# ANTIOXIDANT ACTIVITY, PHYTOCHEMICAL CONTENT AND ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *Aloiampelos ciliaris*

**MAYAU JOHN KIRIMI (B.Ed. Sc.)**
**I56/CE/25294/2018**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Chemistry) in the School of Pure and Applied Sciences of Kenyatta University**

**NOVEMBER, 2025**

## DECLARATION

I declare that this research thesis is my original work and has not been presented for a degree award in any other university.

**Mayau John Kirimi**

**I56/CE/25294/2018**

Signature……Date 22/9/2025

Department of Chemistry

**SUPERVISORS**

We confirm that the candidate carried out the work reported in this thesis under our supervision.

**Prof. Wilson Njue**
Department of Chemistry
Kenyatta University
Signatureee……. Date: 29/10/2025

**Dr. Henry Mwangi**
Department of Chemistry
Kenyatta University
Signature…… Date:8/10/2025

**Dr. John Maingi**
Department Biochemistry, Biotechnology & Microbiology
Kenyatta University
Signature…… Date: 03/11/2018

## DEDICATION

I dedicate this work to my lovely father, siblings, late mum as well as my extended family members. I congratulate them for the immeasurable support, peace, prayers, and unity they gave me.

## ACKNOWLEDGEMENTS

First and foremost, I wish to acknowledge with deepest gratitude the Almighty God for preserving my life throughout the duration of this work and for granting me strength. I am greatly indebted to my supervisors, Prof. Wilson Njue, Dr. Henry Mwangi, and Dr. John Maingi, whose invaluable guidance, unwavering support, encouragement, clear instructions, and tremendous patience were instrumental in the successful completion of this research. I also extend my sincere appreciation to the entire staff of the Department of Chemistry and the Department of Biochemistry, Biotechnology & Microbiology for their continuous guidance and support. My gratitude is further directed to Nancy Kwamboka Maoga and the staff members of the East African Herbarium Botany Department, Parklands, Nairobi, for their assistance in the identification of *A. ciliaris.* In addition, I acknowledge with thanks the contributions of the laboratory technologists, whose expertise and enthusiastic contributions greatly facilitated the research journey. I am equally appreciative of my fellow course mates for their moral support and encouragement throughout this journey. May the ALMIGHTY GOD bless you ALL.

# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ABTS | 2, 2-Azinobis (3-ethylbenzthioazoline-6-sulphonic acid) |
| DCM | Dichloromethane |
| DMSO | Dimethyl Sulfoxide |
| DPPH | 2, 2-Diphenyl-1-Picryhydrazyl Radical |
| GAE | Gallic acid Equivalent |
| GC-MS | Gas chromatography- Mass spectrometer |
| LC-MS | Liquid chromatography mass spectrometer |
| MBC | Minimum Bactericidal Concentration |
| MIC | Minimum Inhibitory Concentrations |
| NACOSTI | National Commission for Science, Technology and Innovation |
| NIST | National Institute of Standards and Technology |
| QE | Quercetin Equivalent |
| UV-VIS | Ultraviolet visible |

## ABSTRACT

Extensive research on medicinal plants has revealed their antibacterial, antifungal, and antioxidant properties. However, the increasing prevalence of bacterial resistance to conventional antibiotics highlights the need to identify new bioactive compounds from medicinal plants. Currently, natural products account for over 50 % of drugs used in the treatment of different infections. Within this context, the *Aloe genus* (family *Asphodelaceae*), comprising approximately 500 *Aloe* species globally and about 50 species in Kenya, has a long-standing history of medicinal use. Notably, *Aloe arborescence*, *Aloe ferox*, and *Aloe perryli* have been extensively documented for their therapeutic potential. Traditionally, Aloe extracts have been employed for anti-malarial, anti-diabetic, anti-inflammatory, ornamental, and green manure purposes. Despite this established knowledge, inadequate information exists regarding the phytochemical composition, therapeutic evaluation, antioxidant activity, and antibacterial properties of *Aloiampelos ciliaris* (*A. ciliaris*). The present study, therefore, aimed to screen for phytochemicals (tannins, phenols, flavonoids, cardiac glycosides), evaluate antioxidant and antibacterial activities, and determine the total phenolic and flavonoid content of *A. ciliaris*. Plant samples were collected from Meru County and macerated in 80 % methanol for 48 hours, followed by solvent partitioning (*n*-hexane, dichloromethane, EtOAc and water). Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging assay, with results ranging from 15.67 % ($IC_{50}$ > 0.0300) to 74.74 % ($IC_{50}$ > 0.0300) at 0.3mg/mL, using ascorbic acid as a standard reference. Total phenolic content ranged from 105.89 ± 0.07 mg GAE/g in the flower crude extract to 1.46 ± 0.04 mg GAE/g in flower DCM extract, with gallic acid as a reference standard. Similarly, total flavonoid content ranged from 182.69 ± 1.64 mg QE/g in root crude extract to 3.59 ± 0.41 mg QE/g in the flower *n*-hexane extract, with quercetin as the reference standard. The antibacterial potential of the extracts was tested against pathogenic Gram-positive (*E. faecalis, B. subtilis*) and Gram-negative bacteria (*E. coli, S. typhi*) using gentamicin and ciprofloxacin as positive controls and 10 % dimethyl sulfoxide (DMSO) as the negative control. Activity ranged from inhibition of *E. coli* by the EtOAc extract (12.0 ± 0.500) to inhibition of *B. subtilis* by the root DCM extract (7.0 ± 0.000). Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, terpenoids, cardiac glycosides, phenols, and saponins. Furthermore, GC-MS analysis facilitated the separation, identification, and quantification of compounds such as methyl-cyclohexane and dodecane. Overall, the findings demonstrate that *A. ciliaris* possesses considerable therapeutic potential, attributable to its diverse secondary metabolites, antioxidant activity, antibacterial effects, and total phenolic and flavonoid contents.

# CHAPTER ONE

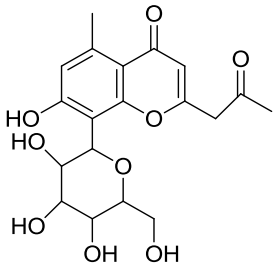
# INTRODUCTION

## 1.1 Background

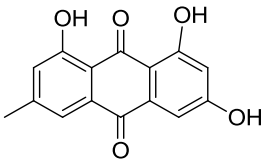
For many years, medicinal plants have been valued as sources of medicines due to their bio-active principles, often considered safer than conventional antibiotics (Mustafa *et al*., 2017). Over eighty percent of the world's population, especially in the third-world countries, depend on plant medicine for their primary health needs (Jamshidi-Kia *et al*., 2017). This reliance is driven by beliefs in minimal side effects, effectiveness, and affordability compared to man-made drugs (Olaokun *et al.*, 2017; Heta and Robo, 2018). Furthermore, medicinal plants contain phytochemicals, crucial for managing human ailments (Wadood, 2013).

More than fifty percent of drugs currently used to treat different infections are derived from natural products (Newman and Cragg, 2020). The Aloeaceae family, especially the genus Aloe, has a significant medicinal history (Tiwari and Upadhayay, 2018) with around 500 species globally and approximately 50 species in Kenya (Egbuna *et al*., 2020). Notably, they include *Aloe vera, Aloiampelos ciliaris, Aloe secundiflora, Aloe marina, Aloe aristata,* and *Aloe polyphylla* (Arunkumar and Muthuselvam, 2009). They are known to possess diverse healing purposes from various backgrounds, cultures, ethnicities, and genders, among others. However, some species like *Aloe eleta*, *Aloe ballyi,* and *Aloe ruspoliana* are primarily used for poisoning rather than healing (East Africa Natural History Society, 2017; Ouchemoukh *et al*., 2012). Aloe species like *Aloe vera, Aloe arborescens, Aloe perryl, Aloe ferox,* and *Aloe barbadense* are extensively researched for their medicinal value (Baby and Justin, 2010; Froldi *et al*., 2019). Others like *Aloe secundiflora* have been treating ailments such as Newcastle disease in chickens, malaria, oedema, and nose bleeding, hence indicating therapeutic properties (Amir *et al*., 2019).

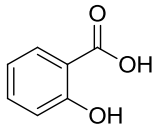
Aloe species contain various phytochemicals like saponins, flavonoids, phenols, terpenes, steroids, and tannins (Kar and Bera, 2018). These natural compounds, synthesized by plants for protection, growth and development, are found in vegetables, grains, and fruits (Bohn, 2014, Martinez *et al*., 2017, Gershenzon and Ullah, 2022). Specific phytochemicals in Aloes include Aloesin (**1**), Emodin (**2**), and Salicylic acid (**3**) among others (Egbuna *et al*., 2020).




**1**




**2**




**3**

Aloe extracts have been found to enhance memory, learning, and cognitive functions, potentially treating Alzheimer's disease (Clementi *et al*., 2015). Aloe glycoprotein (10 kDa) exhibits anti-allergic activity (Sharma *et al*., 2022). Various Aloe species, including *A. arborescens* Mill., *A. striata* Haw., *A. ferox* Mill., *A. clarifora* Strydenburg, *A. mitriformis* Mill, and *A. vera*, show antifungal activity (Zapata *et al*., 2013). *A. vera*, in particular, improves skin elasticity and reduces wrinkles by activating the production of elastic and collagen fibres (Rahmaniah *et al*., 2015). Aloin treats hyperpigmentation, and aloisein inhibits tyrosinase, benefiting hyperpigmentation treatment (Ali *et al*., 2012). *A. vera* gel, combined with antibiotics, has demonstrated antibacterial activity against *H. pylori*, making it an effective natural agent for ulcers (Ratha *et al*., 2015*)*. Aloe species also possess anticancer, antidiuretic, and antibacterial properties (Lawrence *et al*., 2009; Joseph and Raj, 2010; Zhong *et al*., 2013; Suárez *et al.*, 2018).

This study aims to screen secondary metabolites such as phenols, alkaloids, terpenoids, flavonoids, tannins, and cardiac glycosides in the succulent leaves, flowers, and roots of *Aloiampelos ciliaris*, assess their antioxidant and antibacterial activities, and determine the total flavonoid and phenolic content along with the profile of volatile phytochemicals by GC-MS.

### 1.2 Statement of the problem

In rural areas of Kenya, such as Meru, infections like malaria, diarrhea, and tuberculosis (TB) are highly prevalent WHO, (2008-2013). Many plants are traditionally used to treat these ailments, although their effectiveness has not been scientifically validated. The pathogens causing these diseases have developed resistance to allopathic medicines. While certain *Aloe species* like *A. ferox, A. vera,* and *A. arborescens* are well studied and known for their medicinal properties. *Aloiampelos ciliaris* is traditionally used in traditional African societies, such as the Ameru, for treating headaches, stomachaches, malaria, and wounds. However, there is limited work reported on its antimicrobial, antioxidant, and phenolic properties, as well as its volatile compounds. This study aims to scientifically validate the medicinal capability of *Aloiampelos ciliaris*.

### 1.3 Justification of the study

Medicinal plants play a vital role in the treatment of various diseases in developing countries like Kenya (Nankaya *et al*., 2019). The widespread antimicrobial resistance to commonly used antibiotics, such as amoxicillin, has compromised the ability to effectively treat infectious diseases like pneumonia. As a result, exploring new sources of phytochemicals, antioxidant activity, and antibacterial activity, such as those found in *Aloiampelos ciliaris,* is essential. This research will validate its efficacy in combating diseases and use, as a new source of drugs after isolation of pure compounds from it.

## 1.4 Hypotheses

i. There is no significant difference in the phytochemical composition of extracts obtained from different parts of *Aloiampelos ciliaris.*

ii. The levels of total phenolic and flavonoid content do not significantly differ in extracts obtained from different parts of *Aloiampelos ciliaris.*

iii. Extracts obtained from different parts of *Aloiampelos ciliaris* do not significantly differ in their antioxidant properties.

iv. There is no significant difference in antibacterial activities of extracts from different parts of *Aloiampelos ciliaries.*

v. There is no significant difference in the phytochemical profile of volatile compounds from extracts obtained from different parts of *Aloiampelos ciliaris.*

## 1.5 Objectives

### 1.5.1 General objective

To assess the antioxidant activity, phytochemical content, and antibacterial activity in extracts of *Aloiampelos ciliaris*.

### 1.5.2 Specific objectives

i. To determine the presence of flavonoids, alkaloids, tannins, terpenoids, phenols, cardiac glycosides, and saponins in *Aloiampelos ciliaris* extracts.

ii. To determine the total phenolic and flavonoid content in *Aloiampelos ciliaris* extracts.

iii. To determine the antioxidant activity by radical scavenging assays on *Aloiampelos ciliaris* extracts.

iv. To analyze the phytochemical profile of non polar and middle polar volatile compounds in *Aloiampelos ciliaris* crude extracts using GC-MS.

v. To determine the antibacterial effect of *Aloiampelos ciliaris* extracts against bacteria *S. aureus, B. subtilis, S. typhi, E. faecalis,* and *E. coli*.

### 1.6 Significance of the study

The findings of this study will contribute valuable knowledge on potential sources of phytochemicals with antibacterial and antioxidant properties, thereby supporting the discovery of novel drugs derived from *Aloiampelos ciliaris*. In the absence of this research, the phytochemical composition, antioxidant capacity, and antibacterial activity of *A. ciliaris* would remain undocumented, and its traditional use would lack scientific validation.

### 1.7 Scope and limitations of the study

i. The study focused on collecting samples only from some areas of Meru County, Kenya.

ii. Although there are numerous *Aloe* species in the country, this study specifically focused on *Aloiampelos ciliaris*.

iii. Only non-polar and middle polar volatile compounds were separated, identified, and quantified using GC-MS.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Genus *Aloe*

The genus Aloe, characterized by its succulent leaves, has a record of past medicinal use (Adlakha *et al*., 2022). Aloe species like *A. vera*, *A. ferox*, *A. arborescens,* and *A. perryi* are widely recognized for their medicinal properties (Arunkumar and Muthuselvam, 2009). Other Aloe species within the genus also possess therapeutic properties (Babu and Noor, 2020). The medicinal properties of Aloe phytochemicals may be attributed to their antioxidant or prooxidant effects (Aslam *et al.,* 2010; Asamenew *et al*., 2011). Aloe species are known for their anti-inflammatory, antimicrobial, antimalarial, anti-diabetic, antibacterial, and antioxidant activities (Ouchemoukh *et al*., 2012; Zhong *et al*., 2013). In Kenya, communities such as the Kalenjin, Meru, Kamba, and Maasai traditionally use Aloe species for herbal medicine (Kigen *et al*., 2013; Kiplore *et al*., 2014; Cheruiyot and Toroitich, 2019). *Aloe vera,* for instance, is a well-known traditional medicinal herb among tribal people in Kondagattu, India, and Purdue in the United States (Sushen *et al*., 2017). In parts of the Eastern Cape, South Africa, *Aloe ferox* and *Aloe vera* are used for treating sexually transmitted diseases and other medical conditions (Kammoun *et al*., 2011; Tshali, 2023).

### 2.1.1 *Aloiampelos ciliaries*

Previously known as *Aloe ciliaris,* and commonly referred to as the climbing aloe, *Aloiampelos ciliaris* was named in 1825 by Botanist Adrian Harworth (Brandham and Carter, 1990; Grace *et al.*, 2013). *A. ciliries* has various local names: *ishnik* (American), *dahr* (Somali), *kiruma* (Embu), *kihiu* (Kikuyu), *kithunju* (Mbeere), *kipapa* (Taita), *gachukurui* (Meru), *kiluma* (Kamba), *okaka* (Luo) (Brandham and Carter, 1990; Holman, 2002)**.** *Aloiampelos ciliaris* is a climbing succulent plant with slender stems that can reach lengths of approximately 6-8 meters in length (Klopper and Gideon, 2013). Its dark green leaves have

white teeth along the edges and are arranged in open spirals along the stem (Klopper and Gideon, 2013). A distinguishing feature is the presence of teeth on the leaf bases sheathing the stems (Klopper *et al*., 2020). The plant produces reddish-orange tubular flowers, around 25 mm long, pollinated by sunbirds, and has oblong capsule-shaped fruits (Grace *et al*., 2013). Propagation is through cutting a stem segment or planting seeds (Marygun and Codd, 1981). The plant originates from native to the Eastern Cape Province of South Africa, it ranges from Uitenhage to the Kei River mouth in the North East, growing amidst thorny shrubs. In Kenya, it is predominantly found in Meru, Kamba land, Rift valley and Maasai land regions, particularly in dry river valleys with thorny forests (Klopper and Gideon, 2013). Known for its fast growth rate compared to other Aloe species, *Aloiampelos ciliaris's* long stems grow rapidly, pushing through the dense canopies (Klopper and Gideon, 2013).


**Figure 2.1:** *Aloiampelos ciliaris* **Source:** Author

### 2.2 Antioxidant activity and $IC_{50}$.

Antioxidants are compounds that minimize oxidation stress by neutralizing or scavenging reactive oxygen species and free radicals peroxides ($H_2O_2$), hydroperoxides, peroxyl (ROO*), superoxide (O*), hydroxyl (OH) radicals, which can damage tissues (Sharma and Bhat, 2009; Ouchemoukh *et al*., 2012; Ifeanyi, 2018). Reactive oxygen species are highly reactive and

can originate from various sources in biological systems (Singh and Rajini, 2009; Ansari *et al*., 2013). Primary sources (origin) of antioxidants include vegetables (spinach, kale, and amaranth) and fruits (mangoes, lemons, and tomatoes). These contain lycopene, vitamin C, lutein, carotenoids, and phenolic compounds, which inhibit free radicals and lipid peroxidation through metal ion chelation and cycling (Gulcin and Alwasel, 2022; Ivanova *et al*., 2023). Phytochemicals like terpenes, flavonoids, cardiac glycosides, and phenols also have antioxidant properties (Chanda and Ramachandra, 2019).

Antioxidant activity mechanisms include single electron transfer or hydrogen atom transfer (Valko *et al*., 2007; Parcheta *et al*., 2021). Moreover, various assays such as 2, 2-diphenyl-1-picrylhydrazyl radical and 2, 2'-azinobis (3-ethylbenzthioazoline-6-sulphonic acid) (ABTS), are used to assess the radical scavenging activity of foods and plant extracts (Musa *et al*., 2016). These *in vitro* assays are reliable and commonly used because they do not require specialized reactions or equipment (Venskutonins and Van Beek, 2004; Bernard *et. al*., 2018). *In vivo* models, like mice, are also used due to their genetic, biological, and behavioral similarities to humans (Simmon *et al*., 2016).

The inhibitory concentration at 50 % ($IC_{50}$ / $EC_{50}$) measures the amount of a compound needed to inhibit a biological process by 50 % (Jenkinson *et al*., 2020). It provides an informative measure of potency for antagonist drugs in pharmacological research (Sanchez-Moreno *et al*., 1998).

### 2.3 Ethno botanical uses of *Aloe ciliaris*

*Aloiampelos ciliaris* has various ethnobotanical uses, such as being used as green manure to improve soil fertility, a source of firewood, an ornamental plant for its climbing

characteristics, and fodder for animals, in the arid areas (Brigham *et al*., 2003). In Kenya, particularly in Meru and the Rift Valley, it is used to cure diseases like headaches, wounds, stomachaches and malaria (Muthaura *et al*., 2007). *Aloiampelos ciliaris* along with other *Aloe* species, is used globally in countries like Ethiopia, West Africa, Botswana, America and South Africa to manage conditions such as *Diabetes mellitus*, malaria, pain, inflammation and snakebites (Cock, 2015).

**2.4 Total phenolic and flavonoid contents**

Phenolic compounds, coumarins, flavonoids, tannins, and stilbenes are known for their strong antioxidant properties, counteracting free radicals (Vancenzo, 2013). Quantitative assessments of these compounds have been conducted in various food sources and medicinal plants (Aryal *et al*., 2019). Gallic acid and quercetin are commonly used as standard references for phenolic and flavonoid analysis due to their availability and affordability (Mwamatope *et al*., 2020; Tizazu and Bekele, 2024). Folin-Ciocalteu reagent is used to test for phenolics due to the redox reactions involved (Munteanu and Apetrei, 2021). Flavonoids offer health benefits, including anti-cancer, anti-viral, anti-inflammatory, and anti-allergic properties (Clark *et al*., 2015).

**2.5 Phytochemical screening**

Phytochemical screening identifies phytochemical constituents such as phenols, flavonoids, alkaloids, tannins, terpenoids, cardiac glycosides, and saponin in crude extracts (Das and Gezici, 2018). Different compounds display various colors when reacting with specific reagents, indicating their presence (Egbuna *et al*., 2018). For example, alkaloids show a red-brown color when tested with Meyer's reagent (HgCl and KI), forming precipitates from neutral to cream white, and tannins turn blue-black color with 0.1 % Ferric chloride (Egbuna *et al*., 2018). These color changes occur due to electron movement within transition metals,

resulting from specific light wavelength absorption and redox reactions (Limsuwanchote *et al*., 2018). Comparing this study with the one done by Ng *et al*., (2020), *A. ciliaries* has more phytochemical constituents compared to *A. vera.* The analysis of bioactive phytochemical compounds, such as alkaloids, flavonoids, terpenoids, phenolics, and related compounds, requires specialized analytical techniques for accurate identification and quantification. Commonly employed methods include High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Ultraviolet-Visible Spectrophotometry (UV-Vis), and Fourier-Transform Infrared Spectroscopy (FTIR) (Banu and Cathrine, 2015). In the present study, Gas chromatography mass spectrometry was selected due to its availability and proven efficiency in the identification and quantification of non-polar and mid-polar compounds (Banu and Cathrine, 2015).

**2.6 GC-MS analysis**

Crude extracts from roots, leaves, and flowers are cleaned using silica gel to avoid blocking the GC-MS machine (Tuzimski and Petruczynik, 2022). The extraction process uses a range of solvents from nonpolar to polar solvents such as *n*-Hexane, dichloromethane (DCM), acetone, methanol (MeOH), and water (Wong and Kitts, 2006). Gas chromatography Mass spectroscopy (GC-MS) and LC-MS analysis are commonly used to identify and quantify compounds and other secondary metabolites of interest (Sixto *et al*., 2021). Liquid Chromatography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry have key differences based on the sample phase, separation methods, and types of compounds they are best suited for (Pastor *et al*.,2023). LC-MS is best for polar, ionic, or thermally labile compounds that degrade under high heat, like proteins, peptides, nucleic acids, pharmaceuticals, and other large biomolecules, while GC-MS is ideal for volatile, low-molecular-weight organic compounds, unsuitable for heat-sensitive compounds such as

hydrocarbons, solvents, pesticides, and environmental pollutants (Chaudhary *et al*., 2025). Both GC-MS and LC-MS are robust.

### 2.7 Antibacterial activity

Antibacterial activity can be evaluated using standard methods such as agar dilution, disk diffusion, and broth microdilution (Hosseini *et al*., 2024). These *In vitro* techniques assess the activity of extracts against selected microorganisms, with outcomes influenced by factors such as the choice of test organism and the solubility of the extract (Valgas *et al*., 2007; Chikkamadaiah *et al*., 2024). Among these, disk diffusion is the most widely used method (Kim and Kim, 2007; Mayachiew *et al*., 2010). *In vivo* methods, on the other hand, involve the use of living organisms, typically non-human animals like mice (Kilkenny *et al*. 2010). The method is used due to tight control of the physical and chemical environment (Leusch *et al*., 2010). Both gram positive and gram-negative bacteria are known to cause various health issues (Heta and Robo, 2018; Riaz *et al*., 2024). In this study, bacterial strains such as *S. aureus, S. pyogens, B. subtilis, E. maesabitol, S. typhi* and *E. coli, E. faecalis* and *S. mutans* were employed to assess antibacterial activity and to provide insights relevant to human health and gene function.

### 2.8 Data analysis

Data analysis refers to the approaches, techniques and tools applied to examine, clean, transform, and model data with the aim of extracting meaningful insights and supporting informed decision making. Broadly, data analysis methods are classified as either quantitative or qualitative. The choice of methods depends on the type of data, the research objectives, and the analytical tools available. Common approaches include descriptive analysis, inferential analysis, exploratory data analysis (EDA), predictive analysis, content

analysis, and thematic analysis, among others (Islam, 2020). In this research, quantitative analysis was carried out using the MiniTab software, version 17.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Materials, Chemicals, and Apparatus

### 3.1.1 Sampling

After grant of permission by the Kenyatta university (Appendix VI) and NACOSTI (Appendix V), random sampling of *Aloiampelos ciliaris* succulent leaves, flowers, and roots was carried out in their natural habitat in Meru County-Kenya (Tigania East ($0^0$11'51.5" N, $37^0$47'25.4" E) in December 2020. The sampled plants were approximately two and a half years old and were collected using a simple random sampling method during the cool and wet season. The plant specimen was submitted to a taxonomist, Nancy Kwamboka Maoga, at the National Museums of Kenya in Nairobi for verification and identification. A voucher specimen No. MAYAUJK 01 was authenticated, and deposited at the herbarium for future reference, as documented in Appendix VIII.

### 3.1.2 Chemicals and reagents

Analytical-grade reagents and chemicals were utilized in this study comprising; *n*-Hexane (95 %), MeOH (99.9 %), HCl acid (35 %),$H_2SO_4$ (98 %), Glacial acetic acid (99 %), $FeCl_3$, DCM (99.9 %). Additional reagents comprised Quercetin reagent, Gallic acid, ($AlCl_3$), Folin-Ciocalteu (F-C) reagent, mercuric chloride, KI, lead (II) acetate, chloroform (99.00 %), dichloromethane, sodium hydroxide (97 %), bismuth nitrate (99.5 %), sodium chloride (99.999 %), sodium nitrite (99 %). Dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picryhydrazyl (DPPH), Hilton muller agar, Agar powder for Bacteriology, EtOAc, and EtOH (99 %) were also employed. All chemicals and reagents were purchased from ChemExper (United Kingdom) and Sigma-Aldrich (Germany) through a local agent, Kobian.

### 3.1.3 Apparatus and Instruments

The research utilized various equipment, including a Monlinex, 600W blender (Ecully cedex, France), a Heidolph laboratory 4000 rotatory evaporator (USA), GC-MS (ISQ-7000 GC-MS/MS, United State of America) with NIST-17 Search Libraries and UV-VIS Spectrophotometer (A Specord200, Grmany), Autosampler vials, 0.22µm Nylon syringe filters, beakers, conical flasks, separating funnel, micropipettes (200µl and 1000µl) and tips.

## 3.2 Experimentation

### 3.2.1 Material preparation

The collected plant samples (succulent leaves, flowers, and roots) were thoroughly cleaned using running tap $H_2O$ and then rinsed with distilled water. The roots and flowers were dried under the shade, while the leaves were used fresh. The flowers and roots were pulverized using a blender while the leaves were flesh-weighed.

### 3.2.2 Extraction

*Aloiampelos ciliaris* succulent leaves (1600 g), pulverized roots (117 g), and flowers (265 g) were soaked in 500 mL 80 % methanol for 48 hours and then sonicated for 6 hours (Leelasonic ultrasonic bath (Biobase, China). Rotary evaporator (Heidolph Laborata 4000 Rotavapor, United States of America) was used to concentrate the extracts after they had been filtered, to obtain crude. These crude extracts were then partitioned using solvents of increasing polarity: *n*-hexane, DCM, EtOAC and finally $H_2O$.

## 3.3 Phytochemical screening

The established standard protocols were used to analyze initial qualitative phytochemicals.

### 3.3.1 Test for flavonoids

To 1 mL of the extract, three drops of NaOH were added. The formation of an intense yellow color indicates the flavonoids are present, which gradually disappear upon subsequent addition of dilute hydrochloric acid (Biosci *et al*., 2014, Pooja and Vidyasagar, 2016).

### 3.3.2 Test for alkaloids

Approximately 3 mL extract of the plant was combined with 1 mL of 1 % HCl and then treated with 1 mL Meyer's reagent made from KI (5.00 g) and HgCl (1.36 g) in 100 mL of $H_2O$. The formation of a cream white precipitate shows the presence of alkaloids (Dey *et al*., 2010, Pooja and Vidyasagar, 2016).

### 3.3.3 Test for tannins

To 2 mL of crude extract, add basic lead acetate. The formation of a bulky red precipitate due to the precipitation of gelatins indicated the presence of tannins (Archana *et al.*, 2012, Pooja and Vidyasagar, 2016).

### 3.3.4 Test for terpenoids

Approximately 10 mL of ethanol was mixed with a small amount of the extracts. To 5 mL of the filtered sample, 2 mL of $CHCl_3$ was added, followed by 3 mL of $H_2SO_4$. The formation of a reddish-brown color serves as an indication of the presence of terpenoids (Biosci *et al*., 2014, Pooja and Vidyasagar, 2016).

### 3.3.5 Test for phenols

Approximately 5 mL of the plant extract was mixed with a few drops of 5 % (w/v) glacial acetic acid, followed by 5 % (w/v) sodium nitrite ($NaNO_2$) solution. The formation of a muddy brown color indicates the presence of phenols (Archana *et al.*, 2012: Pooja and Vidyasagar, 2016).

### 3.3.6 Test for cardiac glycosides

A mixture of 5 mL of extract plant sample, 2 mL of glacial acetic acid and, 1 drop of $FeCl_3$ (aq) was prepared in a test tube. Subsequently, 1 mL of the conc. $H_2SO_4$ was added. The cardiac glycosides is indicated by formation of a brown ring (Dey *et al*., 2010; Pooja and Vidyasagar, 2016).

### 3.3.7 Test for saponins

10mL of distilled water was used to dilute a small amount of the extract and then vigorously shaken. The formation of a stable foam is attributed to saponins presence (Archana *et al*., 2012, Pooja and Vidyasagar, 2016).

### 3.4 Free Radical Scavenging on DPPH radical

The free radical scavenging activity of plant extracts was evaluated using, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williamset *et al.,* 1995). All extracts were incubated in a dark room to prevent light interference. Varying concentrations of methanolic extracts (0.025, 0.05, 0.1, 0.2, and 0.3 mg/mL) were prepared. A volume of 1.5 mL of the methanolic extract was mixed with 1.5 mL of 0.3 mg/mL of DPPH radical in the bores of the microplate and in triplicates. The mixture (methanolic extract and DPPH) was thoroughly shaken and allowed to stand at room temperature for thirty (30) minutes in a dark room. 50% DMSO was used as a control. A standard reference of ascorbic acid (0.025-0.3 mg/mL) was used, and absorbance was measured at 517 nm after 30 minutes using a UV-VIS Spectrophotometer (Beppu *et al*., 2003; Xu and Chang, 2012). The inhibition percentage of free radical scavenging activity (% RSA) was calculated using the following formula: Inhibition % = Ac-As/Ac*100, where Ac = Control absorbance and As = sample absorbance. The $IC_{50}$ value was calculated from the plot of percentage scavenging against concentrations of plant extract using GraphPad Prism 9 software.

### 3.5 Total phenolic content determination

The total phenolic content was assessed using the F-C colorimetric method by Tumbarski *et al.,* (2019) with slight modifications. A 0.02 g sample extract was prepared in 5 mL of methanol and homogenized for 15 minutes. A set of gallic acid solutions with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL in methanol was used for a standard calibration curve. Subsequently, 1 mL of the resulting crude extract and 2 mL of gallic acid were filled in a 96-well plate in triplicate. A 1 mL of Folin-Ciocalteu reagent and 1mL 5% $Na_2CO_3$ were added to each well. The mixture was incubated at room temperature in a dark room for 2 hours, and then, the absorbance was measured at 765 nm. A standard calibration curve was generated by plotting the absorbance against various concentrations of gallic acid as documented in Appendix IX and the total phenolic content was calculated. The results were expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g). The ANOVA was also carried out as per Appendix II and the data recorded as superscript in table 4.3.

### 3.6 Total flavonoid content determination

The total flavonoid content was determined using the $AlCl_3$ colorimetric method, based on the protocols by Quettier-Deleu *et al*., (2000), Fattahi *et al.,* (2014), and Shraim *et al*., (2021), with slight adjustment. A set of quercetin solutions of concentrations 10, 30, 40, 60, 70, 100, 120, 130, and 150 µg/mL was prepared in methanol (MeOH) to come up with a standard calibration curve shown in the Appendix X. In a test tube, 0.3 mL of the sample was mixed with 3.4 mL of methanol (30%), followed by the addition of 0.15 mL $NaNO_2$ and 0.15 mL of 2% $AlCl_3$. After 5 minutes, 1 mL of 0.5 M NaOH was added, and the mixture was incubated in the dark for 60 minutes. At a wavelength of 347nm, the absorbance was measured using a UV-VIS spectrophotometer (Specord 200, Germany). The total flavonoid content of extracts was calculated by using the calibration curve constructed, and the values were expressed as

milligrams of quercetin equivalents per gram of dry weight (mg QE/g) (Miliauska *et al*., 2004; Cheng *et al*., 2006). The ANOVA for total flavonoids were carried out as per Appendix I, and the data recorded as a superscript in table 4.3.

### 3.7 Antibacterial activity evaluation

Antibacterial assay was accomplished using a modified agar diffusion cup method (Ruparelia *et al*., 2008). Clinical isolates comprising *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC35021), *Streptococcus pyogenes* (ATCC10145, gram-positive), *Salmonella typhi* (ATCC13311), and *Escherichia coli* (ATCC25922) gram-negative bacteria acquired from the Kenyatta University Microbiology laboratory in the Department of Biochemistry, Microbiology and Biotechnology, were tested against *A. ciliaries* extracts. Overnight incubated broth cultures of the test organisms were prepared in nutrient broth media. A 2.0 mL culture of twenty-four-hour-old *S. aureus, S. typhi, S. pyogenes, B. subtilis,* and *E. coli* was spread on respective nutrient agar plates. The concentration of the bacteria was determined using McFarland solution as a reference (GuzmÃ *et al*., 2018). Paper discs (6 mm) were impregnated with different plant extracts and then placed on an agar surface. They were then incubated at 37 °C for 24 hours. Subsequently, the zone of inhibition was measured using a ruler (Gebru *et al*., 2013). Ciprofloxacin and ampicillin were used as standards, while 10% DMSO was used as a control since 10 % DMSO is a polar aprotic solvent that cannot form hydrogen bonds between its own molecules. The plant extracts were further tested to determine their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) (Kahlmeter *et al*., 2003).

### 3.8 GC-MS analysis

Crude extracts of *A. ciliaries* (roots, flowers, and leaves) were fractionated and cleaned up using a 50 ml burette (75 cm) column packed with silica gel. Elution was carried out with

solvents of increasing polarity, namely *n*-hexane, DCM, EtOAc, and MeOH. The fractions were subsequently filtered through 0.22 µm PTFE (Nylon syringe filters) syringe filters and transferred into 1.5 mL auto-sampler vials for GC-MS analysis. Quantitative analysis was performed using a GC-MS/MS instrument equipped with the NIST library (Zhang, 2018). This allowed for the identification and composition determination of the isolated phytochemicals (Cherkaoui *et al*., 2010). Internal calibration was carried out using decafluorobiphenyl while methanol served as the external calibration standard to sample analysis. The lower limit of detection (LLOD) was defined as a signal-to-noise ratio (S/N) ≥3, and the lower limit of quantification (LOQ) was (S/N) ≥10.

For analysis, 1 µL of each sample was injected into an SH-Rxi-5Sil MS capillary GC column (30 m, 0.25 mm ID, 0.25 µm film thickness). Ultrapure helium was used as a carrier gas at a flow rate of 1.0 mL/min. The injector temperature was maintained at 200 $^{o}$C under split mode (5:1). The oven temperature program was set as follows: initial temperature of 50 $^{o}$C (held for 1 minute), followed by an increase of 10 $^{o}$C /min to 250 $^{o}$C (held for 13 minutes), with a total run time of 34 minutes. Data were acquired in full scan mode (mass range of 35-500 m/z), with a similarity index of 70 % and above for compound comparison, and a solvent cut-off time of 3 minutes. The ion source temperature was maintained at 200 $^{0}$ C, and the interface temperature at 250 $^{o}$C. Spectra data of eluted compounds were matched against the NIST-17 Library for compound identification.

**3.9 Data analysis**

The experimental data obtained were analyzed and expressed as mean ± standard deviation (SD). Additionally, descriptive inferential statistics were carried out to analyze data through Minitab software version 17 to determine statistical differences within data set, that is ANOVA test was done.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Crude extracts and partitions

The crude extracts yielded 15.91 g, 10.54 g, and 13.63 g from flowers, leaves, and roots, respectively. The various masses and percentages of partitions are detailed in Table 4.1.

**Table 4.1: Mass in grams and (%) yield of Crude extracts of partitions**

| Sample | *n*-Hexane | | DCM | | EtOAc | | Aqueous | |
|---|---|---|---|---|---|---|---|---|
| | Mass in grams | % yield | Mass in grams | % yield | Mass in grams | % yield | Mass in grams | % yield |
| Flowers | 1.52 | 10.93 | 1.07 | 7.69 | 0.68 | 4.89 | 1.29 | 9.27 |
| Leaves | 0.5 | 5.85 | 0.12 | 1.41 | 0.23 | 2.69 | 0.4 | 4.68 |
| Roots | 0.86 | 7.39 | 0.31 | 2.67 | 0.36 | 3.10 | 1.56 | 13.41 |

The flower crude extract produced the highest yield (15.91 g), followed by root extract (13.63 g) and the leaf extract (10.54 g). Among the solvent partitions, the root aqueous extract exhibited the highest mass (1.56 g), whereas the leaf DCM extract yielded the lowest mass (0.12 g). In general, *n*-hexane generated higher yields across all partitions, except for the root crude extract, where the aqueous fraction produced the highest amount (13.41 %).

For the flower partitions, the yield decreased with increasing solvent polarity: *n*-hexane (10.93 %), DCM (7.69 %), and ethyl acetate (4.89 %). However, the aqueous fraction showed a relatively high yield (9.27 %), likely attributable to the presence of polar sugars stored in flowers. In contrast, the root partitions displayed an increasing yield with solvent polarity: *n*-hexane (7.39 %), DCM (2.67 %), ethyl acetate (3.10 %), aqueous (13.41 %), indicating that non-polar compounds are predominantly stored in the roots. The leaf partitions followed a similar trend, with yields of *n*-hexane (5.85 %), DCM (1.41 %), ethyl acetate (2.69 %),

aqueous (4.6 %), related to the synthesis of mostly polar compounds such as alkaloids (Bhambhani *et al*., 2021).

Overall, variations in percentage yields can be attributed primarily to differences in the varying concentrations of bio-active compounds present in the various plant parts (root, leaves and flowers) and polarity of the solvents used. Non-polar compounds such as *n*-hexane preferentially extract lipophilic compounds whereas polar solvents such as water are more effective in extracting hydrophilic compounds. Comparison of percentage yields across solvents therefore, provides valuable insights into solvent efficiency and the distribution of phytochemicals within the plant.

**4.2 Phytochemical screening**

Several key groups of secondary metabolites were identified in both the crude and partition extracts of *A. ciliaris*. Extracts from the flowers, leaves, and roots all tested positive for the various classes of compounds analyzed. Specifically, alkaloids, flavonoids, phenols, terpenoids, cardiac glycosides, saponins, and tannins were detected across all extracts, as presented in Table 4.2.

**Table 4.2: Phytochemical analysis of *A. ciliaris* extracts**

| Extracts | | **Flavonoids** | **Alkaloids** | **Tannins** | **Terpenoids** | **Phenols** | **Cardiac glycosides** | **Saponins** |
|---|---|---|---|---|---|---|---|---|
| Flower crude extract | | + | + | + | + | + | + | + |
| Leaves crude extract | | + | + | + | + | + | + | + |
| Roots crude extract | | + | + | + | + | + | + | + |
| Flower | *n*-hexane partition | + | + | - | - | - | - | - |
| | Dichloromethane partition | + | + | + | - | - | - | + |
| | Ethyacetate partition | + | + | + | - | + | + | + |
| | Aqueous partition | + | + | + | - | - | + | + |
| Leaves | *n*-hexane partition | + | + | - | - | - | - | + |
| | Dichloromethane partition | + | + | - | - | - | - | + |
| | Ethylacetate partition | + | + | + | + | - | - | + |
| | Aqueous partition | + | - | + | - | - | - | - |
| Roots | *n*-hexane partition | + | + | + | - | - | - | + |
| | Dichloromethane partition | + | + | + | + | + | + | + |
| | Ethylacetate partition | + | + | + | + | + | + | + |
| | Aqueous partition | + | - | + | + | + | + | + |

**Key:** (+) present, (-) absent

The presence of various phytochemicals in the crude extracts of *A. ciliaries* underscores the plant's potential applications as an anti-inflammatory, anticancer, and antimicrobial agent. Flavonoids and alkaloids were detected in nearly all extracts, except alkaloids, in the aqueous root and leaf extracts, and saponins in the aqueous leaf and flower *n*-hexane extracts. Notably, both the DCM and EtOAc root partitions contained all the screened secondary metabolites. However, the DCM partition, despite exhibiting lower total phenolic (1.46 ± 0.04) and flavonoids (4.57 ± 0.48) contents, lacked terpenoids, tannins, and phenols. The extraction efficiency of phytochemicals was strongly influenced by solvent polarity: polar

solvents such as aqueous and EtOAc preferentially extracted polar compounds such as phenols, flavonoids, and cardiac glycosides, whereas nonpolar solvents like *n*-hexane primarily extracted lipophilic compounds, which were comparatively fewer in antioxidant-related constituents. Qualitative screening enabled the identification of specific phytochemicals, while quantitative analysis determined their concentrations; together, these approaches provide a comprehensive understanding of the phytochemical composition of *A. ciliaris*.

### 4.3 Total Phenolic and flavonoid content

#### 4.3.1 Total phenolic and flavonoid contents of *A. ciliaris*

The crude extracts of *A. ciliaris* (flowers, roots, and leaves) were analyzed for their phenolic and flavonoid content, expressed as Gallic acid equivalent (GAE) and quercetin equivalent (QE), respectively. Flavonoids and phenolics were specifically selected for analysis, as they are well-documented in the literature as the principal contributors to antioxidant activity. The results indicated that the extracts contained significant levels of phenolics and flavonoids, as shown in table 4.3

**Table 4.3: Total phenolics and flavonoids content of *A. ciliaris* extracts**

| **Extract** | | **Total Phenolic content (mg GAE/g) (mean ± sd)** | **Total flavonoid content (mg QE/g) (mean ± sd)** |
|---|---|---|---|
| Flower crude extract | | 105.89±0.07[a] | 169.28±0.53[c] |
| Leave crude extract | | 30.47±0.02[d] | 128.61±1.39[d] |
| Root crude extract | | 83.64±0.02[b] | 182.69±1.64[a] |
| Flower | *n*-hexane partition | 2.32±0.01[n] | 3.59±0.41[l] |
| | DCM partition | 1.46±0.04[o] | 4.57±0.48[l] |
| | EtOAc partition | 15.63±0.02[h] | 102.11±3.63[e] |
| | Aqueous partition | 9.48±0.03[k] | 35.01± 0.56[ij] |
| Leave | *n*-hexane partition | 8.23±0.01[m] | 36.42±0.42[i] |
| | DCM partition | 9.11±0.01[l] | 63.86±0.83[h] |
| | EtOAc partition | 40.45±0.02[c] | 177.95±2.02[b] |
| | Aqueous partition | 11.26±0.04[j] | 26.23±0.26[k] |
| Root | *n*-hexane partition | 29.42±0.01[e] | 71.54±0.73[g] |
| | DCM partition | 17.95±0.10[g] | 33.29±1.12[j] |
| | EtOAc partition | 20.17±0.01[f] | 77.94±0.82[f] |
| | Aqueous partition | 13.93±0.01[i] | 76.65±0.92[f] |

*A similar superscript means no significant difference in ANOVA analysis.*

Flower extracts exhibited the highest phenolic content (105.89 ± 0.07 mg GAE/g), roots (83.64 ± 0.02 mg GAE/g), and leaf extracts (30.47 ± 0.02 mg GAE/g). In contrast, the highest flavonoid concentration was observed in the root extracts (182.69 ± 1.64 mg QE/g), followed by the flowers (83.64 ± 0.02 mg QE/g) and leaves (30.47 ± 0.02 mg QE/g). The high levels of flavonoids and phenolics are consistent with significant antioxidant activity (Xu *et al*., 2003; D`Archivio *et al*., 2007).

Notably, the DCM and EtOAc partitions of the root extracts contained all screened secondary metabolites, although the DCM partition exhibited the lowest phenolic and flavonoid contents. The detection of these phytochemicals substantiates the medicinal potential of *A. ciliaris*, particularly in anti-inflammatory, anticancer, and antimicrobial applications (Agati *et al*., 2012). Comparatively, *A. ciliaris* demonstrated higher flavonoid and phenolic levels than *A. vera*. Overall, the findings highlight a strong relationship between antioxidant activity and total phenolic and flavonoid content, with higher concentrations corresponding to greater bioactivity.

### 4.4 Antioxidant activity by free radical scavenging

#### 4.4.1 Antioxidant activity by free radical scavenging of crude extracts

The plant extracts exhibited concentration-dependent increases in antioxidant activities, as illustrated in Figure 4.1 (a) and (b).

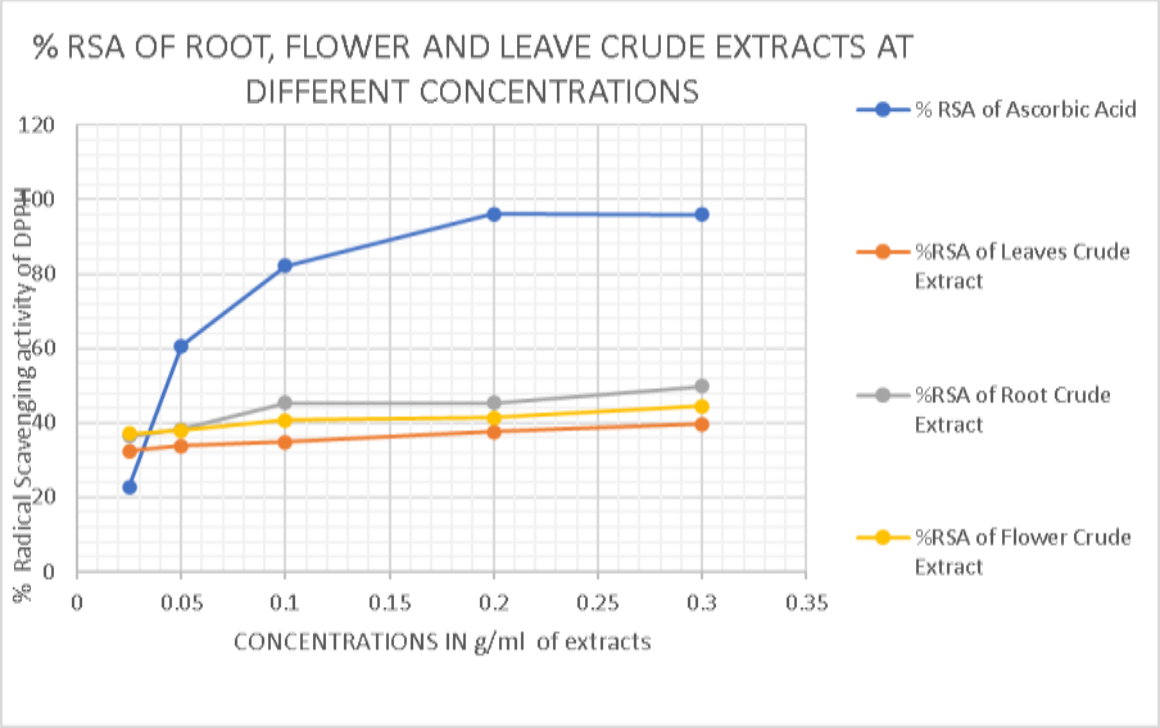



**Figure 4.1(a):** Antioxidant activity of roots, flowers and leave extracts.

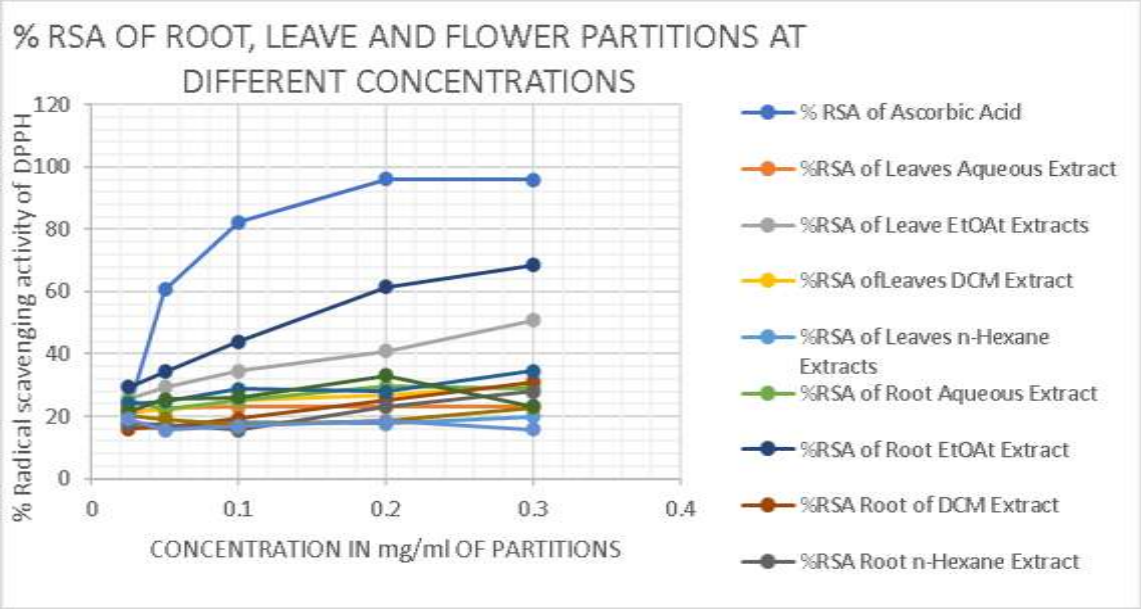



**Figure 4.1 (b):** Antioxidant activity of root, flower, and leaf crude extracts partitions

The crude extracts of *A. ciliaris* roots, flowers, and leaves demonstrated DPPH radical scavenging activity ranging from 39.68% to 49.73%, compared to 96% for the standard reference, ascorbic acid, at 0.3 mg/mL. Antioxidant activity generally increased with concentration, reaching a maximum at 0.3 mg/mL. Notably, *A. vera* leaf extract displayed higher activity (50%) than *A. ciliaris* (39.68%) (Ozsoy *et al*., 2009). Among the *A. ciliaris* extracts, the root exhibited the highest radical scavenging activity, likely attributable to nutrient absorption from the soil, while the leaves recorded the lowest.

To further evaluate phytochemical distribution, the extracts were partitioned using solvents of increasing polarity (*n*-hexane, DCM, EtOAc, and aqueous). The DPPH radical scavenging activity of these fractions ranged from 15.67% to 74.74%, compared to 96% for ascorbic acid at 0.3 mg/mL. The root EtOAc fraction demonstrated the highest antioxidant activity, consistent with its elevated flavonoid and phenolic content, whereas the flower *n*-hexane fraction exhibited the lowest. In general, plant parts with higher total phenolic and flavonoid levels corresponded with higher antioxidant activity. The root EtOAc fraction showed the greatest radical scavenging potential (74.74%, $IC_{50}$ = 0.140 mg/mL), followed by leaf EtOAc

extract (50.73%, $IC_{50}$ = 0.29 mg/mL). Other notable results included root crude extract (49.73%, $IC_{50}$ = 0.300 mg/mL), leaf crude extract (39.68%, $IC_{50}$ > 0.300 mg/mL), and flower crude extract (44.49%, $IC_{50}$ > 0.300 mg/mL). Overall, root demonstrated superior antioxidant activity, likely due to direct nutrient absorption, while leaves showed reduced activity as metabolites were translocated to storage sites. A strong correlation was observed between antioxidant activity, total phenolic ($R^2$=0.9997) and flavonoid ($R^2$=0.9991) content.

Inhibition concentration ($IC_{50}$) values confirmed this relationship, with higher antioxidant activity associated with lower $IC_{50}$ values. The findings, summarized in Table 4.4, highlight the significant antioxidant potential of *A. ciliaris* extracts.

**Table 4.4: $IC_{50}$ and antioxidant activity of the extracts**

| **Extract** | | **% RSA on DPPH of Extract** | **$IC_{50}$(mg/ml)** |
|---|---|---|---|
| Ascorbic Acid | | 96.00 | 0.045 |
| Flower Crude extract | | 44.49 | >0.300 |
| Leave crude Extract | | 39.68 | >0.300 |
| Root crude extract | | 49.73 | 0.300 |
| Flower | *n*-hexane partition | 15.67 | >0.300 |
| | DCM partition | 23.20 | >0.300 |
| | EtOA partition | 34.46 | >0.300 |
| | Aqueous partition | 22.72 | >0.300 |
| Leave | *n*-hexane partition | 19.80 | >0.300 |
| | DCM partition | 30.28 | >0.300 |
| | EtOAc partition | 50.73 | 0.297 |
| | Aqueous partition | 29.98 | >0.300 |
| Root | *n*-hexane partition | 28.02 | 0.135 |
| | DCM partition | 31.03 | >0.300 |
| | EtOAc partition | 74.74 | 0.140 |
| | Aqueous partition | 29.03 | >0.300 |

Interestingly, the root *n*-hexane partition exhibited moderate activity (28 %) but the lowest $IC_{50}$ value of 0.135, underscoring that antioxidant potential depends not only on compound quantity but also on structural characteristics, synergistic effects, and the types of phenolics present. Although the root EtOAc fraction had the highest activity at 0.3 mg/mL (74.74%, $IC_{50}$ = 0.140 mg/mL), it was still lower than that of ascorbic acid (96%, $IC_{50}$ = 0.045 mg/mL). Other results included root crude extract (49.73%, $IC_{50}$ = 0.300 mg/mL) and leaves EtOAc extract (50.73%, $IC_{50}$ = 0.295 mg/mL).

**4.5 Antibacterial assessment**

The plant extracts showed significant antibacterial activity against various clinical bacterial strains, including the gram-negative species *E. coli* and *S. typhi*, as well as the gram-positive species *E. faecalis*, *B. subtilis*, *E. maesabitol, S. mutans, S. pyogenes,* and *S. aureus.* Among the extracts, the root aqueous extract exhibited the highest bioactivity, producing a significant zone of inhibition (11.0 ±0.50 mm) against *E. faecalis.* This was followed by flower *n*-hexane (10.0 ±0.00 mm) and the root DCM extract (10.0±0.50). The leaf DCM extract was most effective against *B. subtilis*, showing a zone of inhibition of 10.0±0.500. Notably, the flower EtOAc partition extract was the only fraction that exhibited inhibitory activity against *E. coli*, a gram-negative bacterium, as summarized in Table 4.5.1.

**Table 4.5.1: Inhibition zones of *A. ciliaris* extracts on *E. coli*, *E. faecalis* and *B. subtilis.***

| Extracts | | Zones of inhibition *(mm)* | | |
|---|---|---|---|---|
| | | *E. faecalis* | *B. subtilis* | *E. coli* |
| Flower | EtOAc partition | - | $7.0\pm0.000^{H}$ | $12.0\pm0.500^{E}$ |
| | *n*-hexane partition | $10.0\pm0.00^{EFG}$ | - | - |
| leaves DCM partition | | - | $10.0\pm0.500^{EFG}$ | - |
| Root | DCM partition | $10.0\pm0.500^{EFG}$ | - | - |
| | EtOAc partition | - | $9.3\pm0.577^{FGH}$ | - |
| | aqueous partition | $11.0\pm0.500^{GH}$ | $8.0\pm0.500^{D}$ | - |
| 10% DMSO (Negative control) | | - | - | - |
| Gentamicin (positive control) | | $21.7\pm2.08^{D}$ | $27.7\pm0.577^{C}$ | $20.3\pm0.577^{D}$ |
| Ciprofloxacin (positive control) | | $30.0\pm1.000^{BC}$ | $33.3\pm0.577^{A}$ | $31.0\pm1.000^{AB}$ |

**Key: (-)** No activity

*Similar superscript letters imply no significant difference, while different superscript letters mean there is a significant difference at 95 % confidence level and p-value (Turkey P<0.05).*

Other bacterial strains like *S. typhi*, *S. aureus*, *S. mutans*, *S. pyogens* and *E. maesabitol* exhibited no observable zones of inhibition. In general, Gram positive bacteria (*E. faecalis* and *B. subtili)* demonstrated greater susceptibility, showing zones of inhibition across more extracts when compared to the Gram-negative bacterium (*E. coli*). This difference is likely attributable to the thick peptidoglycan layer in Gram-positive bacteria, which facilitates the absorption of antimicrobial agents. By contrast, Gram negative bacteria like *E. coli* possess an outer membrane rich in lipopolysaccharides, membrane which restricts the entry of many phytochemicals, thereby conferring increased resistance. Plate 4.1 illustrates the activity of root and leaf ethyl acetate extracts against *B. subtilis.*

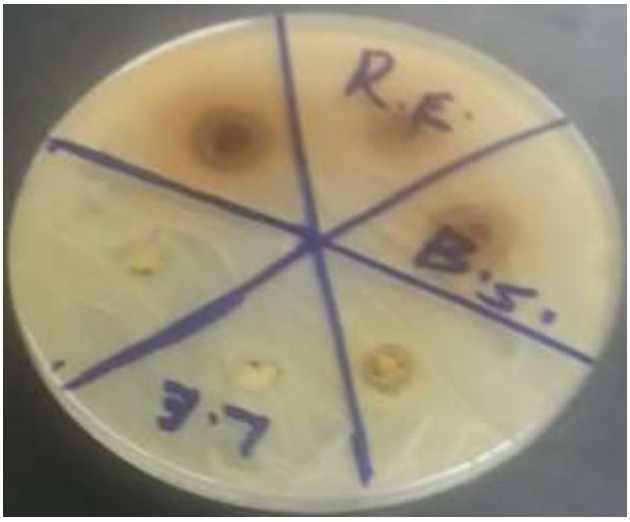



**Plate 4.1:** Root EtOAc (R. E): *B. subtilis* (B.s) 9.3 mm and Leave EtOAC: B.s 6 mm

The observed variations in inhibition levels were attributed to differences in bacterial cell membrane composition which influence interactions with membrane-associated proteins (Gebru *et al*., 2013). Among the tested strains, *E. faecalis* a Gram-positive bacterium, exhibited the highest inhibition zone (11.0 ± 0.500 mm). However, when compared to the positive controls (Gentamicin and Ciprofloxacin), all plant extracts demonstrated lower inhibition zones. This disparity is expected, as crude plant extracts generally contain relatively low concentrations of bioactive compounds compared to purified antibiotics like gentamicine and ciprofloxacin. The positive controls produced markedly higher inhibition zones against *E. faecalis* (21.7 ± 2.08 mm; 30.0 ± 1.000 mm) and *B. subtilis* (27.7 ± 0.577 mm; 33.3 ± 0.577 mm) respectively. None of the extracts exhibited inhibition zones below that of the negative control (10 % DMSO.

Minimum inhibitory concentration (MIC) values against *B. subtilis,* were 0.25 mg/mL for the root EtOAc partition, 0.56 mg/mL for the flower EtOAc partition, and 0.43 mg/mL for the root aqueous extract (Table 4.5.2).

**Table 4.5.2: The significant MIC values for the three partitions against *B. subtilis* and E. *faecalis*.**

| Partition/Bacteria | *B. subtilis* | *E. faecalis* |
|---|---|---|
| Root EtOAc partition | 0.25 mg/mL | N/A |
| Root aqueous partition | 0.56 mg/mL | N/A |
| Flower EtOAc partition | N/A | 0.43 mg/mL |

N/A**:** not applicable

Other extracts, such as the flower partitions, did not yield positive results, and *E. coli* exhibited no measurable inhibition in MIC determinations. Extracts with detectable MIC values were subjected to minimum bactericidal concentration (MBC) testing. The root EtOAc partition exhibited an MBC of 0.25 mg/mL, while root aqueous extract recorded an MBC of 0.46 mg/mL. The flower EtOAc partition did not demonstrate any bactericidal activity. Nevertheless, the flower EtOAc partition produced an inhibition zone of 12.0 ± 0.500 mm against *E. coli*, although this was notably lower than the inhibition observed with the positive controls.

The inhibitory effects of the extracts were attributed to phytochemicals such as dodecane, tetradecane, tridecane, heneicosane, *n*-Hexadecanoic acid, and hexadecanoic acid methyl ester, all of which are known for antimicrobial properties against both gram-positive and gram-negative bacteria (Solanki *et al*., 2008). These compounds were identified from different parts of *A. ciliaries* extracts through GC-MS. MIC, defined as the lowest concentration of an extract that inhibits visible bacterial growth, and MBC, the lowest concentration that results in bacterial cell death, were determined for *B. subtilis* and *E. faecalis*. These two strains were selected because they consistently exhibited measurable

zones of inhibition across multiple extracts, indicating sufficient susceptibility for further MIC/MBC assays.

Invariably, some extracts did not yield measurable results, likely due to factors such as compound volatility, low extract concentration, or high inoculum density. Overall, the roots and flowers of *A. ciliaris* demonstrated the most promising antimicrobial activity. However, considering concerns related to plant extinction and the seasonality of flowers, the leaves of *A. ciliaris* are recommended as the primary sample source material for future investigations.

### 4.6 The Gas chromatography mass spectroscopy (GC-MS) analysis of *A. cilliaris*

The crude extracts of *A. ciliaris* (roots, leaves, and flowers) were partitioned to prevent blockage of the GC-MS/MS instrument. These fractions were subsequently analyzed using GC-MS/MS for the quantification and identification of volatile compounds. A sample of leaf GC-MS profIle spectrum is presented in Figure 4.2 for the DCM leaf fraction, in Appendix XI .

The similarity indices (SI) of the identified compounds ranged from 73 to 98, indicating strong correspondence with reference standards in the National Institute of Standards and Technology (NIST) library. The sharp chromatographic peaks facilitated accurate quantitative analysis of compound concentrations within the samples. Key compounds identified in samples include; 2,4-Di-tert-butylphenol (53.79 % in flower methanol), Butane, 1,1-dibutoxy (4.92 % in root *n*-hexane), 9-Octadecenamide, (Z) (33.87 % in leaves DCM and 27.87 % in flower DCM), 1-Butanone- 1-cyclohexyl (4.92 % in root *n*-hexane), 2-Pentanone-4-hydroxy-4-methyl (59.09 % in root EtOAc and 69.52 % in root EtOAc), Pentadecafluorooctanoic acid- undecyl ester (53.6 % in leaf *n*-hexane) and β-D-Glucopyranoside, methyl (22.76 % in root methanol and 22.76 % in leaf DCM).

Retention time, a crucial parameter in gas chromatography, varied across compounds. For example, 1-Hexacosanol (1.46 %, RT: 30.945 min in flower DCM) exhibited the longest elution time, while Pentadecafluorooctanoic acid- undecyl ester (53.6 %, RT: 3.04 minutes, leaf *n*-hexane) eluted the fastest. Different partition extracts displayed different compounds under GC-MS analysis as documented in the Appendix IV. Previous studies have reported that compounds like Heneicosane (RT: 18.542), *n*-Hexadecane (RT: 13.44), 2-hydroxy-1-(hydroxymethyl) ethyl ester Hexadecanoic acid (RT: 28.514) exhibit antibacterial activity, several of which were identified in these partitions (Chen *et al*., 2022). The root EtOAc fraction demonstrated biological activity against *B. subtilis*, while the root methanol fraction exhibited activity against both *B. subtilis* and *E. faecalis*, underscoring their potential medicinal value. Overall, the volatile compounds in *A. cilliaries* possess significant pharmacological properties, warranting further detailed investigation.

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

The crude extracts of *A. ciliaris* (flowers, roots, and leaves), together with their respective partitions, were found to contain significant phytochemicals, like alkaloids, tannins, flavonoids, terpenoids, phenols, saponins, and cardiac glycosides. Flavonoids were consistently present across all the extracts, indicating their substantial contribution to the overall phytochemical profile, while alkaloids, tannins, and saponins were also well represented.

The total phenolic and total flavonoid contents varied among the plant parts and their partitions. The flower crude extract exhibited the highest total phenolic content (105.89 mg GAE/g), whereas the flower DCM fraction recorded the lowest (1.46 mg GAE/g).In contrast, the root crude extract had the highest total flavonoid content (182.69 mg QE/g), while the flower *n*-hexane fraction contained the lowest (3.59 mg QE/g).

Antioxidant activity also varied depending on the polarity of the extracting solvents, ranging from 15.67 % in the flower *n*-hexane fraction to 74.74 % in the root EtOAc fraction. All plant parts (flowers, roots, and leaves) demonstrated significant antioxidant activity against free radicals, although this activity was slightly lower than that of the ascorbic acid control. The results further indicated that lower $IC_{50}$ values corresponded to higher antioxidant activity, underscoring the role of these plant parts as valuable natural sources of antioxidants and potential therapeutic agents.

When tested against both Gram-positive and Gram-negative bacteria (*E. faecalis*, *B. subtilis*, and *E. coli*), the crude extracts and their partitions displayed measurable antibacterial activity. Their inhibition zones exceeded that of the negative control (10% DMSO, 6.0 mm) but were smaller than those of the positive control (gentamicin, 27.7 mm), thereby suggesting their potential medicinal significance.

GC-MS analysis of all the extracts revealed numerous compounds across the different plant parts. Among them, the flowers and roots exhibited higher antioxidant activity, as well as greater total phenolic and flavonoid contents, compared to the leaves. These findings indicate that the flowers and roots of *A. ciliaris* represent the most promising sources for the discovery of new natural antioxidants.

## 5.2 Recommendations

### 5.2.1 Recommendations from the study

i. The significant radical scavenging activity and the presence of diverse secondary metabolites in the flowers, roots, and leaves of *Aloiampelos ciliaris* highlight their potential for medicinal applications. To fully realize this potential, the specific bioactive compounds responsible for the observed pharmacological effects should be isolated and characterized for possible use in therapeutic formulations.

ii. While the current study successfully identified volatile compounds through GC-MS analysis, the identification of polar, non-volatile constituents may be more effectively achieved using LC-MS, thereby providing a more comprehensive profile of the plant's phytochemical composition.

### 5.2.2 Recommendations for further research

Further investigation is necessary to isolate, characterize, and identify the specific phytochemical constituents responsible for the antioxidant activity, total phenolic content,

and flavonoid content observed in *A. ciliaris*. Sampling the species from diverse geographical regions within the country and conducting comparative analyses would provide a more comprehensive understanding of its phytochemical profile and biological activity. Moreover, comparative studies with other species within the Aloe genus would be valuable, given their diverse biological roles, which range from therapeutic and healing properties to potentially toxic effects. Since the current study focused exclusively on bacterial pathogens, future research should extend to evaluating the extracts against other microorganisms, including fungi and viruses, to establish a broader spectrum of antimicrobial potential

## REFERENCES

Adlakha, K., Koul, B. and Kumar, A. (2022). Value-added products of Aloe species: Panacea for several maladies. *South African journal of botany*, *147*, 1124-1135.

Agati, G., Azzarrello, E., Pollastri, S. and Tattin, M. (2012). Flavonoids as antioxidants in plants. *Location and fuctional significance, plants Sciences,* 196:67-76.

Ali, S.A., Galgut, J.M. and Choudhary, R.K. (2012). On the novel action of the melanolysis by a leaf extract of *Aloe vera* and its active ingredient aloin, potent skin depigmenting agents. *Planta Medicinal,* 78:767-771.

Amir, H. M., Grace, O. M., Wabuyele, E., & Manoko, M. L. K. (2019). Ethnobotany of aloe L. (Asphodelaceae) in Tanzania. *South African Journal of Botany*, *122*, 330-335.

Ansari, A. Q., Ahmed, S.A., MA, Waheed and Sayyed Jumed, A. (2013). Extraction and determination of antioxidant activity of *Withania somnifera. Dunal. European Journal of Experimental Biology,* 3(5):502-507.

Archana, P., Samantha, T., Mahithan, B., Chamundeswari and Ramaswammy (2012). Plant biotechnology research group, Department of Biotechnology Kakatiya University, *Waranga 1-50 6009(AP), India.*

Arunkumar, S. and Muthuselvam, M. (2009). Analysis of phytochemical constituents and antimicrobial activitiesof *Aloe vera* L. against clinical pathogens. *World Journal Agricultural Sciences,* 5(5):572-576.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, *8*(4), 96.

Asamenew, S., Bisrat D., Mazumder A. and Asres, K. (2011). Invitro antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlans* Reynolds. *Phytotherapy Research,* 25:1756-1760.

Aslam, K., Khosa, M.K., Jahan, N. and Nosheen, S. (2010). Synthesis and applications of coumarin. *Pakistan Journal Pharmaceutical Sciences,* 23:449-454.

Babu, S. N. and Noor, A. (2020). Bioactive constituents of the genus Aloe and their potential therapeutic and pharmacological applications: A review. *Journal of Applied Pharmaceutical Science*, *10* (11), 133-145.

Baby, J. and Justin, S.R. (2010). Phamacognostic and phytochemical properties of *Aloe vera* linn. An overview. *International Journal of Phamarceutical Sciences review and Research,* 4:106.

Beppu, H., Koike, T., Shimpo K., Chihara, T., Hoshino, M., Ida, C. and Kazuya, H (2003). Radical scavenging effects of *Aloe arborescens* miller on prevention of pancreatic islet B-cell destruction in rats. *Journal of Ethnophamarcol,* 89:37-45.

Bernard, M., Jubeli, E., Pungente, M. D. and Yagoubi, N. (2018). Biocompatibility of polymer-based biomaterials and medical devices–regulations, in vitro screening and risk-management. *Biomaterials science*, *6*(8), 2025-2053.

Banu, K. S. and Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, *2*(4), 25-32.

Bhambhani, S., Kondhare, K. R. and Giri, A. P. (2021). Diversity in chemical structures and biological properties of plant alkaloids. *Molecules*, *26*(11), 3374.

Biosci, I.J., Abdul, hafeez, E.Y., Karamova, N.S. and Ilinskaya, O.N. (2014). Antioxidant activity and total phenolic compounds content of certain medicinal plants. *International Journal of Biosciences*; 5(9):213-222.

Bohn, T. (2014). Dietary factors that affecting polyphenol bioavailability. *Nutrition reviews*,72 (7):429-52.

Brandham, P.E. and Carter, S. (1990). A revesion of the *Aloe tidmarshii/A. cilliaries* complex in South Africa. *Kew Bulletin*, 45(4):637-643.

Brigham, S. and Don, W. (2003). Ornamental trees of San Diego. *Journal of the Arnold Arboretum ISBN*, 13: 978093479720

Cardarell,i M., Rouphael, Y., Pellizzoni, M., Colla, G. and Lucini, L. (2017). Profile of bioactive secondary metabolites and antioxidant capacity of leaf exudates from eighteen *Aloe* species. *Indigeneous crop production*, 108:44-51.

Chaudhary, S., Passi, A., Jindal, S. and Goyal, K. (2025). LC-MS/MS: a powerful tool for modern analytical science: Fundamentals, techniques, applications and innovations. *Journal of Liquid Chromatography and Related Technologies*, 1-11.

Chanda, S. and Ramachandra, T. V. (2019). A review on some Therapeutic aspects of Phytochemicals present in Medicinal plants. *International Journal of Pharmacy and Life Sciences*, *10*(1).

Chen, J. J., Cheng, M. J, Lee T. H., Kuo, Y. H. and Lu, C. T. (2022). Secondary Metabolites with Anti-Inflammatory from the Roots of *Cimicifuga taiwanensis*. *Molecules*, 27 (18):6035.

Cheng, Z., Moore, J. and Yu, L. (2006). High throughput relative DPPH radical scavenging capacity assay. *Journal of Agriculture and Food Chemistry*, 54(20):7429-7436.

Cherkaoui, A., Hibbis, J., Emonet, S., Tangoma, M., Francois, P. and Schrenzel, J. (2010). Comparison of two matrix-assisted laser desorption ionization time of flight mass spectroscopy methods with convectional phenotypic for identification of of bacterial to the species level. *Journal of Clinical Microbiology*, 48:1169-1175.

Cheruiyot, E., and Toroitich, R. (2019). Technologies Used to Document and Disseminate Indigenous Knowledge on Food Preservation among the Kalenjins of Kenya. *Digital Technologies FoR Information*, 429.

Chikkamadaiah, M., Venkatesha, D., Dilkalal, A., Narayana, R. K., Narayanappa, V. H., and Anandan, S. (2024). Assessment of antibacterial, anti-inflammatory, and anti-cancer activities of Melia azedarach L. leaf extract. *Cancer Plus*, *6*(1), 2763.

Clark, J. L, Zahradka, P. and Taylor, C. G (2015). Efficacy of flavonoids in the management of high blood pressure. *Nutrition Review*, 73(12):799-822.

Clementi, M.E., Tringali, G., TRiggiani, D. and Giardina, B. (2015). *Aloe arborescens* extract protects IMR-32 cells against Alzheimer Amyloid Beta peptide through inhibition of radical peroxide production. *Naural Product Communications,* 10(11):1993-5.

Cock, I.E. (2015). Phytochemistry and therapeutic uses including treatments for gastrointestinal conditions and chronic inflammation. *Progress in Drugs research,* 70:179-235.

D`Archivio, M., Filesi, C., Di Benedetto, R., Giovanninin, C. and Masella, R. (2007). Polyphenols, dietary sources and bioavailability. *The Annali dell'Istituto Superiore di Sanita,* 43:348-361.

Das, K. and Gezici, S. (2018). Secondary plant metabolites, their separation and identification, and role in human disease prevention. *Annals of Phytomedicine An-International Journals*, *7*, 13-24.

Dey, S.N., De, S.H., and Ghosh, A.K. (2010). Evaluation of analgesic activity of methanolic extract of amorphophallus paeoniifolius tuber by tail flick and acetic-induced writhing response method. *International Journal of Pharma and Biosciences,* 1:1662-8.

Egbuna, C., Amadi, C. N., Patrick-Iwuanyanwu, K. C., Ezzat, S. M., Awuchi, C. G., Ugonwa, P. O. and Orisakwe, O. E. (2021). Emerging pollutants in Nigeria. *A systematic review, Environmental toxicology and pharmacology*, *85*, 103638.

Egbuna, C., Gupta, E., Ezzat, S. M., Jeevanandam, J., Mishra, N., Akram, M. and Onyekere, P. F. (2020). Aloe species as valuable sources of functional bioactives. *Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations*, 337-387.

Fattahi, S., Akhavan-Niaki, H., Pourbagher, R., Zabihi, E., Abedian, Z. and Mostafazadeh, A. (2014). Total Phenolic and Flavonoids contents of aqueous Extract of Stinging Nettle and In Vitro Antiproliferative Effect on Hela and BT-474 Cell Lines. *International Journal of Molecular and Cellular Medicine;* 3;102-7.

Froldi, G., Baronchelli, F., Marin, E., Grison, M. and Molecules (2019). Antiglycation activity and HT-29 cellular uptake of Aloe Emodin, Aloin and *Aloe arborescens* leaf extracts. *Molecules*, 5;24(11):2128.

Gebru, H., Taddesse, A., Kaushal, J. and Yadav, O. (2013). Green synthesis of AgNPs and their antibacterial activity. *Journal of Surface Science and Technology,* 29 (1-2), 47-66.

Gershenzon J. and Ullah C. (2022). Plants protect themselves from herbivores by optimizing the distribution of chemical defenses. *Proceeding of the National Academy of Sciences of USA*, 119(4).

Gulcin, İ. and Alwasel, S. H. (2022). Metal ions, metal chelators and metal chelating assay as antioxidant method. *Processes*, 10(1), 132.

GuzmÃ, L., Ramirez, B. S., Maribel, C. F., Pescador, M. G. N. and Cruz, F. J. M. (2018). Low accuracy of the McFarland method for estimation of bacterial populations. *African Journal of Microbiology Research*, 12(31), 736-740.

Grace, O.M., Klopper, R.R., Smith, G.F., Crouch, N.R., Figneiredo, E., Ronsted, N. and Vanwyk, A.E. (2013). A revised generic classification for aloe (xanthrorhoeceae subfam. Asphodeloideae). *Phytotaxa,* 76 (1):7-14.

Heta, S. and Robo, I. (2018). The side effects of the most commonly used group of antibiotics in periodontal treatments. *Medical Sciences*, *6*(1), 6.

Holman, E.W. (2002). The relation between folk and scientific classification of plants and animals. *Journal of Classification,* 19:131-59.

Hosseini, F., Habibi Najafi, M. B., Rasool Oromiehie, A., Nasiri Mahalati, M. and Yavarmanesh, M. (2024). Study on synergistic effects of curcumin and bixin against foodborne pathogens. *Food Science and Nutrition*.

Ifeanyi, O. E. (2018). A review on free radicals and antioxidants. *International Journal Current Research Medical Science*, 4(2), 123-133.

Islam, M. (2020). Data analysis: types, process, methods, techniques and tools. *International Journal on Data Science and Technology*, *6*(1), 10-15.

Ivanova, T., Marchev, A., Chervenkov, M., Bosseva, Y., Georgiev, M., Kozuharova, E., & Dimitrova, D. (2023). Catching the green—Diversity of ruderal spring plants traditionally consumed in bulgaria and their potential benefit for human health. *Diversity*, *15*(3), 435.

Jamshidi-Kia, F., Lorigooini, Z. and Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1), 1-7.

Jenkinson, S., Schmidt, F., Ribeiro, L. R., Delaunois, A. and Valentin, J. P. (2020). A practical guide to secondary pharmacology in drug discovery. *Journal of Pharmacological and Toxicological Methods*, *105*, 106869.

Joseph, B. and Raj, S. (2010). Phamarcognostic and phytochemical properties of *Aloe vera* linn-an. Overview. *International Journal Phamaceutical Science Review and Ressearch,* 4:106-110.

Kahlmeter G., Derek F., Fred W., Johan W., Anders O., Arne R., Martin S., Pavla U. and Alkiviadis V. (2003). European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria.*Journal of Antimicrobial Chemotherapy,* 52, 145–148.

Kammoun, M., Miladi, S., Ali, Y.B., Damark, M., Gargouri, Y. and Bezzine, S. (2011). In vitro study of the PLA2 inhibition and antioxidant activities of *Aloe vera* leaf skin extracts. *Lipids and Health Diseases,* 10:30.

Kar, S.K. and Bera, T.K. (2018). Phytochemical constituents of *Aloe vera* and their multifunctional properties: A comprehensive review. *International Journal of Scientific Research,* 9(4):1416-23.

Kigen, G. K., Ronoh, H. K., Kipkore, W. K., & Rotich, J. K. (2013). Current trends of traditional herbal medicine practice in Kenya: a review. *African Journal of Pharmacology and Therapeutics*, *2*(1).

Kilkenny C., Browne, W. Cuthill, I. C., Emerson, M. and Altman, D. G. (2010). Animal research, reporting *in vivo* experiments. *British Journal of pharmacology,*106(7)1577-1579

Kim, J. S. and Kim, Y. H. (2007). The inhibitory effect of natural bioactives on the growth of pathogenic bacteria. *Nutrition Research and Practice,* 1: 273-278.

Kiplore, W., Wanjohi, B., Ronoh, H. and Kigen, G.A. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. *Journal Ethnobiology and Ethnomedicine,* 10:24.

Klopper, R. R., Crouch, N. R., Smith, G. F. and van Wyk, A. E. (2020). A synoptic review of the aloes (Asphodelaceae, Alooideae) of KwaZulu-Natal, an ecologically diverse province in eastern South Africa. *PhytoKeys*, *142*, 1.

Klopper and Gideon, F.S. (2013). *Aloiampelos ciliaries* var. redacta (S. Carter). *Phytotaxa,* 76:10.

Lawrence, R., Tripathi, P. and Jeyakumar, E. (2009). Isolation, purification and evaluation of antibacterial agents from *Aloe vera*. *Brazilian Journal of Microbiology,* 40:906-915.

Leusch, F. D., De Jager, C., Levi, Y., Lim, R., Puijker, L., Sacher, F. and Chapman, H. F. (2010). Comparison of five in vitro bioassays to measure estrogenic activity in environmental waters. *Environmental Science and Technology*, *44*(10), 3853-3860.

Li, X., Krumholz, H. M., Yip, W., Cheng, K. K., De Maeseneer, J., Meng, Q. and Hu, S. (2020). Quality of primary health care in China: challenges and recommendations. *The Lancet*, *395*(10239), 1802-1812.

Limsuwanchote, S., Putalun, W., Tanaka, H., Morimoto, S., Keawpradub, N. and Wungsintaweekul, J. (2018). Development of an immunochromatographic strip incorporating anti- mitragynine monoclonal antibody conjugated to colloidal gold for kratom alkaloids detection. *Drug Testing and Analysis*, *10*(7), 1168-1175.

Martinez, K. B., Mackert, J. D. and McIntosh, M. K. (2017). Polyphenols and intestinal health. In *Nutrition and functional foods for healthy aging* (pp. 191-210). Academic Press.

Marygunn and Codd L.E.W. (1981). Botanical exploration of Southern Africa, Balkema, Cape Town. *CRC Press: ISBN* 0869611291, 9780869611296.

Mayachiew, P., Devahastin, S., Mackey, B. M. and Niranjan, K. (2010). Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract. *Food Research International* 43: 125-132.

Miladi, S. and Demak, M. (2008). In vitro antioxidant activities of Aloe vera leaf skinextracts. *Journal de la societe chimiquede tunisie,* 10:101-109.

Miliauska, G., Venskutonins, P.R. and Van Beek, T.A. (2004). Screening of radical activity of some medical and aromatic plant extracts. *Food Chemistry,* 85(2):231-237.

Munteanu, I. G. and Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity. *Review, International journal of molecular sciences*, *22*(7), 3380.

Musa, K. H., Abdullah, A. and Al-Haiqi, A. (2016). Determination of DPPH free radical scavenging activity: application of artificial neural networks. *Food chemistry, 194*, 705-711.

Mustafa, G., Arif, R., Atta, A., Sharif, S., and Jamil, A. (2017). Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. *Matrix Science Pharma*, *1*(1), 17-26.

Muthaura, C. N, Rukunga, G. M, Chhabra, S.C, Omar S. A, Guantai, A. N, Gathirwa, J. W, Tolo, F. M, Mwitari, P. G, Keter, L. K, Kirira, P. G, Kimani, C. W, Mungai, G. M and Njagi, E. N. (2007) Antimalarial activity of some plants traditionally used in treatment of malaria in Kwale district of Kenya. *Journal of Ethnopharmacology*,112(3):545-51.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E. and Nyirenda, C. (2020). Total phenolic contents and antioxidant activity of Senna singueana, Melia azedarach, Moringa oleifera and Lannea discolor herbal plants. *Scientific African*, *9*, e00481.

Nankaya, J., Gichuki, N., Lukhoba, C. and Balslev, H. (2019). Medicinal plants of the Maasai of Kenya: a review. *Plants*, *9*(1), 44.

Newman, D. J. and Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of natural products*, *83*(3), 770-803.

Ng, Z. X., Yong, P. H. and Lim, S. Y. (2020). Customized drying treatments increased the extraction of phytochemicals and antioxidant activity from economically viable medical plants. *Industrial crops and products*,155,112815

Ouchemoukh, S., Hachoud, S., Boudraham, M.A., Mokrani, A. and Louaileche, H. (2012). Antioxidant activities of some dried fruits consumed in Algeria. *LWT-Food Science and Technology*, 49(2):329-332.

Olaokun, O.O., Mkolo, N.M., Mogale, M.A. and King, P.H. (2017). Phytochemical screening, antioxidant, anti-inflammatory and glucose utilization activities of three South African plants used traditionally to treat diseases. *Biomedical (Aligarh)*, 9:412.

Ozsoy, N., Candoken, E. and Akev, N. (2009). Implications for degenerative disorders: Antioxidant activity, total phenols, flavonoids, ascorbic acid, β-carotene and β-tocopherol in *Aloe vera*. *Oxidative medicine and cellular longevity*,2:99-106.

Pastor, K., Ilić, M., Vujić, D., Ačanski, M., Kravić, S., Stojanović, Z., and Đurović, A. (2023). Gas chromatography and mass spectrometry: the technique. In *Emerging Food Authentication Methodologies Using GC/MS* (pp. 3-31). Cham: Springer International Publishing.

Parcheta, M., Świsłocka, R., Orzechowska, S., Akimowicz, M., Choińska, R. and Lewandowski, W. (2021). Recent developments in effective antioxidants: The structure and antioxidant properties. *Materials*, 14(8), 1984.

Pooja, S., and Vidyasagar, G. M. (2016). Phytochemical screening for secondary metabolites of Opuntia dillenii Haw. *Journal of Medicinal Plants Studies*, *4*(5), 39-43.

Quettier-Deleu C., Gressier B., Vasseur, J., Dine, T., Brunet C., Luyckx, M.C., Cazin, J.C., Bailleul, F and Trotin, F. (2000): Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, 72(11), 35-42.

Rahmaniah, A.H. Aldebasi, Y.H., Srikar, S., Khan, A.A. and Aly, S.M. (2015). Potential candidate in health management through modulation of biological activities. *Phamarcognosy Review,* 9(18):120-6.

Ratha, M.H. and Laxmipriya, M.P. (2015). Evaluation of biological properties and clinical effectiveness of *Aloe vera*. *Journal of Traditional Complementary Medicine,* 5(1):21-6.

Riaz, M., Javed, M., Bukhari, S., Shaheen, F., Khan, T., Khalid, H. and Zulfiqar, R. (2024). Bloodstream infections and trends of antimicrobial sensitivity pattern: a laboratory based study. *Zhonghua er bi yan hou tou jing wai ke za zhi= Chinese journal of otorhinolaryngology head and neck surgery*, *55*, 1421-1428.

Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P. and Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia*, 4 (3), 707-716.

Sanchez-Moreno, C., Larrauri, J.A. and Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of Agriculture and Food Chemistry,* 47(6), 425-431.

Simmon, R.D., Kumar, S. and Jo, H. (2016). The role of endothelial mechanosensitive genes in antherosclerosis and omics approaches. *Archieves of Biochemestry and Biophysics,* 591:111-31.

Singh, N. and Rajini, P.S. (2004). Free radical scavenging of an aqueous extracts of potato peel. *Food Chemistry,* 85(4):611-616.

Sixto, A., Pérez-Parada, A., Niell, S., & Heinzen, H. (2019). GC–MS and LC–MS/MS workflows for the identification and quantitation of pyrrolizidine alkaloids in plant extracts, a case study: Echium plantagineum. *Revista Brasileira de Farmacognosia*, *29*, 500-503.

Sharma, D., Purohit, N., Chaubey, S., Tiwari, M., Bhadauriya, M., Bhasker, N. and Kumar, D. (2022). A Review on Pharmacological and Therapeutic Potential of Aloe barbadensis Miller. *European Journal of Medicinal Plants*, *33*(6), 23-43.

Sharma, O.P. and Bhat, T.K. (2009). DPPH antioxidant assay revisited. *Food Chemistry,* 113(4):1202-1204.

Shraim, A. M., Ahmed, T. A., Rahman, M. M., and Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *Lwt*, *150*, 111932.

Solanki, R., Khanna, M. and Lal, R. (2008). Bioactive compounds from marine *Actinomyces*. *Indian Journal of microbiology,* 48:410-431.

Suárez, A. I. and Chávez, K. (2018). Appraisal of medicinal plants with anticancer properties in South America. *Anticancer plants: Properties and Application: Volume 1*, 229-283.

Sushen, U., Unnithan, C., Rajan, S., Chouhan, R., Couhan, S., Uddin, F. and Kowsaoya, R. (2017). *Aloe vera*. A potential herb used as traditional medicine by tribal people of Kondagatu and Purdue. *Pharmaceutical, Medical and Biological Sciences*, 4(7), 831-820

Tizazu, A. and Bekele, T. (2024). A review on the medicinal applications of flavonoids from aloe species. *European Journal of Medicinal Chemistry Reports*, 100135.

Tiwari, M. and Upadhayay, M. (2018). The medicinal plant components and applications (Aloe vera). *Journal of Medicinal Plants Studies*, *6*(3), 89-95.

Tumbarski, Y., Nikolova, R., Petkova N., Ivanov, I. and Lante, A. (2019). Biopreservation of fresh strawberries by carboxylmethyl cellulose edible coating enriched with bacteriocin from bacillus methylotropicus BM47. *Food Technology and Biotechnology,* 57:1-19.

Tshali, N. (2023). *Phytochemical screening and anti-bacterial activity of medicinal plants used to treat skin diseases in the Eastern Cape, South Africa* (Doctoral dissertation, Mangosuthu University of Technology (MUT)).

Tuzimski, T., and Petruczynik, A. (2022). Determination of anti-Alzheimer's disease activity of selected plant ingredients. *Molecules*, *27*(10), 3222.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Telser, J. (2007). Free radicals and antioxidant in normal physiological functions and human disease. *The international Journal of Biochemistry and Cell Biology,* 9(1):44-84.

Valgas, C., Souza, S. M. d. and Smania, E. F. A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, 38: 369-380.

Vancenzo Lantanzo (2013). Natural products. *Phytochemistry, Botany and Metabolism of Alkaloids, Phenols and Terpene,* 1543-1580.

Xu, B. and Chang, S.K.C. (2012). Comparative study on antiproliferatoin properties and cellular antioxidants activities of commonly consumed food legumes against nine human cancer cell lines. *Food Chemistry,* 134: 1287-1296.

Xu, Y., Xu, J. and Hu, Q. (2003). Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbandesis* Miller) extracts. *Journal of Agricultural and food chemistry,* 51:7788-7791.

Wadood, A. (2013). Phytochemical analysis of medicinal plants occurring in local areas of Mardan. *Biochemistry and analytical biochemistry,* 2 (4); 2-5.

Wong, P.Y. and Kitts, D.D. (2006). Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselimum crispum*) and cilantro (*Criandrum sativum*) extracts. *Food Chemistry,* 97(3):505-515.

Zapata, P., Nararro, D., Guillen, F., Castillo, S., Martinez –Ronevo, D. and Serrano, M. (2013). Characterization of gels from different Aloe species as antifungal treatment. *Potential crops for industrial applications Industrial Crops and Products,* 42:223-230.

Zhang, D. H. (2018). Volatile analysis of several nitrogen fixingcyanobacteriaisolated from rice fields using a Gas chromatography mass spectromer. *Journal of Anhui Agricultural Sciences,* 46:145-148.

Zhong, J., Huang, Y., Ding., Wu, X., Wan, J. and Luo, H. (2013). Chemical constituents of *Aloe barbadensis* miller and their inhibitory effects on Phosphodiesterase-4D. *Fitoterapia*, 91:159-165.

# APPENDICES

## Appendix I: One-way analysis of variance (ANOVA) of different *Aloiampelos ciliaries* extracts (total flavonoids) at different mean concentrations

Method

Null hypothesis: All means are equal
Alternative hypothesis: At least one mean is different
Significance level: α = 0.05

Equal variances were assumed for the analysis.

Factor Information

| Factor | Levels | Values |
|---|---|---|
| samples | 15 | Flower Aqueous Extract, Flower Crude Extract, Flower DCM Extract, Flower EtOAt Extract, Flower n-Hexane Extract, Leave Aqueous Extract, Leave Crude Extract, Leave DCM Extract, Leave EtOAt Extract, Leave n-Hexane Extract, Root Aqueous Extract, Root Crude Extract, Root DCM Extract, Root EtOAc Extract, Root n-Hexane Extract |

Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| samples | 14 | 155477 | 11105.5 | 6144.49 | 0.000 |
| Error | 30 | 54 | 1.8 | | |
| Total | 44 | 155531 | | | |

Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 1.34439 | 99.97% | 99.95% | 99.92% |

Means

| samples | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| Flower Aqueous Extract | 3 | 35.014 | 0.556 | ( 33.429, 36.599) |
| Flower Crude Extract | 3 | 169.282 | 0.532 | (167.696, 170.867) |
| Flower DCM Extract | 3 | 4.572 | 0.479 | ( 2.987, 6.157) |
| Flower EtOAc Extract | 3 | 102.11 | 3.63 | ( 100.53, 103.70) |
| Flower n-Hexane Extract | 3 | 3.589 | 0.413 | ( 2.004, 5.174) |
| Leave Aqueous Extract | 3 | 26.231 | 0.257 | ( 24.646, 27.816) |
| Leave Crude Extract | 3 | 128.605 | 1.392 | (127.019, 130.190) |
| Leave DCM Extract | 3 | 63.860 | 0.825 | ( 62.275, 65.446) |
| Leave EtOAc Extract | 3 | 177.95 | 2.02 | ( 176.36, 179.53) |
| Leave n-Hexane Extract | 3 | 36.421 | 0.420 | ( 34.836, 38.006) |
| Root Aqueous Extract | 3 | 76.647 | 0.923 | ( 75.062, 78.232) |
| Root Crude Extract | 3 | 182.690 | 1.638 | (181.105, 184.276) |
| Root DCM Extract | 3 | 33.292 | 1.115 | ( 31.707, 34.877) |
| Root EtOAc Extract | 3 | 77.936 | 0.820 | ( 76.351, 79.522) |
| Root n-Hexane Extract | 3 | 71.543 | 0.735 | ( 69.958, 73.128) |

Pooled StDev = 1.34439

## Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

| samples | N | Mean | Grouping |
|---|---|---|---|
| Root Crude Extract | 3 | 182.690 | A |
| Leave EtOAc Extract | 3 | 177.95 | B |
| Flower Crude Extract | 3 | 169.282 | C |
| Leave Crude Extract | 3 | 128.605 | D |
| Flower EtOAt Extract | 3 | 102.11 | E |
| Root EtOAc Extract | 3 | 77.936 | F |
| Root Aqueous Extract | 3 | 76.647 | F |
| Root n-Hexane Extract | 3 | 71.543 | G |
| Leave DCM Extract | 3 | 63.860 | H |
| Leave n-Hexane Extract | 3 | 36.421 | I |
| Flower Aqueous Extract | 3 | 35.014 | I |
| Root DCM Extract | 3 | 33.292 | I |
| Leave Aqueous Extract | 3 | 26.231 | J |
| Flower DCM Extract | 3 | 4.572 | K |
| Flower n-Hexane Extract | 3 | 3.589 | K |

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

| Adjusted Difference of Levels | Difference of Means | SE of Difference | 95% CI | T-Value | P-Value |
|---|---|---|---|---|---|
| Flower Crude - Flower Aqueo | 134.27 | 1.10 | ( 130.22, 138.31) | 122.32 | 0.000 |
| Flower DCM E - Flower Aqueo | -30.44 | 1.10 | ( -34.49, -26.40) | -27.73 | 0.000 |
| Flower EtOAc - Flower Aqueo | 67.10 | 1.10 | ( 63.05, 71.14) | 61.13 | 0.000 |
| Flower n-Hex - Flower Aqueo | -31.43 | 1.10 | ( -35.47, -27.38) | -28.63 | 0.000 |
| Leave Aqueou - Flower Aqueo | -8.78 | 1.10 | ( -12.83, -4.74) | -8.00 | 0.000 |
| Leave Crude - Flower Aqueo | 93.59 | 1.10 | ( 89.55, 97.63) | 85.26 | 0.000 |
| Leave DCM Ex - Flower Aqueo | 28.85 | 1.10 | ( 24.80, 32.89) | 26.28 | 0.000 |
| Leave EtOAc - Flower Aqueo | 142.93 | 1.10 | ( 138.89, 146.98) | 130.21 | 0.000 |
| Leave n-Hexa - Flower Aqueo | 1.41 | 1.10 | ( -2.64, 5.45) | 1.28 | 0.991 |
| Root Aqueous - Flower Aqueo | 41.63 | 1.10 | ( 37.59, 45.68) | 37.93 | 0.000 |
| Root Crude E - Flower Aqueo | 147.68 | 1.10 | ( 143.63, 151.72) | 134.53 | 0.000 |
| Root DCM Ext - Flower Aqueo | -1.72 | 1.10 | ( -5.77, 2.32) | -1.57 | 0.953 |
| Root EtOAc E - Flower Aqueo | 42.92 | 1.10 | ( 38.88, 46.97) | 39.10 | 0.000 |
| Root n-Hexan - Flower Aqueo | 36.53 | 1.10 | ( 32.49, 40.57) | 33.28 | 0.000 |
| Flower DCM E - Flower Crude | -164.71 | 1.10 | (-168.75, -160.67) | -150.05 | 0.000 |
| Flower EtOAc - Flower Crude | -67.17 | 1.10 | ( -71.21, -63.13) | -61.19 | 0.000 |
| Flower n-Hex - Flower Crude | -165.69 | 1.10 | (-169.74, -161.65) | -150.95 | 0.000 |
| Leave Aqueou - Flower Crude | -143.05 | 1.10 | (-147.09, -139.01) | -130.32 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Leave Crude - Flower Crude | -40.68 | 1.10 | ( -44.72, -36.63) | -37.06 | 0.000 |
| Leave DCM Ex - Flower Crude | -105.42 | 1.10 | (-109.47, -101.38) | -96.04 | 0.000 |
| Leave EtOAc - Flower Crude | 8.67 | 1.10 | ( 4.62, 12.71) | 7.89 | 0.000 |
| Leave n-Hexa - Flower Crude | -132.86 | 1.10 | (-136.90, -128.82) | -121.04 | 0.000 |
| Root Aqueous - Flower Crude | -92.63 | 1.10 | ( -96.68, -88.59) | -84.39 | 0.000 |
| Root Crude E - Flower Crude | 13.41 | 1.10 | ( 9.36, 17.45) | 12.22 | 0.000 |
| Root DCM Ext - Flower Crude | -135.99 | 1.10 | (-140.03, -131.95) | -123.89 | 0.000 |
| Root EtOAc E - Flower Crude | -91.35 | 1.10 | ( -95.39, -87.30) | -83.22 | 0.000 |
| Root n-Hexan - Flower Crude | -97.74 | 1.10 | (-101.78, -93.69) | -89.04 | 0.000 |
| Flower EtOAc - Flower DCM E | 97.54 | 1.10 | ( 93.50, 101.58) | 88.86 | 0.000 |
| Flower n-Hex - Flower DCM E | -0.98 | 1.10 | ( -5.03, 3.06) | -0.90 | 1.000 |
| Leave Aqueou - Flower DCM E | 21.66 | 1.10 | ( 17.62, 25.70) | 19.73 | 0.000 |
| Leave Crude - Flower DCM E | 124.03 | 1.10 | ( 119.99, 128.08) | 112.99 | 0.000 |
| Leave DCM Ex - Flower DCM E | 59.29 | 1.10 | ( 55.24, 63.33) | 54.01 | 0.000 |
| Leave EtOAc - Flower DCM E | 173.38 | 1.10 | ( 169.33, 177.42) | 157.95 | 0.000 |
| Leave n-Hexa - Flower DCM E | 31.85 | 1.10 | ( 27.81, 35.89) | 29.01 | 0.000 |
| Root Aqueous - Flower DCM E | 72.08 | 1.10 | ( 68.03, 76.12) | 65.66 | 0.000 |
| Root Crude E - Flower DCM E | 178.12 | 1.10 | ( 174.07, 182.16) | 162.27 | 0.000 |
| Root DCM Ext - Flower DCM E | 28.72 | 1.10 | ( 24.68, 32.76) | 26.16 | 0.000 |
| Root EtOAc E - Flower DCM E | 73.36 | 1.10 | ( 69.32, 77.41) | 66.84 | 0.000 |
| Root n-Hexan - Flower DCM E | 66.97 | 1.10 | ( 62.93, 71.02) | 61.01 | 0.000 |
| Flower n-Hex - Flower EtOAt | -98.52 | 1.10 | (-102.57, -94.48) | -89.75 | 0.000 |
| Leave Aqueou - Flower EtOAt | -75.88 | 1.10 | ( -79.92, -71.84) | -69.13 | 0.000 |
| Leave Crude - Flower EtOAt | 26.49 | 1.10 | ( 22.45, 30.54) | 24.13 | 0.000 |
| Leave DCM Ex - Flower EtOAt | -38.25 | 1.10 | ( -42.30, -34.21) | -34.85 | 0.000 |
| Leave EtOAc - Flower EtOAt | 75.84 | 1.10 | ( 71.79, 79.88) | 69.09 | 0.000 |
| Leave n-Hexa - Flower EtOAt | -65.69 | 1.10 | ( -69.73, -61.65) | -59.84 | 0.000 |
| Root Aqueous - Flower EtOAt | -25.46 | 1.10 | ( -29.51, -21.42) | -23.20 | 0.000 |
| Root Crude E - Flower EtOAt | 80.58 | 1.10 | ( 76.53, 84.62) | 73.41 | 0.000 |
| Root DCM Ext - Flower EtOAt | -68.82 | 1.10 | ( -72.86, -64.78) | -62.70 | 0.000 |
| Root EtOAc E - Flower EtOAt | -24.18 | 1.10 | ( -28.22, -20.13) | -22.02 | 0.000 |
| Root n-Hexan - Flower EtOAt | -30.57 | 1.10 | ( -34.61, -26.52) | -27.85 | 0.000 |
| Leave Aqueou - Flower n-Hex | 22.64 | 1.10 | ( 18.60, 26.69) | 20.63 | 0.000 |
| Leave Crude - Flower n-Hex | 125.02 | 1.10 | ( 120.97, 129.06) | 113.89 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Leave DCM Ex - Flower n-Hex | 60.27 | 1.10 | ( 56.23, | 64.32) | 54.91 |
| 0.000 | | | | | |
| Leave EtOAc - Flower n-Hex | 174.36 | 1.10 | ( 170.31, | 178.40) | 158.84 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower n-Hex | 32.83 | 1.10 | ( 28.79, | 36.88) | 29.91 |
| 0.000 | | | | | |
| Root Aqueous - Flower n-Hex | 73.06 | 1.10 | ( 69.01, | 77.10) | 66.56 |
| 0.000 | | | | | |
| Root Crude E - Flower n-Hex | 179.10 | 1.10 | ( 175.06, | 183.15) | 163.16 |
| 0.000 | | | | | |
| Root DCM Ext - Flower n-Hex | 29.70 | 1.10 | ( 25.66, | 33.75) | 27.06 |
| 0.000 | | | | | |
| Root EtOAc E - Flower n-Hex | 74.35 | 1.10 | ( 70.30, | 78.39) | 67.73 |
| 0.000 | | | | | |
| Root n-Hexan - Flower n-Hex | 67.95 | 1.10 | ( 63.91, | 72.00) | 61.91 |
| 0.000 | | | | | |
| Leave Crude - Leave Aqueou | 102.37 | 1.10 | ( 98.33, | 106.42) | 93.26 |
| 0.000 | | | | | |
| Leave DCM Ex - Leave Aqueou | 37.63 | 1.10 | ( 33.59, | 41.67) | 34.28 |
| 0.000 | | | | | |
| Leave EtOAc - Leave Aqueou | 151.72 | 1.10 | ( 147.67, | 155.76) | 138.21 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave Aqueou | 10.19 | 1.10 | ( 6.15, | 14.23) | 9.28 |
| 0.000 | | | | | |
| Root Aqueous - Leave Aqueou | 50.42 | 1.10 | ( 46.37, | 54.46) | 45.93 |
| 0.000 | | | | | |
| Root Crude E - Leave Aqueou | 156.46 | 1.10 | ( 152.42, | 160.50) | 142.53 |
| 0.000 | | | | | |
| Root DCM Ext - Leave Aqueou | 7.06 | 1.10 | ( 3.02, | 11.10) | 6.43 |
| 0.000 | | | | | |
| Root EtOAc E - Leave Aqueou | 51.71 | 1.10 | ( 47.66, | 55.75) | 47.10 |
| 0.000 | | | | | |
| Root n-Hexan - Leave Aqueou | 45.31 | 1.10 | ( 41.27, | 49.36) | 41.28 |
| 0.000 | | | | | |
| Leave DCM Ex - Leave Crude | -64.74 | 1.10 | ( -68.79, | -60.70) | -58.98 |
| 0.000 | | | | | |
| Leave EtOAc - Leave Crude | 49.34 | 1.10 | ( 45.30, | 53.39) | 44.95 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave Crude | -92.18 | 1.10 | ( -96.23, | -88.14) | -83.98 |
| 0.000 | | | | | |
| Root Aqueous - Leave Crude | -51.96 | 1.10 | ( -56.00, | -47.91) | -47.33 |
| 0.000 | | | | | |
| Root Crude E - Leave Crude | 54.09 | 1.10 | ( 50.04, | 58.13) | 49.27 |
| 0.000 | | | | | |
| Root DCM Ext - Leave Crude | -95.31 | 1.10 | ( -99.36, | -91.27) | -86.83 |
| 0.000 | | | | | |
| Root EtOAc E - Leave Crude | -50.67 | 1.10 | ( -54.71, | -46.62) | -46.16 |
| 0.000 | | | | | |
| Root n-Hexan - Leave Crude | -57.06 | 1.10 | ( -61.11, | -53.02) | -51.98 |
| 0.000 | | | | | |
| Leave EtOAc - Leave DCM Ex | 114.09 | 1.10 | ( 110.04, | 118.13) | 103.93 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave DCM Ex | -27.44 | 1.10 | ( -31.48, | -23.40) | -25.00 |
| 0.000 | | | | | |
| Root Aqueous - Leave DCM Ex | 12.79 | 1.10 | ( 8.74, | 16.83) | 11.65 |
| 0.000 | | | | | |
| Root Crude E - Leave DCM Ex | 118.83 | 1.10 | ( 114.79, | 122.87) | 108.25 |
| 0.000 | | | | | |
| Root DCM Ext - Leave DCM Ex | -30.57 | 1.10 | ( -34.61, | -26.52) | -27.85 |
| 0.000 | | | | | |
| Root EtOAc E - Leave DCM Ex | 14.08 | 1.10 | ( 10.03, | 18.12) | 12.82 |
| 0.000 | | | | | |
| Root n-Hexan - Leave DCM Ex | 7.68 | 1.10 | ( 3.64, | 11.73) | 7.00 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave EtOAt | -141.53 | 1.10 | (-145.57, | -137.48) | -128.93 |
| 0.000 | | | | | |
| Root Aqueous - Leave EtOAt | -101.30 | 1.10 | (-105.34, | -97.26) | -92.28 |
| 0.000 | | | | | |

```
Root Crude E - Leave EtOAt         4.74        1.10  (   0.70,    8.79)     4.32
0.011
Root DCM Ext - Leave EtOAt      -144.66        1.10  (-148.70, -140.61)  -131.78
0.000
Root EtOAt E - Leave EtOAt      -100.01        1.10  (-104.05,  -95.97)   -91.11
0.000
Root n-Hexan - Leave EtOAt      -106.40        1.10  (-110.45, -102.36)   -96.93
0.000
Root Aqueous - Leave n-Hexa       40.23        1.10  (  36.18,   44.27)    36.65
0.000
Root Crude E - Leave n-Hexa      146.27        1.10  ( 142.23,  150.31)   133.25
0.000
Root DCM Ext - Leave n-Hexa       -3.13        1.10  (  -7.17,    0.91)    -2.85
0.275
Root EtOAc E - Leave n-Hexa       41.52        1.10  (  37.47,   45.56)    37.82
0.000
Root n-Hexan - Leave n-Hexa       35.12        1.10  (  31.08,   39.17)    32.00
0.000
Root Crude E - Root Aqueous      106.04        1.10  ( 102.00,  110.09)    96.61
0.000
Root DCM Ext - Root Aqueous      -43.36        1.10  ( -47.40,  -39.31)   -39.50
0.000
Root EtOAc E - Root Aqueous        1.29        1.10  (  -2.75,    5.33)     1.17
0.996
Root n-Hexan - Root Aqueous       -5.10        1.10  (  -9.15,   -1.06)    -4.65
0.005
Root DCM Ext - Root Crude E     -149.40        1.10  (-153.44, -145.35)  -136.10
0.000
Root EtOAc E - Root Crude E     -104.75        1.10  (-108.80, -100.71)   -95.43
0.000
Root n-Hexan - Root Crude E     -111.15        1.10  (-115.19, -107.10)  -101.26
0.000
Root EtOAc E - Root DCM Ext       44.64        1.10  (  40.60,   48.69)    40.67
0.000
Root n-Hexan - Root DCM Ext       38.25        1.10  (  34.21,   42.30)    34.85
0.000
Root n-Hexan - Root EtOAc E       -6.39        1.10  ( -10.44,   -2.35)    -5.82
0.000

Individual confidence level = 99.91%
```

## Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

samples                  N     Mean  Grouping
Root Crude Extract       3  182.690  A
Leave EtOAc Extract      3   177.95    B
Flower Crude Extract     3  169.282      C
Leave Crude Extract      3  128.605        D
Flower EtOAc Extract     3   102.11          E
Root EtOAc Extract       3   77.936            F
Root Aqueous Extract     3   76.647            F
Root n-Hexane Extract    3   71.543              G
Leave DCM Extract        3   63.860                H
Leave n-Hexane Extract   3   36.421                  I
Flower Aqueous Extract   3   35.014                  I J
Root DCM Extract         3   33.292                    J
Leave Aqueous Extract    3   26.231                      K
Flower DCM Extract       3    4.572                        L
Flower n-Hexane Extract  3    3.589                        L

Means that do not share a letter are significantly different.


Fisher Individual Tests for Differences of Means
```

| Adjusted Difference of Levels | Difference of Means | SE of Difference | 95% CI | T-Value | P-Value |
|---|---|---|---|---|---|
| Flower Crude - Flower Aqueo | 134.27 | 1.10 | ( 132.03, 136.51) | 122.32 | 0.000 |
| Flower DCM E - Flower Aqueo | -30.44 | 1.10 | ( -32.68, -28.20) | -27.73 | 0.000 |
| Flower EtOAc - Flower Aqueo | 67.10 | 1.10 | ( 64.86, 69.34) | 61.13 | 0.000 |
| Flower n-Hex - Flower Aqueo | -31.43 | 1.10 | ( -33.67, -29.18) | -28.63 | 0.000 |
| Leave Aqueou - Flower Aqueo | -8.78 | 1.10 | ( -11.02, -6.54) | -8.00 | 0.000 |
| Leave Crude - Flower Aqueo | 93.59 | 1.10 | ( 91.35, 95.83) | 85.26 | 0.000 |
| Leave DCM Ex - Flower Aqueo | 28.85 | 1.10 | ( 26.60, 31.09) | 26.28 | 0.000 |
| Leave EtOAc - Flower Aqueo | 142.93 | 1.10 | ( 140.69, 145.17) | 130.21 | 0.000 |
| Leave n-Hexa - Flower Aqueo | 1.41 | 1.10 | ( -0.84, 3.65) | 1.28 | 0.210 |
| Root Aqueous - Flower Aqueo | 41.63 | 1.10 | ( 39.39, 43.87) | 37.93 | 0.000 |
| Root Crude E - Flower Aqueo | 147.68 | 1.10 | ( 145.43, 149.92) | 134.53 | 0.000 |
| Root DCM Ext - Flower Aqueo | -1.72 | 1.10 | ( -3.96, 0.52) | -1.57 | 0.127 |
| Root EtOAc E - Flower Aqueo | 42.92 | 1.10 | ( 40.68, 45.16) | 39.10 | 0.000 |
| Root n-Hexan - Flower Aqueo | 36.53 | 1.10 | ( 34.29, 38.77) | 33.28 | 0.000 |
| Flower DCM E - Flower Crude | -164.71 | 1.10 | (-166.95, -162.47) | -150.05 | 0.000 |
| Flower EtOAc - Flower Crude | -67.17 | 1.10 | ( -69.41, -64.93) | -61.19 | 0.000 |
| Flower n-Hex - Flower Crude | -165.69 | 1.10 | (-167.93, -163.45) | -150.95 | 0.000 |
| Leave Aqueou - Flower Crude | -143.05 | 1.10 | (-145.29, -140.81) | -130.32 | 0.000 |
| Leave Crude - Flower Crude | -40.68 | 1.10 | ( -42.92, -38.44) | -37.06 | 0.000 |
| Leave DCM Ex - Flower Crude | -105.42 | 1.10 | (-107.66, -103.18) | -96.04 | 0.000 |
| Leave EtOAc - Flower Crude | 8.67 | 1.10 | ( 6.42, 10.91) | 7.89 | 0.000 |
| Leave n-Hexa - Flower Crude | -132.86 | 1.10 | (-135.10, -130.62) | -121.04 | 0.000 |
| Root Aqueous - Flower Crude | -92.63 | 1.10 | ( -94.88, -90.39) | -84.39 | 0.000 |
| Root Crude E - Flower Crude | 13.41 | 1.10 | ( 11.17, 15.65) | 12.22 | 0.000 |
| Root DCM Ext - Flower Crude | -135.99 | 1.10 | (-138.23, -133.75) | -123.89 | 0.000 |
| Root EtOAc E - Flower Crude | -91.35 | 1.10 | ( -93.59, -89.10) | -83.22 | 0.000 |
| Root n-Hexan - Flower Crude | -97.74 | 1.10 | ( -99.98, -95.50) | -89.04 | 0.000 |
| Flower EtOAc - Flower DCM E | 97.54 | 1.10 | ( 95.30, 99.78) | 88.86 | 0.000 |
| Flower n-Hex - Flower DCM E | -0.98 | 1.10 | ( -3.22, 1.26) | -0.90 | 0.378 |
| Leave Aqueou - Flower DCM E | 21.66 | 1.10 | ( 19.42, 23.90) | 19.73 | 0.000 |
| Leave Crude - Flower DCM E | 124.03 | 1.10 | ( 121.79, 126.27) | 112.99 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Leave DCM Ex - Flower DCM E | 59.29 | 1.10 | ( 57.05, | 61.53) | 54.01 |
| 0.000 | | | | | |
| Leave EtOAc - Flower DCM E | 173.38 | 1.10 | ( 171.13, | 175.62) | 157.95 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower DCM E | 31.85 | 1.10 | ( 29.61, | 34.09) | 29.01 |
| 0.000 | | | | | |
| Root Aqueous - Flower DCM E | 72.08 | 1.10 | ( 69.83, | 74.32) | 65.66 |
| 0.000 | | | | | |
| Root Crude E - Flower DCM E | 178.12 | 1.10 | ( 175.88, | 180.36) | 162.27 |
| 0.000 | | | | | |
| Root DCM Ext - Flower DCM E | 28.72 | 1.10 | ( 26.48, | 30.96) | 26.16 |
| 0.000 | | | | | |
| Root EtOAc E - Flower DCM E | 73.36 | 1.10 | ( 71.12, | 75.61) | 66.84 |
| 0.000 | | | | | |
| Root n-Hexan - Flower DCM E | 66.97 | 1.10 | ( 64.73, | 69.21) | 61.01 |
| 0.000 | | | | | |
| Flower n-Hex - Flower EtOAt | -98.52 | 1.10 | (-100.76, | -96.28) | -89.75 |
| 0.000 | | | | | |
| Leave Aqueou - Flower EtOAt | -75.88 | 1.10 | ( -78.12, | -73.64) | -69.13 |
| 0.000 | | | | | |
| Leave Crude - Flower EtOAt | 26.49 | 1.10 | ( 24.25, | 28.73) | 24.13 |
| 0.000 | | | | | |
| Leave DCM Ex - Flower EtOAt | -38.25 | 1.10 | ( -40.49, | -36.01) | -34.85 |
| 0.000 | | | | | |
| Leave EtOAc - Flower EtOAt | 75.84 | 1.10 | ( 73.59, | 78.08) | 69.09 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower EtOAt | -65.69 | 1.10 | ( -67.93, | -63.45) | -59.84 |
| 0.000 | | | | | |
| Root Aqueous - Flower EtOAt | -25.46 | 1.10 | ( -27.71, | -23.22) | -23.20 |
| 0.000 | | | | | |
| Root Crude E - Flower EtOAt | 80.58 | 1.10 | ( 78.34, | 82.82) | 73.41 |
| 0.000 | | | | | |
| Root DCM Ext - Flower EtOAt | -68.82 | 1.10 | ( -71.06, | -66.58) | -62.70 |
| 0.000 | | | | | |
| Root EtOAc E - Flower EtOAt | -24.18 | 1.10 | ( -26.42, | -21.93) | -22.02 |
| 0.000 | | | | | |
| Root n-Hexan - Flower EtOAt | -30.57 | 1.10 | ( -32.81, | -28.33) | -27.85 |
| 0.000 | | | | | |
| Leave Aqueou - Flower n-Hex | 22.64 | 1.10 | ( 20.40, | 24.88) | 20.63 |
| 0.000 | | | | | |
| Leave Crude - Flower n-Hex | 125.02 | 1.10 | ( 122.77, | 127.26) | 113.89 |
| 0.000 | | | | | |
| Leave DCM Ex - Flower n-Hex | 60.27 | 1.10 | ( 58.03, | 62.51) | 54.91 |
| 0.000 | | | | | |
| Leave EtOAc - Flower n-Hex | 174.36 | 1.10 | ( 172.12, | 176.60) | 158.84 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower n-Hex | 32.83 | 1.10 | ( 30.59, | 35.07) | 29.91 |
| 0.000 | | | | | |
| Root Aqueous - Flower n-Hex | 73.06 | 1.10 | ( 70.82, | 75.30) | 66.56 |
| 0.000 | | | | | |
| Root Crude E - Flower n-Hex | 179.10 | 1.10 | ( 176.86, | 181.34) | 163.16 |
| 0.000 | | | | | |
| Root DCM Ext - Flower n-Hex | 29.70 | 1.10 | ( 27.46, | 31.94) | 27.06 |
| 0.000 | | | | | |
| Root EtOAc E - Flower n-Hex | 74.35 | 1.10 | ( 72.11, | 76.59) | 67.73 |
| 0.000 | | | | | |
| Root n-Hexan - Flower n-Hex | 67.95 | 1.10 | ( 65.71, | 70.20) | 61.91 |
| 0.000 | | | | | |
| Leave Crude - Leave Aqueou | 102.37 | 1.10 | ( 100.13, | 104.62) | 93.26 |
| 0.000 | | | | | |
| Leave DCM Ex - Leave Aqueou | 37.63 | 1.10 | ( 35.39, | 39.87) | 34.28 |
| 0.000 | | | | | |
| Leave EtOAc - Leave Aqueou | 151.72 | 1.10 | ( 149.47, | 153.96) | 138.21 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave Aqueou | 10.19 | 1.10 | ( 7.95, | 12.43) | 9.28 |
| 0.000 | | | | | |
| Root Aqueous - Leave Aqueou | 50.42 | 1.10 | ( 48.17, | 52.66) | 45.93 |
| 0.000 | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Root Crude E - Leave Aqueou | 156.46 | 1.10 | ( 154.22, | 158.70) | 142.53 |
| 0.000 | | | | | |
| Root DCM Ext - Leave Aqueou | 7.06 | 1.10 | ( 4.82, | 9.30) | 6.43 |
| 0.000 | | | | | |
| Root EtOAc E - Leave Aqueou | 51.71 | 1.10 | ( 49.46, | 53.95) | 47.10 |
| 0.000 | | | | | |
| Root n-Hexan - Leave Aqueou | 45.31 | 1.10 | ( 43.07, | 47.55) | 41.28 |
| 0.000 | | | | | |
| Leave DCM Ex - Leave Crude | -64.74 | 1.10 | ( -66.99, | -62.50) | -58.98 |
| 0.000 | | | | | |
| Leave EtOAc - Leave Crude | 49.34 | 1.10 | ( 47.10, | 51.58) | 44.95 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave Crude | -92.18 | 1.10 | ( -94.43, | -89.94) | -83.98 |
| 0.000 | | | | | |
| Root Aqueous - Leave Crude | -51.96 | 1.10 | ( -54.20, | -49.72) | -47.33 |
| 0.000 | | | | | |
| Root Crude E - Leave Crude | 54.09 | 1.10 | ( 51.84, | 56.33) | 49.27 |
| 0.000 | | | | | |
| Root DCM Ext - Leave Crude | -95.31 | 1.10 | ( -97.55, | -93.07) | -86.83 |
| 0.000 | | | | | |
| Root EtOAc E - Leave Crude | -50.67 | 1.10 | ( -52.91, | -48.43) | -46.16 |
| 0.000 | | | | | |
| Root n-Hexan - Leave Crude | -57.06 | 1.10 | ( -59.30, | -54.82) | -51.98 |
| 0.000 | | | | | |
| Leave EtOAc - Leave DCM Ex | 114.09 | 1.10 | ( 111.85, | 116.33) | 103.93 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave DCM Ex | -27.44 | 1.10 | ( -29.68, | -25.20) | -25.00 |
| 0.000 | | | | | |
| Root Aqueous - Leave DCM Ex | 12.79 | 1.10 | ( 10.54, | 15.03) | 11.65 |
| 0.000 | | | | | |
| Root Crude E - Leave DCM Ex | 118.83 | 1.10 | ( 116.59, | 121.07) | 108.25 |
| 0.000 | | | | | |
| Root DCM Ext - Leave DCM Ex | -30.57 | 1.10 | ( -32.81, | -28.33) | -27.85 |
| 0.000 | | | | | |
| Root EtOAc E - Leave DCM Ex | 14.08 | 1.10 | ( 11.83, | 16.32) | 12.82 |
| 0.000 | | | | | |
| Root n-Hexan - Leave DCM Ex | 7.68 | 1.10 | ( 5.44, | 9.92) | 7.00 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave EtOAt | -141.53 | 1.10 | (-143.77, | -139.28) | -128.93 |
| 0.000 | | | | | |
| Root Aqueous - Leave EtOAt | -101.30 | 1.10 | (-103.54, | -99.06) | -92.28 |
| 0.000 | | | | | |
| Root Crude E - Leave EtOAt | 4.74 | 1.10 | ( 2.50, | 6.98) | 4.32 |
| 0.000 | | | | | |
| Root DCM Ext - Leave EtOAt | -144.66 | 1.10 | (-146.90, | -142.41) | -131.78 |
| 0.000 | | | | | |
| Root EtOAc E - Leave EtOAt | -100.01 | 1.10 | (-102.25, | -97.77) | -91.11 |
| 0.000 | | | | | |
| Root n-Hexan - Leave EtOAt | -106.40 | 1.10 | (-108.65, | -104.16) | -96.93 |
| 0.000 | | | | | |
| Root Aqueous - Leave n-Hexa | 40.23 | 1.10 | ( 37.98, | 42.47) | 36.65 |
| 0.000 | | | | | |
| Root Crude E - Leave n-Hexa | 146.27 | 1.10 | ( 144.03, | 148.51) | 133.25 |
| 0.000 | | | | | |
| Root DCM Ext - Leave n-Hexa | -3.13 | 1.10 | ( -5.37, | -0.89) | -2.85 |
| 0.008 | | | | | |
| Root EtOAc E - Leave n-Hexa | 41.52 | 1.10 | ( 39.27, | 43.76) | 37.82 |
| 0.000 | | | | | |
| Root n-Hexan - Leave n-Hexa | 35.12 | 1.10 | ( 32.88, | 37.36) | 32.00 |
| 0.000 | | | | | |
| Root Crude E - Root Aqueous | 106.04 | 1.10 | ( 103.80, | 108.29) | 96.61 |
| 0.000 | | | | | |
| Root DCM Ext - Root Aqueous | -43.36 | 1.10 | ( -45.60, | -41.11) | -39.50 |
| 0.000 | | | | | |
| Root EtOAc E - Root Aqueous | 1.29 | 1.10 | ( -0.95, | 3.53) | 1.17 |
| 0.249 | | | | | |
| Root n-Hexan - Root Aqueous | -5.10 | 1.10 | ( -7.35, | -2.86) | -4.65 |
| 0.000 | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Root DCM Ext - Root Crude E | -149.40 | 1.10 | (-151.64, -147.16) | -136.10 | 0.000 |
| Root EtOAc E - Root Crude E | -104.75 | 1.10 | (-107.00, -102.51) | -95.43 | 0.000 |
| Root n-Hexan - Root Crude E | -111.15 | 1.10 | (-113.39, -108.91) | -101.26 | 0.000 |
| Root EtOAc E - Root DCM Ext | 44.64 | 1.10 | ( 42.40, 46.89) | 40.67 | 0.000 |
| Root n-Hexan - Root DCM Ext | 38.25 | 1.10 | ( 36.01, 40.49) | 34.85 | 0.000 |
| Root n-Hexan - Root EtOAt E | -6.39 | 1.10 | ( -8.64, -4.15) | -5.82 | 0.000 |

Simultaneous confidence level = 24.08%

## Appendix II: One-way ANOVA of different *Aloiampelos ciliaries* extract (total phenolic) at different mean concentrations

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.
Factor Information
Factor   Levels  Values
Samples      15  Flower Aqueous Extract, Flower Crude Extract, Flower DCM Extract,
Flower
                 EtOAt Extract, Flower n-Hexane Extract, Leave Aqueous Extract,
Leave Crude
                 Extract, Leave DCM Extract, Leave EtOAt Extract, Leave n-Hexane
Extract,
                 Root Aqueous Extract, Root Crude Extract, Root DCM Extract, Root
EtOAt
                 Extract, Root n-Hexane Extract

Analysis of Variance

Source   DF   Adj SS   Adj MS     F-Value  P-Value
Samples  14  37633.6  2688.12  1877869.40    0.000
Error    30      0.0     0.00
Total    44  37633.7


Model Summary

        S     R-sq  R-sq(adj)  R-sq(pred)
0.0378348  100.00%    100.00%     100.00%


Means

Samples                  N     Mean    StDev        95% CI
Flower Aqueous Extract   3   9.4842   0.0328  ( 9.4395,  9.5288)
Flower Crude Extract     3  105.889    0.073  (105.844, 105.934)
Flower DCM Extract       3   1.4640   0.0375  ( 1.4194,  1.5086)
Flower EtOAc Extract     3  15.6285   0.0164  (15.5839, 15.6731)
Flower n-Hexane Extract  3  2.32130  0.00821  (2.27668, 2.36591)
Leave Aqueous Extract    3  11.2607   0.0358  (11.2160, 11.3053)
Leave Crude Extract      3  30.4706   0.0246  (30.4259, 30.5152)
Leave DCM Extract        3  9.11464  0.00821  (9.07003, 9.15925)
Leave EtOAc Extract      3  40.4521   0.0164  (40.4075, 40.4967)
Leave n-Hexane Extract   3  8.23349  0.00821  (8.18888, 8.27811)
Root Aqueous Extract     3  13.9278   0.0142  (13.8832, 13.9724)
Root Crude Extract       3  83.6377   0.0246  (83.5931, 83.6823)
Root DCM Extract         3  17.9545   0.0998  (17.9099, 17.9991)
Root EtOAc Extract       3  20.1668   0.0142  (20.1222, 20.2115)
Root n-Hexane Extract    3  29.4236   0.0082  (29.3790, 29.4682)

Pooled StDev = 0.0378348
```

## Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Samples                  N     Mean  Grouping
Flower Crude Extract     3  105.889  A
Root Crude Extract       3  83.6377    B
```

| | | | |
|---|---|---|---|
| Leave EtOAc Extract | 3 | 40.4521 | C |
| L eave Crude Extract | 3 | 30.4706 | D |
| Root n-Hexane Extract | 3 | 29.4236 | E |
| Root EtOAc Extract | 3 | 20.1668 | F |
| Root DCM Extract | 3 | 17.9545 | G |
| Flower EtOAc Extract | 3 | 15.6285 | H |
| Root Aqueous Extract | 3 | 13.9278 | I |
| Leave Aqueous Extract | 3 | 11.2607 | J |
| Flower Aqueous Extract | 3 | 9.4842 | K |
| Leave DCM Extract | 3 | 9.11464 | L |
| Leave n-Hexane Extract | 3 | 8.23349 | M |
| Flower n-Hexane Extract | 3 | 2.32130 | N |
| Flower DCM Extract | 3 | 1.4640 | O |

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

| Difference of Levels | Difference of Means | SE of Difference | 95% CI | T-Value | Adjusted P-Value |
|---|---|---|---|---|---|
| Flower Crude - Flower Aqueo | 96.4048 | 0.0309 | ( 96.2910, 96.5186) | 3120.71 | 0.000 |
| Flower DCM E - Flower Aqueo | -8.0202 | 0.0309 | ( -8.1340, -7.9064) | -259.62 | 0.000 |
| Flower EtOAc - Flower Aqueo | 6.1443 | 0.0309 | ( 6.0305, 6.2581) | 198.90 | 0.000 |
| Flower n-Hex - Flower Aqueo | -7.1629 | 0.0309 | ( -7.2767, -7.0490) | -231.87 | 0.000 |
| Leave Aqueou - Flower Aqueo | 1.7765 | 0.0309 | ( 1.6627, 1.8903) | 57.51 | 0.000 |
| Leave Crude - Flower Aqueo | 20.9864 | 0.0309 | ( 20.8726, 21.1002) | 679.35 | 0.000 |
| Leave DCM Ex - Flower Aqueo | -0.3695 | 0.0309 | ( -0.4833, -0.2557) | -11.96 | 0.000 |
| Leave EtOAc - Flower Aqueo | 30.9680 | 0.0309 | ( 30.8542, 31.0818) | 1002.46 | 0.000 |
| Leave n-Hexa - Flower Aqueo | -1.2507 | 0.0309 | ( -1.3645, -1.1369) | -40.48 | 0.000 |
| Root Aqueous - Flower Aqueo | 4.4436 | 0.0309 | ( 4.3298, 4.5574) | 143.84 | 0.000 |
| Root Crude E - Flower Aqueo | 74.1536 | 0.0309 | ( 74.0397, 74.2674) | 2400.41 | 0.000 |
| Root DCM Ext - Flower Aqueo | 8.4704 | 0.0309 | ( 8.3566, 8.5842) | 274.19 | 0.000 |
| Root EtOAc E - Flower Aqueo | 10.6827 | 0.0309 | ( 10.5689, 10.7965) | 345.81 | 0.000 |
| Root n-Hexan - Flower Aqueo | 19.9395 | 0.0309 | ( 19.8256, 20.0533) | 645.46 | 0.000 |
| Flower DCM E - Flower Crude | -104.425 | 0.031 | (-104.539, -104.311) | -3380.33 | 0.000 |
| Flower EtOAc - Flower Crude | -90.2605 | 0.0309 | (-90.3743, -90.1467) | -2921.81 | 0.000 |
| Flower n-Hex - Flower Crude | -103.568 | 0.031 | (-103.681, -103.454) | -3352.58 | 0.000 |
| Leave Aqueou - Flower Crude | -94.6283 | 0.0309 | (-94.7421, -94.5145) | -3063.20 | 0.000 |
| Leave Crude - Flower Crude | -75.4184 | 0.0309 | (-75.5322, -75.3046) | -2441.36 | 0.000 |
| Leave DCM Ex - Flower Crude | -96.7743 | 0.0309 | (-96.8882, -96.6605) | -3132.67 | 0.000 |
| Leave EtOAc - Flower Crude | -65.4369 | 0.0309 | (-65.5507, -65.3230) | -2118.25 | 0.000 |
| Leave n-Hexa - Flower Crude | -97.6555 | 0.0309 | (-97.7693, -97.5417) | -3161.19 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Root Aqueous - Flower Crude | -91.9612 | 0.0309 | (-92.0750, -91.8474) | -2976.86 | 0.000 |
| Root Crude E - Flower Crude | -22.2513 | 0.0309 | (-22.3651, -22.1375) | -720.29 | 0.000 |
| Root DCM Ext - Flower Crude | -87.9345 | 0.0309 | (-88.0483, -87.8207) | -2846.51 | 0.000 |
| Root EtOAc E - Flower Crude | -85.7221 | 0.0309 | (-85.8359, -85.6083) | -2774.90 | 0.000 |
| Root n-Hexan - Flower Crude | -76.4654 | 0.0309 | (-76.5792, -76.3516) | -2475.25 | 0.000 |
| Flower EtOAt - Flower DCM E | 14.1645 | 0.0309 | ( 14.0507, 14.2783) | 458.52 | 0.000 |
| Flower n-Hex - Flower DCM E | 0.8573 | 0.0309 | ( 0.7435, 0.9711) | 27.75 | 0.000 |
| Leave Aqueou - Flower DCM E | 9.7967 | 0.0309 | ( 9.6829, 9.9105) | 317.13 | 0.000 |
| Leave Crude - Flower DCM E | 29.0066 | 0.0309 | ( 28.8928, 29.1204) | 938.97 | 0.000 |
| Leave DCM Ex - Flower DCM E | 7.6507 | 0.0309 | ( 7.5369, 7.7645) | 247.66 | 0.000 |
| Leave EtOAc - Flower DCM E | 38.9882 | 0.0309 | ( 38.8744, 39.1020) | 1262.08 | 0.000 |
| Leave n-Hexa - Flower DCM E | 6.7695 | 0.0309 | ( 6.6557, 6.8833) | 219.14 | 0.000 |
| Root Aqueous - Flower DCM E | 12.4638 | 0.0309 | ( 12.3500, 12.5776) | 403.46 | 0.000 |
| Root Crude E - Flower DCM E | 82.1737 | 0.0309 | ( 82.0599, 82.2876) | 2660.03 | 0.000 |
| Root DCM Ext - Flower DCM E | 16.4905 | 0.0309 | ( 16.3767, 16.6044) | 533.81 | 0.000 |
| Root EtOAc E - Flower DCM E | 18.7029 | 0.0309 | ( 18.5891, 18.8167) | 605.43 | 0.000 |
| Root n-Hexan - Flower DCM E | 27.9596 | 0.0309 | ( 27.8458, 28.0735) | 905.08 | 0.000 |
| Flower n-Hex - Flower EtOAt | -13.3072 | 0.0309 | (-13.4210, -13.1934) | -430.76 | 0.000 |
| Leave Aqueou - Flower EtOAt | -4.3678 | 0.0309 | ( -4.4816, -4.2540) | -141.39 | 0.000 |
| Leave Crude - Flower EtOAt | 14.8421 | 0.0309 | ( 14.7283, 14.9559) | 480.45 | 0.000 |
| Leave DCM Ex - Flower EtOAt | -6.5138 | 0.0309 | ( -6.6276, -6.4000) | -210.86 | 0.000 |
| Leave EtOAc - Flower EtOAt | 24.8237 | 0.0309 | ( 24.7098, 24.9375) | 803.56 | 0.000 |
| Leave n-Hexa - Flower EtOAt | -7.3950 | 0.0309 | ( -7.5088, -7.2812) | -239.38 | 0.000 |
| Root Aqueous - Flower EtOAt | -1.7007 | 0.0309 | ( -1.8145, -1.5869) | -55.05 | 0.000 |
| Root Crude E - Flower EtOAt | 68.0092 | 0.0309 | ( 67.8954, 68.1230) | 2201.52 | 0.000 |
| Root DCM Ext - Flower EtOAt | 2.3260 | 0.0309 | ( 2.2122, 2.4398) | 75.30 | 0.000 |
| Root EtOAc E - Flower EtOAt | 4.5384 | 0.0309 | ( 4.4246, 4.6522) | 146.91 | 0.000 |
| Root n-Hexan - Flower EtOAt | 13.7951 | 0.0309 | ( 13.6813, 13.9089) | 446.56 | 0.000 |
| Leave Aqueou - Flower n-Hex | 8.9394 | 0.0309 | ( 8.8256, 9.0532) | 289.37 | 0.000 |
| Leave Crude - Flower n-Hex | 28.1493 | 0.0309 | ( 28.0355, 28.2631) | 911.22 | 0.000 |
| Leave DCM Ex - Flower n-Hex | 6.7933 | 0.0309 | ( 6.6795, 6.9071) | 219.91 | 0.000 |
| Leave EtOAc - Flower n-Hex | 38.1308 | 0.0309 | ( 38.0170, 38.2446) | 1234.33 | 0.000 |
| Leave n-Hexa - Flower n-Hex | 5.9122 | 0.0309 | ( 5.7984, 6.0260) | 191.38 | 0.000 |
| Root Aqueous - Flower n-Hex | 11.6065 | 0.0309 | ( 11.4927, 11.7203) | 375.71 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Root Crude E - Flower n-Hex | 81.3164 | 0.0309 | ( 81.2026, | 81.4302) | 2632.28 |
| 0.000 | | | | | |
| Root DCM Ext - Flower n-Hex | 15.6332 | 0.0309 | ( 15.5194, | 15.7470) | 506.06 |
| 0.000 | | | | | |
| Root EtOAc E - Flower n-Hex | 17.8456 | 0.0309 | ( 17.7317, | 17.9594) | 577.68 |
| 0.000 | | | | | |
| Root n-Hexan - Flower n-Hex | 27.1023 | 0.0309 | ( 26.9885, | 27.2161) | 877.33 |
| 0.000 | | | | | |
| Leave Crude - Leave Aqueou | 19.2099 | 0.0309 | ( 19.0961, | 19.3237) | 621.84 |
| 0.000 | | | | | |
| Leave DCM Ex - Leave Aqueou | -2.1460 | 0.0309 | ( -2.2598, | -2.0322) | -69.47 |
| 0.000 | | | | | |
| Leave EtOAc - Leave Aqueou | 29.1915 | 0.0309 | ( 29.0777, | 29.3053) | 944.95 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave Aqueou | -3.0272 | 0.0309 | ( -3.1410, | -2.9134) | -97.99 |
| 0.000 | | | | | |
| Root Aqueous - Leave Aqueou | 2.6671 | 0.0309 | ( 2.5533, | 2.7809) | 86.34 |
| 0.000 | | | | | |
| Root Crude E - Leave Aqueou | 72.3771 | 0.0309 | ( 72.2632, | 72.4909) | 2342.91 |
| 0.000 | | | | | |
| Root DCM Ext - Leave Aqueou | 6.6939 | 0.0309 | ( 6.5801, | 6.8077) | 216.69 |
| 0.000 | | | | | |
| Root EtOAt E - Leave Aqueou | 8.9062 | 0.0309 | ( 8.7924, | 9.0200) | 288.30 |
| 0.000 | | | | | |
| Root n-Hexan - Leave Aqueou | 18.1630 | 0.0309 | ( 18.0491, | 18.2768) | 587.95 |
| 0.000 | | | | | |
| Leave DCM Ex - Leave Crude | -21.3559 | 0.0309 | (-21.4697, | -21.2421) | -691.31 |
| 0.000 | | | | | |
| Leave EtOAc - Leave Crude | 9.9816 | 0.0309 | ( 9.8678, | 10.0954) | 323.11 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave Crude | -22.2371 | 0.0309 | (-22.3509, | -22.1233) | -719.83 |
| 0.000 | | | | | |
| Root Aqueous - Leave Crude | -16.5428 | 0.0309 | (-16.6566, | -16.4290) | -535.50 |
| 0.000 | | | | | |
| Root Crude E - Leave Crude | 53.1671 | 0.0309 | ( 53.0533, | 53.2810) | 1721.07 |
| 0.000 | | | | | |
| Root DCM Ext - Leave Crude | -12.5160 | 0.0309 | (-12.6299, | -12.4022) | -405.16 |
| 0.000 | | | | | |
| Root EtOAc E - Leave Crude | -10.3037 | 0.0309 | (-10.4175, | -10.1899) | -333.54 |
| 0.000 | | | | | |
| Root n-Hexan - Leave Crude | -1.0470 | 0.0309 | ( -1.1608, | -0.9331) | -33.89 |
| 0.000 | | | | | |
| Leave EtOAc - Leave DCM Ex | 31.3375 | 0.0309 | ( 31.2237, | 31.4513) | 1014.42 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave DCM Ex | -0.8811 | 0.0309 | ( -0.9950, | -0.7673) | -28.52 |
| 0.000 | | | | | |
| Root Aqueous - Leave DCM Ex | 4.8131 | 0.0309 | ( 4.6993, | 4.9269) | 155.81 |
| 0.000 | | | | | |
| Root Crude E - Leave DCM Ex | 74.5231 | 0.0309 | ( 74.4093, | 74.6369) | 2412.38 |
| 0.000 | | | | | |
| Root DCM Ext - Leave DCM Ex | 8.8399 | 0.0309 | ( 8.7261, | 8.9537) | 286.15 |
| 0.000 | | | | | |
| Root EtOAc E - Leave DCM Ex | 11.0522 | 0.0309 | ( 10.9384, | 11.1660) | 357.77 |
| 0.000 | | | | | |
| Root n-Hexan - Leave DCM Ex | 20.3090 | 0.0309 | ( 20.1952, | 20.4228) | 657.42 |
| 0.000 | | | | | |
| Leave n-Hexa - Leave EtOAc | -32.2186 | 0.0309 | (-32.3324, | -32.1048) | -1042.95 |
| 0.000 | | | | | |
| Root Aqueous - Leave EtOAc | -26.5244 | 0.0309 | (-26.6382, | -26.4106) | -858.62 |
| 0.000 | | | | | |
| Root Crude E - Leave EtOAc | 43.1856 | 0.0309 | ( 43.0718, | 43.2994) | 1397.95 |
| 0.000 | | | | | |
| Root DCM Ext - Leave EtOAc | -22.4976 | 0.0309 | (-22.6114, | -22.3838) | -728.27 |
| 0.000 | | | | | |
| Root EtOAt E - Leave EtOAc | -20.2853 | 0.0309 | (-20.3991, | -20.1715) | -656.65 |
| 0.000 | | | | | |
| Root n-Hexan - Leave EtOAc | -11.0285 | 0.0309 | (-11.1423, | -10.9147) | -357.00 |
| 0.000 | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Root Aqueous - Leave n-Hexa | 5.6943 | 0.0309 | ( 5.5805, 5.8081) | 184.33 | 0.000 |
| Root Crude E - Leave n-Hexa | 75.4042 | 0.0309 | ( 75.2904, 75.5180) | 2440.90 | 0.000 |
| Root DCM Ext - Leave n-Hexa | 9.7210 | 0.0309 | ( 9.6072, 9.8348) | 314.68 | 0.000 |
| Root EtOAc E - Leave n-Hexa | 11.9334 | 0.0309 | ( 11.8195, 12.0472) | 386.29 | 0.000 |
| Root n-Hexan - Leave n-Hexa | 21.1901 | 0.0309 | ( 21.0763, 21.3039) | 685.94 | 0.000 |
| Root Crude E - Root Aqueous | 69.7099 | 0.0309 | ( 69.5961, 69.8237) | 2256.57 | 0.000 |
| Root DCM Ext - Root Aqueous | 4.0267 | 0.0309 | ( 3.9129, 4.1405) | 130.35 | 0.000 |
| Root EtOAc E - Root Aqueous | 6.2391 | 0.0309 | ( 6.1253, 6.3529) | 201.96 | 0.000 |
| Root n-Hexan - Root Aqueous | 15.4958 | 0.0309 | ( 15.3820, 15.6096) | 501.61 | 0.000 |
| Root DCM Ext - Root Crude E | -65.6832 | 0.0309 | (-65.7970, -65.5694) | -2126.22 | 0.000 |
| Root EtOAc E - Root Crude E | -63.4709 | 0.0309 | (-63.5847, -63.3571) | -2054.61 | 0.000 |
| Root n-Hexan - Root Crude E | -54.2141 | 0.0309 | (-54.3279, -54.1003) | -1754.96 | 0.000 |
| Root EtOAt E - Root DCM Ext | 2.2123 | 0.0309 | ( 2.0985, 2.3261) | 71.62 | 0.000 |
| Root n-Hexan - Root DCM Ext | 11.4691 | 0.0309 | ( 11.3553, 11.5829) | 371.26 | 0.000 |
| Root n-Hexan - Root EtOAc E | 9.2568 | 0.0309 | ( 9.1430, 9.3706) | 299.65 | 0.000 |

Individual confidence level = 99.91%

## Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

| Samples | N | Mean | Grouping |
|---|---|---|---|
| Flower Crude Extract | 3 | 105.889 | A |
| Root Crude Extract | 3 | 83.6377 | B |
| Leave EtOAt Extract | 3 | 40.4521 | C |
| Leave Crude Extract | 3 | 30.4706 | D |
| Root n-Hexane Extract | 3 | 29.4236 | E |
| Root EtOAt Extract | 3 | 20.1668 | F |
| Root DCM Extract | 3 | 17.9545 | G |
| Flower EtOAt Extract | 3 | 15.6285 | H |
| Root Aqueous Extract | 3 | 13.9278 | I |
| Leave Aqueous Extract | 3 | 11.2607 | J |
| Flower Aqueous Extract | 3 | 9.4842 | K |
| Leave DCM Extract | 3 | 9.11464 | L |
| Leave n-Hexane Extract | 3 | 8.23349 | M |
| Flower n-Hexane Extract | 3 | 2.32130 | N |
| Flower DCM Extract | 3 | 1.4640 | O |

Means that do not share a letter are significantly different.

Fisher Individual Tests for Differences of Means

| Difference of Levels | Difference of Means | SE of Difference | 95% CI | T-Value | Adjusted P-Value |
|---|---|---|---|---|---|
| Flower Crude - Flower Aqueo | 96.4048 | 0.0309 | ( 96.3417, 96.4679) | 3120.71 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Flower DCM E - Flower Aqueo | -8.0202 | 0.0309 | ( -8.0833, | -7.9571) | -259.62 |
| 0.000 | | | | | |
| Flower EtOAc - Flower Aqueo | 6.1443 | 0.0309 | ( 6.0812, | 6.2074) | 198.90 |
| 0.000 | | | | | |
| Flower n-Hex - Flower Aqueo | -7.1629 | 0.0309 | ( -7.2259, | -7.0998) | -231.87 |
| 0.000 | | | | | |
| Leave Aqueou - Flower Aqueo | 1.7765 | 0.0309 | ( 1.7134, | 1.8396) | 57.51 |
| 0.000 | | | | | |
| Leave Crude - Flower Aqueo | 20.9864 | 0.0309 | ( 20.9233, | 21.0495) | 679.35 |
| 0.000 | | | | | |
| Leave DCM Ex - Flower Aqueo | -0.3695 | 0.0309 | ( -0.4326, | -0.3064) | -11.96 |
| 0.000 | | | | | |
| Leave EtOAc - Flower Aqueo | 30.9680 | 0.0309 | ( 30.9049, | 31.0311) | 1002.46 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower Aqueo | -1.2507 | 0.0309 | ( -1.3137, | -1.1876) | -40.48 |
| 0.000 | | | | | |
| Root Aqueous - Flower Aqueo | 4.4436 | 0.0309 | ( 4.3805, | 4.5067) | 143.84 |
| 0.000 | | | | | |
| Root Crude E - Flower Aqueo | 74.1536 | 0.0309 | ( 74.0905, | 74.2166) | 2400.41 |
| 0.000 | | | | | |
| Root DCM Ext - Flower Aqueo | 8.4704 | 0.0309 | ( 8.4073, | 8.5335) | 274.19 |
| 0.000 | | | | | |
| Root EtOAc E - Flower Aqueo | 10.6827 | 0.0309 | ( 10.6196, | 10.7458) | 345.81 |
| 0.000 | | | | | |
| Root n-Hexan - Flower Aqueo | 19.9395 | 0.0309 | ( 19.8764, | 20.0025) | 645.46 |
| 0.000 | | | | | |
| Flower DCM E - Flower Crude | -104.425 | 0.031 | (-104.488, | -104.362) | -3380.33 |
| 0.000 | | | | | |
| Flower EtOAc - Flower Crude | -90.2605 | 0.0309 | (-90.3236, | -90.1974) | -2921.81 |
| 0.000 | | | | | |
| Flower n-Hex - Flower Crude | -103.568 | 0.031 | (-103.631, | -103.505) | -3352.58 |
| 0.000 | | | | | |
| Leave Aqueou - Flower Crude | -94.6283 | 0.0309 | (-94.6914, | -94.5652) | -3063.20 |
| 0.000 | | | | | |
| Leave Crude - Flower Crude | -75.4184 | 0.0309 | (-75.4815, | -75.3553) | -2441.36 |
| 0.000 | | | | | |
| Leave DCM Ex - Flower Crude | -96.7743 | 0.0309 | (-96.8374, | -96.7113) | -3132.67 |
| 0.000 | | | | | |
| Leave EtOAc - Flower Crude | -65.4369 | 0.0309 | (-65.4999, | -65.3738) | -2118.25 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower Crude | -97.6555 | 0.0309 | (-97.7186, | -97.5924) | -3161.19 |
| 0.000 | | | | | |
| Root Aqueous - Flower Crude | -91.9612 | 0.0309 | (-92.0243, | -91.8981) | -2976.86 |
| 0.000 | | | | | |
| Root Crude E - Flower Crude | -22.2513 | 0.0309 | (-22.3144, | -22.1882) | -720.29 |
| 0.000 | | | | | |
| Root DCM Ext - Flower Crude | -87.9345 | 0.0309 | (-87.9976, | -87.8714) | -2846.51 |
| 0.000 | | | | | |
| Root EtOAc E - Flower Crude | -85.7221 | 0.0309 | (-85.7852, | -85.6590) | -2774.90 |
| 0.000 | | | | | |
| Root n-Hexan - Flower Crude | -76.4654 | 0.0309 | (-76.5285, | -76.4023) | -2475.25 |
| 0.000 | | | | | |
| Flower EtOAc - Flower DCM E | 14.1645 | 0.0309 | ( 14.1014, | 14.2276) | 458.52 |
| 0.000 | | | | | |
| Flower n-Hex - Flower DCM E | 0.8573 | 0.0309 | ( 0.7942, | 0.9204) | 27.75 |
| 0.000 | | | | | |
| Leave Aqueou - Flower DCM E | 9.7967 | 0.0309 | ( 9.7336, | 9.8598) | 317.13 |
| 0.000 | | | | | |
| Leave Crude - Flower DCM E | 29.0066 | 0.0309 | ( 28.9435, | 29.0697) | 938.97 |
| 0.000 | | | | | |
| Leave DCM Ex - Flower DCM E | 7.6507 | 0.0309 | ( 7.5876, | 7.7138) | 247.66 |
| 0.000 | | | | | |
| Leave EtOAc - Flower DCM E | 38.9882 | 0.0309 | ( 38.9251, | 39.0513) | 1262.08 |
| 0.000 | | | | | |
| Leave n-Hexa - Flower DCM E | 6.7695 | 0.0309 | ( 6.7064, | 6.8326) | 219.14 |
| 0.000 | | | | | |
| Root Aqueous - Flower DCM E | 12.4638 | 0.0309 | ( 12.4007, | 12.5269) | 403.46 |
| 0.000 | | | | | |

| | | | | |
|---|---|---|---|---|
| Root Crude E - Flower DCM E | 82.1737 | 0.0309 | ( 82.1107, 82.2368) | 2660.03 |
| 0.000 | | | | |
| Root DCM Ext - Flower DCM E | 16.4905 | 0.0309 | ( 16.4275, 16.5536) | 533.81 |
| 0.000 | | | | |
| Root EtOAc E - Flower DCM E | 18.7029 | 0.0309 | ( 18.6398, 18.7660) | 605.43 |
| 0.000 | | | | |
| Root n-Hexan - Flower DCM E | 27.9596 | 0.0309 | ( 27.8966, 28.0227) | 905.08 |
| 0.000 | | | | |
| Flower n-Hex - Flower EtOAc | -13.3072 | 0.0309 | (-13.3703, -13.2441) | -430.76 |
| 0.000 | | | | |
| Leave Aqueou - Flower EtOAc | -4.3678 | 0.0309 | ( -4.4309, -4.3047) | -141.39 |
| 0.000 | | | | |
| Leave Crude - Flower EtOAc | 14.8421 | 0.0309 | ( 14.7790, 14.9052) | 480.45 |
| 0.000 | | | | |
| Leave DCM Ex - Flower EtOAc | -6.5138 | 0.0309 | ( -6.5769, -6.4508) | -210.86 |
| 0.000 | | | | |
| Leave EtOAc - Flower EtOAc | 24.8237 | 0.0309 | ( 24.7606, 24.8867) | 803.56 |
| 0.000 | | | | |
| Leave n-Hexa - Flower EtOAc | -7.3950 | 0.0309 | ( -7.4581, -7.3319) | -239.38 |
| 0.000 | | | | |
| Root Aqueous - Flower EtOAc | -1.7007 | 0.0309 | ( -1.7638, -1.6376) | -55.05 |
| 0.000 | | | | |
| Root Crude E - Flower EtOAc | 68.0092 | 0.0309 | ( 67.9461, 68.0723) | 2201.52 |
| 0.000 | | | | |
| Root DCM Ext - Flower EtOAc | 2.3260 | 0.0309 | ( 2.2629, 2.3891) | 75.30 |
| 0.000 | | | | |
| Root EtOAc E - Flower EtOAc | 4.5384 | 0.0309 | ( 4.4753, 4.6015) | 146.91 |
| 0.000 | | | | |
| Root n-Hexan - Flower EtOAc | 13.7951 | 0.0309 | ( 13.7320, 13.8582) | 446.56 |
| 0.000 | | | | |
| Leave Aqueou - Flower n-Hex | 8.9394 | 0.0309 | ( 8.8763, 9.0024) | 289.37 |
| 0.000 | | | | |
| Leave Crude - Flower n-Hex | 28.1493 | 0.0309 | ( 28.0862, 28.2124) | 911.22 |
| 0.000 | | | | |
| Leave DCM Ex - Flower n-Hex | 6.7933 | 0.0309 | ( 6.7303, 6.8564) | 219.91 |
| 0.000 | | | | |
| Leave EtOAc - Flower n-Hex | 38.1308 | 0.0309 | ( 38.0677, 38.1939) | 1234.33 |
| 0.000 | | | | |
| Leave n-Hexa - Flower n-Hex | 5.9122 | 0.0309 | ( 5.8491, 5.9753) | 191.38 |
| 0.000 | | | | |
| Root Aqueous - Flower n-Hex | 11.6065 | 0.0309 | ( 11.5434, 11.6696) | 375.71 |
| 0.000 | | | | |
| Root Crude E - Flower n-Hex | 81.3164 | 0.0309 | ( 81.2533, 81.3795) | 2632.28 |
| 0.000 | | | | |
| Root DCM Ext - Flower n-Hex | 15.6332 | 0.0309 | ( 15.5701, 15.6963) | 506.06 |
| 0.000 | | | | |
| Root EtOAc E - Flower n-Hex | 17.8456 | 0.0309 | ( 17.7825, 17.9086) | 577.68 |
| 0.000 | | | | |
| Root n-Hexan - Flower n-Hex | 27.1023 | 0.0309 | ( 27.0392, 27.1654) | 877.33 |
| 0.000 | | | | |
| Leave Crude - Leave Aqueou | 19.2099 | 0.0309 | ( 19.1468, 19.2730) | 621.84 |
| 0.000 | | | | |
| Leave DCM Ex - Leave Aqueou | -2.1460 | 0.0309 | ( -2.2091, -2.0829) | -69.47 |
| 0.000 | | | | |
| Leave EtOAc - Leave Aqueou | 29.1915 | 0.0309 | ( 29.1284, 29.2546) | 944.95 |
| 0.000 | | | | |
| Leave n-Hexa - Leave Aqueou | -3.0272 | 0.0309 | ( -3.0902, -2.9641) | -97.99 |
| 0.000 | | | | |
| Root Aqueous - Leave Aqueou | 2.6671 | 0.0309 | ( 2.6040, 2.7302) | 86.34 |
| 0.000 | | | | |
| Root Crude E - Leave Aqueou | 72.3771 | 0.0309 | ( 72.3140, 72.4401) | 2342.91 |
| 0.000 | | | | |
| Root DCM Ext - Leave Aqueou | 6.6939 | 0.0309 | ( 6.6308, 6.7569) | 216.69 |
| 0.000 | | | | |
| Root EtOAc E - Leave Aqueou | 8.9062 | 0.0309 | ( 8.8431, 8.9693) | 288.30 |
| 0.000 | | | | |
| Root n-Hexan - Leave Aqueou | 18.1630 | 0.0309 | ( 18.0999, 18.2260) | 587.95 |
| 0.000 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Leave DCM Ex - Leave Crude | -21.3559 | 0.0309 | (-21.4190, -21.2928) | -691.31 | 0.000 |
| Leave EtOAc - Leave Crude | 9.9816 | 0.0309 | ( 9.9185, 10.0447) | 323.11 | 0.000 |
| Leave n-Hexa - Leave Crude | -22.2371 | 0.0309 | (-22.3002, -22.1740) | -719.83 | 0.000 |
| Root Aqueous - Leave Crude | -16.5428 | 0.0309 | (-16.6059, -16.4797) | -535.50 | 0.000 |
| Root Crude E - Leave Crude | 53.1671 | 0.0309 | ( 53.1041, 53.2302) | 1721.07 | 0.000 |
| Root DCM Ext - Leave Crude | -12.5160 | 0.0309 | (-12.5791, -12.4530) | -405.16 | 0.000 |
| Root EtOAc E - Leave Crude | -10.3037 | 0.0309 | (-10.3668, -10.2406) | -333.54 | 0.000 |
| Root n-Hexan - Leave Crude | -1.0470 | 0.0309 | ( -1.1100, -0.9839) | -33.89 | 0.000 |
| Leave EtOAc - Leave DCM Ex | 31.3375 | 0.0309 | ( 31.2744, 31.4006) | 1014.42 | 0.000 |
| Leave n-Hexa - Leave DCM Ex | -0.8811 | 0.0309 | ( -0.9442, -0.8181) | -28.52 | 0.000 |
| Root Aqueous - Leave DCM Ex | 4.8131 | 0.0309 | ( 4.7500, 4.8762) | 155.81 | 0.000 |
| Root Crude E - Leave DCM Ex | 74.5231 | 0.0309 | ( 74.4600, 74.5862) | 2412.38 | 0.000 |
| Root DCM Ext - Leave DCM Ex | 8.8399 | 0.0309 | ( 8.7768, 8.9030) | 286.15 | 0.000 |
| Root EtOAc - Leave DCM Ex | 11.0522 | 0.0309 | ( 10.9891, 11.1153) | 357.77 | 0.000 |
| Root n-Hexan - Leave DCM Ex | 20.3090 | 0.0309 | ( 20.2459, 20.3721) | 657.42 | 0.000 |
| Leave n-Hexa - Leave EtOAc | -32.2186 | 0.0309 | (-32.2817, -32.1555) | -1042.95 | 0.000 |
| Root Aqueous - Leave EtOAc | -26.5244 | 0.0309 | (-26.5874, -26.4613) | -858.62 | 0.000 |
| Root Crude E - Leave EtOAc | 43.1856 | 0.0309 | ( 43.1225, 43.2487) | 1397.95 | 0.000 |
| Root DCM Ext - Leave EtOAc | -22.4976 | 0.0309 | (-22.5607, -22.4345) | -728.27 | 0.000 |
| Root EtOAc E - Leave EtOAc | -20.2853 | 0.0309 | (-20.3484, -20.2222) | -656.65 | 0.000 |
| Root n-Hexan - Leave EtOAc | -11.0285 | 0.0309 | (-11.0916, -10.9654) | -357.00 | 0.000 |
| Root Aqueous - Leave n-Hexa | 5.6943 | 0.0309 | ( 5.6312, 5.7574) | 184.33 | 0.000 |
| Root Crude E - Leave n-Hexa | 75.4042 | 0.0309 | ( 75.3411, 75.4673) | 2440.90 | 0.000 |
| Root DCM Ext - Leave n-Hexa | 9.7210 | 0.0309 | ( 9.6579, 9.7841) | 314.68 | 0.000 |
| Root EtOAc E - Leave n-Hexa | 11.9334 | 0.0309 | ( 11.8703, 11.9964) | 386.29 | 0.000 |
| Root n-Hexan - Leave n-Hexa | 21.1901 | 0.0309 | ( 21.1270, 21.2532) | 685.94 | 0.000 |
| Root Crude E - Root Aqueous | 69.7099 | 0.0309 | ( 69.6468, 69.7730) | 2256.57 | 0.000 |
| Root DCM Ext - Root Aqueous | 4.0267 | 0.0309 | ( 3.9636, 4.0898) | 130.35 | 0.000 |
| Root EtOAc E - Root Aqueous | 6.2391 | 0.0309 | ( 6.1760, 6.3022) | 201.96 | 0.000 |
| Root n-Hexan - Root Aqueous | 15.4958 | 0.0309 | ( 15.4327, 15.5589) | 501.61 | 0.000 |
| Root DCM Ext - Root Crude E | -65.6832 | 0.0309 | (-65.7463, -65.6201) | -2126.22 | 0.000 |
| Root EtOAc E - Root Crude E | -63.4709 | 0.0309 | (-63.5339, -63.4078) | -2054.61 | 0.000 |
| Root n-Hexan - Root Crude E | -54.2141 | 0.0309 | (-54.2772, -54.1510) | -1754.96 | 0.000 |
| Root EtOAc E - Root DCM Ext | 2.2123 | 0.0309 | ( 2.1492, 2.2754) | 71.62 | 0.000 |

```
Root n-Hexan - Root DCM Ext     11.4691      0.0309  ( 11.4060,  11.5322)    371.26
0.000
Root n-Hexan - Root EtOAc E      9.2568      0.0309  (  9.1937,   9.3198)    299.65
0.000

Simultaneous confidence level = 24.08%
```

## Appendix III: % scavenging activity of extracts on DPPH

| Conc. mg/ml | % RSA of Ascorbic Acid | %RSA of Leaves Aq. Extract | %RSA of Leave EtOAt Extracts | %RSA of Leaves DCM Extract | %RSA of Leaves n-Hexane Extracts | %RSA of Root Aq. Extract | %RSA of Root EtOAt Extract | %RSA Root of DCM Extract | %RSA Root n-Hexane Extract | %RSA of Flower Aq. Extract | % RSA of Flower EtOAt Extract | % RSA of Flower DCM Extract | % RSA of Flower n-Hexane Extract | %RSA of Root Crude Extract | %RSA of Flower Crude Extract | %RSA of Leaves Crude Extract |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.3 | 96.06 | 22.98 | 50.73 | 30.28 | 19.80 | 29.028 | 74.47 | 31.03 | 28.02 | 22.72 | 34.46 | 23.20 | 15.67 | 49.73 | 44.49 | 39.68 |
| 0.2 | 96.00 | 23.03 | 40.88 | 26.77 | 17.56 | 29.66 | 61.46 | 25.19 | 23.15 | 18.50 | 27.86 | 33.15 | 18.45 | 45.29 | 41.39 | 37.55 |
| 0.1 | 82.22 | 23.00 | 34.56 | 25.32 | 17.98 | 25.06 | 43.97 | 19.20 | 15.67 | 17.24 | 28.73 | 25.99 | 16.90 | 45.40 | 40.70 | 34.85 |
| 0.05 | 60.72 | 22.70 | 29.28 | 21.69 | 16.03 | 22.23 | 34.37 | 16.55 | 17.33 | 19.03 | 24.94 | 25.37 | 15.51 | 38.22 | 37.97 | 33.73 |
| 0.025 | 22.76 | 21.08 | 25.62 | 21.88 | 17.47 | 24.99 | 29.34 | 16.07 | 17.73 | 20.20 | 24.09 | 21.64 | 19.17 | 36.41 | 37.03 | 32.39 |

## Appendix IV: 4.7: Tables of GC-MS analysis of *A. ciliaries*

**Table 4.7.1: The GCMS results of flower *n*-hexane fraction of *Aloiampelos ciliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.084 | 161104 | 1.42 | Cyclohexane, methyl- | 97 |
| 2 | 3.169 | 143979 | 1.27 | Methyl Isobutyl Ketone | 95 |
| 3 | 3.521 | 4252658 | 37.48 | Toluene | 98 |
| 4 | 3.815 | 38673 | 0.34 | Cyclopentanol, 1-methyl- | 92 |
| 5 | 3.897 | 31189 | 0.27 | Octane <n-> | 89 |
| 6 | 4.052 | 57995 | 0.51 | Acetate <butyl-> | 93 |
| 7 | 5.295 | 163720 | 1.44 | 2-Propenoic acid, butyl ester | 80 |
| 8 | 5.399 | 52010 | 0.46 | Nonane <n-> | 94 |
| 9 | 5.463 | 188933 | 1.67 | Pentan-2-ol, 4-allyloxy-2-methyl- | 85 |
| 10 | 5.771 | 212833 | 1.88 | 2,5-Dimethyl-4-hydroxy-3-hexanone | 84 |
| 11 | 6.075 | 70315 | 0.62 | Hydroperoxide, 1-ethylbutyl | 89 |
| 12 | 6.23 | 81758 | 0.72 | Hydroperoxide, 1-methylpentyl | 93 |
| 13 | 6.465 | 96552 | 0.85 | Benzene, 1-ethyl-2-methyl- | 95 |
| 14 | 6.507 | 457492 | 4.03 | 1-Butanone, 1-cyclohexyl- | 86 |
| 15 | 6.604 | 94340 | 0.83 | 1,2,3-trimethyl- Benzene | 92 |
| 16 | 6.771 | 47377 | 0.42 | 1-ethyl-3-methyl- Benzene | 93 |
| 17 | 7.045 | 421228 | 3.71 | Mesitylene | 96 |
| 1 8 | 7.09 | 95288 | 0.84 | Decane <n-> | 95 |
| 19 | 7.46 | 29272 | 0.26 | Heptane, 3,3,5-trimethyl- | 88 |
| 20 | 7.526 | 265394 | 2.34 | Benzene, 1-ethyl-3-methyl- | 96 |
| 21 | 7.718 | 98978 | 0.87 | 2-Pyrrolidinone, 1-methyl- | 86 |
| 22 | 8.008 | 48113 | 0.42 | Benzene, 1-methyl-3-propyl- | 94 |
| 23 | 8.107 | 180058 | 1.59 | 1,4-diethyl- Benzene | 91 |
| 24 | 8.435 | 87803 | 0.77 | 4-ethyl-1,2-dimethyl- Benzene | 96 |
| 25 | 8.484 | 95303 | 0.84 | o-Cymene | 95 |
| 26 | 8.59 | 223604 | 1.97 | Benzene, 2-ethyl-1,4-dimethyl- | 96 |
| 27 | 8.787 | 169825 | 1.5 | Dodecane <n-> | 96 |
| 28 | 8.933 | 59455 | 0.52 | 4-ethyl-1,2-dimethyl- Benzene | 96 |
| 29 | 9.139 | 326781 | 2.88 | 1,2,4,5-tetramethyl- Benzene | 96 |
| 30 | 9.2 | 494828 | 4.36 | 1,2,3,4-tetramethyl- Benzene | 95 |
| 31 | 9.481 | 36974 | 0.33 | 1,19-Eicosadiene | 78 |
| 32 | 9.561 | 64029 | 0.56 | Benzene, 1-methyl-2-(2-propenyl)- | 92 |
| 33 | 9.736 | 258184 | 2.28 | 1,2,3,4-tetramethyl-5-methylene-1,3-Cyclopentadiene | 93 |
| 34 | 9.843 | 42260 | 0.37 | 1-Chloroeicosane | 79 |
| 35 | 9.953 | 41338 | 0.36 | Benzenepropanoic acid, decyl ester | 73 |
| 36 | 10.372 | 150381 | 1.33 | Azulene | 97 |
| 37 | 10.429 | 163938 | 1.45 | Dodecane | 84 |
| 38 | 11.193 | 602902 | 5.31 | Butane, 1,1-dibutoxy- | 97 |
| 41 | 14.932 | 1033016 | 9.11 | 2,4-Di-tert-butylphenol | 97 |
| 39 | 11.915 | 27513 | 0.24 | Cyclohexasiloxane, dodecamethyl- | 88 |
| 40 | 13.445 | 177613 | 1.57 | Hexadecane <n-> | 96 |

**Table 4.7.2: The GCMS results of flower DCM fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.555 | 176202 | 0.5 | Toluene | 98 |
| 2 | 4.494 | 143012 | 0.41 | 4-hydroxy-4-methyl-2-Pentanone | 97 |
| 3 | 6.949 | 304118 | 0.86 | Dec-1-ene | 97 |
| 4 | 8.872 | 89507 | 0.25 | Nonanal | 97 |
| 5 | 9.001 | 48322 | 0.14 | (2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl) tetrahydro-2H-pyran | 97 |
| 6 | 9.149 | 58279 | 0.17 | 1,2,4,5-tetramethyl- Benzene | 96 |
| 7 | 9.212 | 114138 | 0.32 | 1,2,4,5-tetramethyl- Benzene | 95 |
| 8 | 10.298 | 1054781 | 3 | 1-Dodecanol | 96 |
| 9 | 10.434 | 53049 | 0.15 | Tridecane <n-> | 94 |
| 10 | 11.199 | 548633 | 1.56 | Butane, 1,1-dibutoxy- | 96 |
| 11 | 13.336 | 1960839 | 5.58 | 1-Tetradecene | 96 |
| 12 | 13.449 | 105570 | 0.3 | Tetradecane | 97 |
| 13 | 14.937 | 3722054 | 10.58 | 2,4-Di-tert-butylphenol | 97 |
| 14 | 16.045 | 2785131 | 7.92 | 1-Nonadecene | 97 |
| 15 | 16.137 | 90211 | 0.26 | Heptadecane | 97 |
| 16 | 18.1 | 60179 | 0.17 | Dodecanamide | 94 |
| 17 | 18.478 | 3044567 | 8.66 | 1-Nonadecene | 97 |
| 18 | 19.713 | 78407 | 0.22 | methyl ester, (Z)- 9-Hexadecenoic acid | 93 |
| 19 | | 355695 | 1.01 | methyl ester Hexadecanoic acid, | 95 |
| 20 | 20.676 | 2834875 | 8.06 | Eicosene | 95 |
| 21 | 21.744 | 463568 | 1.32 | (Z, Z)-, methyl ester9,12-Octadecadienoic acid | 95 |
| 22 | 21.814 | 573213 | 1.63 | Linolenate <methyl-> | 93 |
| 23 | 22.747 | 575755 | 1.64 | Tetradecanamide | 93 |
| 24 | 22.928 | 2349001 | 6.68 | 1-Hexacosanol | 95 |
| 25 | 25.478 | 9802543 | 27.87 | 9-Octadecenamide, (Z)- | 92 |
| 26 | 25.898 | 849486 | 2.42 | 9-Octadecenamide, (Z)- | 89 |
| 27 | 26.034 | 1990559 | 5.66 | 1-Hexacosanol | 95 |
| 28 | 28.271 | 262755 | 0.75 | Hexadecanoic acid, octyl ester | 86 |
| 29 | 28.572 | 160831 | 0.46 | 2-hydroxy-1-(hydroxymethyl)ethyl ester Hexadecanoic acid, | 88 |
| 30 | 30.945 | 512974 | 1.46 | Hexacosanol | 94 |

**Table 4.7.3: The GCMS results of flower EtOAc fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.063 | 951721 | 1.56 | Perfluorotributylamine | 90 |
| 2 | 3.203 | 218531 | 0.36 | Methyl Isobutyl Ketone | 95 |
| 3 | 3.545 | 811365 | 1.33 | Toluene | 98 |
| 4 | 3.772 | 67868 | 0.11 | Methyl Isobutyl Ketone | 86 |
| 5 | 4.079 | 129554 | 0.21 | Acetate <butyl-> | 97 |
| 6 | 4.483 | 224486 | 0.37 | 2-Pentanone, 4-hydroxy-4-methyl- | 97 |
| 7 | 4.839 | 205906 | 0.34 | Ethylbenzene | 98 |
| 8 | 4.979 | 394149 | 0.65 | o-Xylene | 98 |
| 9 | 5.338 | 240346 | 0.4 | o-Xylene | 93 |
| 10 | 6.085 | 77712 | 0.13 | Hydroperoxide, 1-ethylbutyl | 90 |
| 11 | 6.239 | 90560 | 0.15 | Hydroperoxide, 1-methylpentyl | 91 |
| 12 | 6.681 | 102821 | 0.17 | Cyclotetrasiloxane, octamethyl- | 96 |
| 13 | 6.938 | 435234 | 0.72 | 1-Decene | 97 |
| 14 | 7.049 | 109066 | 0.18 | Mesitylene | 96 |
| 15 | 7.529 | 65620 | 0.11 | Mesitylene | 95 |
| 16 | 8.589 | 64038 | 0.11 | 2-ethyl-1,4-dimethyl- Benzene | 94 |
| 17 | 8.86 | 133460 | 0.22 | Nonanal | 97 |
| 18 | 8.99 | 79387 | 0.13 | (2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl) tetrahydro-2H-pyran | 97 |
| 19 | 9.137 | 106601 | 0.18 | 1,2,3,4-tetramethyl- Benzene | 96 |
| 20 | 9.2 | 193114 | 0.32 | 1,2,3,4-tetramethyl- Benzene | 96 |
| 21 | 9.266 | 167338 | 0.28 | Decamethylcyclopentasiloxane | 94 |
| 22 | 9.734 | 116457 | 0.19 | Benzene, 1,2,4,5-tetramethyl- | 92 |
| 23 | 10.287 | 1414458 | 2.32 | 1-Dodecanol | 96 |
| 24 | 10.369 | 89004 | 0.15 | Azulene | 96 |
| 25 | 10.423 | 135003 | 0.22 | Dodecane <n-> | 94 |
| 26 | 11.187 | 730817 | 1.2 | Butane, 1,1-dibutoxy- | 97 |
| 27 | 11.908 | 97235 | 0.16 | Cyclohexasiloxane, dodecamethyl- | 92 |
| 28 | 13.325 | 2586806 | 4.25 | 1-Pentadecene | 96 |
| 29 | 13.438 | 175149 | 0.29 | Tetradecane | 97 |
| 30 | 14.271 | 345268 | 0.57 | Cycloheptasiloxane, tetradecamethyl- | 90 |
| 31 | 14.926 | 4884201 | 8.03 | 2,4-Di-tert-butylphenol | 97 |
| 32 | 16.036 | 3913009 | 6.43 | Octadec-1-ene | 97 |
| 33 | 16.099 | 538209 | 0.88 | Diethyleneglycol dimethacrylate | 94 |

| | | | | | |
|---|---|---|---|---|---|
| 34 | 16.192 | 79619 | 0.13 | Tridecane, 3-methylene- | 88 |
| 35 | 16.416 | 427726 | 0.7 | Cyclooctasiloxane, hexadecamethyl- | 92 |
| 36 | 16.814 | 91621 | 0.15 | Cinnamaldehyde, alpha-pentyl- | 94 |
| 37 | 16.989 | 98765 | 0.16 | Tetradecanamide | 84 |
| 38 | 18.092 | 182329 | 0.3 | Dodecanamide | 94 |
| 39 | 18.191 | 105024 | 0.17 | 4-hydroxybenzaldehyde-3,5-di-tert-Butyl | 86 |
| 40 | 18.247 | 244273 | 0.4 | Cyclononasiloxane, octadecamethyl- | 85 |
| 41 | 18.467 | 4170890 | 6.85 | 1-Nonadecene | 97 |
| 42 | 18.543 | 181289 | 0.3 | Heneicosane | 95 |
| 43 | 18.616 | 77163 | 0.13 | 2-Undecene, 3-methyl-, (Z)- | 86 |
| 44 | 19.705 | 162925 | 0.27 | Methyl hexadec-9-enoate | 94 |
| 45 | 19.822 | 105297 | 0.17 | oxaspiro (4,5) deca-6,9-diene-2,8-dione7,9-Di-tert-butyl-1- | 79 |
| 46 | 19.919 | 658498 | 1.08 | Hexadecanoic acid, methyl ester | 94 |
| 47 | 20.262 | 122823 | 0.2 | Hexadecanoic acid <n-> | 92 |
| 48 | 20.31 | 64361 | 0.11 | Dibutyl phthalate | 86 |
| 49 | 20.433 | 122851 | 0.2 | 1-Hexadecanol, 2-methyl- | 85 |
| 50 | 20.537 | 62769 | 0.1 | Cyclopropane, 1-(1,2-dimethylpropyl)-1-methyl-2-nonyl- | 83 |
| 51 | 20.668 | 4041537 | 6.64 | Eicosene | 96 |
| 52 | 20.81 | 80672 | 0.13 | 1-Decen-3-one | 81 |
| 53 | 20.844 | 100873 | 0.17 | cis-10-Heptadecenoic acid, methyl ester | 83 |
| 54 | 21.732 | 699777 | 1.15 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 96 |
| 55 | 21.802 | 945308 | 1.55 | Linolenate <methyl-> | 93 |
| 56 | 22.077 | 60098 | 0.1 | Methyl stearate | 93 |
| 57 | 22.65 | 237995 | 0.39 | Dodecanoic acid, n.-octyl ester | 89 |
| 58 | 22.735 | 1170156 | 1.92 | Tetradecanamide | 94 |
| 59 | 22.916 | 3624258 | 5.96 | 1-Hexacosanol | 95 |
| 60 | 25.171 | 97112 | 0.16 | [1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)- | 81 |
| 61 | 25.35 | 357380 | 0.59 | Linoleyl acetate | 81 |
| 62 | 25.469 | 15725637 | 25.84 | 9-Octadecenamide, (Z)- | 93 |
| 63 | 25.88 | 1587719 | 2.61 | Tetradecanamide | 90 |
| 64 | 26.018 | 3463722 | 5.69 | 1-Hexacosanol | 95 |
| 65 | 28.247 | 462363 | 0.76 | Octan-2-yl palmitate | 87 |
| 66 | 28.525 | 394868 | 0.65 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 88 |
| 67 | 30.911 | 944032 | 1.55 | 1-Hexacosanol | 94 |

**Table 4.7.4: The GC-MS results of flower methanol fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 4.547 | 36376 | 1.36 | 4-hydroxy-4-methyl-2-Pentanone | 96 |
| 2 | 4.806 | 78988 | 2.95 | Lactic acid | 94 |
| 3 | 6.459 | 153794 | 5.74 | Glycerin | 97 |
| 4 | 7.257 | 60615 | 2.26 | Monomethyl malonate | 91 |
| 5 | 7.956 | 65618 | 2.45 | Phenol, 2-methyl- | 96 |
| 6 | 8.799 | 97534 | 3.64 | Dodecane | 96 |
| 7 | 9.536 | 97708 | 3.64 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 89 |
| 8 | 11.49 | 29723 | 1.11 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 79 |
| 9 | 14.937 | 1442411 | 53.79 | 2,4-Di-tert-butylphenol | 95 |
| 10 | 15.61 | 62570 | 2.33 | .alpha.-D-Glucopyranoside, methyl | 95 |
| 11 | 16.316 | 28607 | 1.07 | .beta.-D-Glucopyranoside, methyl | 87 |
| 12 | 16.852 | 34092 | 1.27 | Benzene, 1-methyl-2-(phenylmethoxy)- | 95 |
| 13 | 18.32 | 56812 | 2.12 | Sorbitol | 89 |
| 14 | 19.801 | 56688 | 2.11 | Dibutyl phthalate | 94 |
| 15 | 20.174 | 97593 | 3.64 | Inositol, 1-deoxy- | 82 |
| 16 | 20.268 | 76997 | 2.87 | 2,6-dihexadecanoate -l-(+)-Ascorbic acid | 91 |
| 17 | 21.644 | 45702 | 1.7 | n-Nonadecanol-1 | 95 |
| 18 | 29.121 | 159766 | 5.96 | Bis(2-ethylhexyl) phthalate | 94 |

**Table 4.7.5: The GCMS results of root n-hexane fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.085 | 147807 | 1.26 | Cyclohexane, methyl- | 97 |
| 2 | 3.17 | 140379 | 1.2 | Methyl Isobutyl Ketone | 95 |
| 3 | 3.522 | 4044829 | 34.55 | Toluene | 98 |
| 4 | 3.828 | 41844 | 0.36 | Cyclopentanol, 1-methyl- | 91 |
| 5 | 3.897 | 35758 | 0.31 | Octane <n-> | 87 |
| 6 | 4.053 | 58586 | 0.5 | Acetate <butyl-> | 94 |
| 7 | 4.966 | 52919 | 0.45 | o-Xylene | 95 |
| 8 | 5.103 | 32494 | 0.28 | n-Butyl ether | 92 |
| 9 | 5.301 | 71577 | 0.61 | 2-Propenoic acid, butyl ester | 88 |
| 10 | 5.355 | 78004 | 0.67 | Cyclohexanone | 84 |
| 11 | 5.4 | 49553 | 0.42 | Nonane <n-> | 93 |
| 12 | 5.465 | 200400 | 1.71 | Pentan-2-ol, 4-allyloxy-2-methyl- | 86 |
| 1 3 | 5.772 | 233835 | 2 | 2,5-Dimethyl-4-hydroxy-3-hexanone | 85 |
| 14 | 6.076 | 78324 | 0.67 | Hydroperoxide, 1-ethylbutyl | 90 |
| 15 | 6.231 | 89202 | 0.76 | Hyd roperoxide, 1-methylpentyl | 93 |
| 16 | 6.508 | 575759 | 4.92 | 1-Butanone, 1-cyclohexyl- | 83 |
| 17 | 6.606 | 90362 | 0.77 | 1,2,3-trimethyl- Benzene | 92 |
| 18 | 6.772 | 34173 | 0.29 | 1-ethyl-3-methyl- Benzene | 93 |
| 19 | 6.975 | 23842 | 0.2 | Butanoate <butyl-> | 86 |
| 20 | 7.046 | 381954 | 3.26 | Mesitylene | 96 |
| 21 | 7.088 | 89230 | 0.76 | Decane <n-> | 95 |
| 22 | 7.466 | 22019 | 0.19 | Heptane, 3,3,5-trimethyl- | 86 |
| 23 | 7.527 | 246117 | 2.1 | Benzene, 1-ethyl-3-methyl- | 96 |
| 24 | 7.709 | 124520 | 1.06 | 2-Pyrrolidinone, 1-methyl- | 94 |
| 25 | 7.949 | 31363 | 0.27 | 1,2-diethyl- benzene | 88 |
| 26 | 8.009 | 53791 | 0.46 | 1-methyl-3-propyl- benzene | 94 |
| 27 | 8.109 | 152040 | 1.3 | Cyclopentene <3,5-dimethylene-1,4,4-trimethyl-> | 90 |
| 28 | 8.27 | 44855 | 0.38 | oxy-2-phenyl-Ethane, 1-(9-borabicyclo[3.3.1]non-9-yl) | 80 |
| 29 | 8.436 | 76813 | 0.66 | 4-ethyl-1,2-dimethyl- Benzene | 95 |
| 30 | 8.485 | 83602 | 0.71 | o-Cymene | 95 |
| 31 | 8.591 | 195567 | 1.67 | Benzene, 2-ethyl-1,4-dimethyl- | 96 |
| 32 | 8.789 | 145601 | 1.24 | Undecane <n-> | 96 |
| 33 | 8.934 | 47653 | 0.41 | 4-ethyl-1,2-dimethyl- benzene | 96 |
| 34 | 9.14 | 283811 | 2.42 | 1,2,4,5-tetramethyl- Benzene | 96 |
| 35 | 9.202 | 432781 | 3.7 | 1,2,4,5-tetramethyl- Benzene, | 96 |
| 36 | 9.488 | 31009 | 0.26 | 10-Chloro-1-decanol, pentafluoropropionate | 73 |

| | | | | | |
|---|---|---|---|---|---|
| 37 | 9.563 | 62348 | 0.53 | Benzene, 1-methyl-2-(2-propenyl)- | 92 |
| 38 | 9.736 | 256497 | 2.19 | 1,3-Cyclopentadiene, 1,2,3,4-tetramethyl-5-methylene- | 93 |
| 39 | 9.844 | 46160 | 0.39 | 1-Chloroeicosane | 77 |
| 40 | 9.953 | 46755 | 0.4 | Benzenepropanoic acid, decyl ester | 73 |
| 41 | 10.374 | 151212 | 1.29 | Azulene | 97 |
| 42 | 10.429 | 168010 | 1.44 | Dodecane | 86 |
| 43 | 10.626 | 38299 | 0.33 | 1-Hexene, 2-(O-anisyl)-4-methyl- | 72 |
| 44 | 11.195 | 584713 | 4.99 | Butane, 1,1-dibutoxy- | 96 |
| 45 | 11.979 | 35048 | 0.3 | Tetradecane | 96 |
| 46 | 13.332 | 50019 | 0.43 | 1-Tetradecanol | 94 |
| 47 | 13.446 | 235138 | 2.01 | Hexadecane <n-> | 96 |
| 48 | 14.297 | 26116 | 0.22 | Cycloheptasiloxane, tetradecamethyl- | 83 |
| 49 | 14.827 | 20571 | 0.18 | Heptadecane | 93 |
| 50 | 14.934 | 1078122 | 9.21 | 2,4-Di-tert-butylphenol | 97 |
| 51 | 16.043 | 75796 | 0.65 | 1-Nonadecene | 95 |
| 52 | 16.136 | 48242 | 0.41 | Heptadecane | 96 |
| 53 | 18.474 | 64158 | 0.55 | 1-Nonadecene | 96 |
| 54 | 18.551 | 30624 | 0.26 | Heneicosane | 95 |
| 55 | 19.929 | 30262 | 0.26 | Hexadecanoic acid, methyl ester | b94 |
| 56 | 20.083 | 36351 | 0.31 | | 0 |
| 57 | 20.674 | 28505 | 0.24 | 9-Tricosene, (Z)- | 94 |
| 58 | 21.63 | 21494 | 0.18 | n-Pentadecanol-9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 86 |
| 59 | 21.743 | 23145 | 0.2 | | 89 |
| 60 | 21.805 | 26922 | 0.23 | 7-Hexadecenal, (Z)- | 85 |

**Table 4.7.6: The GCMS results of root DCM fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 6.942 | 240348 | 0.53 | 1-Decene | 97 |
| 2 | 8.993 | 46359 | 0.1 | (2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl) tetrahydro-2H-pyran | 97 |
| 3 | 9.206 | 65453 | 0.14 | Benzene, 1,2,4,5-tetramethyl- | 92 |
| 4 | 10.29 | 1012885 | 2.23 | 1-Dodecanol | 97 |
| 5 | 11.19 | 400275 | 0.88 | Butane, 1,1-dibutoxy- | 96 |
| 6 | 13.327 | 2120910 | 4.67 | 1-Pentadecene | 96 |
| 7 | 13.44 | 105510 | 0.23 | Hexadecane <n-> | 96 |
| 8 | 14.927 | 4487891 | 9.88 | 2,4-Di-tert-butylphenol | 96 |
| 9 | 16.036 | 3151062 | 6.94 | 1-Nonadecene | 97 |
| 10 | 18.09 | 112493 | 0.25 | Dodecanamide | 94 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | 18.466 | 3400163 | 7.49 | 1-Nonadecene | 97 |
| 12 | 18.542 | 68370 | 0.15 | Heneicosane | 95 |
| 13 | 20.664 | 3170013 | 6.98 | Eicosene | 95 |
| 14 | 22.643 | 236029 | 0.52 | Dodecanoic acid, n.-octyl ester | 90 |
| 15 | 22.729 | 1240687 | 2.73 | Hexadecanamide | 93 |
| 16 | 22.912 | 2934026 | 6.46 | 1-Hexacosanol | 95 |
| 17 | 23.094 | 438021 | 0.96 | 3,7,11,15-Tetramethylhexadec-2-ene | 88 |
| 18 | 25.455 | 15381952 | 33.87 | 9-Octadecenamide, (Z)- | 94 |
| 19 | 25.87 | 1733838 | 3.82 | Tetradecanamide | 90 |
| 20 | 26.009 | 3195394 | 7.04 | 1-Hexacosanol | 94 |
| 21 | 28.234 | 381629 | 0.84 | Octan-2-yl palmitate | 88 |
| 22 | 28.514 | 652941 | 1.44 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 88 |
| 23 | 30.897 | 836468 | 1.84 | 1-Hexacosanol | 94 |

**Table 4.7.7: The GCMS results of root EtOAc fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.043 | 136953 | 0.69 | 1,4-Dimethyl-5-oxabicyclo[2.1.0]pentane | 84 |
| 2 | 3.145 | 37239 | 0.19 | 2,3-Dimethyl-1-hexene | 76 |
| 3 | 3.215 | 96747 | 0.49 | Cyclohexene, 4-methyl- | 90 |
| 4 | 3.738 | 39304 | 0.2 | CH3C(O)CH2CH2OH | 89 |
| 5 | 3.863 | 424465 | 2.13 | 3-Penten-2-one, 4-methyl- | 97 |
| 6 | 4.135 | 227645 | 1.14 | 4-hydroxy-2-Pentanone | 95 |
| 7 | 4.471 | 13824053 | 69.52 | 4-hydroxy-4-methyl-2-Pentanone | 98 |
| 8 | 5.479 | 51605 | 0.26 | Pentan-2-ol, 4-allyloxy-2-methyl- | 84 |
| 9 | 5.646 | 163471 | 0.82 | 3-Oxetanol, 2,2,3-trimethyl- | 86 |
| 10 | 5.785 | 57637 | 0.29 | 3-Hydroxy-3-methylvaleric acid | 83 |
| 11 | 6.515 | 161581 | 0.81 | 1-Butanone, 1-cyclohexyl- | 85 |
| 12 | 6.82 | 40233 | 0.2 | 2-Hexanone, 4-hydroxy-5-methyl- | 96 |
| 13 | 7.175 | 53846 | 0.27 | 2-Butanol, 3-methyl-, acetate | 90 |
| 14 | 9.508 | 122872 | 0.62 | 2,6-Dimethyl-6-nitro-2-hepten-4-one | 87 |
| 15 | 10.264 | 124655 | 0.63 | - 2,3:5,6-diepoxy-2,6-dimethyl-4-Heptanone | 86 |
| 16 | 12.198 | 104974 | 0.53 | .alpha.-d-Ribopyranoside, methyl | 95 |
| 17 | 12.971 | 60588 | 0.3 | .alpha.-d-Lyxofuranoside-1,2,4,5-tetraethyl methyl | 94 |
| 18 | 13.024 | 44967 | 0.23 | Cyclohexane, (1.alpha.,2.alpha.,4.alpha.,5.alpha.)- | 85 |
| 19 | 13.324 | 272382 | 1.37 | 1-Pentadecene | 96 |

| | | | | | |
|---|---|---|---|---|---|
| 20 | 13.44 | 39003 | 0.2 | Tetradecane | 93 |
| 21 | 13.656 | 24111 | 0.12 | 1,7-Dimethyl-4-(1-methylethyl)cyclodecane | 88 |
| 22 | 14.22 | 49778 | 0.25 | Cyclohexane, octyl- | 96 |
| 23 | 14.925 | 1139435 | 5.73 | 2,4-Di-tert-butylphenol | 97 |
| 24 | 16.034 | 393292 | 1.98 | 1-Nonadecene | 96 |
| 25 | 16.127 | 42202 | 0.21 | Heneicosane | 93 |
| 26 | 18.464 | 315055 | 1.58 | 1-Nonadecene | 97 |
| 27 | 18.872 | 47912 | 0.24 | (1-pentyloctyl)- | 87 |
| 28 | 19.263 | 89123 | 0.45 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester Benzene | 96 |
| 29 | 19.378 | 160407 | 0.81 | 9-Heptadecanone | 89 |
| 30 | 19.786 | 212248 | 1.07 | Dibutyl phthalate | 95 |
| 31 | 19.971 | 39314 | 0.2 | Phthalic acid, isobutyl 2-pentyl ester | 91 |
| 32 | 20.255 | 77234 | 0.39 | n-Hexadecanoic acid | 94 |
| 33 | 20.303 | 207568 | 1.04 | Dibutyl phthalate | 96 |
| 34 | 20.488 | 115773 | 0.58 | Dibutyl phthalate | 93 |
| 35 | 20.661 | 213738 | 1.07 | 1-Heptacosanol | 94 |
| 36 | 21.627 | 76422 | 0.38 | 13-Tetradecen-1-ol acetate | 89 |
| 37 | 21.725 | 38409 | 0.19 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 86 |
| 38 | 21.793 | 41794 | 0.21 | 6-Octadecenoic acid, methyl ester, (Z)- | 87 |
| 39 | 22.913 | 73111 | 0.37 | 1-Heptacosanol | 90 |
| 40 | 29.08 | 444061 | 2.23 | Bis(2-ethylhexyl) phthalate | 95 |

**Table 4.7.8: The GCMS results of root methanol fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 4.317 | 41124 | 1.05 | Glyceraldehyde | 89 |
| 2 | 4.524 | 462859 | 11.83 | 2-Pentanone, 4-hydroxy-4-methyl- | 97 |
| 3 | 4.806 | 39321 | 1 | Lactic acid | 84 |
| 4 | 5.248 | 30420 | 0.78 | Dihydroxyacetone | 74 |
| 5 | 6.423 | 118572 | 3.03 | Glycerin | 98 |
| 6 | 7.758 | 42346 | 1.08 | 2-Pyrrolidinone, 1-methyl- | 94 |
| 7 | 7.955 | 53889 | 1.38 | Phenol, 2-methyl- | 96 |
| 8 | 8.793 | 94684 | 2.42 | Undecane <n-> | 96 |
| 9 | 9.274 | 77863 | 1.99 | 2,3-dihydroxy-, Propanal -(S)- | 80 |
| 10 | 9.537 | 21305 | 0.54 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 82 |
| 11 | 11.009 | 75700 | 1.93 | 1,2,3-Propanetriol, 1-acetate | 90 |
| 12 | 11.477 | 21210 | 0.54 | Heptanoic acid, 6-oxo- | 78 |

| | | | | | |
|---|---|---|---|---|---|
| 13 | 12.217 | 37374 | 0.95 | .alpha.-d-Ribopyranoside, methyl | 89 |
| 14 | 12.439 | 28473 | 0.73 | Butanedioic acid, 2-hydroxy-2-methyl-, (S)- | 82 |
| 15 | 12.994 | 21982 | 0.56 | .beta.-Methyl xyloside | 91 |
| 16 | 14.726 | 84116 | 2.15 | DL-Arabinitol | 89 |
| 17 | 14.936 | 762257 | 19.48 | 2,4-Di-tert-butylphenol | 96 |
| 18 | 15.513 | 472158 | 12.06 | .alpha.-D-Glucopyranoside, methyl | 95 |
| 19 | 15.638 | 480367 | 12.27 | .beta.-D-Glucopyranoside, methyl | 97 |
| 20 | 16.095 | 169426 | 4.33 | Methyl-.beta.-D-thiogalactoside | 89 |
| 21 | 16.239 | 310612 | 7.94 | .alpha.-Methyl-l-sorboside | 78 |
| 22 | 16.848 | 25647 | 0.66 | Benzene, 1-methyl-2-(phenylmethoxy)- | 94 |
| 23 | 18.195 | 22241 | 0.57 | 3,5-di-tert-Butyl-4-hydroxybenzaldehyde | 78 |
| 24 | 19.805 | 77105 | 1.97 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 90 |
| 25 | 19.927 | 30138 | 0.77 | Hexadecanoic acid, methyl ester | 91 |
| 26 | 19.988 | 18799 | 0.48 | 1,2-Benzenedicarboxylic acid, mono(1-methylethyl) ester | 70 |
| 27 | 20.268 | 55504 | 1.42 | Hexadecanoic acid <n-> | 92 |
| 28 | 20.31 | 33260 | 0.85 | Dibutyl phthalate | 88 |
| 29 | 21.644 | 27462 | 0.7 | n-Pentadecanol | 91 |
| 30 | 21.739 | 37288 | 0.95 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 88 |
| 31 | 21.812 | 23091 | 0.59 | methyl ester-6-Octadecenoic acid (Z)- | 81 |
| 32 | 22.465 | 20287 | 0.52 | Octadecanoic acid | 82 |
| 33 | 29.111 | 96811 | 2.47 | Phthalic acid, bis(2-ethylhexyl) ester (6CI,8CI) | 93 |

**Table 4.7.9: The GCMS results of leaves *n*-hexane fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.044 | 17670145 | 53.6 | Pentadecafluorooctanoic acid, undecyl ester | 77 |
| 2 | 3.514 | 5028386 | 15.25 | Toluene | 98 |
| 3 | 4.045 | 70271 | 0.21 | Acetate <butyl-> | 92 |
| 4 | 5.29 | 191494 | 0.58 | 2-Propenoic acid, butyl ester | 78 |
| 5 | 5.394 | 56913 | 0.17 | Nonane <n-> | 92 |
| 6 | 5.457 | 220648 | 0.67 | Pentane, 3-ethyl-2,4-dimethyl- | 85 |
| 7 | 5.766 | 255167 | 0.77 | 1-Pentanol, 2,2-dimethyl- | 83 |
| 8 | 6.07 | 80326 | 0.24 | Hydroperoxide, 1-ethylbutyl | 88 |
| 9 | 6.225 | 97757 | 0.3 | Hydroperoxide, 1-methylpentyl | 90 |
| 10 | 6.46 | 112704 | 0.34 | 1-ethyl-2-methyl- Benzene | 95 |
| 11 | 6.502 | 541034 | 1.64 | 1-Butanone, 1-cyclohexyl- | 87 |
| 12 | 6.601 | 103359 | 0.31 | 1,2,3-trimethyl- Benzene | 92 |

| | | | | | |
|---|---|---|---|---|---|
| 13 | 7.04 | 504822 | 1.53 | Mesitylene | 96 |
| 14 | 7.083 | 105058 | 0.32 | Decane <n-> | 94 |
| 15 | 7.523 | 302362 | 0.92 | Benzene, 1-ethyl-2-methyl- | 96 |
| 16 | 8.104 | 210082 | 0.64 | 4-ethyl-1,2-dimethyl- Benzene | 90 |
| 17 | 8.431 | 103330 | 0.31 | Benzene, 4-ethyl-1,2-dimethyl- | 96 |
| 18 | 8.48 | 112466 | 0.34 | o-Cymene | 95 |
| 19 | 8.587 | 254727 | 0.77 | Benzene, 2-ethyl-1,4-dimethyl- | 96 |
| 20 | 8.784 | 166343 | 0.5 | Dodecane <n-> | 96 |
| 21 | 9.134 | 374036 | 1.13 | 1,2,3,4-tetramethyl benzene | 96 |
| 22 | 9.197 | 556937 | 1.69 | 1,2,3,4-tetramethyl benzene | 96 |
| 23 | 9.265 | 89908 | 0.27 | Decamethylcyclopentasiloxane | 91 |
| 24 | 9.558 | 66379 | 0.2 | 1-methyl-2-(2-propenyl) benzene | 91 |
| 25 | 9.731 | 277436 | 0.84 | 1,3-Cyclopentadiene, 1,2,3,4-tetramethyl-5-methylene- | 93 |
| 26 | 10.368 | 174779 | 0.53 | Azulene | 96 |
| 27 | 10.424 | 191488 | 0.58 | Dodecane | 84 |
| 28 | 11.188 | 678535 | 2.06 | Butane, 1,1-dibutoxy- | 97 |
| 29 | 11.911 | 114579 | 0.35 | Cyclohexasiloxane, dodecamethyl- | 89 |
| 30 | 13.44 | 230740 | 0.7 | Heptadecane <n-> | 96 |
| 31 | 14.252 | 477494 | 1.45 | Cycloheptasiloxane, tetradecamethyl- | 90 |
| 32 | 14.3 | 89238 | 0.27 | Cycloheptasiloxane, tetradecamethyl- | 86 |
| 33 | 14.929 | 1288532 | 3.91 | 2,4-Di-tert-butylphenol | 97 |
| 34 | 15.07 | 80485 | 0.24 | Dodecanoic acid, methyl ester | 96 |
| 35 | 15.247 | 89119 | 0.27 | | 0 |
| 36 | 15.992 | 70745 | 0.21 | Phthalic acid, ethyl neopentyl ester | 76 |
| 37 | 16.093 | 659962 | 2 | Diethyleneglycol dimethacrylate | 95 |
| 38 | 16.416 | 828428 | 2.51 | Cyclooctasiloxane, hexadecamethyl- | 92 |
| 39 | 18.251 | 346338 | 1.05 | Cyclononasiloxane, octadecamethyl- | 86 |
| 40 | 19.875 | 95624 | 0.29 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- | 78 |

**Table 4.7.10: The GCMS results of leaves DCM fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 6.942 | 240348 | 0.53 | 1-Decene | 97 |
| 2 | 8.993 | 46359 | 0.1 | (2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran | 97 |
| 3 | 9.206 | 65453 | 0.14 | Benzene, 1,2,4,5-tetramethyl- | 92 |
| 4 | 10.29 | 1012885 | 2.23 | 1-Dodecanol | 97 |
| 5 | 11.19 | 400275 | 0.88 | Butane, 1,1-dibutoxy- | 96 |
| 6 | 13.327 | 2120910 | 4.67 | 1-Pentadecene | 96 |
| 7 | 13.44 | 105510 | 0.23 | Hexadecane <n-> | 96 |

| | | | | | |
|---|---|---|---|---|---|
| 8 | 14.927 | 4487891 | 9.88 | 2,4-Di-tert-butylphenol | 96 |
| 9 | 16.036 | 3151062 | 6.94 | 1-Nonadecene | 97 |
| 10 | 18.09 | 112493 | 0.25 | Dodecanamide | 94 |
| 11 | 18.466 | 3400163 | 7.49 | 1-Nonadecene | 97 |
| 12 | 18.542 | 68370 | 0.15 | Heneicosane | 95 |
| 13 | 20.664 | 3170013 | 6.98 | Eicosene | 95 |
| 14 | 22.643 | 236029 | 0.52 | Dodecanoic acid, n.-octyl ester | 90 |
| 15 | 22.729 | 1240687 | 2.73 | Hexadecanamide | 93 |
| 16 | 22.912 | 2934026 | 6.46 | 1-Hexacosanol | 95 |
| 17 | 23.094 | 438021 | 0.96 | 3,7,11,15-Tetramethylhexadec-2-ene | 88 |
| 18 | 25.455 | 15381952 | 33.87 | 9-Octadecenamide, (Z)- | 94 |
| 19 | 25.87 | 1733838 | 3.82 | Tetradecanamide | 90 |
| 20 | 26.009 | 3195394 | 7.04 | 1-Hexacosanol | 94 |
| 21 | 28.234 | 381629 | 0.84 | Palmitateoctan-2-yl | 88 |
| 22 | 28.514 | 652941 | 1.44 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 88 |
| 23 | 30.897 | 836468 | 1.84 | Hexacosan-1-ol | 94 |

**Table 4.7.11: The GCMS results of leaves EtOAc fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 3.865 | 420706 | 1.64 | 3-Penten-2-one, 4-methyl- | 97 |
| 2 | 4.135 | 248857 | 0.97 | 2-Pentanone, 4-hydroxy- | 95 |
| 3 | 4.474 | 15200695 | 59.09 | 2-Pentanone, 4-hydroxy-4-methyl- | 98 |
| 4 | 5.646 | 181886 | 0.71 | 2-Pentanone, 5-hydroxy- | 86 |
| 5 | 6.389 | 122648 | 0.48 | Glycerin | 98 |
| 6 | 6.516 | 87155 | 0.34 | 1-Butanone, 1-cyclohexyl- | 84 |
| 7 | 6.82 | 50940 | 0.2 | 2-Hexanone, 4-hydroxy-5-methyl- | 96 |
| 8 | 7.175 | 67876 | 0.26 | 2-Butanol, 3-methyl-, acetate | 91 |
| 9 | 7.712 | 71773 | 0.28 | 2-Pyrrolidinone, 1-methyl- | 96 |
| 10 | 9.509 | 208888 | 0.81 | 2,6-Dimethyl-6-nitro-2-hepten-4-one | 87 |
| 11 | 10.266 | 194982 | 0.76 | , 2,3:5,6-diepoxy-2,6-dimethyl-4-heptanone | 86 |
| 12 | 11.145 | 69689 | 0.27 | Cyclohexane, octyl- | 94 |
| 13 | 12.974 | 90260 | 0.35 | .alpha.-d-Lyxofuranoside, methyl | 94 |
| 14 | 13.025 | 87011 | 0.34 | Cyclohexane, 1,1'-hexylidenebis- | 86 |
| 15 | 13.325 | 554170 | 2.15 | 1-Tetradecanol | 96 |
| 16 | 13.657 | 49785 | 0.19 | 1,7-Dimethyl-4-(1-methylethyl)cyclodecane | 89 |
| 17 | 14.925 | 1320420 | 5.13 | 2,4-Di-tert-butylphenol | 97 |
| 18 | 15.663 | 2108684 | 8.2 | .alpha.-D-Glucopyranoside, methyl | 96 |
| 19 | 16.034 | 840573 | 3.27 | 1-Nonadecene | 96 |

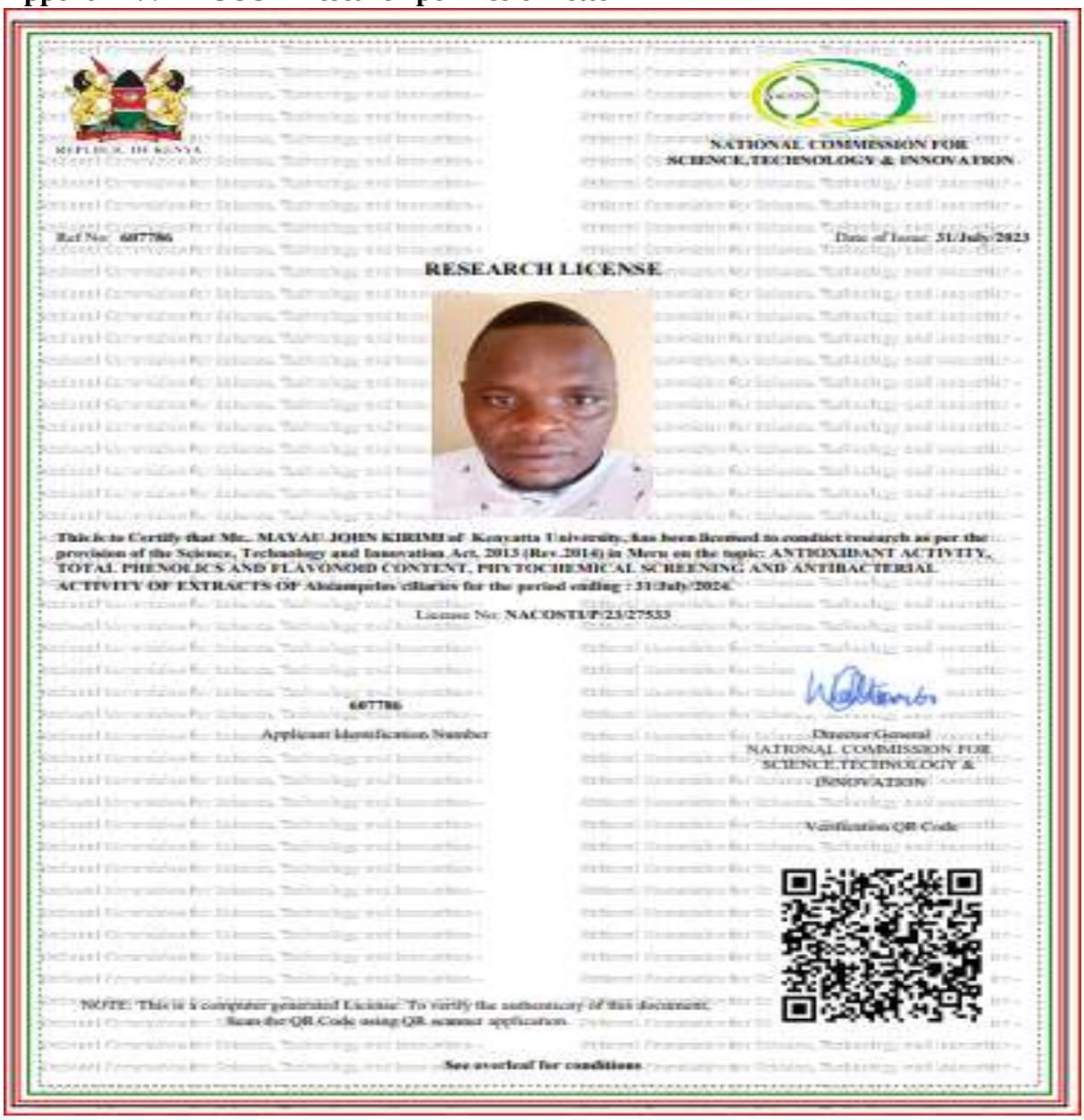
| | | | | | |
|---|---|---|---|---|---|
| 20 | 16.128 | 439429 | 1.71 | .alpha.-D-Glucopyranoside, methyl | 89 |
| 21 | 18.465 | 656367 | 2.55 | 1-Nonadecene | 97 |
| 22 | 19.264 | 180714 | 0.7 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 96 |
| 23 | 19.379 | 274187 | 1.07 | 9-Heptadecanone | 89 |
| 24 | 19.787 | 365881 | 1.42 | Dibutyl phthalate | 94 |
| 25 | 20.303 | 360876 | 1.4 | Dibutyl phthalate | 96 |
| 26 | 20.663 | 380700 | 1.48 | 1-Heptacosanol | 94 |
| 27 | 21.625 | 116255 | 0.45 | 2-Cyclohexylnonadecane | 89 |
| 28 | 22.912 | 166257 | 0.65 | 1-Heptacosanol | 93 |
| 29 | 29.076 | 808046 | 3.14 | Bis(2-ethylhexyl) phthalate | 96 |

**Table 4.7.12: The GCMS results of leaves MeOH fraction of *Aloiampelos cilliaries* indicating the serial number, retention time, type of compounds, peak area, percent area and similarity index.**

| s/n | Retention time (min) | Peak Area | Area % | Compound name | Similarity index |
|---|---|---|---|---|---|
| 1 | 4.532 | 206600 | 4.18 | 2-Pentanone, 4-hydroxy-4-methyl- | 97 |
| 2 | 7.876 | 27275 | 0.55 | 1-Butanamine, 3-methyl-N-(3-methylbutylidene)- | 83 |
| 3 | 7.956 | 97139 | 1.96 | Phenol, 2-methyl- | 96 |
| 4 | 8.255 | 40983 | 0.83 | 1,3,5-Triazine-2,4,6-triamine | 79 |
| 5 | 8.794 | 106125 | 2.15 | Dodecane <n-> | 96 |
| 6 | 9.544 | 146625 | 2.96 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 95 |
| 7 | 10.358 | 84904 | 1.72 | Malic Acid | 83 |
| 8 | 10.755 | 62718 | 1.27 | 5-Hydroxymethylfurfural | 95 |
| 9 | 11.015 | 30077 | 0.61 | Propan-1,2,3-triol, 1-acetate | 85 |
| 10 | 11.489 | 203510 | 4.11 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 79 |
| 11 | 13.446 | 74075 | 1.5 | 2,3,4,4-Tetramethyl-pentane-1,3-diol | 78 |
| 12 | 14.935 | 1040067 | 21.03 | 2,4-Di-tert-butylphenol | 96 |
| 13 | 15.692 | 1125757 | 22.76 | .alpha.-D-Glucopyranoside, methyl | 96 |
| 14 | 15.894 | 108391 | 2.19 | .alpha.-Methyl-D-mannopyranoside | 90 |
| 15 | 16.145 | 377522 | 7.63 | Methyl-.beta.-D-thiogalactoside | 96 |
| 16 | 16.273 | 296275 | 5.99 | Quinic acid | 73 |
| 17 | 16.849 | 49971 | 1.01 | Benzene, 1-methyl-2-(phenylmethoxy)- | 96 |
| 18 | 17.706 | 121814 | 2.46 | L-Mannose | 81 |
| 19 | 19.27 | 33304 | 0.67 | Phthalate <di-isobutyl> | 92 |
| 20 | 19.798 | 59421 | 1.2 | Dibutyl phthalate | 95 |
| 21 | 20.082 | 32462 | 0.66 | | 0 |
| 22 | 20.268 | 79021 | 1.6 | Hexadecanoic acid <n-> | 91 |
| 23 | 20.314 | 79694 | 1.61 | Dibutyl phthalate | 93 |
| 24 | 20.5 | 41934 | 0.85 | Dibutyl phthalate | 88 |
| 25 | 20.804 | 42044 | 0.85 | 1-Naphthalenecarbonitrile, 4-amino- | 71 |

| | | | | | |
|---|---|---|---|---|---|
| 26 | 21.644 | 41418 | 0.84 | n-Nonadecanol-1 | 94 |
| 27 | 23.18 | 96027 | 1.94 | 9-Hexacosene | 85 |
| 28 | 29.112 | 241197 | 4.88 | Bis(2-ethylhexyl) phthalate | 94 |

## Appendix V: NACOSTI research permission letter



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 607786

Date of Issue: 31/July/2023

RESEARCH LICENSE

This is to Certify that Mr., MAYAE JOHN KIRIMI of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Meru on the topic: ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS AND FLAVONOID CONTENT, PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF EXTRACTS OF Abdiampelos ciliaris for the period ending : 31/July/2024.

License No: NACOSTI/P/23/27553

607786

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated Licence. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

**Appendix VI: Authorization letter**

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: 156/25294/2018

DATE: 25th October, 2022

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. MAYAU JOHN KIRIMI - REG. NO. 156/25294/18

I write to introduce Mr. Mayau John Kirimi who is a Postgraduate Student of this University. He is registered for M.Sc. degree programme in the Department of Chemistry.

Mr. Mayau intends to conduct research for a M.Sc. thesis Proposal entitled, "Antioxidant Activity, Total Phenolic and Flavonoid Content Phytochemical Screening and Antibacterial Activity of Extracts of *Aloiampelos ciliaries*."

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

## Appendix VII: Referred publication

Vol. 18(7), pp. 107-117, September, 2024
DOI: 10.5897/JMPR2024.7351
Article Number: D03751972610
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS

Journal of Medicinal Plants Research

*Full Length Research Paper*

# Antioxidant activity, total phenolic and flavonoid contents, phytochemical screening and antibacterial activity of extracts of *Aloiampelos ciliaris*

**John Kirimi Mayau[1], Wilson Njue[1], Henry Mwangi[1]* and John Maingi[2]**

[1]Department of Chemistry, Kenyatta University, P. O. Box 43844-0100, Nairobi, Kenya.
[2]Department of Biochemistry, Biotechnology & Microbiology, Kenyatta University, P. O. Box 43844-0100, Nairobi, Kenya.

Received 25 May, 2024; Accepted 4 July, 2024

**Research on medicinal plants has extensively explored their antibacterial, antifungal, and antioxidant properties due to rising bacterial antibiotic resistance. Aloe species have traditionally been used for their therapeutic benefits. This study focused on *Aloiampelos ciliaris*, conducting phytochemical screening and evaluating its antioxidant and antibacterial activities, along with total phenolic and flavonoid content. Samples from Meru County, Kenya, were extracted using 80% methanol and partitioned with solvents of increasing polarity. The DPPH assay measured antioxidant activity, ranging from 15.67% ($IC_{50}$ > 0.0300) to 74.74% ($IC_{50}$ > 0.0300). Total phenolic content, expressed as Gallic acid equivalent (GAE), varied from 105.89 ± 0.07 mg GAE/g in flower crude extract to 1.46 ± 0.04 mg GAE/g in flower DCM extract. Total flavonoid content, expressed as quercetin equivalent (QE), ranged from 182.69 ± 1.64 mg QE/g in root crude extract to 3.59 ± 0.41 mg QE/g in flower n-hexane extract. Antibacterial tests against Gram-positive and Gram-negative bacteria showed inhibition zones, with *Escherichia coli* in EtOAc exhibiting a 112.0 ± 0.500 mm zone of inhibition and *Bacilus subtilis* with a 7.0±0.000 zone. Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, terpenoids, cardiac glycosides, phenols, and saponins. GC-MS detected compounds such as methyl-cyclohexane and dodecane. The bioactive compounds in *A. ciliaris* highlight its potential as a valuable natural resource for treating various diseases.**

**Key words**: *Aloiampelos ciliaries*, extracts, Antibacterial, antioxidant, phytochemical screening.

## INTRODUCTION

Medicinal plants, with their valuable bioactive compounds, have been valued for centuries as safer alternatives to conventional antibiotics (Gershenzon and Ullah, 2002). Over 80% of the world's population, particularly in developing countries, relies on plant-based medicine for primary health needs due to their minimal side effects, effectiveness, and affordability compared to synthetic drugs (Bodeker et al., 2005; Olaokun et al., 2017). Additionally, these plants contain phytochemicals that help manage human ailments (Wadood, 2013). Natural

*Corresponding author. E-mail: henry.mwangi@ku.ac.ke. Tel: +254722484282.

## Appendix VIII: Plant Identification

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

**12 November 2024**

REF: NMK/BOT/CTX/2/ID/19/2024

Mayau John Kirimi,

P.O. Box 43844 - 00100
Nairobi.
Mobile No.-0703987557.

Dear Mr. Mayau,

**PLANT IDENTIFICATION**

The plant specimen which you brought to EA Herbarium on 11th November 2024 for identification was determined as follows;

**MAYAU JK-01**

**Scientific name:** *Aloiampelos ciliaris* (Haw.) Klopper & Gideon F.Sm. in Asphodelaceae family

**Common name**: Climbing Aloe

Thank you for consulting the Herbarium for your plant identification and confirmation

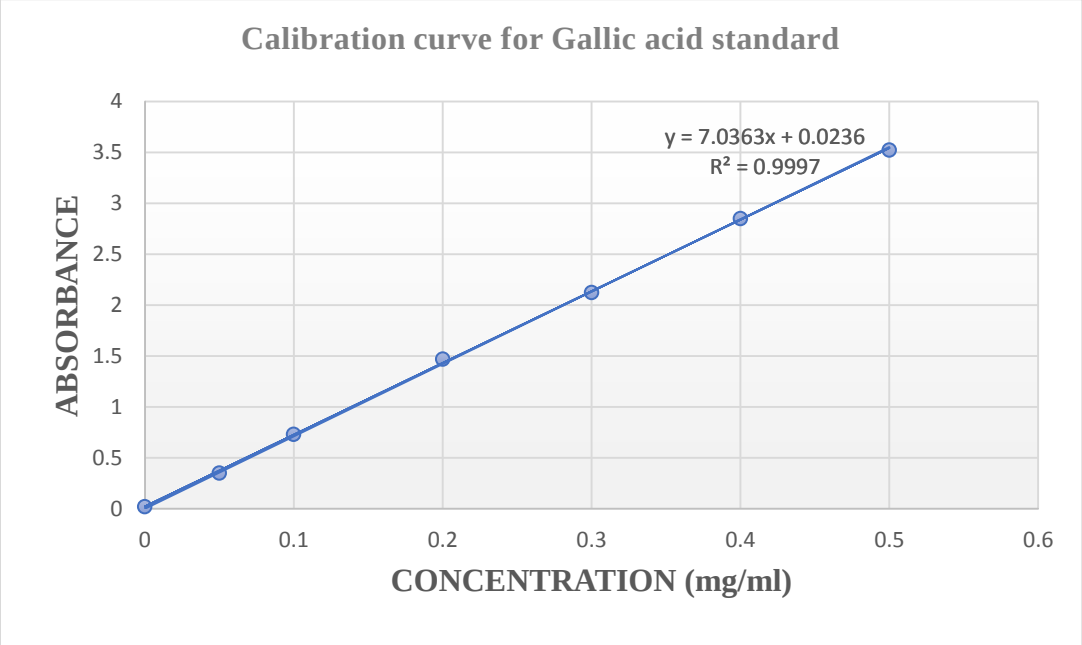
Yours Sincerely

NATIONAL MUSEUMS OF KENYA
12 NOV 2024
HERBARIUM NAIROBI

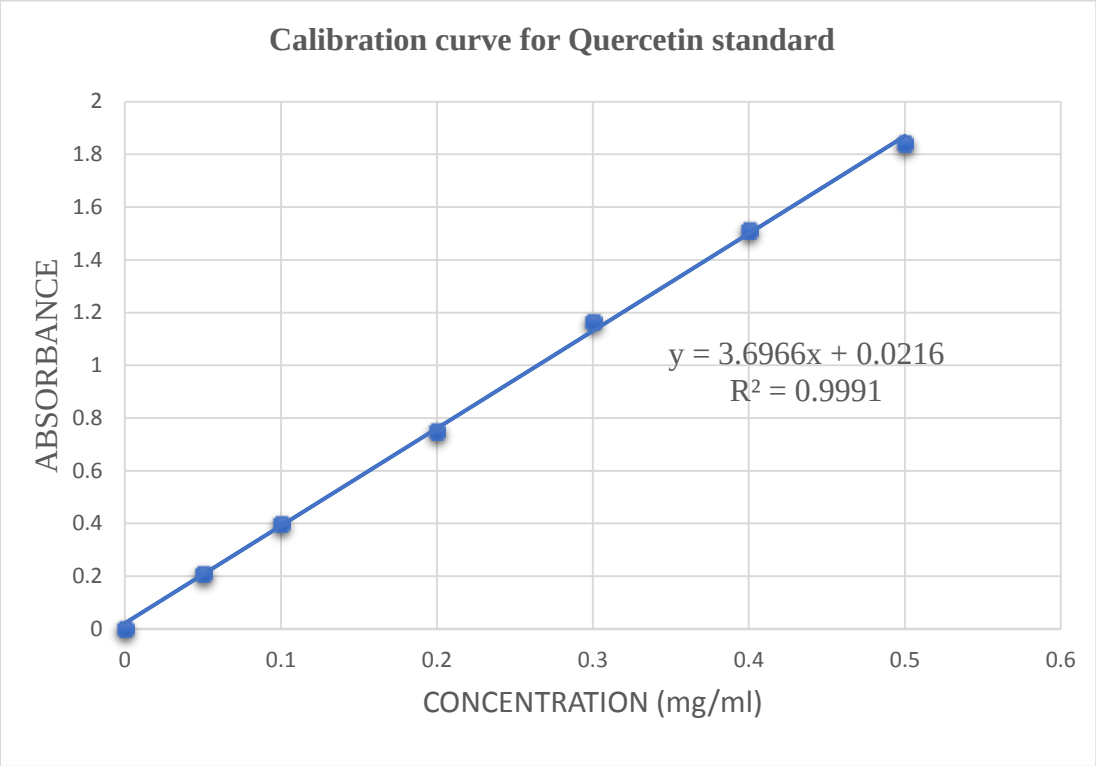
Nancy Kwamboka Maoga
**For: Head, Botany Department**

Address P.O. Box 40658 00100 GPO Nairobi Telephone +254 20 4448930/3. +254 20 3742161/4. +254 20 3742131/4. Fax +254 20 3741424. Website www.museums.or.ke

**Appendix IX: Calibration curves for Gallic acid standard**



**Appendix X: Calibration curves for Quercetin standard**




**Appendix XI: Figure 4.2: The GC-MS profile spectra of the DCM leaf fraction**