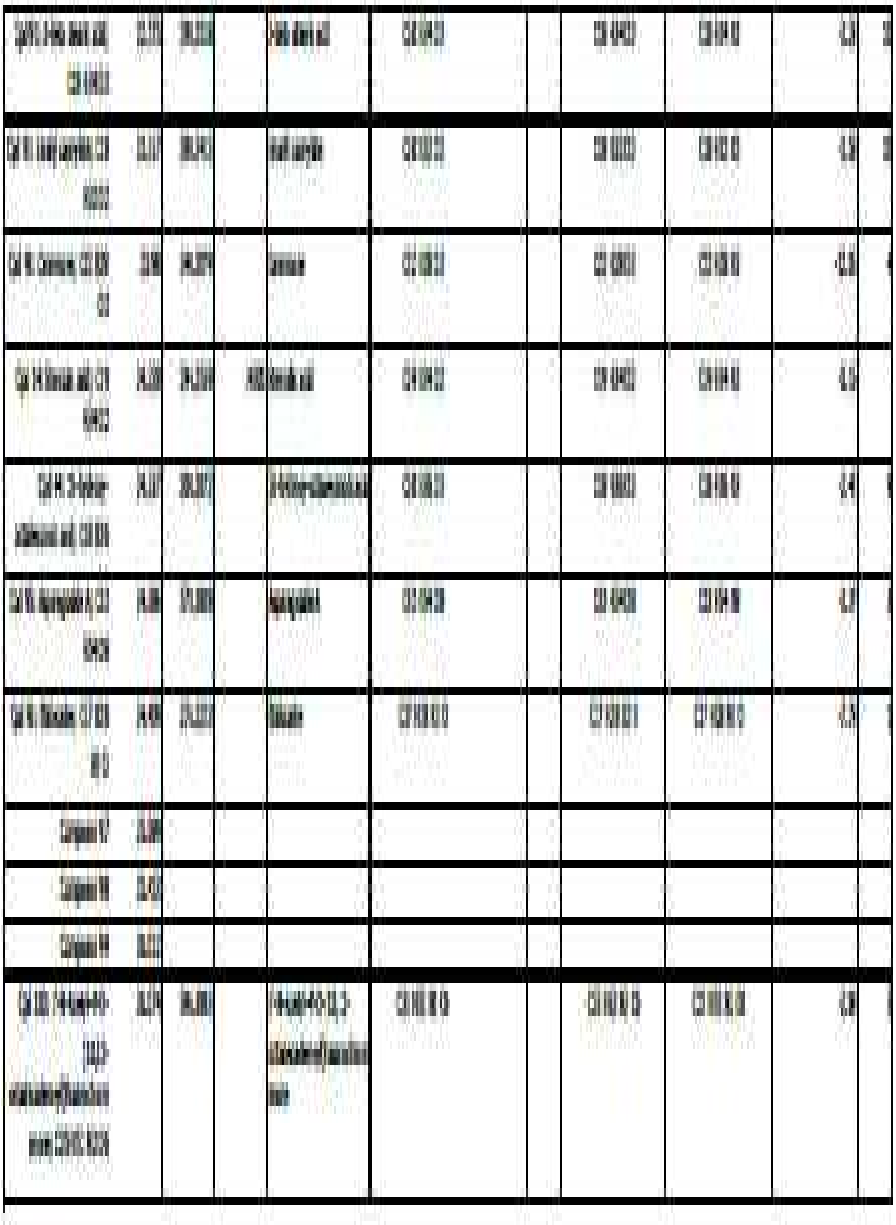
APPENDIX III

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

Cpd 50: Di(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/16:4(6Z,8E,12Z,14Z)(2R,3S)); C43 H64 O5	12.115	660.4691		Di(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/16:4(6Z,8E,12Z,14Z)(2R,3S))	C43 H64 O5	C43 H64 O5	C43 H64 O5	0.71	1
Compound 51	12.158								
Compound 52	12.212								
Cpd 53: Jervine; C27 H39 N O3	12.240	425.2937		Jervine	C27 H39 N O3	C27 H39 N O3	C27 H39 N O3	-1.72	1
Compound 54	12.288								
Cpd 55: 2-Hexadecenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol; C28 H50 O4	12.466	578.4223		2-Hexadecenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol	C28 H50 O4	C28 H50 O4	C28 H50 O4	17.02	1
Compound 56	12.510								
Cpd 57: Glutamyl-lysine; C11 H21 N3 O5	12.512	275.1474		Glutamyl-lysine	C11 H21 N3 O5	C11 H21 N3 O5	C11 H21 N3 O5	0.60	4
Compound 58	12.562		60713						
Compound 59	12.726								
Compound 60	12.927		148868						
Compound 61	13.106		90673						
Compound 62	13.151								
Compound 63	13.151								
Compound 64	13.202								
Compound 65	13.291								
Compound 66	13.463								
Compound 67	13.478								
Compound 68	13.514								
Compound 69	13.722		186766						
Compound 70	13.771								
Compound 71	13.781								
Cpd 72: Pantomine; C21 H25 N O	13.791	307.2675		Pantomine	C21 H25 N O	C21 H25 N O	C21 H25 N O	13.82	1
Compound 73	13.792								
Compound 74	13.796								
Compound 75	13.848								
Compound 76	13.940								
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	9.24	1
Compound 80	14.138								
Compound 81	14.21								
Compound 82	14.3								
Compound 83	14.227								
Compound 84	14.272								
Compound 85	14.275								
Compound 86	14.706								
Compound 87	14.722								
Compound 88	14.727								
Compound 89	14.809								
Compound 90	14.871								
Compound 91	14.976								
Compound 92	15.107								
Compound 93	15.754								
Compound 94	16.240								
Cpd 95: Septanitrolline; C27 H52 N2 O11	16.864	768.3543		Septanitrolline	C27 H52 N2 O11	C27 H52 N2 O11	C27 H52 N2 O11	4.07	1
Compound 96	20.047								
Compound 97	20.422								
Cpd 98: Di(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(4Z,7Z,10Z,13Z,16Z)); C47 H72 O5	22.140	716.5377	32215	Di(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(4Z,7Z,10Z,13Z,16Z))	C47 H72 O5	C47 H72 O5	C47 H72 O5	14.26	4
Cpd 99: Lisuride; C22 H25 N5 O4	22.564	375.1952	32150	Lisuride	C22 H25 N5 O4	C22 H25 N5 O4	C22 H25 N5 O4	10.31	1
Compound 100	23.211		55731						

APPENDIX IV

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



Data File	[redacted]	Sample Name	[redacted]
Solution Type	Unknown	Position	P1-H1
Instrument Name	QTOF	User Name	[redacted]
Acq. Method	[redacted]	Acquired Time	11/7/2024 1:36:37 AM
ESI Calibration Status	[redacted]	Int. Method	Default.m
Comment			

Sample Group	[redacted]
Acquisition SW	4240 series TOF/6560 series
Version	D-TOF B.05.01 (B0115.3)

L-CITRULLINE_1MIN

Compound Label	RT	Mass	Abund	Name	Formula	MFG Formula	DB Formula	DB diff (ppm)	Qual (DB)
Compound 1	0.218								
Compound 2	1.196								
Compound 3	1.448		95571						
Compound 4	1.462								
Compound 5	1.598								
Compound 6	1.698								
Cpd 7: 2P Hydroxy pentanedioic acid; C5 H8 O5 (a)	2.109	340.0377	18917	2P Hydroxy pentanedioic acid	[redacted]	[redacted]	[redacted]	4.29	1
Compound 8	2.299		28533						
Compound 9	2.441		70007						
Compound 10	2.479		32157						
Cpd 11: Mineur; (*1) (C) (*2)	3.65	346.1473	46778	Mineur	(*1) (C) (*2)	(*1) (C) (*2)	(*1) (C) (*2)	-11.12	1
Cpd 12: Pterostilbene acid; C7 H7 N O6	3.692	207.0854		Pterostilbene acid	C9 H17 N O6	C9 H17 N O6	C9 H17 N O6	30.69	7
Cpd 13: 2 Methylpyrrolidine; C5 H11 N	3.752	99.0917	16553.0	2 Methylpyrrolidine	C5 H11 N	C5 H11 N	C5 H11 N	1.42	2
Cpd 14: 2-Hexthalenol 2-aminobutanoic; C17 H13 N O2	3.734	263.2904	17529.0	2-Hexthalenol 2-aminobutanoic	C17 H13 N O2	C17 H13 N O2	C17 H13 N O2	4.6	4
Cpd 15: N (5 Methyl 3 methoxyphenyl; (*1) H16 N O	3.798	166.1221	54849.0	N (5 Methyl 3 methoxyphenyl	C11 H17 N O	C11 H17 N O	C11 H17 N O	11.42	2
Cpd 16: L-alpha-Hexidine; C7 H12 N2 O2	3.852	172.2831		L-alpha-Hexidine	C7 H12 N2 O2	C7 H12 N2 O2	C7 H12 N2 O2	9.82	2
Compound 17	3.941		26470						
Cpd 18: 2 Methylpyrrolidine; C5 H11 N	4.007	99.0917		2 Methylpyrrolidine	C5 H11 N	C5 H11 N	C5 H11 N	1.42	2
Cpd 19: Alanylvaline dipeptide; C8 H16 N2 O3 (-1)	4.111	187.2761		Alanylvaline dipeptide	C8 H15 N2 O3 (-1)	C8 H15 N2 O3 (-1)	C8 H15 N2 O3 (-1)	7.83	2
Compound 20	4.186		34848						
Cpd 21: Theanine; C7 H14 N2 O3	4.243	146.0915	116588	Theanine	C7 H14 N2 O3	C7 H14 N2 O3	C7 H14 N2 O3	0.43	1
Compound 22	4.287		12280						
Cpd 23: (3S)-3-[(2,4-Methylenedioxyphenyl)-2-methoxypropionyl]-2-oxazolidinone; C13 H13 N O5	4.313	334.0862	28733	(3S)-3-[(2,4-Methylenedioxyphenyl)-2-methoxypropionyl]-2-oxazolidinone	C13 H13 N2 O5 (-1)	C13 H13 N2 O5 (-1)	C13 H13 N2 O5 (-1)	0.04	2
Cpd 24: Beanine; C21 H29 N O	4.348	311.335	31121	Beanine	C21 H29 N O	C21 H29 N O	C21 H29 N O	-0.42	2
Cpd 25: Lidine; C20 H17 N O2	4.392	303.3364		Lidine	C20 H17 N O2	C20 H17 N O2	C20 H17 N O2	0.33	2
Cpd 26: 4-[3,4-Dimethoxyphenyl]-1-methoxy-pyridazine; C13 H16 N2 O3	4.407	164.116		4-[3,4-Dimethoxyphenyl]-1-methoxy-pyridazine	C13 H16 N2	C13 H16 N2	C13 H16 N2	4.39	1
Cpd 27: Heteroidine; C16 H10 N2 O2	4.431	245.2963		Heteroidine	C16 H13 N2 O2	C16 H13 N2 O2	C16 H13 N2 O2	-2.86	2
Cpd 28: 4-(Thiouracil)-3,4-dethiotine; C12 H16 O3 N2	4.851	171.1014	76898.0	4-(Thiouracil)-3,4-dethiotine	C11 H16 O3 N2 (-1)	C11 H16 O3 N2 (-1)	C11 H16 O3 N2 (-1)	-0.9	1
Cpd 29: Maclabin; C24 H32 N2 O2	4.872	380.5177	23561	Maclabin	C24 H32 N2 O2	C24 H32 N2 O2	C24 H32 N2 O2	-0.38	2
Cpd 30: 3 (2 Caffoyl) 1 (3 methylbutyl) acid; C17 H20 O6	5.071	320.1311	202162.0	3 (2 Caffoyl) 1 (3 methylbutyl) acid	C17 H20 O6	C17 H20 O6	C17 H20 O6	0.44	66
Cpd 31: Dolastatin; C24 H30 N2 O2	5.072	378.5417	23561	Dolastatin	C24 H30 N2 O2	C24 H30 N2 O2	C24 H30 N2 O2	-2.23	2
Cpd 32: Kolavenol; C22 H34 O2	5.244	330.5003	44668	Kolavenol	C22 H34 O2 (-1)	C22 H34 O2 (-1)	C22 H34 O2 (-1)	1.79	1
Compound 33	5.348		39470						
Cpd 34: Flavanol; C18 H20 N2	5.512	264.3345	40830	Flavanol	C18 H20 N2	C18 H20 N2	C18 H20 N2	-0.08	2
Compound 35	5.870		19256						
Cpd 36: Stilbenetine; C14 H16 N4	5.943	242.1411	11736	Stilbenetine	C14 H16 N4	C14 H16 N4	C14 H16 N4	-14.47	1
Compound 37	5.977		12491						
Compound 38	6.023		17013						
Compound 39	6.160		16056						
Compound 40	6.270								
Compound 41	6.356		13475						
Cpd 42: Thiaxanglyfy; (*1) H10 N2 O4 S	6.402	191.0856	11669	Thiaxanglyfy	(*1) (C) (*2) (*3)	(*1) (C) (*2) (*3)	(*1) (C) (*2) (*3)	-1.96	0
Cpd 43: Dolabrybyn; C21 H34 O2	6.502	318.5103		Dolabrybyn	C21 H31 O2	C21 H31 O2	C21 H31 O2	0.79	1
Cpd 44: Monbenonic; (*2) H18 N2 O	6.540	301.1483	11607	Monbenonic	(*2) (C) (*1)	(*2) (C) (*1)	(*2) (C) (*1)	4.14	1
Cpd 45: Northexahydryn; C33 H54 N2 O4 S	6.548	238.2667	14462	Northexahydryn	C33 H54 N2 O4 S	C33 H54 N2 O4 S	C33 H54 N2 O4 S	2.85	0
Compound 46	6.648		51502						
Cpd 47: 3,3' Bisangustofuran; C28 H22 O6	6.815	510.1552		3,3' Bisangustofuran	C28 H22 O6	C28 H22 O6	C28 H22 O6	0.21	1

APPENDIX V

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

Compound 48	6.932		11254						
Compound 49	7.092		10566						
Compound 50	7.34		10240						
Compound 51	7.376		12210						
Compound 52	7.401		10792						
Cpd 53: 2',7-Dihydroxy-4'-methoxy-6-prenylflavan; C21 H24 O4	7.578	340.1669		2',7-Dihydroxy-4'-methoxy-6-prenylflavan	C21 H24 O4	C21 H24 O4	C21 H24 O4	-4.26	1
Compound 54	7.657		21465						
Compound 55	7.843		17996						
Compound 56	7.91								
Cpd 57: Gentamicin; C21 H43 N5 O7	8.039	477.3169		Gentamicin	C21 H43 N5 O7	C21 H43 N5 O7	C21 H43 N5 O7	-7.42	1
Compound 58	8.159		18066						
Compound 59	8.227		11560						
Compound 60	8.336		33750						
Cpd 61: 4-O-alpha-D-Galactopyranosylcalystegine B2; C13 H23 N O9	8.767	337.1361	11179	4-O-alpha-D-Galactopyranosylcalystegine B2	C13 H23 N O9	C13 H23 N O9	C13 H23 N O9	3.46	4
Compound 62	8.852		11716						
Compound 63	8.968		10866						
Cpd 64: 3-Undecyl-4(1H)-quinolinone; C20 H29 N O	9.39	299.221		3-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	-7.34	2
Cpd 66: Sulawesine C; C29 H47 N5 O5	9.712	665.364		Sulawesine C	C29 H47 N5 O5	C29 H47 N5 O5	C29 H47 N5 O5	-12.4	3
Compound 67	9.884		34722						
Compound 68	10.012								
Cpd 69: Sulawesine A; C40 H49 N5 O6	10.434	687.374		Sulawesine A	C40 H49 N5 O6	C40 H49 N5 O6	C40 H49 N5 O6	-11.08	1
Compound 70	10.908								
Cpd 71: C8-108; C37 H57 N O14	11.139	739.3928		C8-108	C37 H57 N O14	C37 H57 N O14	C37 H57 N O14	-8.83	2
Cpd 72: Kuwanjidine; C20 H21 N O3	11.261	323.1557	11582	Kuwanjidine	C20 H21 N O3	C20 H21 N O3	C20 H21 N O3	-11.12	4
Compound 73	11.332		17804						
Compound 74	11.448		19965						
Compound 75	11.581		21410						
Cpd 76: Kuwanjidine; C20 H21 N O3	11.894	323.1554	17762	Kuwanjidine	C20 H21 N O3	C20 H21 N O3	C20 H21 N O3	-10.04	4
Compound 77	11.994		13425						
Compound 78	12.221								
Compound 79	12.376								
Compound 80	12.467								
Compound 81	12.644								
Compound 82	14.04								
Compound 83	14.279								
Compound 84	14.617								
Compound 85	15.216		11872						
Cpd 86: 1-Oxo-3-yl pitavastatin; C25 H24 O10	15.62	482.2139		1-Oxo-3-yl pitavastatin	C25 H24 O10	C25 H24 O10	C25 H24 O10	3.02	3
Compound 87	15.749								
Cpd 88: Nalbinolate; C26 H26 N2 O3	15.774	414.1953		Nalbinolate	C26 H26 N2 O3	C26 H26 N2 O3	C26 H26 N2 O3	-2.27	3
Cpd 89: CAS 20599; C25 H40 O N O3	15.793	497.2754		CAS 20599	C25 H40 O N O3	C25 H40 O N O3	C25 H40 O N O3	-1.7	1
Cpd 90: Octanoylglucuronide; C14 H24 O8	16.239	320.1451	31519	Octanoylglucuronide	C14 H24 O8	C14 H24 O8	C14 H24 O8	6.38	2
Cpd 91: LysoPE(24:0/0:0); C29 H60 N O7 P	16.31	565.4089		LysoPE(24:0/0:0)	C29 H60 N O7 P	C29 H60 N O7 P	C29 H60 N O7 P	2.21	2
Cpd 92: 2,6-Di-tert-butyl-4-ethylphenol; C16 H26 O	16.58	234.1984		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-Desmethyldosmiptan; C13	20.9	273.1474		N-Desmethyldosmiptan	C13 H19 N3 O2	C13 H19 N3 O2	C13 H19 N3 O2	1.26	2
Compound 95	21.726								
Compound 96	22.372								
Compound 97	24.146								
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	-3.66	4
Compound 99	24.32								
Compound 100	24.817								

Data File	[illegible]	Sample Name	[illegible]
Sample Type	Sample	Position	[illegible]
Instrument Name	QTOF	User Name	
Acq Method	[illegible]	Acquired Time	[illegible]
IRM Calibration Status	[illegible]	DA Method	[illegible]
Comment			

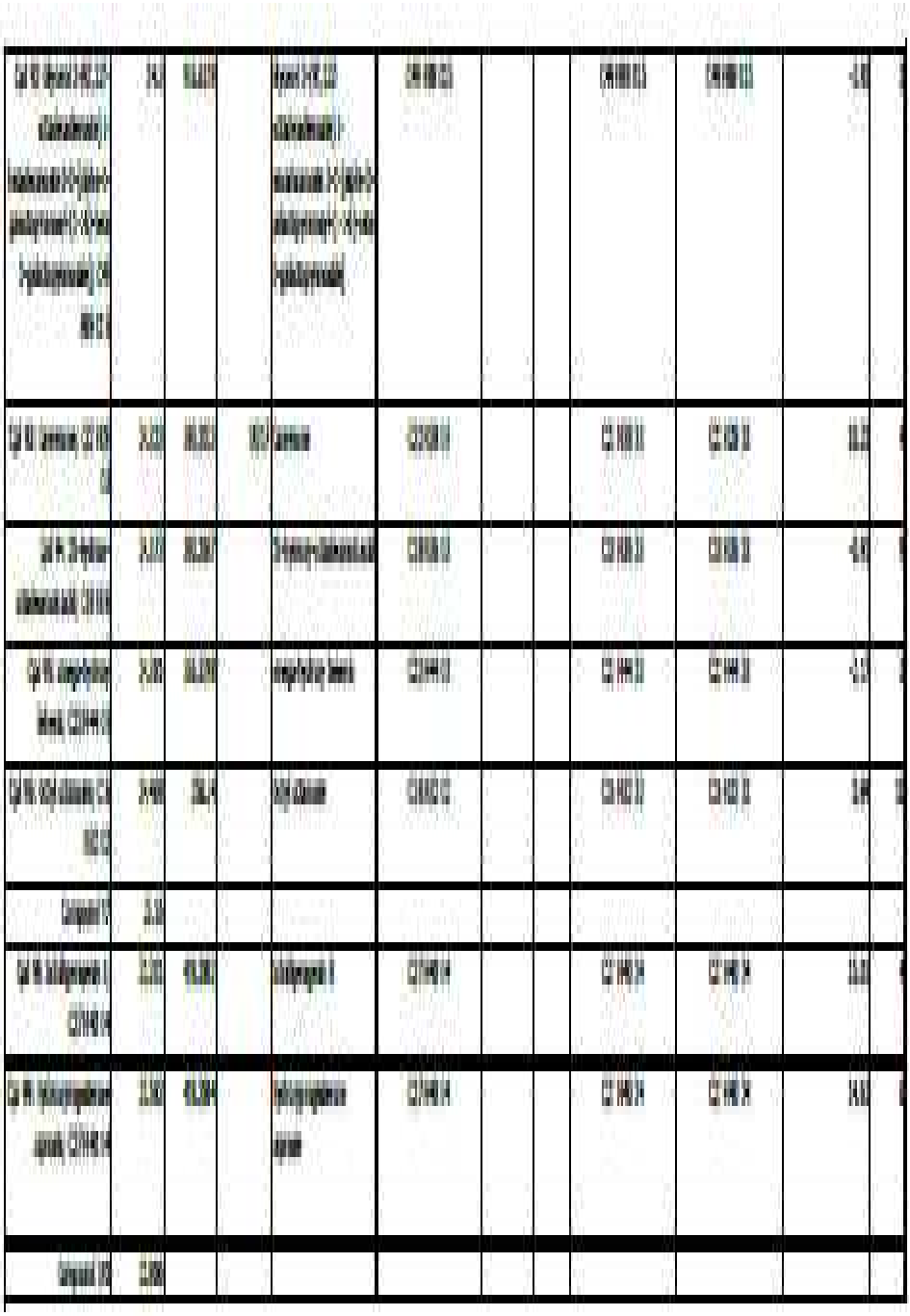
Sample Group	[illegible]
Acquisition SW Version	[illegible]

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

APPENDIX VI

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



Data File	TI-BMC-CD-20.d	Sample Name	TI-BMC-CD-20
Sample Type	Sample	Position	P1-E
Instrument Name	QTOF	User Name	
Acq Method	metabolite_ESI_+VE_POPS.m	Acquired Time	11/7/2024 2:34:06 AM
DA Method	Default.m		
IRM Calibration Status	Success		
Comment			

Sample Group: Info.

Acquisition SW: 6230 series TOF/6530 series

Mass Hunter: (LTOF) 1.05.01 (B0115.1)

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	MFE Formula	DB formula	DB Diff (ppm)	MSMS (DB)
Compound 1	1.39								
Compound 2	1.40								
Compound 3	1.5								
Compound 4	1.76		8328						
Compound 5	1.88		5076						
Compound 6	2.13								
Cpd 7: l-proline-l-glycine; C7 H12 N2 O3	2.46	172.0831	4586	L-valyl-L-glycine	C7 H12 N2 O3	C7 H12 N2 O3	C7 H12 N2 O3	9.92	2
Cpd 8: l-proline-l-glycine; C7 H12 N2 O3	2.75	172.0835	5408	L-valyl-L-glycine	C7 H12 N2 O3	C7 H12 N2 O3	C7 H12 N2 O3	7.46	2
Cpd 9: Isoleonin; C19 H20 N2 O3	4.75	324.1474	2898	Isoleonin	C19 H20 N2 O3	C19 H20 N2 O3	C19 H20 N2 O3	-4.7	9
Cpd 10: Harmine; C13 H12 N2	5.13	224.1047	7862	Harmine	C13 H12 N2	C13 H12 N2	C13 H12 N2	-7.61	2
Cpd 11: Linoleic acid glucuronide; C21 H32 O8	5.24	412.2151		Linoleic acid glucuronide	C21 H32 O8	C21 H32 O8	C21 H32 O8	-5.62	4
Cpd 12: Isoleonine; C19 H18 N4	5.4	334.1426	1001	Isoleonine	C19 H18 N4	C19 H18 N4	C19 H18 N4	-12.59	1
Compound 13	5.72								
Cpd 14: 9-Hexacosenoyldeoxycytidine; C35	5.83	468.2222		9-Hexacosenoyldeoxycytidine	C34 H34 O9	C34 H34 O9	C34 H34 O9	-4.16	2
Cpd 15: Isoleonine; C19 H18 N4	6.07	334.1484	23448	Isoleonine	C19 H18 N4	C19 H18 N4	C19 H18 N4	-12.91	1
Cpd 16: Scleropthyrin; C33 H24 O10	6.22	580.1318		Scleropthyrin	C33 H24 O10	C33 H24 O10	C33H24 O10	8.95	1
Cpd 17: Isoleonidin; C25 H30 N4 O9 S2	6.28	594.1467		Isoleonidin	C25 H30 N4 O9 S2	C25 H30 N4 O9 S2	C25 H30 N4 O9 S2	-2.13	10
Cpd 18: Scleropthyrin; C33 H24 O10	6.45	580.1326		Scleropthyrin	C33 H24 O10	C33 H24 O10	C33H24 O10	7.33	1
Cpd 19: 1,7-Eleangulone; C38 H22 O4	6.56	542.1571		1,7-Eleangulone	C38 H22 O4	C38 H22 O4	C38 H22 O4	-4.8	1
Compound 20	6.67								
Compound 21	6.848								
Cpd 22: 1,7-Eleangulone; C38 H22 O4	7.024	542.1599		1,7-Eleangulone	C38 H22 O4	C38 H22 O4	C38 H22 O4	-7.02	1
Compound 23	7.03								
Cpd 24: 7a-Hydroxy-3-carbamoyl-deacetylcephalosporin C; C15	7.32	402.0971	5117	7a-Hydroxy-3-carbamoyl-deacetylcephalosporin C	C15 H20 N4 O8 S	C15 H20 N4 O8 S	C15 H20 N4 O8 S	-4.39	1
Cpd 25: Isoleonidin; C25 H30 N4 O9 S2	7.66	594.1467		Isoleonidin	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	7.66	411.3122	24052	Cyclopamine	C27 H41 N O2	Q7 H41 N O2	C27 H41 N O2	3.64	1
Cpd 27: Mormacidine; C23 H33 N O2	7.71	345.2509		Mormacidine	C23 H33 N O2	C23 H33 N O2	C23 H33 N O2	1.77	2
Cpd 28: 4''-Methyl-4''-(3,4-dihydroxy-5-(isovaleryloxymethyl)-4-(isovaleryloxy)tetrahydropyran-2-yloxy)-3'',4''-dihydropyran-2''-yloxy)-5-hydroxy-2-(4-hydroxyphenyl)-7-(3-methylbut-2-enyloxy)chromen-4-one; C31 H34 O14	7.88	624.1971		4''-Methyl-4''-(3,4-dihydroxy-5-(isovaleryloxymethyl)tetrahydropyran-2-yloxy)-3'',4''-dihydropyran-2''-yloxy)-5-hydroxy-2-(4-hydroxyphenyl)-7-(3-methylbut-2-enyloxy)chromen-4-one	C31 H34 O14	C31 H34 O14	C31H34 O14	-14.86	1
Compound 29	7.94		4313						
Cpd 30: 11,12,14-trihydroxy-7-methoxy-6,11,13-eleazamine; C23 H28 O5	8.03	376.1913	13412	11,12,14-trihydroxy-7-methoxy-6,11,13-eleazamine-10-one	C23 H28 O5	C23 H28 O5	C23 H28 O5	-7.29	1
Cpd 31: 2,4,6-trihydroxy-1,3,5-hexane; C9 H18 O3	8.17	174.1259	30088	2,4,6-trihydroxy-1,3,5-hexane	C9 H18 O3	C9 H18 O3	C9H18 O3	9.77	1
Cpd 32: N-[[(Ethoxycarbonyl)methyl]-p-menthane-3-carboxamide; C15 H27 N O3	8.19	269.1991		N-[[(ethoxycarbonyl)methyl]-p-menthane-3-carboxamide	C15 H27 N O3	C15 H27 N O3	C15 H27 N O3	10.04	1
Compound 33	8.36		20826						
Compound 34	8.46		5814						
Compound 35	8.58		30834						
Cpd 36: Ginkgolide C; C20 H24 O11	8.69	440.1256		Ginkgolide C	C20 H24 O11	C20 H24 O11	C20H24 O11	13.82	1
Cpd 37: 1-Carbazol-9-yl-3-(3,5-dimethoxyoxan-1-yl)-oxan-2-ol; C20 H21 N2 O	8.86	319.1709	10548	1-Carbazol-9-yl-3-(3,5-dimethoxyoxan-1-yl)-oxan-2-ol	C20 H21N2 O	C20 H21 N2 O	C20 H21 N2 O	-8.66	1
Cpd 38: Isoleonin; C22 H25 N2 O	8.96	347.2019	13472	Isoleonin	C22 H25N2 O	Q2 H25 N2 O	C22 H25 N2 O	-6.18	1
Cpd 39: N'-Acetylgeranoid; C19 H26 N4 O11	9.24	486.343		N'-Acetylgeranoid	C19 H26 N4 O11	C19 H26 N4 O11	C19 H26 N4 O11	-10.05	1
Cpd 40: Isleonine C; C39 H47 N5 O5	9.62	665.3594		Isleonine C	C39 H47 N5 O5	C39 H47 N5 O5	C39 H47 N5 O5	-12.33	1
Cpd 41: Isoleonin; C22 H25 N2 O	9.69	347.2016	40448	Isoleonin	C22 H25N2 O	Q2 H25 N2 O	C22 H25 N2 O	-0.92	1
Compound 42	10.18		9264						
Cpd 43: Isleonine A; C40 H49 N5 O6	10.25	687.3701		Isleonine A	C40 H49 N5 O6	C40 H49 N5 O6	C40 H49 N5 O6	-13.94	1
Compound 44	10.44								
Compound 45	10.56		13562						

APPENDIX VII

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

Data File	TU-ERC-CD-20 -VE.d	Sample Name	TU-ERC-CD-20
Sample Type	Sample	Position	P1-B2
Instrument Name	QTOF	User Name	
Acq Method	metabolite_ISO_-VE_KOPRI.m	Acquired Time	11/8/2024 6:50:02 AM
IDM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition IDM Version	500 series TOF/MS/MS series QTOF 6.80.83 (B0135.2)

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	Diff (ppm)	MS1 Formula	MS Formula	MS Diff (ppm)	Note (MS)
Cpd 32: Malic acid; C4 H6 O5	1.479	134.0221	276058	Malic acid	C4 H6 O5			C4 H5 O5	C4 H5 O5	2.23	
Cpd 12: Tetrahydropalmatrubine-9-Ethyl Ether; C22 H29 N O4	5.211	371.2091		Tetrahydropalmatrubine-9-Ethyl Ether	C22 H29 N O4			C22 H29 N O4	C22 H29 N O4	0.43	
Cpd 1: C7 H6 O2	5.212	122.0364	326		C7 H6 O2	122.0368	-1.36	C7 H6 O2	C7 H6 O2		
Cpd 9: C7 H6 O2	5.212	122.0364	326		C7 H6 O2	122.0368	-1.36	C7 H6 O2	C7 H6 O2		
Cpd 22: C9 H10 O4	5.63	182.0579	4105		C9 H10 O4	182.0579	0.42	C9 H10 O4	C9 H10 O4		
Cpd 14: Homolycopodine; C28 H51 N O10 S2	5.711	617.3217		Homolycopodine	C28 H51 N O10 S2			C28 H51 N O10 S2	C28 H51 N O10 S2	9.1	
Compound 4	5.758										
Cpd 35: Quercetin 3-rhamnoside 7-glucoside; C27 H30 O16	6.774	610.1532		Quercetin 3-rhamnoside 7-glucoside	C27 H30 O16			C27 H29 O16	C27 H30 O16	-2.46	
Cpd 17: Kaempferol 3-O-rutinoside; C28 H32 O15	7.074	608.1743		Kaempferol 3-O-rutinoside	C28 H32 O15			C28 H31 O15	C28 H32 O15	-5.3	
Cpd 18: Quercetin 3,7-dirhamnoside; C27 H30 O16	7.139	594.1634		Quercetin 3,7-dirhamnoside	C27 H30 O16			C27 H29 O16	C27 H30 O16	-5.39	
Cpd 19: Senega root; C40 H76 O19 Ba O5	7.207	932.4847		Senega root	C40 H76 O19 Ba O5			C40 H75 O19 Ba O5	C40 H76 O19 Ba O5	1.1	
Cpd 20: Ephedroxin A; C20 H28 O11	7.264	556.1993		Ephedroxin A	C20 H28 O11			C20 H27 O11	C20 H28 O11	3.23	
Cpd 21: Chrysoeriol; C27 H28 O16	7.271	608.1517		Chrysoeriol	C27 H28 O16			C27 H27 O16	C27 H28 O16	15.07	
Cpd 23: Diosmetin 7-O-beta-D-glucuronopyranoside; C22 H20 O12	7.378	476.0963		Diosmetin 7-O-beta-D-glucuronopyranoside	C22 H20 O12			C22 H19 O12	C22 H20 O12	-0.98	
Compound 3	7.374										
Cpd 24: Rutinoside; C28 H32 O15	7.441	608.1743		Rutinoside	C28 H32 O15			C28 H31 O15	C28 H32 O15	-5.46	
Cpd 25: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	7.444	564.1532		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H27 O14	C26 H28 O14	-5.41	
Cpd 26: Ephedroxin A; C20 H28 O11	7.533	556.1993		Ephedroxin A	C20 H28 O11			C20 H27 O11	C20 H28 O11	3.51	
Cpd 27: Genistein 4',7-di-O-glucoside; C27 H30 O15	7.619	922.2272		Genistein 4',7-O-diglucoside	C27 H30 O15			C27 H29 O15	C27 H30 O15	15.32	
Compound 16	7.636										
Compound 29	7.82										
Compound 28	7.879										
Compound 30	7.883										
Compound 31	7.783										
Cpd 33: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	7.777	564.1532		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H27 O14	C26 H28 O14	-5.43	
Cpd 34: Quercetin 3,7-dirhamnoside; C27 H30 O16	7.870	594.1634		Quercetin 3,7-dirhamnoside	C27 H30 O16			C27 H29 O16	C27 H30 O16	5.47	
Compound 35	7.879										
Cpd 36: Luteolin 4'-O-glucoside; C21 H20 O11	7.960	448.1006		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H19 O11	C21 H20 O11	-5.28	
Compound 37	7.93										
Cpd 38: Genistein 4',7-di-O-diglucoside; C27 H30 O15	7.940	922.2274		Genistein 4',7-O-diglucoside	C27 H30 O15			C27 H29 O15	C27 H30 O15	15.47	
Cpd 39: Diosmetin 7-O-beta-D-glucuronopyranoside; C22 H20 O12	7.957	476.0955		Diosmetin 7-O-beta-D-glucuronopyranoside	C22 H20 O12			C22 H19 O12	C22 H20 O12	0.77	
Cpd 40: Quercetin 3,7-dirhamnoside; C27 H30 O16	8.066	610.1583		Quercetin 3,7-dirhamnoside	C27 H30 O16			C27 H29 O16	C27 H30 O16	-3.03	
Cpd 42: Quercetin 3-rhamnoside 7-glucoside; C27 H30 O16	8.080	610.1583		Quercetin 3-rhamnoside 7-glucoside	C27 H30 O16			C27 H29 O16	C27 H30 O16	-3.06	
Cpd 43: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	8.099	564.1532		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H27 O14	C26 H28 O14	-5.42	
Cpd 45: Tricetin; C15 H10 O7	8.177	302.0424		Tricetin	C15 H10 O7			C15 H9 O7	C15 H10 O7	5.47	
Cpd 44: Luteolin 4'-O-glucoside; C21 H20 O11	8.221	448.1006		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H19 O11	C21 H20 O11	-5.83	
Cpd 47: Genistein 8-C-glucoside; C21 H20 O10	8.426	432.1057		Genistein 8-C-glucoside	C21 H20 O10			C21 H19 O10	C21 H20 O10	-6.81	
Cpd 46: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	8.427	564.1587		Kaempferol 3-rhamnoside 7-xyloside	C26 H28 O14			C26 H27 O14	C26 H28 O14	-3.36	
Cpd 48: Quercetin 3,7-dirhamnoside; C27 H30 O16	8.544	594.1634		Quercetin 3,7-dirhamnoside	C27 H30 O16			C27 H29 O16	C27 H30 O16	-4.04	
Cpd 49: Isothiazole 4,5-diglucoside; C27 H30 O14	8.556	578.1685		Isothiazole 4,5-diglucoside	C27 H30 O14			C27 H29 O14	C27 H30 O14	-5.48	
Cpd 50: DMP-4-glucohydroxamoside; C20 H31 N O12 P	8.571	507.1611		DMP-4-glucohydroxamoside	C20 H31 N O12 P			C20 H31 N O12 P	C20 H31 N O12 P	8.31	
Cpd 51: Quercetin 4'-O-glucoside; C21 H20 O12	8.660	464.0955	5618	Quercetin 4'-O-glucoside	C21 H20 O12			C21 H19 O12	C21 H20 O12	2.16	
Cpd 52: Luteolin 4'-O-glucoside; C21 H20 O11	8.741	448.1006		Luteolin 4'-O-glucoside	C21 H20 O11			C21 H19 O11	C21 H20 O11	-2.76	
Cpd 53: Isoorientin; C21 H20 O11	8.75	462.1163	13082	Isoorientin	C21 H22 O11			C21 H21 O11	C21 H22 O11	-6.83	

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% DPPHsolution 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 to10-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.

वन तथा वातावरण मन्त्रालय
वनस्पति विभाग
राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशाला
गोदावरी, ललितपुर

पत्र संख्या: ०८०।०८९
च.नं. ६४४

मिति २०८०/०६/२६

विषयः नमूना पहिचान सम्बन्धमा।

श्री बीरेन्द्र बहुगुणी क्याम्पस,
भरतपुर, चितवन।

प्रस्तुत विषयमा तहाँ क्याम्पसको 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 प्रविधिक विश्लेषणको प्रतिवेदन (पाना १) यसै पत्रसाथ संलग्न गरी पठाइएको व्यहोरा अनुरोध छ।

२०८०।६।२६
सुभाष खत्री
वरिष्ठ अनुसन्धान अधिकृत
(१६३६८१)
कार्यालय प्रमुख

फोन नं: ४१७४२७७, ४१७४०४७, फ्याक्स: ९७७-०१-४१७४३४९, पोष्ट बक्स नं: ३७०८
Website: www.kath.gov.np

प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमूना परिक्षण गर्न पठाउने व्यक्ति/निकाय:- डी.एफ.ओ. वन्यजन्तु क्याम्पस भरतपुर, चितवन।

१(क) विद्यार्थीहरुको नाम:- श्री अजय महतो (नि.नि. दर्ता नं. ५-२-१९-५७७-२०११)

२. प्राप्त नमूनाको विवरण:- वनस्पतिको नमूना थान-१

३. यस कार्यालयमा प्राप्त मिति:- २०८०/०६/२५

४. परिक्षणका आधारहरु:- (क) हर्बेरियमका भएका नमूनाहरु संगको तुलनात्मक अध्ययन
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

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

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

६. परिक्षण गर्ने अधिकारी:-

रिता चौधरी
अनुसन्धान अधिकृत
(१२८२००)

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Bharatpur, Chitwan

पत्रसंख्या :
च. नं.(Ref.) :

मिति : २०८१।१०।२०
Date :

जो जस सँग सम्बन्धित छ ।

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