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Phytochemical Investigation of Bioactive Components from the Stem Bark of *Syzygium guineense* for Antibacterial and Antioxidant Conducts

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Abstract

Plants provide numerous benefits, like medicine, food, shelter, clothing, fuel wood, and building materials. They contribute to soil fertility, recycle ecosystem nutrients, and protect water catchment regions. Phytochemicals produced by plants provide health benefits beyond macronutrients and micronutrients. Primary metabolites contain natural sugars, amino acids, proteins, and purines, while secondary metabolites protect plants from environmental hazards and diseases. Reports reveal metabolites from *S. guineense* organs, including isoprenoids, arjulonic acids, and asiatic acids. This study aimed to investigate different bioactive constituents' different extraction methods using ethanol as solvent, namely: reflux extraction, ultrasonication extraction, and maceration extraction, using high-resolution UPLC-MS techniques. The stem bark extract of *S. guineense* showed promising activity against *S. aureus* and *E. coli*, but as time intervals increased, the bacterial strain became more resistant. The extracts also showed DPPH radical scavenging action, with R values of 0.9972, indicating their potential as natural medicinal compounds for antioxidant and antibacterial disease management.

Keywords: Phytochemical; Bioactive component; *S. guineense*; Antibacterial; Antioxidant

Introduction

Plants are essential to practically all life on earth and are of great value. They offer a variety of benefits to people, including medicine, food, shelter, clothes, fuel wood for cooking, building materials, cutlery, and cattle feed. In addition to developing soils and preserving soil fertility, they recycle vital ecosystem nutrients and safeguard water catchment regions [1]. Over a long period of time, humans have explored many natural resources looking for remedies of various ailments. Traditional medicines have played an intrinsic role in human life for thousands of years, with people depending on medicinal plants and their products as dietary supplements as well as using them therapeutically for treatment of chronic disorders, such as cancer, malaria, diabetes, arthritis, inflammation, and liver and cardiac disorders [2]. In recent years, herbal prescriptions have received considerable attention as an alternative way to compensate for perceived deficiencies in orthodox pharmacotherapy worldwide. Despite a lack of medical evidence to support their therapeutic efficacy and toxicological effects, the use of herbal medicine has increased considerably. According to World Health Organization (WHO), up to 80% of the world's population in underdeveloped and developing countries relies on traditional medicine practices for their primary health care needs [3]. Natural products are resources derived from living organisms, such as plants, animals and microorganisms. The chemicals produced by plants may be defined as "phytochemicals" [2,4]. Phytochemicals in plants have undoubtedly been a resource of medicinal treatment for human diseases for a long time. They played a key role in primary health care of nearly 75–80% of the world's population according to the World Health Organization [5].

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [6]. Phytochemicals in a plant can be explored by using various methods such as extraction, separation, purification, identification, and structure elucidation, determination of physical and chemical properties, biosynthesis and quantification. The phytochemicals could be classified as primary and secondary metabolites. Primary metabolites involved natural sugars, amino acids, proteins, purines and pyrimidines of nucleic acids and chlorophyll. Secondary metabolites are the remaining plant chemicals such as glycosides, alkaloids, terpenoids, flavonoids, lignans, steroids, curcumines, saponins and phenolic and others [6]. The secondary metabolites are primary for plants to protect themselves from environmental hazards such as pollution, UV exposure; stress, drought and pathogenic attack, as well as researchers have reported that phytochemicals can protect them from human diseases [3,6]. The secondary metabolites have biological properties such as antioxidant activity, anticancer property, antimicrobial effect, anti-inflammatory and stimulant to the immune system [7].

Literature review

Additionally, there are reports that demonstrate the identification and isolation of metabolites from several *S. guineense* organs. Oladosu reported isolation of 3-- hydroxylupane-type isoprenoids: betulinic acid methylenediol ester (1) and betulinic acid (2) from the chloroform extract of stem bark of Syzygium guineense [8]. Isolation of 2- hydroxyoleanolic acid (3), 2-hydroxyursolic acid (4), arjulonic acid (5), asiatic acid (6), terminolic acid (7), 6- hydroxy asiatic acid (8), arjulonic acid 28-β-glycopyranosyl ester (9) and asiatic acid 28-β-glycopyranosyl ester (10) were reported from the leaves of Syzygium guineense [9]. Abok and Manulu reported detection of twelve compounds from n-hexane extract of leaves of Syzygium guineense using TLC and GC-MS analyses [10]. Some of the compounds were 1-ethyl-2-methylbenzene (11), Ylangene (12), decahydro-4a-methyl-

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1-methylene-7- (1-methylethynyl) - naphthalene (γ-muurolone) (13), 4-dimethyl-7- (1- methylethenyl) azulene (14) and caryophyllene oxide (15) (Abok & Manulu, 2016) (Figure 1) [11].

Some phytochemical constituents from Syzygium genus

Phloretin (1), myrigalone-G (2), myrigalone B (3) [12], 2′,4′-dihydroxy-6′-methoxy-3′-methyldihydrochalcone (4), 2′-hydroxy-4′,6′-dimethoxy-3′-methyldihydrochalcone (5), 2′,4′-dihydroxy-6′-methoxy3′,5′-dimethyldihydrochalcone (6) [13], 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone or stercurensin (7), 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (8) [13], 2′,4′-dihydroxy-6′-methoxy-3′,5′dimethylchalcone (9) [14], 2′,4′-dihydroxy-3′,5′-dimethyl-6′-methoxychalcone (10), 2′,4′-dihydroxy-6′-methoxchalcone or cardamonin (11) [15], pinocembrin (12), (−)-strobopinin (13), 8-methylpinocembrin (14), demethoxymatteutcinol (15), 7-hydroxy-5-methoxy-6,8-dimethylfoavanone (16) [16], 7,8,3′,4′-tetrahydroxy-3,5-dimethoxyflavone (17) [17], 7-hydroxy-5-methoxy-6,8-dimethylflavanone (18), quercetin (19) [18], kaempferol (20) [19], gallocatechin (21), myricetin (22) [15], (−)-epigallocatechin (23), (−)-epigallocatechin 3-Ogallate (24), samarangenin A (25), samarangenin B (26), prodelphinidin B-2 3″-O-gallate (27) and prodelphinidin B-23,3″-O-gallate (28) [20] are presented in (Figure 2).

Determination of antibacterial activity

The three main areas of concern in the current situation are illness prevention, protection, and therapy. One of them is microbial infection because of the development of multi-resistant bacteria in therapeutic trials. Additionally, researchers are working to find ways to treat these illnesses without harming the body in any way [21]. The WHO also suggested using plant-based treatments to treat certain illnesses. The *Syzygium* species is a member of the Myrtaceae family, which has a wide range of pharmacological, antioxidant, and phytochemical activities. To evaluate its effectiveness against some bacterial strains linked to diarrheal disorders, Tsakala studied the antibacterial activity of *S. guineense* extract [22]. Significant action against gram-positive and gram-negative bacteria as well as fungi has been observed in studies on *S. cumini* and *S. jambos* [23]. Jamun showed a promising antibacterial activity against *Klebsiella* pneumonia, according to

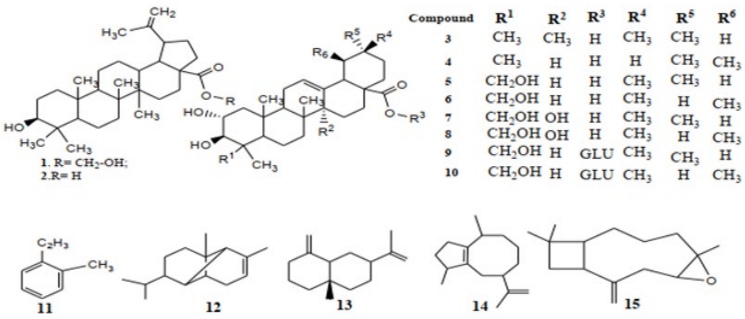


Figure 1: The chemical structures of compounds isolated/detected from *S. guineense.*

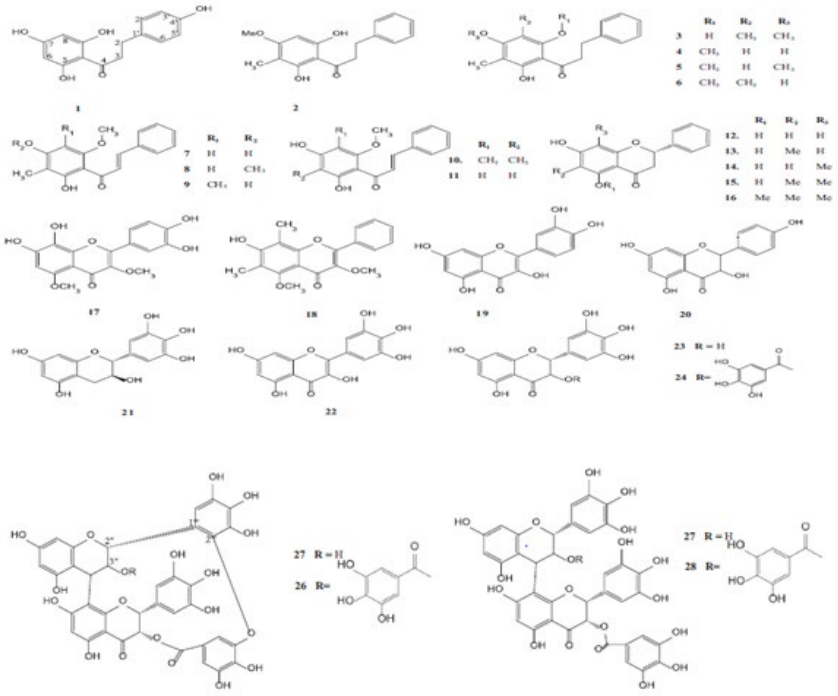


Figure 2: Compounds from various parts of *S. guineense, S. aqueum, S. samarangense, S. aromaticum,* and *S. cumini.*

[24]. Monoterpene aldehydes are suggested to be the cause of the photoactivity seen in *S. cumini* [25]. According to Ahmad and Beg, gram-positive bacteria are thought to be more sensitive than gram-negative bacteria due of variations in the architecture of their cell walls [26]. *S. cumini* has been shown to have antibacterial activity by [27], who also noted the presence of wide zones of inhibition against *S. aureus* and *B. subtilis* [28]. The antibacterial properties of the leaf essential oils of *S. cumini* and *S. travancoricum* were reported [29]. *S. caryophyllatum* is a Myrtaceae species that is in danger of extinction and has a high concentration of phytochemicals with antioxidant and hypoglycaemic properties. For this species, there is currently a lack of information about its antibacterial activity. Therefore, the focus of the current investigation was on the plant extract's antibacterial efficacy against gram positive and gram negative microorganisms [21].

Determination of antioxidant activity

Free radical scavenging activity by 1, 1-diphenyl-2- picrylhydrazyl (DPPH): The build-up of free radicals is linked to numerous human diseases. Free radicals can be neutralized by antioxidants, reducing their negative effects. Therefore, it is crucial to look for naturally occurring antioxidants with plant origin [30].

There is compelling evidence linking the build-up of free radicals to a number of harmful pathophysiological processes, including cancer, diabetes, cardiovascular, and neurodegenerative illnesses [31,32]. An unstable atom or molecule with an unpaired electron is known as a free radical. In healthy human cells, this unstable radical has a propensity to bond with biological macromolecules including proteins, lipids, and DNA to become stable, resulting in damage to the DNA and proteins [31]. Due to decreased cellular antioxidant safeguards, such radical-induced cell damage may spread more widely. Antioxidant protective mechanisms that eliminate damaged molecules are built into all biological systems; however they are not always effective. Antioxidants must therefore be consumed through diet in order to shield cells from free radical damage. Antioxidants are compounds that protect and stabilise the cellular damage brought on by free radicals by giving electrons to the harmed cells. Additionally, antioxidants convert free radicals into waste by-products that the body excretes. Fruits and vegetables high in antioxidants are known to reduce the risk of numerous diseases brought on by free radicals [4,33,30]. The loss of DPPH absorbance at 515 nm was continuously monitored for 10 minutes in order to determine the kinetics and stoichiometry of reactions involving the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) stable radical and 25 antioxidant compounds with various structures, molecular weights, numbers of OH groups, and redox potential. The linear response ranges and reaction saturation points were investigated for a variety of antioxidant concentrations [34].

The scavenging activity percentage (RSA %) was determined according to the following equation:

$$RSA\% = \frac{Ac - As}{Ac} * 100$$

Where, Ac = Absorbance of control and As = Absorbance of sample

Materials and Methods

Plant materials

Direct interviews with the native healers provided information about the medicinal plant. In December 2021, Misha Wereda shero kebele, Hadiya Zone, South Nation Nationalities Peoples' Region (SNNPR), Ethiopia was to be the place where the stem bark of *S. guineense* would be collected. The region is around 320 kilo meters from Ethiopia's capital city of Addis Ababa. Botany experts at the Department of Biological Sciences identify plants there. The stored voucher specimen was given both scientific and common names. In order to prevent direct sunlight from degrading some of the chemicals in the samples, the freshly cut stem bark was washed, chopped into small pieces, and allowed to air dry within the research lab. It is then dispersed and frequently turned over to prevent fermentation and degradation. To get it to dry, this was done for nearly four weeks. Using an electric grinder, the dried material was crushed into a fine powder. The powder was measured, placed in sample bags with labels on them, and kept at room temperature.

Extraction

The plant material was extracted with three different extraction methods using ethanol as solvent, namely: (a) Reflux extraction, using 10g of powdered stem bark of *S. guineense* socked with 80 mL of ethanol for 2 hours. (b) Ultra-sonication extraction, using 10g of powdered stem bark of *S. guineense* socked with 80 mL of ethanol for 1 hour. (c) Maceration extraction used 33mg of powdered stem bark of *S. guineense* socked with 2 mL of ethanol for six days with manual shacking. Then, both reflux and ultrasonication extracts were filtered by using filter paper, while maceration extract first centrifuged for 10 minutes at 8000 rpm and filtered by using syringe. Finally, all of them were filtered with a 0.22µm syringe (Simple Pure, Nylon filter, Membrane Solutions) into the LCMS analysing vials (1.5mL) containing 1mL of each extract.

UPLC-ESI-Q-TOF/MS analysis

High-resolution UPLC-MS analysis was performed using a Waters Xevo G2-S- Quadrupole time-of-flight (Q-TOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA). The following MS settings were applied in the positive mode: cone voltage of 30V, cone gas flow at 50 L/h, desolvation gas flow at 1000 L/h, and desolvation temperature of 450°C. Data were acquired by scanning from 50 to 1200 m/ z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, firstly at low collision energy (4 V) and secondly using collision energy ramp (30-60V) to obtain fragmentation data. Leucine enkaphalin (C28H37N5O7 (m/z 556.2766 +ve)) was used as the reference mass for accurate mass determination, and the instrument was calibrated with sodium format. Separation was analysed on an Acquity BEH C18 column (2.1×100mm, 1.7µm particle size) fitted with a PDA and Xevo G2-S-QToF (QTOF) MS mass spectrometer (Waters, Milford, Ireland). The solvent system consisted of 16mL of acetonitrile (ACN) (Solvent B), and 4mL of water (Milli-Q waters) (Solvent A), 200µL of 10% formic acid and 100µL of 0.1mol/L NaOH (Table 1).

Result and Discussion

Maceration extraction

In this study three different extraction methods were used firstly, maceration extraction and it was afforded two different compounds as shown in the following (Table 2). The chromatograph of each compound was listed in the following (Figure 3) (Diagram 1 and Diagram 2).

Reflux extraction

Secondly, Reflux extraction method, this was afforded totally six bioactive chemical constituents these are mentioned below in the

Table 1: Show the gradients used.

S. No.	Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
1	0.00	0.300	98.0	2.0	Initial
2	5.00	0.300	95.0	5.0	6
3	7.00	0.300	80.0	20.0	6
4	8.50	0.300	70.0	30.0	6
5	9.00	0.300	20.0	80.0	6
6	9.50	0.300	98.0	2.0	6
7	10.50	0.300	98.0	2.0	6

Table 2: Shows identified compound from the stem bark of *S. guineense* by maceration method.

S. No.	Components	Observed m/z	Observed RT (min)	Adduct Ions
1	4-[(1,3-Dioxo-1,3-dihydro-2-benzofuran-5-yl)ethynyl]-1H-pyrazole-5-carbonitrile	301.9969	8.22	+K
2	(4R)-1-{[(3R,4R,5R)-4,5-Dihydroxy-3-(hydroxymethyl)tetrahydro-1(2H)-pyridazinyl]acetyl}-4-hydroxy-L-prolyl-(4R)-4-hydroxy-L-prolyl-L-phenylalaninamide	579.2778	9.73	+H

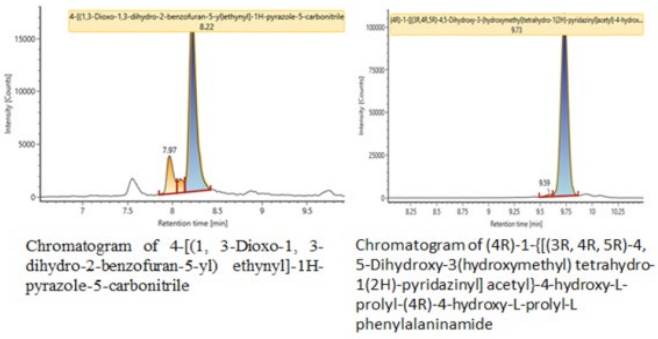


Figure 3: The chromatograph of each compound..

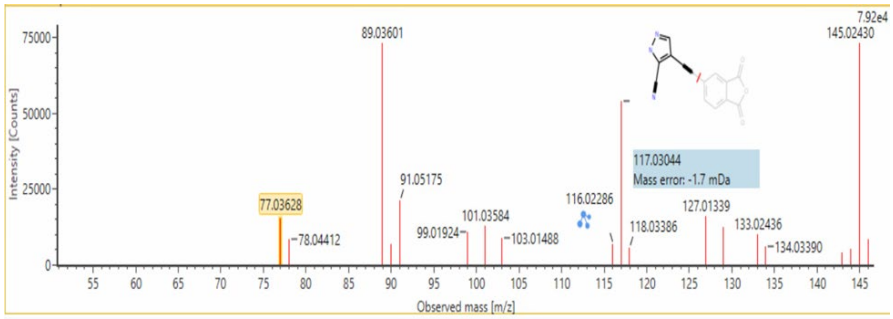


Diagram 1: Mass spectrum of 4-[(1, 3-Dioxo-1, 3-dihydro-2-benzofuran-5-yl) ethynyl]-1H-pyrazole-5-carbonitrile.

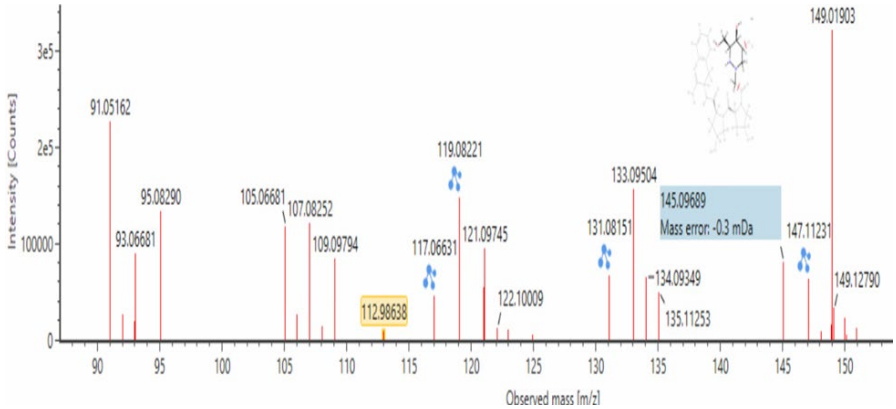


Diagram 2: Mass spectrum of (4R)-1-{[(3R, 4R, 5R)-4, 5-Dihydroxy-3(hydroxymethyl) tetrahydro-1(2H)-pyridazinyl] acetyl}-4-hydroxy-L-prolyl-(4R)-4-hydroxy-L-prolyl-L phenylalaninamide.

(Table 3). The chromatograph of each compound was listed in the following (Figure 4) (Diagram 3, Diagram 4, Diagram 5, Diagram 6, Diagram 7 and Digaram 8).

Sonication extraction

The third extraction was sonication method this also yielded five bioactive chemical compounds these are listed in the following (Table 4). The chromatograph of each constituent was displayed in following (Figure 5) (Diagram 9, Diagram 10 and Diagram 11) (Table 5).

Conducting antibacterial activity test

The antibacterial activity of both Gram-positive and Gram-negative bacterial strains, *S. aureus* and *E. coli* was analysed (Chart 1 and Chart 2) respectively.

Table 3: Shows those bioactive chemical constituents extracted by reflux method.

S. No.	Components	Observed m/z	Observed RT (min)	Adduct Ions
1	Conessine	374.3514	9.77	$+NH_4$
2	Swainsonine	191.1376	9.77	$+NH_4$
3	Brefeldin A	303.1555	9.77	+Na
4	Lobeline	355.2379	9.93	$+NH_4$
5	Vinpocetin	351.2053	9.93	+H
6	Caffeine	212.1125	8.21	$+NH_4$, +H

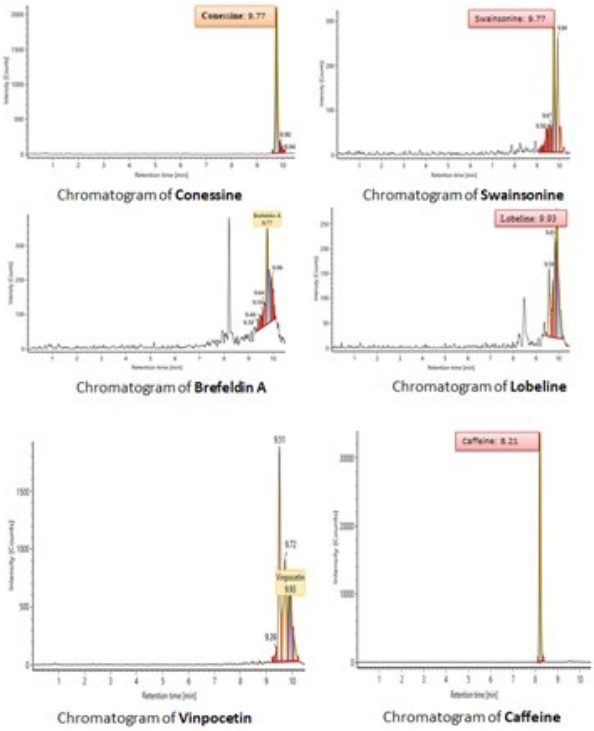


Figure 4: The chromatograph of each compound.

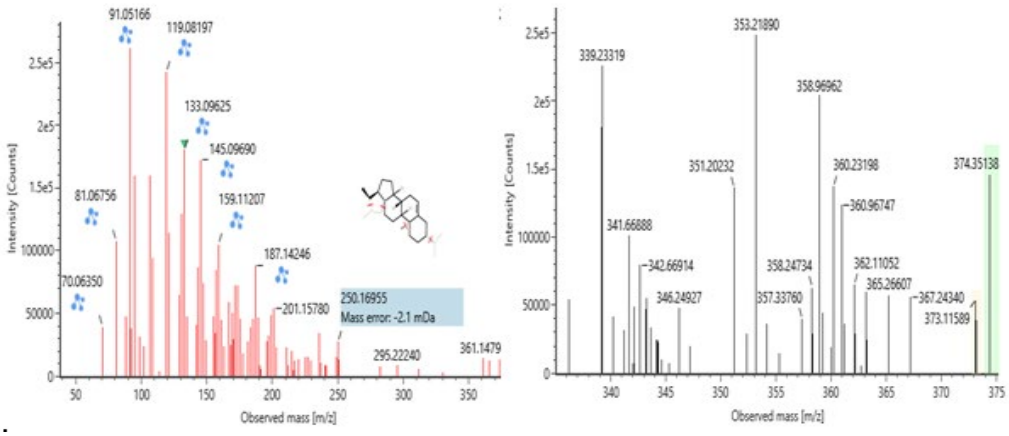


Diagram 3: Mass spectrum of Conessine.

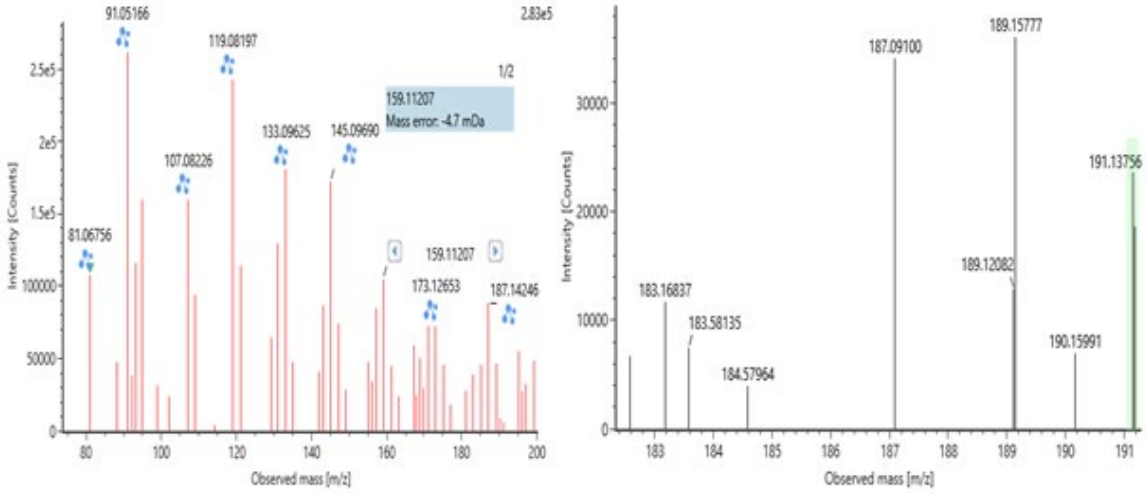


Diagram 4: Mass spectrum of Swainsonine.

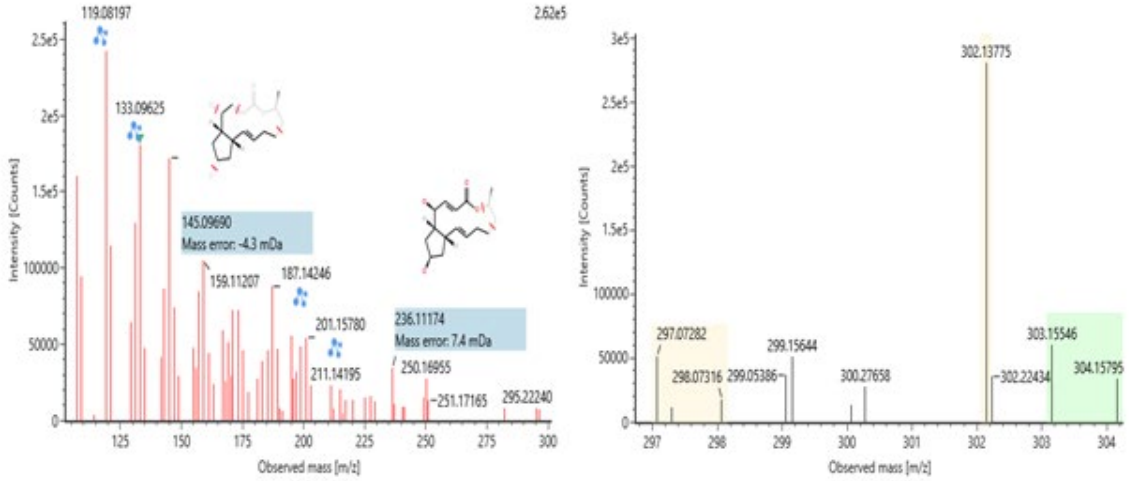


Diagram 5: Mass spectrum of Brefeldin A.

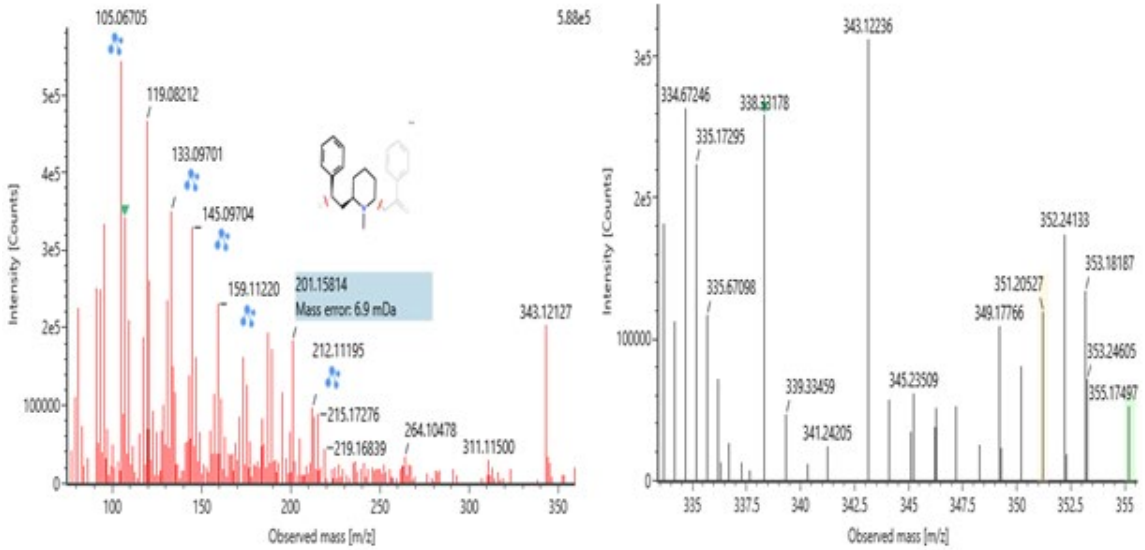


Diagram 6: Mass spectrum of Lobeline.

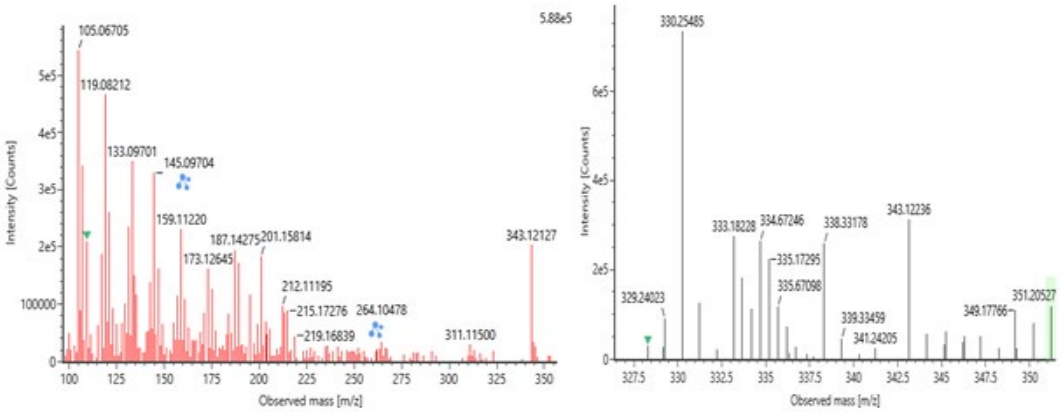


Diagram 7: Mass spectrum of Vinpocetin.

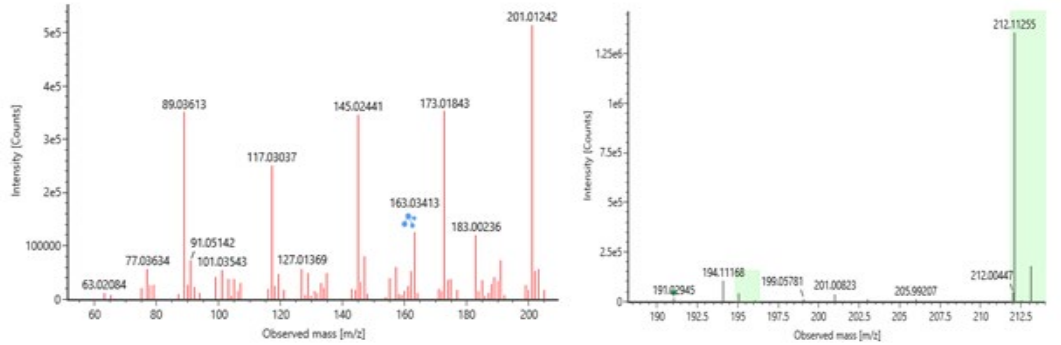


Diagram 8: Mass spectrum of Caffeine.

Table 4: Show that bioactive chemical constituents extracted by sonication method.

S. No.	Components	Observed m/z	Observed RT (min)	Adduct Ions
1	Lagochiline	379.2423	9.93	+Na
2	Degeulin	412.1779	9.77	+NH4
3	Bavachinin A	361.1403	8.22	+Na
4	Coumermycin A1	1127.4132	3.84	+NH4
5	Nonactin	759.4335	9.93	+Na

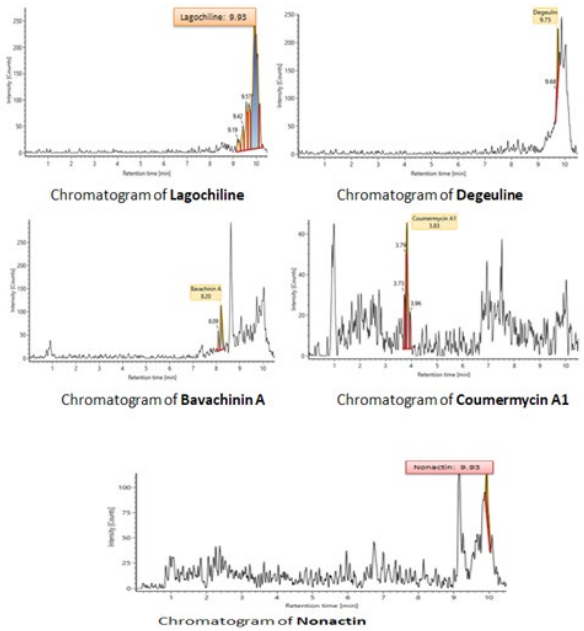


Figure 5: The chromatograph of each constituent.

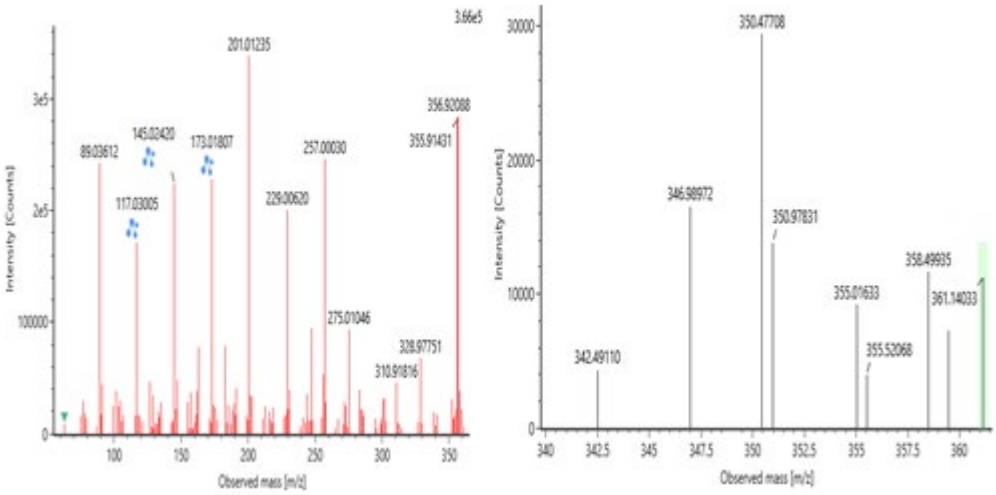


Diagram 9: Mass spectrum of Bavachinin A.

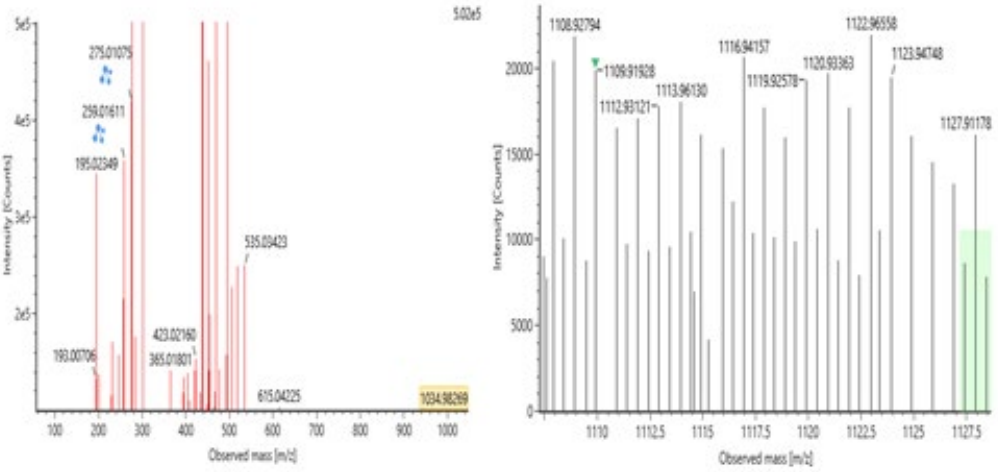


Diagram 10: Mass spectrum of Coumermycin A1.

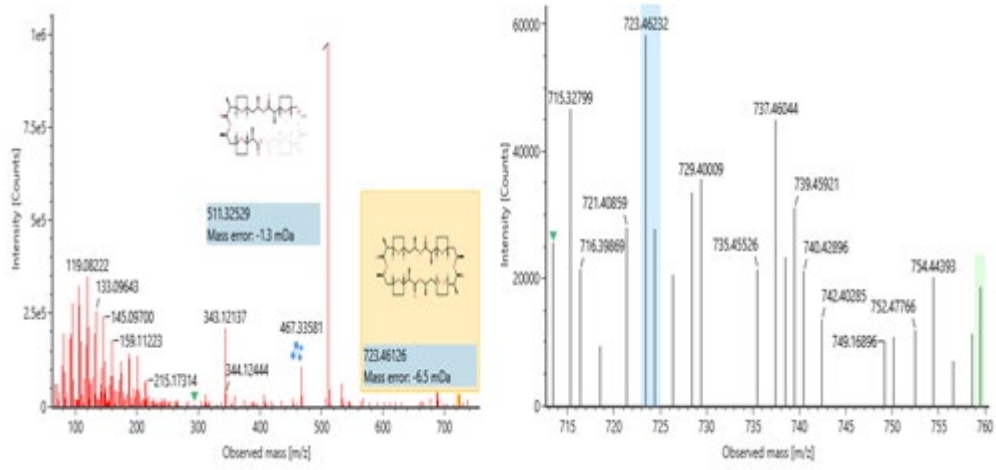
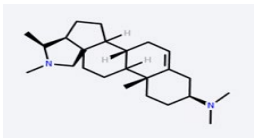
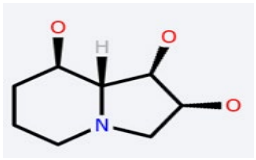
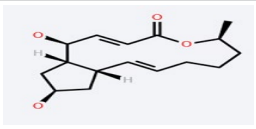
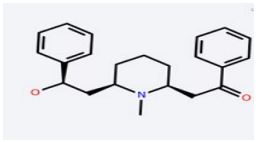
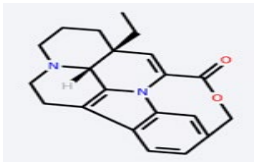
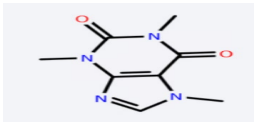
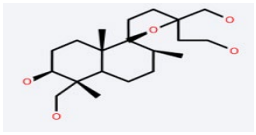
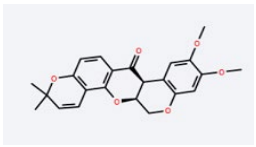
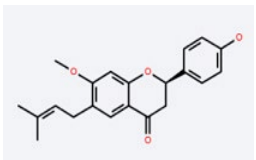
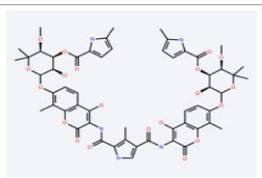
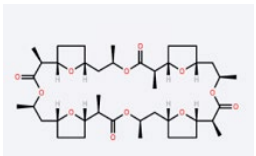


Diagram 11: Mass spectrum of Nonactin.

Table 5: Shows their representative structure with their bioactivities reference.

S. No.	Components Name	Structure	Molecular Formula	Reference for Bioactivities
1	Conessine		$C_{24}H_{40}N_2$	[35,36]
2	Swainsonine		$C_8H_{15}NO_3$	[37]
3	Brefeldin		$C_{16}H_{24}O_4$	[38]
4	Lobeline		$C_{22}H_{27}NO_2$	[39]
5	Vinpocetin		$C_{22}H_{26}N_2O_2$	[40]
6	Caffeine		$C_8H_{10}N_4O_2$	[41]
7	Lagochiline		$C_{20}H_{36}O_5$	[42]
8	Degeulin		$C_{23}H_{22}O_6$	[43]
9	Bavachinin A		$C_{21}H_{22}O_4$	[44]
10	Coumermycin A1		$C_{55}H_{59}N_5O_{20}$	[45]
11	Nonactin		$C_{40}H_{64}O_{12}$	[46]

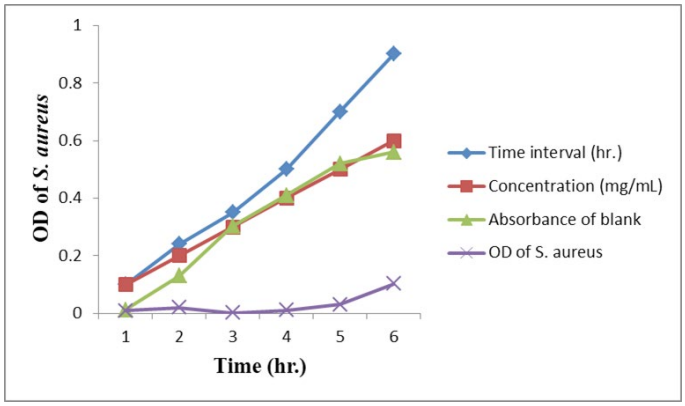


Chart 1: Demonstrate the antibacterial activity of stem bark extract of *S. guineense* for *S. aureus*.

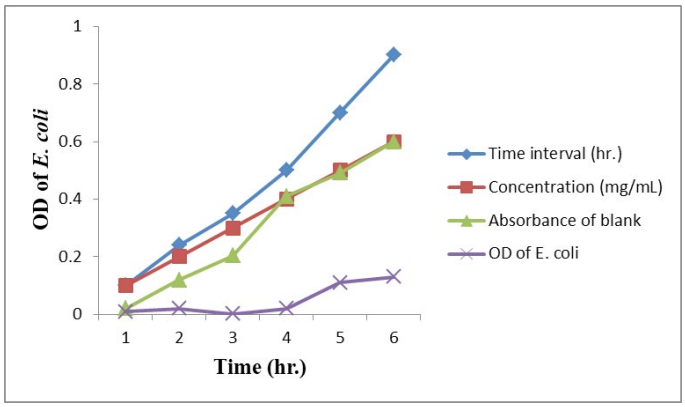


Chart 2: Demonstrate the antibacterial activity of stem bark extract of *S. guineense* for *E. coli*.

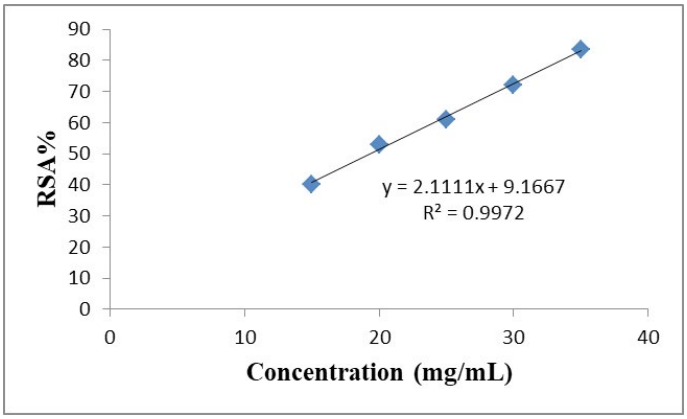


Chart 3: Show the antioxidant activity of stem bark extract of *S. guineense*.

Analysing antioxidant activity (Table 6) (Chart 3)

Conclusion

At the beginning time intervals, the stem bark extract of *S. guineense* demonstrated encouraging activity against *S. aureus* and *E. coli*. However, as the time interval increased, the bacterial strain became more resistant, with the selectively negative strain becoming more resistant than the positive strain. Additionally, the extracts demonstrated encouraging results for DPPH radical scavenging action at different doses, specifically as absorbance declined and concentration

Table 6: Illustrates the antioxidant activity of stem bark extract of *S. guineense*.

S. No	Concentration (mg/mL)	Absorbance of Control	Absorbance of Sample	RSA%
1	15	0.72	0.43	40.28
2	20	0.72	0.34	52.78
3	25	0.72	0.28	61.11
4	30	0.72	0.2	72.22
5	35	0.72	0.12	83.33

increased. With R values of 0.9972, which is extremely close to one, these confirmed that *S. guineense* had exceptional results at minimal absorbance, suggesting their ability to scavenge free radicals. These results showed that stem bark from *S. guineense* may contain natural medicinal compounds useful in the management of antioxidant and antibacterial diseases. Because *S. guineense's* stem bark contains a variety of phytochemical components, Consequently, *S. guineense*, which demonstrates the plant's value as a medicinal agent and is used to treat a variety of illnesses.

Authors Contribution Statement

This study was carried out in cooperation among all authors. Author Teshale Ayano Begeno designed the study, collected the data, investigated and implemented the statistical analysis, wrote the etiquette and wrote the first draft of the manuscript. Authors Haoyue An and Jehangir Khan, rewriting, reviewing and editing. Authors Zhen-Xia Du, and Haiyue Hou, managed the analyses of the study, interpretation of the data and critical revisions and supervisions of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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