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DEPARTMENT OF CHEMISTRY

MSc THESIS

ON

PHYTOCHEMICAL INVESTIGATION, ANTIMICROBIAL AND HEPATOPROTECTIVE PROPERTIES OF THE ROOT OF *Asparagus africanus* Lam.

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PHYTOCHEMICAL INVESTIGATION, ANTIMICROBIAL AND HEPATOPROTECTIVE PROPERTIES OF THE ROOT OF *Asparagus africanus* Lam.

A THESIS SUBMITTED TO THE SCHOOL OF GRADUTE STUDIES, JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (ORGANIC)

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Declaration!

I declare that the thesis titled 'Phytochemical investigation, antimicrobial and hepatoprotective properties of the root extract of *Asparagus africanus* Lam. is original and my blueprint. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and list of references is given. The research is accepted for the fulfillment of the requirement for the degree of Master of Science in Chemistry (Organic).

Acknowledgement

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List of abbreviations and acronyms

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate transaminase
ATCC	American Type Culture Collection
^{13}C-NMR	Carbon Thirteen Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
DILI	Drug induced liver injury
1D-NMR	One-Dimensional Nuclear Magnetic Resonance
DMSO	Dimethyl Sulfoxide
^{1}H-NMR	Proton Nuclear Magnetic Resonance
IC_{50}	50% Inhibitory Concentration
INH	Isoniazid
MHA	Muller Hinton Agar
MHz	Mega Hertz
MIC	Minimum Inhibitory Concentration
NMR	Nuclear Magnetic Resonance
NIH	National Institute of Health
RIF	Rifampicin
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
WHO	World Health Organization

Abstract

The emergency of resistance pathogens limits the therapeutic uses of many of the drugs that are in the market and undesirable side effects associated to certain antibiotics, thus this study was aimed to search alternative drugs for antimicrobial agent and liver disease from root extracts of *Asparagus africanus*. The air dried plant materials were ground and extracted sequentially with petroleum ether, chloroform, acetone and methanol by maceration technique at room temperature to give crude extracts of 4 g (0.4%), 13 g (1.3%), 8 g (0.8%) and 50 g (5%), respectively. The phytochemical screening of the extracts revealed the presence of various secondary metabolites such as saponin, terpenoids, flavonoid, tannin and steroids. Two separate columns were prepared for the isolation of compounds, first for the methanol extract (20 g) and second one is for the combined extracts of chloroform (10 g) and acetone (6 g) leading to the isolation of six compounds (**AA-1**, **AA-2**, **AA-3**, **AA-4**, **AA-5**, and **AA-6**) and further purified by gel filtration with Sephadex LH-20. Only two compounds **AA-5** and **AA-6** were characterized using ^{1}H and ^{13}C NMR, named as stigmasterol and nyasol, respectively. The crude extracts and isolated compounds were evaluated for *in vitro* antimicrobial activities using disk diffusion assay and the methanolic extract were also evaluated for *in vivo* hepatoprotective effects on twenty five male Swiss albino mice for 28 days by inducing isoniazide and rifampicin hepatotoxicity. The antimicrobial activity of methanol and acetone extract showed the marginal zone of inhibition against two bacterial strains *E. coli* (12.5±0.7, 12.5±0.9) mm, *B. subtilus* (11.8±0.8, 11.8±0.8) mm and one fungal strain *C. albicans* (12.5 ±1.0, 11.8±0.7) mm, respectively compared to chloroform and petroleum ether extracts. Compounds **AA-5** (stigmasterol) and **AA-6** (nyasol) were showed a marginal zone of inhibition against two bacterial strains (*E.coli*, 8.0, 8.0) mm, (*S. typhi*; 9.0, 8.0) mm and one fungal strains (*C.albicanus*; 10.0, 8.0) mm, respectively. The *in vivo* hepatoprotective study showed the elevation of the serum alanine aminotransferase, aspartate transaminase, alkaline phosphatase, total bilirubin level of toxicant drug induced group and decrease the serum albumin compared to normal control group, but both the treatment (200 and 400 mg/kg) groups were significantly maintained the level of serum liver biomarkers to the normal with dose dependent manner $P<0.05$.

***Key words**: - Asparagus africanus, stigmasterol, Nyasol, Antimicrobial, Hepatoprotective*.

1. Introduction

1.1. Background of the study

Medicinal plants are the major remedy in traditional system of medicine for thousand years and have made a great contribution to maintain human health in many parts of the world, especially in rural areas of developing countries as a primary source[1]. The use of medicinal plants is almost as old as the history of mankind. Historical evidence showed that medicinal plants were traditionally used as early as 5000 to 4000 B.C. in China and 1600 B.C. by Syrians, Babylonians, Hebrews and Egyptians[2]. Ethiopia also has a long history of conventional medicines and has found ways of using them to fight diseases. In fact, up to 80% of the population in Ethiopia uses traditional medicine because of the cultural acceptability of the healers and local pharmacopoeias, the relatively low cost of traditional medicine, and the difficulty of accessing modern health care facilities[3]. Overall, this highlights the need to focus on medicinal plants for their reliability and affordability of traditional medicine used into local level[4]. Millions of plant species are considered to have medicinal value and are used to treat various diseases worldwide[3, 4]. However, the traditional medical systems are characterized by variations in ecological difference of the countries, biodiversity, socio-cultural backgrounds of different ethnic groups and historical developments that related to migration, introduction of foreign culture and religion[5]. Most of the noble medicinal products used in modern medicinal products are made indirectly from medicinal plants, with about 90% of the raw materials coming from wild sources. Medicinal plants have been used as conventional treatments for infectious diseases for thousands of years in many parts of the world as well[6].

Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses or fungi and have impacted both developed and developing countries. Infectious diseases are the number one cause of death globally, accounting for more than 50% of deaths in tropical countries, according to the WHO[7]. The control of microbial infection has been remarkably effective since the discovery of antimicrobial drugs[8]. However, the emergency of resistance pathogens limits the therapeutic uses of many of the drugs that are in the market and undesirable side effects associated to certain antibiotics have led to the search of new antimicrobial agents from medicinal plants[9].

Nowadays, owing to the widespread use of drugs due to the treatment of different diseases and the rapid research of new drugs, the various types of clinical drugs have gradually increased and the incidence of DILI has increased year by year[10]. Approximately 50% of liver was damaged due to over doses of certain medicinal drugs, industrial chemicals, herbal remedies, and even dietary supplements[11]. Liver is one of the principal organs in the contribution of the major drug metabolize/eliminate, exposes the organ to toxic injury and make it most frequently targeted organ in terms of drug toxicity[12]. Drug-induced hepatotoxicity is the most common cause of acute liver failure in many countries and it caused by hepatotoxic injury induced by drugs or their metabolites or due to hepatic hypersensitivity to drugs and their metabolites during medication and might be also due to over dose of drugs[9]. Certain drugs might even cause such injuries within recommended doses and the severity of liver injury greatly increased if drug is continued after the onset of symptoms[11]. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests and a substantial number of new antibiotics introduced in to the market are obtained from natural or semi-synthetic resources[13]. This fascinated the researchers to search out alternative sources of natural products with wide spectra of biological activities[14].

Now a day, there is an increasing interest in the study of natural products, especially as part of drug discovery programs, as it represents a formidable reservoir of potentially useful leads for new medicines. Natural product could be defined as a chemical compound that produced naturally by plants, animals and microorganisms[15,16]. The secondary metabolite as cryptotanshinone (**1**) derived from the roots of *Salvia miltiorrhiza Bunge,* phyllanthin (**2**) from *Phyllanthus amarus*, Silymarin (**3**) isolated from the seed and fruits of *Silybum marianum* and *p*-methoxy benzoic acid (**4**) from *Capparis spinosa are reported as* hepatoprotective activity[17]. quinine (**5**) isolated from the bark of *Cinchona succirubra*, were the natural product to show efficacy against malaria[18]. salicin (**6**) isolated from the bark of the willow tree *Salix alba*[19]. Anticancer, Bruceatin (**7**), from *Brucea antidysentrica* of Ethiopian plant are a pointer that medicinal plants remain a good source of novel and effective drugs[20, 21] (Figure **1**). Therefore, this study focused on the isolation and characterization of compounds from the roots of *A. africans* Lam.and evaluate for the isolated compounds and crude extracts for antimicrobial activity and hepatoprotective effects.

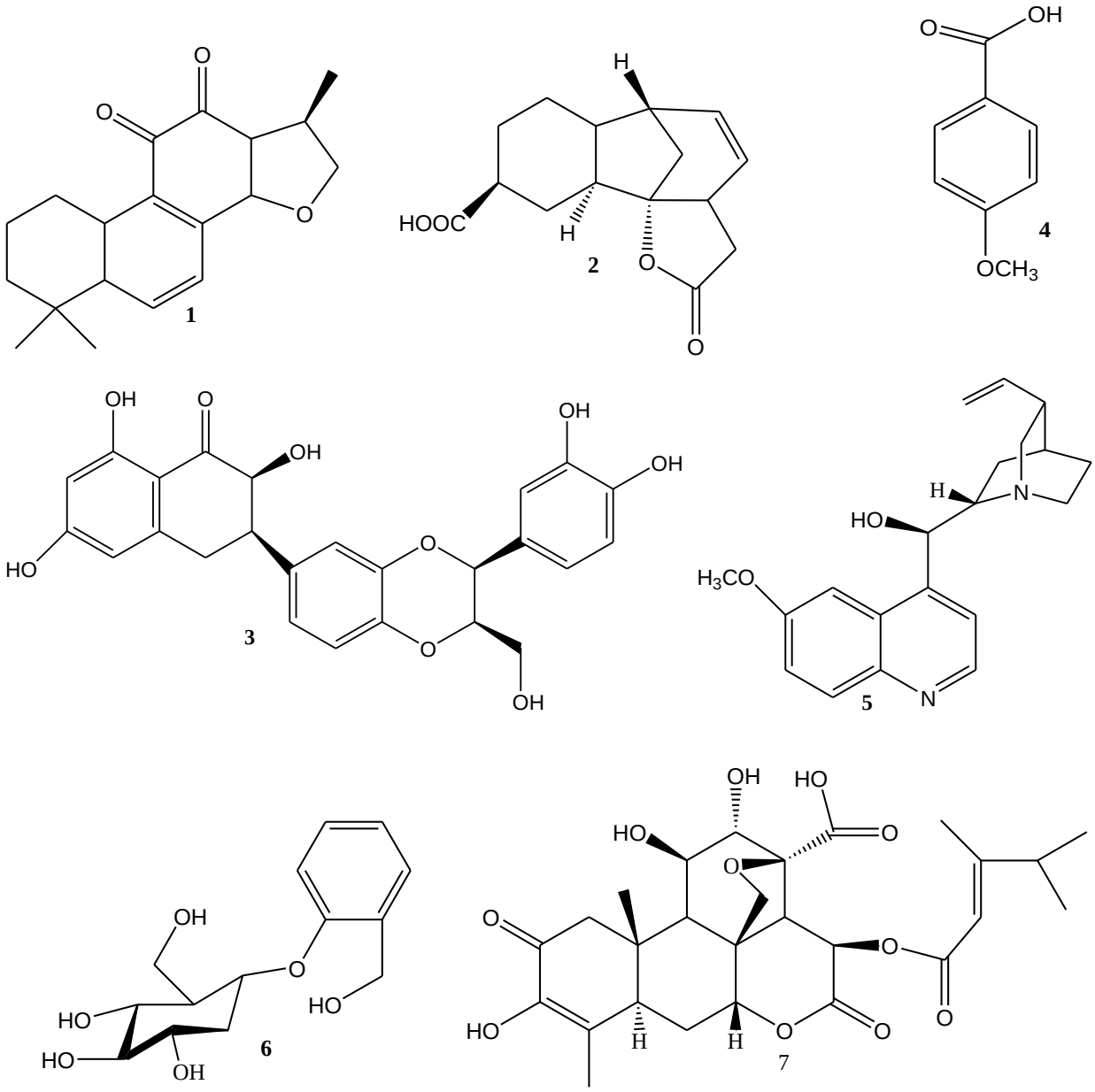


Figure 1. Chemical structure of some selected drugs derived from natural products.

1.2. Statement of the problem

The world is still facing enormous problems due to several ailments since time immemorial. Among these ailments, infectious diseases took a tremendous part affecting both humans and livestock throughout the world. An estimated 57 million annual deaths worldwide, or 15 million (>25%), are directly linked to infectious diseases[7]. The development and spread of antibiotic resistance are of great concern to the global health community, as is the evolution of new strains of disease-causing agents and undesirable side effects associated with certain antibiotics led to the search of new antimicrobial agents from medicinal plants[22]. Moreover, despite advances in modern medicine, there is no effective drug available that stimulates liver function, offers protection to the liver from damage or helps to regenerate hepatic cells. There is an urgent need for effective drugs to replace/supplement those in current use[9,23]. Many plant species have been tested for antimicrobial and hepatoprotective activity, and still the vast majority have not been adequately evaluated. Therefore, the development of alternatives antimicrobial and hepatoprotective agents from medicinal plants is of great interest to curb the current health associated problems.

1.3. Objectives of the study

1.3.1. General objective

To assess the phytochemical constituents, antimicrobial activity and hepatoprotective effect of the root extracts of *A. africanus* Lam.

1.3.2. Specific objectives

- To extract phytochemicals from the root extract of *A. africanus* using petroleum ether, chloroform, acetone and methanol.
  - To screen the phytochemical constituents of crude root extracts of *A. africanus* using chemical test methods.
  - To isolate compounds from root extract of *A. africanus by* using column chromatography.
  - To elucidate the structures of isolated compounds based on physical data and spectroscopic techniques such as (^{1}H NMR, ^{13}C NMR and DEPT-135).
  - To evaluate the antimicrobial activities of the crude extracts and isolated compounds from the roots of *A. africanus* against four pathogenic bacteria strains (*Staphylococcus aureus, Escherichia coli, Salmonella thyphimurium* and *Bacillus subtilus*) and one fungus strain (*Candida albicans*) using disk diffusion method.
  - To evaluate hepatoprotective effect of the crude methanolic extract against common liver markers (AST, ALT, ALP, total bilirubin and serum albumin) and liver histopathology.

1.4. Significance of the study

Traditionally, different parts of *A. africanus* has been used by the local people for the treatment of liver disease and microbial infections. Therefore, the outcome of this study would have the following significance:-

- Give information about the presences of various phytochemicals in the species, guide as plants are the source of drug discovery, exploit indigenous knowledge of mankind and it examined society can get such drug in local, for low cost and protect the plant as their own healing.
- Comprehend compounds that are responsible for antibacterial and antifungal agents of the plant.
- Support the awareness of the society use of the root extract of *A. africanus* as antimicrobial and hepatoprotective agent.
- The chemical constituents of this plant provide baseline information for future pharmacological and bio prospecting studies.
- Document the obtained result for further study related to chemical profile, isolation and purification of active principles.

2. Review of related literature

2.1. Botanical information of *A. africanus* Lam.

2.1.1. The family Asparagaceae

The family of Asparagaceae has 143 genera and 3632 species, distributed nearly worldwide, especially natural in tropical, sub-tropical, temperate area and contains medicinal, aromatic, vegetable and ornamental plants and its distributed worldwide[24]. The family is represented by with two sub-genera, *Asparagus* and *Myrsiphyllum*. Shrubby, tree-like or rhizomatous herbs; sometimes climbers, epiphytic or succulent. Leaves basally aggregated when stemless or along stem, simple, sometimes scale-like (e.g. *Asparagus* L., *Hemiphylacus* S. Watson, and *Ruscus* L.)[25]. Flowers are bisexual or rarely unisexual with identical sepals and petals, as well as stamens in multiples of three. A black pigment, phytomelanin, is present across this family, creating very black colored seed coats. Some of the more famous edible family members are *Asparagus*, agave and yucca. The APG III system of 2009 proposed *Asparagaceae sensu lato* seven subfamilies, these are: Agavoideae, Aphyllanthoideae, Lomandroideae, Asparagoideae, Brodiaeoideae, Nolinoideae and Scilloideae. A wide variety of interesting plants are classified in this family, with many having great economic, horticultural, and cultural significance[26, 27].

2.1.2. The genus *Asparagus*

A large and very diverse flowering plant family, comprising thousands of species and spread almost across the world. The genus Asparagus has been classified in the Asparagaceae family rather than in the Liliaceae by modern taxonomists[27] and it's include about 370 species around the world. The genus *Asparagus* is common at low altitudes in shade and in tropical climates throughout India, Asia, Australia and Africa. Most species are found in semi-arid to arid areas, but also found in Mediterranean climate. The members of this family are mostly bulbous, cormous or rhizomatous perennial herbs, as well as some shrubs and tree-like plants. Most species are used as food or especially as ornamental plants. The garden *Asparagus* (*Asparagus officinalis* L.) has been cultivated since ancient Greek times as a vegetable. The main popular ornamental plants are the

"agave (*Agave* L.), hyacinth (*Hyacinthus* L.), grape hyacinth (*Muscari* Mill.), ruscus (*Ruscus* L.). Many species of the genus *Asparagus* are important in traditional medicines. Many of its species and their active principles have been studied for phytochemical screening, biological and pharmacological purposes. Some of them have shown efficacy in the prevention and treatment of diseases such as for liver, kidney, heart and bladder problems; their toxic effects have also been evaluated[4, 27, 26].

2.1.3. *Asparagus africanus* Lam.

A. africanus "Saritti" in (Afaan Oromo) and ''yest kest'' in (Amharic) belonging to the family *Asparagaceae* is medicinal shrub valued for its medicinal properties. It is widely distributed throughout Africa, including Ethiopia, parts of Europe, Asia and Australia. *A. africanus,* with a common name African *Asparagus* is a perennial shrub with stems up to 6 meters high growing between 700 and 3800 m above sea level[28]. However, it is widely distributed and suitably grows higher up to 6 m at the altitude range of 1450 and 2900 m[29].

Figure 2. *Asparagus africanus* Lam. (Photography taken from study area, Bench Maji, SNNPR, Ethiopia, August, 2019).

2.2. Ethno medicinal uses of *A. africanus* Lam.

A. africanus Lam. is known as a folk medicine in Ethiopian. It is also used traditionally for management of diabetes mellitus. *A. africanus* widely used as traditional medicine for treatment of different diseases in Ethiopia (Table. **1**).

Table 1. Some selected etnomedicinal use of *A. africanus* Lam.

Plant scientific name	Disease Treated	Plant Parts	Reference
A. africanus Lam	Antibacterial, sexual impotency, gonorrhea, & syphilis	R,L	(30)
	Antimalarial, insecticidal and repellent properties	R	(31)
	Tuberculosis	S	(32)
	Liver disease	R	(33)
	Vomiting, nausea, diarrhoea	R	(34)
	Uterine cancer, Breast cancer	R	(35)
	Snake bite, Diabetes, Poisoning	L,R	(26,36)
	Skin infections, UTIs	L, R	(37)
	Haemorrhoid, syphilis	L, R	(38)

Keys: S=shoots, R= Root, L=Leave.

2.3. Phytochemical constituents of *Asparagus* species

Phytochemical screening indicated several bioactive constituents, such as flavonoids, tannins, steroids, terpenoids and saponin contained in *A. africanus* Lam. steroidal saponin are the major active component of the genus *Asparagus*[39].

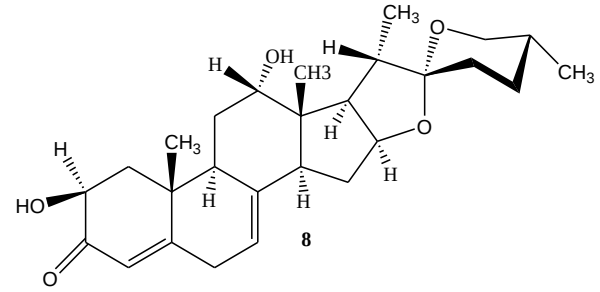
I) Saponin: are a class of substances with a rigid skeleton of at least four hydrocarbon rings to which sugars in groups of one or two are attached (usually not more than 10 units). They are subdivided into tri terpenoids and steroid glycosides. Steroidal saponin are mainly compounds containing 27 carbon atoms forming the core structures: spirostan and furostan[40]. Saponin, glycosides widely distributed in the plant kingdom, include a diverse group of compounds characterized by their structure containing a steroidal or tri terpenoids aglycone and one or more sugar chains. Plant extracts containing saponin widely used in food and other

industrial applications mainly as foaming agents and considered as “ant nutritional factors”[41].

Table 2. Saponin and its derivatives isolated from the genus *Asparagus*.

Steroidal Saponins	Species	Plant	Reference
Muzanzagenin**(8)**	*A. africanus*	R	(42)
Shatavaroside B **(9)** (1*S*,2*R*,3*S*,8*S*,9*S*,10*S*,13*S*,14*S*,16*S*,17*R*,22*R*,25*R*)-21-nor-18β, 27 α-dimethyl-1β, 2β,3β trihydroxy-25-spirost-4-en-19β-oic acid(**10**)	*A. racemosus*	R	(43)
Sarsasapogenin(**11**)	*A. officinalis*	R	(44)
Asparagosides (**12**)			
Blechnoside B **(13)**	*A. dumosus*	L	(45)

Keys: R= Root, L=Leave



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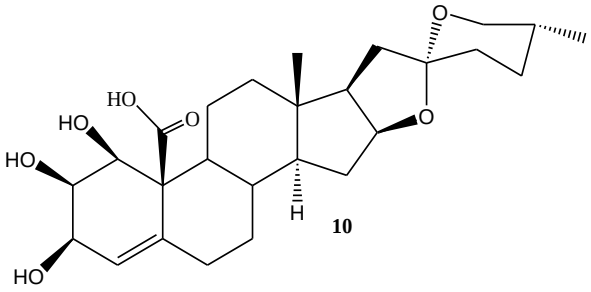

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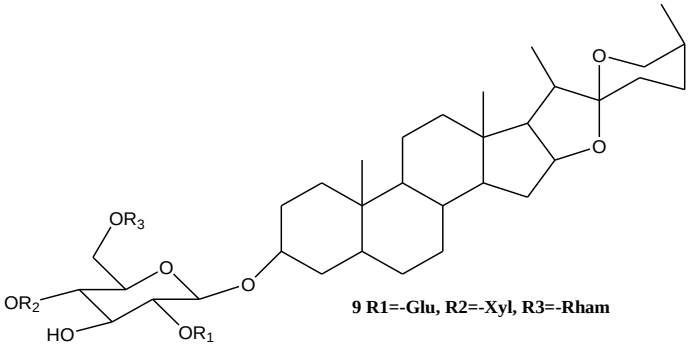
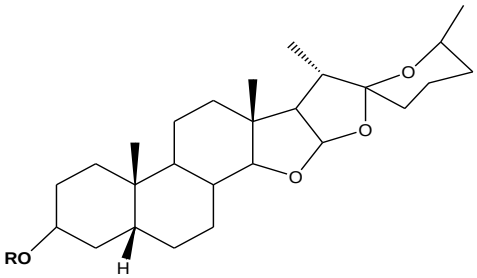


9 R1=-Glu, R2=-Xyl, R3=-Rham



11 R=H
12 R = Glue(4-1) Rhal (Glue, hatvarin

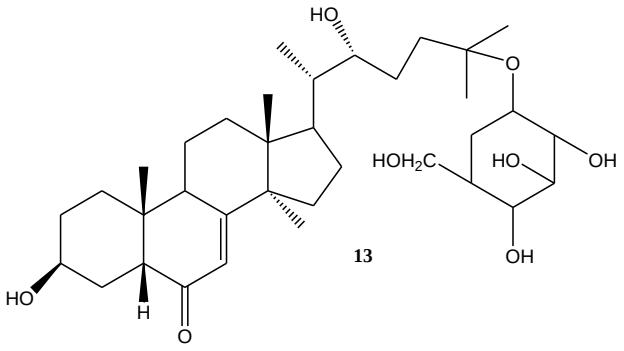


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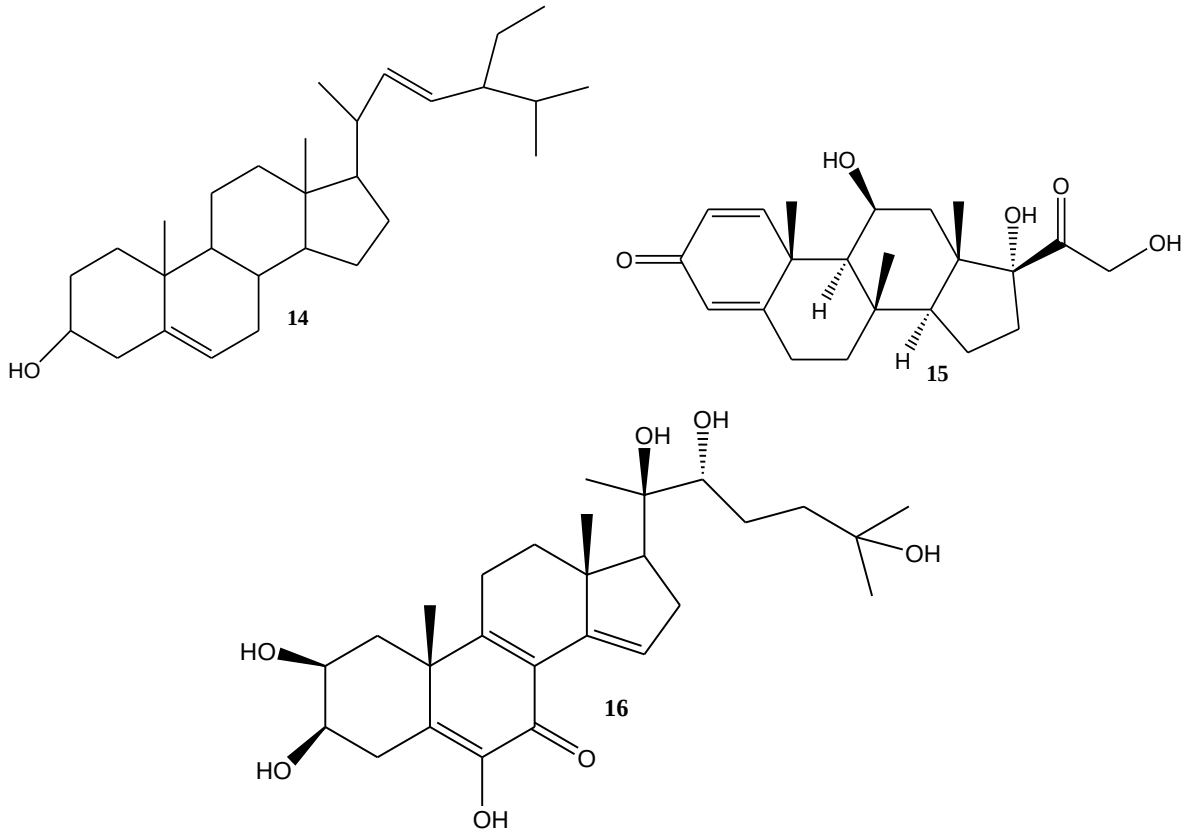
Figure 3. Saponin and its derivatives isolated from the genus *Asparagus*.

II) Terpenes: are belong to the biggest class of secondary metabolites and basically consist of five carbon isoprene units which are assembled to each other (many isoprene units) by thousands of ways. Terpenes are simple hydrocarbons, while terpenoids are modified class of terpenes with different functional groups and the oxidized methyl group moved or removed at various positions. Terpenoids are divided into monoterpenes (consist of 10 carbon atoms with two isoprene units and molecular formula $C_{10}H_{16}$), sesquiterpenes (consisting of three isoprene units ($C_{15}H_{24}$)), diterpenes (having $C_{20}H_{32}$ molecular formula and four isoprene units), sesterpenes (consist of 25 carbon atoms with 5 isoprene units and molecular formula $C_{25}H_{40}$), and triterpenes (contains 30 carbon atoms consisting of 6 isoprene units). Most of the terpenoids with the variation in their structures are biologically active and used worldwide for the treatment diseases. Many terpenoids inhibited different human cancer cells and are used as anticancer drugs such as Taxol and its derivatives. Many flavorings and nice fragrances are consisting on turbines because of its nice aroma. Terpenes and its derivatives are used as antimalarial drugs such as artemisinin and related compounds. Terpenoids play a diverse role in the field of foods, drugs, cosmetics, hormones and vitamins[46].

Table 3. Tetracyclic triterpenoid and its derivatives isolated from the genus *Asparagus*.

Triterpenoid	**Species**	**Plant part**	**Reference**
Stigmasterol (**14**)	*A. racemosus*	*R*	(47)
Diosgenin**(15)**	*A. officinalis*	*R*	(44)
Calonysterone**(16)**	*A. dumosus B*	*L*	(45**)**



Figures 4. Tetracyclic triterpenoid isolated from the genus *Asparagus.*

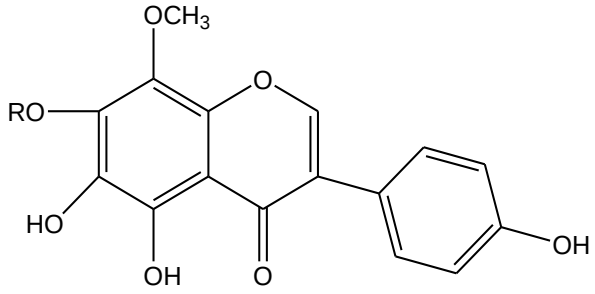
II) Flavonoids

Flavonoids are polyphenolic compounds which possess a C_{15} ($C_6C_3C_6$) framework. They contain chroman ring (C-ring) with a second aromatic ring (B-ring) at the C-2, C-3, or C-4 position. The heterocyclic six-membered C-ring is sometimes replaced by a five-membered ring (e.g., aurones) or the acyclic form (chalcones). The oxidation state of C-ring is used to classify flavonoids into different categories, of which typical examples are flavan-3-ols, flavanones, flavones and flavonols. Flavonoids are classified into many classes like flavones, flavonols, flavonones, anthocyanin's, isoflavonoids, chalcones etc. each classes of flavonoids are subdivided into many subclasses[48].

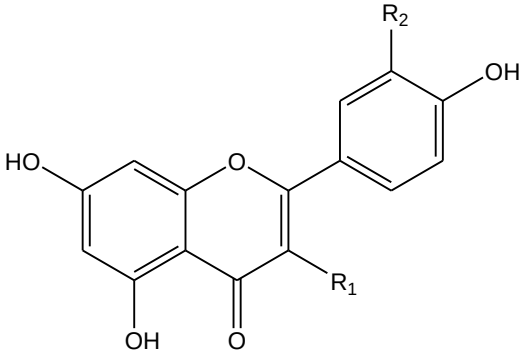
Table 4. Flavonoids and its derivatives isolated from the genus *Asparagus.*

Flavonoid	Species	plant part	Reference
8-methoxy-5, 6, 4‟ trihydroxyisoflavone-7-***O***-ß-D glucopyranoside **(17)**		R	(49)
Quercetin **(18)**			
Apigenin **(19)**	*A. racemosus*	F, Fr	(47)
Kaempferol **(20)**			
Quercetin-3-O-rhamnosyl glycosides **(22)**	*A. officinalis*	R	(50)
Cyanidin-3-galactoside(**23**),	*A. racemosus*	R	(47)
Cyanidin-3 glucorhamnoside **(24)**	*A. officinalis*		
Cyanidin 3-rutinoside **(25)**		R	(39)

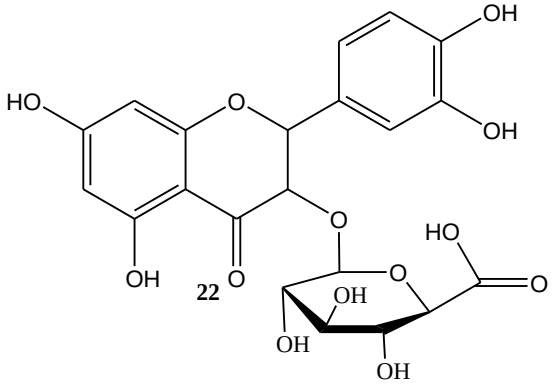
Keys: R= Root, L=Leave, F= Flower, Fr=Fruit



17 R=b-Dglu(pyr)



18 R1=OH, R2=OH **19 R1=H, R2=H**
20 R1=OH, R2=H **21 R1=O-rutinose. R2=OH**



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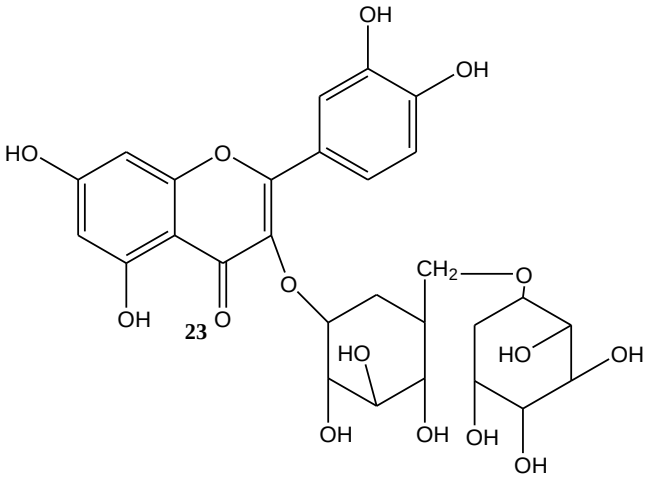

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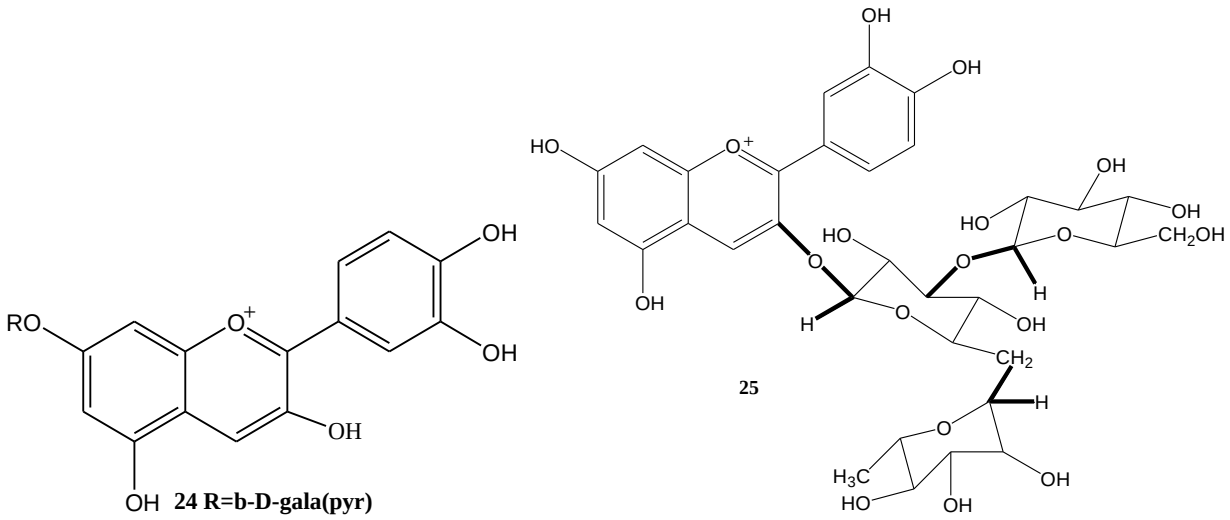


Figure 5. Flavonoid and its derivatives isolated from some of the genus *Asparagus*.

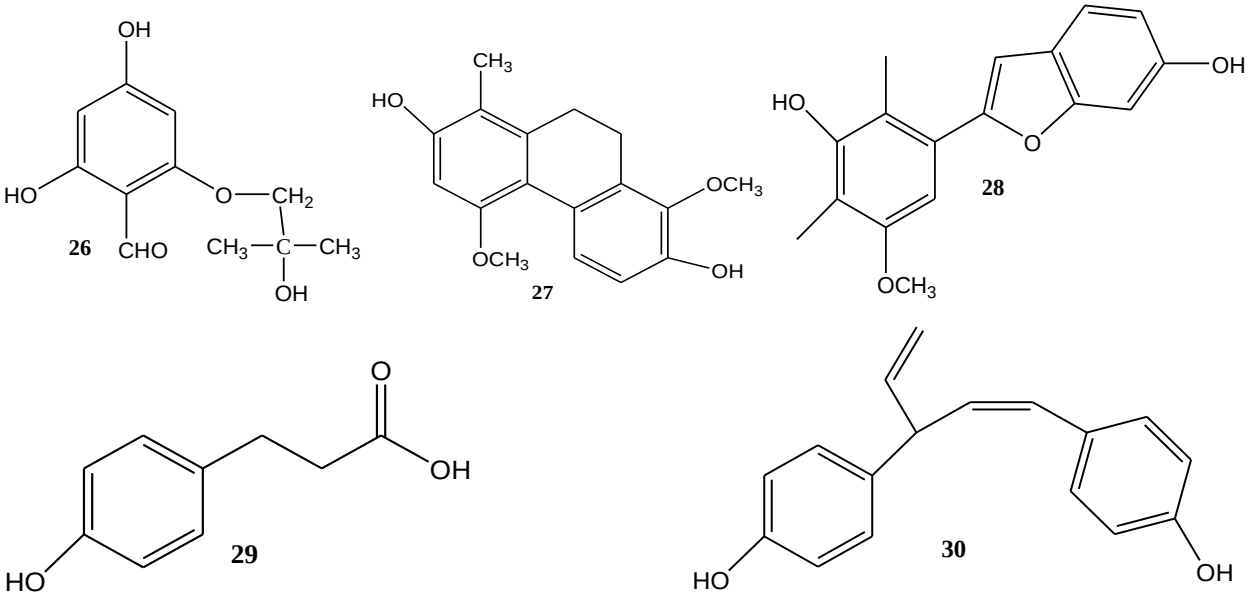
III). Phenolic and norlignans

Phenolic compounds are phytochemicals present in most plant tissues, including fruits and vegetables, which are ubiquitously dispersed. Via the shikimic acid and phenyl propanoid pathways. In plants, phenolic compounds are primary class of secondary metabolites and are classified into phenolic acids and polyphenols[51]. These compounds, related to one or more phenolic groups, are present in combination with mono- and polysaccharides or may occur as derivatives, such as esters or methyl esters[49]. There are some of the compound isolated from the genus *Asparagus* such as phenolic compound and norlignans (Table. **5**).

Table 5. Phenolic and norlignans isolated from different part of the genus *Asparagus.*

Phenolic compound	Species	Plant part	Reference
4, 6-dihydroxy-2-O-(2‟‟-hydroxyisobutyl) benzaldehyde **(26)**	*A. racemosus*	R	(51)
Racemosol (**27**)			
Racemofuran (**28**)			(49)
P-cumaric acid**(29)**	*A. officinalis*	R	(44)
Norlignans			
Nyasol **(30)**	*A. Africanus*	R	(42)
Iso-agatheresinol **(31)**	*A. officinalis*	R	(44)
Gobicusin A **(32)**			

Keys: R= Root, L=Leave, P=peels



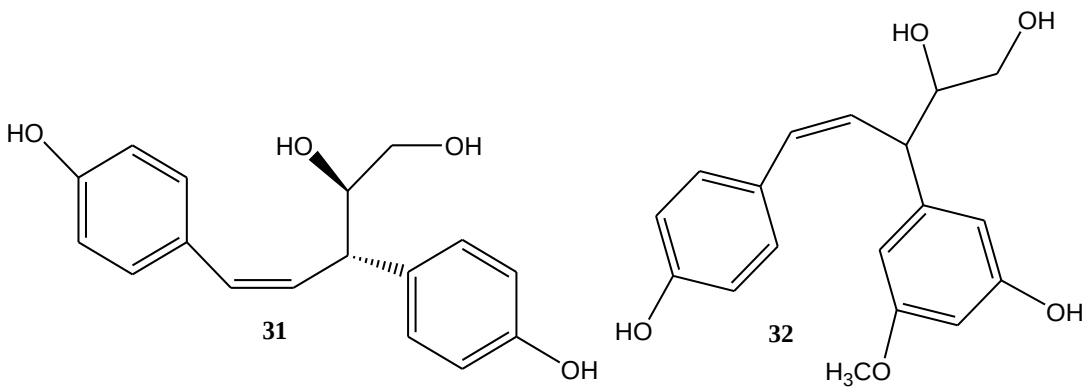


Figure 6. Phenolic and norlignans isolated from some of the genus *Asparagus*.

2.4. Some reported biological activities of *Asparagus africanus* Lam.

A. africanus Lam. is used to treat various human ailments in Ethiopia and other African countries. The extract of this plant exhibit effective in treating: bilharzias, syphilis, gonorrhea, leishmaniasis, malaria, inflammation, impotence which was confirmed scientifically[52], anti-fertility as birth canal dilator[53].

2.4.1. Antidiabetic activity

The root extract of *A. africanus* demonstrated anti-diabetic actions, particularly at the dose of 200 mg/kg in experimental rodents supporting the traditional claim. The plant extract showed anti-diabetic activity in all employed rodent models, in non-dose dependent but time dependent manner[52,54]. In the same genus Streptozotocin induced diabetic rats were treated with a methanolic extract of the seeds (250 and 500 mg/kg per day) for 28 days. Treatment of the diabetic rats with the *A. Officinalis* extract at doses of 250 and 500 mg/kg suppressed the elevated blood glucose in a dose and time dependent manner[29].

2.4.2. Antiprotozoal activity

Leishmaniasis and malaria are protozoan diseases that are responsible for widespread morbidity and mortality. Two antiprotozoal compounds have been isolated from the roots of *A. africanus*, which was named as muzanzagenin (**9**) and the lignan Nyasol (**31**) potently inhibited the growth of leishmaniasis major promastigotes, the IC_{50} being 12 µM, and moderately inhibits *Plasmodium falciparum* schizont with the IC_{50} 49 µM. These concentrations only moderately affect the proliferation of human lymphocytes. Two antiprotozoal compounds, a sapogenin (muzanzagenin) and lignan ((+)-nyasol) were isolated and reported to be responsible for the antimalarial and anti leishmania activity[42].

2.4.3. Anti-inflammatory and analgesic activity

The methanolic root extract of *A. africanus,* which contained mainly saponin and carbohydrate showed significant analgesic and anti-inflammatory activities ($P<0.05$) in the tail- flick/hotplate test and egg albumen-induced rat paw edema tests that were comparable to the test drugs (morphine 20 mg/kg and indomethacin 50 mg/kg respectively). These results indicate that the extract possesses analgesic and anti-inflammatory properties. But it is dose-dependent[55].

2.4.4. Antifertility

The aqueous extracts of the leaves and the roots showed antifertility activities of the aqueous and ethanol extracts were 40% (for leaves), 60% (for roots) and 20% (for leaves), 40% (for roots), respectively. All the extracts have resulted in significant ($P<0.05$) reduction in the number of implants as compared with their respective controls. Each extract potentiated acetylcholine induced uterine contractions in a concentration dependent manner significantly ($P<0.05$). Steroidal saponin is the major bioactive chemical constituent of *A. africanus* and it contribute to the anti-fertility effect[53].

2.4.5. Antimicrobial activities

The isolated compounds from the genus *Asparagus* have a significant activity on different bacterial strain. *A. africanus* Lam. leaves extract showed greater antimycobacterial activity on *Mycobacterium aurum* A^+, with moderate antibacterial activity against *Klebsiella pneumonia* due to the saponin present in this plant. It is reported that Saponin have also known to have a broad spectrum of pharmacological and antimicrobial activities[32]. The MIC of petroleum ether and dichloro methane root extracts of *A. africanus* against *S. aureus, Klebsiella pneumoniae* and *mycobacterium aurum* A^+ were 12.5, 12.5 and 6.25 mg/mL respectivly[56].

2.4.6. Antioxidant activity

The phytochemistry of the genus *Asparagus* are rich in phenolic compound, flavonoid and saponin and those are significant role for the radical scavenging activity. The root extract of *A. racemosus* extracts appear to be composed of saponin, the contents of which are richer in extracts from polar compared to non-polar solvents. Successive extraction resulted in fractions having different antioxidant activities[57,58]. The root extract of *A. africanus* could be a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing or slowing down the progress of ageing-associated oxidative stress related to degenerative diseases[4].

2.4.7. Toxicity

A.africanus has been described as absolutely safe for long term use except for a pregnant women due to uterine contractile property that cause abortion. The methanolic extract of the roots of *A. africanus* did not cause mortality and abnormality up to the dose of 5000 mg/kg orally and thus considered to be none toxic[55]. The previous study report also revealed that methanolic extract of *A. africanus* caused no mortality, no behavioral abnormality and no significant biochemical alteration in rats and it could be deduced that the extract was probably be safe up to 2,000 mg/kg BW[59].

2.5. Biosynthesis of sterols and norlignans

2.5.1. Biosynthesis of the sterols

Sterol shares a similar rigidity based on the cyclopentane-perhydro-phenantrene ring system with C10 and C13 methyl substituents, the C3 hydroxyl group and the 8-10 carbon atom side chain attached to C17. Although animals only produce cholesterol, it is also released in small amounts by plants. Plant synthesize a complex mixture of sterols and β-sitostrol, stigmasterol and campesterol the most abundant. Most of the reaction with in the sterol biosynthesis pathway take place in enzymatic reaction. However, sterols mainly accumulate in the plasma membrane (PM), which suggests the existence of some transport mechanism between these two membranous systems[60]. Sterols are produced via the MVA pathway following more than 30 enzymatic reactions which are common in all eukaryotes until the generation of sterol precursor 2, 3-oxidosequalene (OS). From this point, the plant sterol biosynthesis pathway diverge from yeast or mammalian pathway and it's characterized by specific steps that are restricted to plants[60, 61].

The first particularly in plant appears in the cyclization of OS in to cycloartenole by cycloartenole synthase. Cycloartenole is the first metabolite with in the pathway that is susceptible to be metabolized by two different branch point enzymes: sterol side chain reductase 2 (SSR2) and sterol- methyltransferase 1(SMT1). On the other hand, SMT1 catalyze the alkylation of cycloartanol to 24-methylene cycloartenole, leading to the generation of precursor for the plant specific strolls (β-sitostrol, stigmasterol, and campesterol). Further reaction down stream of SMT1 are essentially linear until reaching 24-metylenelophenol, where another branching point is found. The branch point enzyme sterol-methyltransferase 2 and 3 (SMT2) transforms it into 24-ethylidenlophenol, conferring to the plant kingdom the ability of producing 24-ethyl Sterols as the major molecular species by directing carbon toward β-sitostrol, stigmasterol biosynthesis[61]. *Key enzymes*: GPPS, geranyl pyrophosphate synthase; FPPS, farnesyl pyrophosphate synthase; SS, squalene synthase; SE, squalene epoxidase, C22DES, sterol C22desaturase; SMT1, sterol methyltransferase 1; SMT2/3, sterol methyltransferase 2/3.

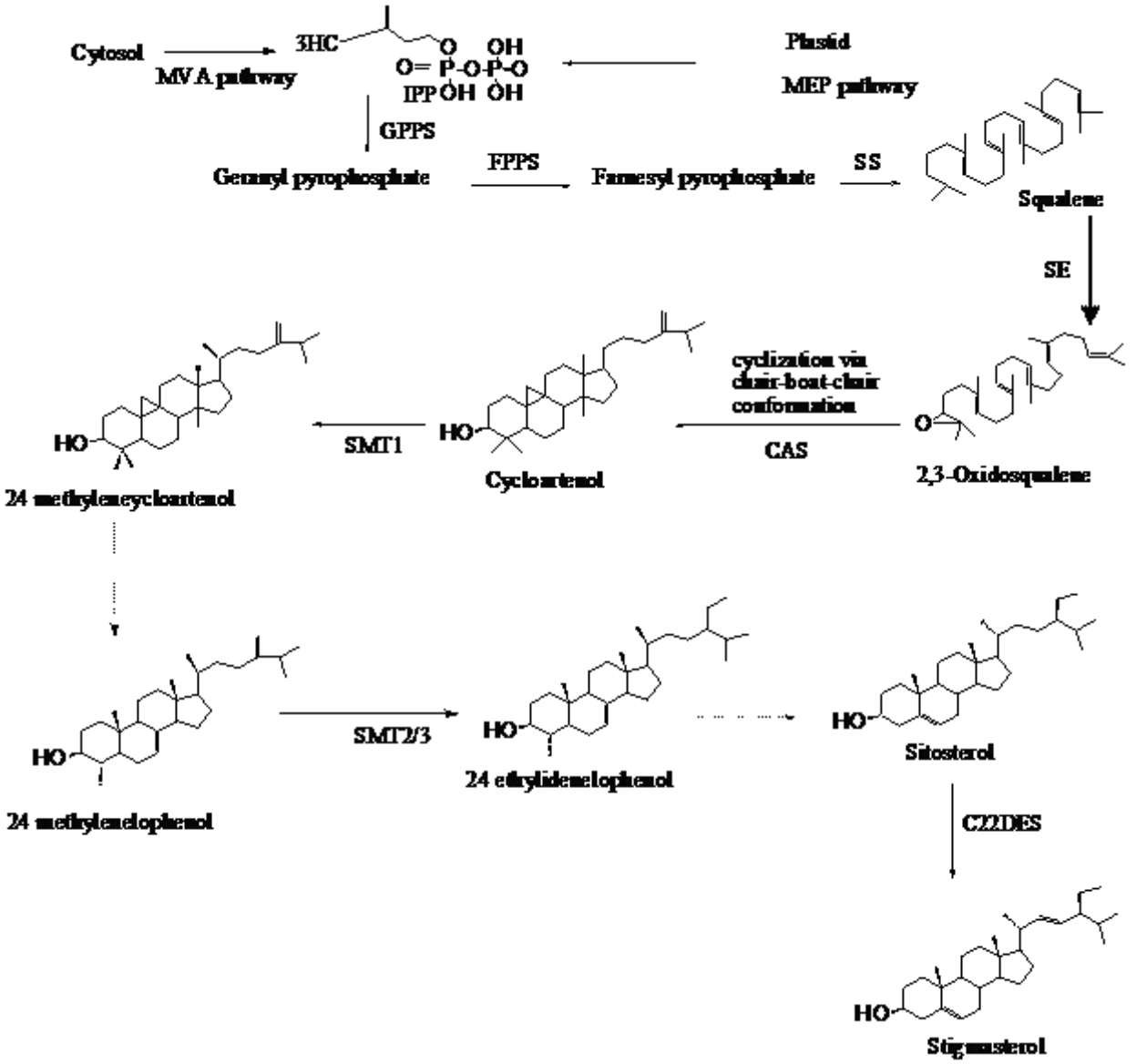


Figure 7. Biosynthesis of the selected Sterols: solid arrow represent single enzymatic step and dotted arrows indicate multiple reactions.

2.5.2. Biosynthesis of norlignans

The chemical structures of norlignans are apparently composed of phenyl propane (C6-C3) and phenyl ethane (C6-C2) units. Based on the linkage position between the two units, the chemical structures are classified into three groups: (1) C7-C8′ linkage type, (2) C8-C8′ linkage type, and (3) C9-C8′ linkage type. Norlignans have two aromatic rings and a side chain with five carbons, it had been hypothesized that norlignans biosynthesis was partly related to lignan biosynthesis.

However, the following points must be discriminated from lignan formation. First, lignin have a C8-C8′ linkage but typical norlignans have a C8-C7′ linkage. Second, one carbon atom must be lost in norlignans biosynthesis if norlignans are derived from two phenyl propane units. Norlignans [(*Z*) - and (*E*)-hinokiresinols/Nyasol] are formed from *p*-cumarylalchol and *p*-cumarayl CoA as monomers via *p*-coumaryl *p*-coumarate as a dimeric intermediate[62]. (E)-Hinokiresinol is the source of *C. japonica* and is typically found in conifer heartwood together with other norlignans in contrast to the distribution of (Z)-hinokiresinol in monocotyledonous herbs in the genus *Asparagus* including *A. officinalis*[63].

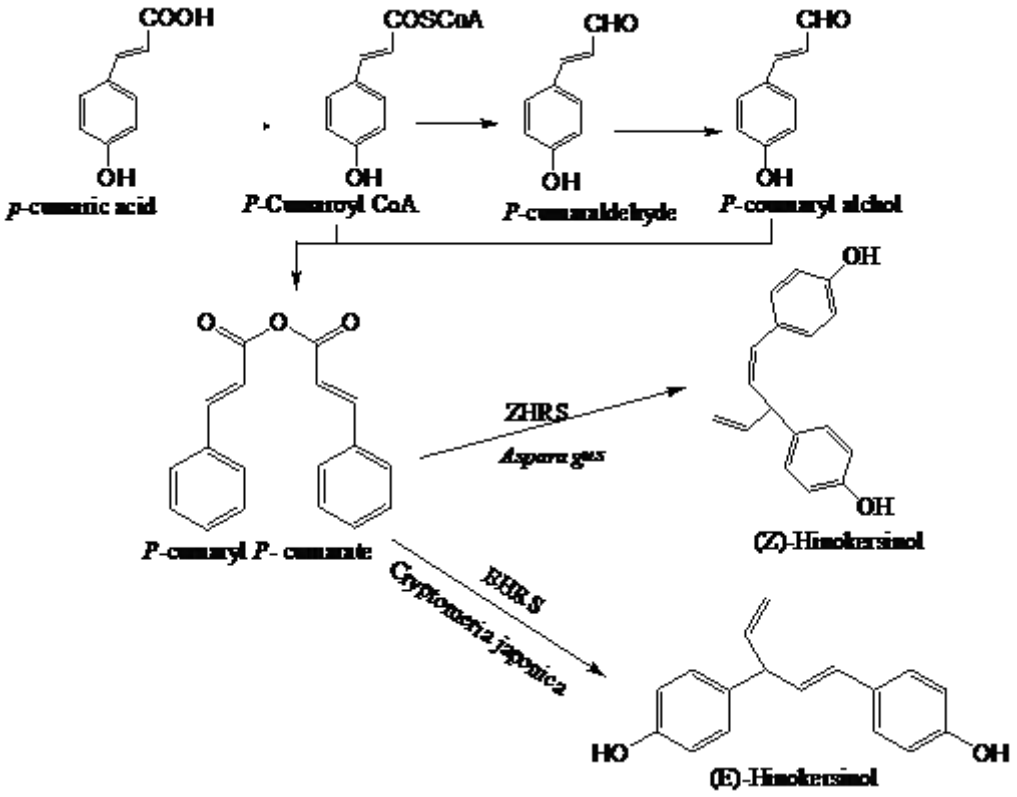


Figure-8. Biosynthesis of selected norlignans biosynthetic routes for (E) - and (Z)-hinokiresinols (Nyasol) in *C. japonica* and *Asparagus*: Key enzymes catalyzing the reactions: HRS (hinokiresinol synthase).

2.6. Microbial infections, management strategies and the challenges

Infectious diseases are caused by pathogenic microorganisms including bacteria, viruses, fungi, protozoans and multicellular parasites. In developed countries, it is the second leading cause of death for both children and adults worldwide and the third leading cause of death[64]. An estimated 57 million annual deaths worldwide, or 15 million (>25%), are directly linked to infectious diseases. However, antimicrobial resistance was discovered at the same time, accompanied by increased transmission of disease and reduced production of new antibiotics. This led to the emergence of multi-resistance that created a situation[65]. To prevent and control infectious diseases, it is of vital interest and concern in both developed and developing countries to come up with new remedies for such diseases[64].

Bacteria are widely distributed and can adapt to a number of conditions, such as soil, water and air, and are the most diverse group of single-cell organisms on earth. They are beneficial as well as dangerous to humans. In Gram-positive and Gram-negative bacteria, the composition of the cell wall varies. The presence of an outer membrane consisting of lipopolysaccharides makes Gram-negative bacteria distinct from Gram-positive bacteria. Gram-negative bacteria are distinct from Gram-positive bacteria by the presence of an outer membrane consisting of lipopolysaccharides. It also includes specific proteins for the transport of hydrophilic molecules and covers the peptidoglycan layer that is bound to the outer membrane by lipoprotein. There are more peptidoglycan layers without periplasm in Gram-positive bacteria[66]. *Bacillus subtilus* is a Gram-positive bacterium with an endospore-forming rod shape that occurs in chainlike formations. Its main habitat is the soil and food poisoning is responsible for it[67]. *Staphylococcus aureus* is a gram-positive spherical parasitic bacterium that causes diseases ranging from mild skin infections and abscesses to life-threatening diseases such as pneumonia, meningitis, Helicobacter pylori septicemia and can cause gastric ulcers. *Salmonella thyphimurium* is a Gram-negative, typhoid-causing bacterium. *Escherichia coli*: - is a gram-negative bacterium commonly found in humans and other animals in the intestinal tract. Sometimes, *Escherichia coli* can be pathogenic, causing diarrhoea, wounds, and urinary infections, posing a danger to the safety of food[66]. Besides bacterial infections, human is also fighting fungal infection and associated with increasing morbidity and mortality in immunocompromised and severely ill patients[68]. However, a few fungal pathogens are

causative agents of infectious diseases, for example, *Candida albicans*. *Candida albicans* (a form of yeast) is the major causative agent of fungal infections[69]. It is capable of causing 90% of fungal infections by colonizing the mucosal surface of the gastrointestinal tract, female genital tract and infects the skin and mucous membranes of the vagina (vaginitis), head of the penis (balanitis), mouth (oral thrush) or rectum[67]. Ninety percent of HIV/AIDS patients suffer from fungal infections and 20% of them die as a direct consequence of such infections[67,69]. For the treatment of microbial infections, many antibiotics are available, but some microbials have led to the emergence of resistance and side effects. Sequential proof of resistance is shown by *Staphylococcus aureus*. *Staphylococcus aureus* exhibits sequential evidence of resistance. In the 1940s, it was resistant to sulfates, penicillin in the 1950s, methicillin in the 1980s and recently vancomycin[70]. *Escherichia coli* in some inpatients in the US and UK showed resistance to sulfamides and ampicillin-sulbactam[71]. Several antifungal medications have recently approved, but systemic fungal infections remain deadly[72]. The highest prevalence of antibiotic resistance is attributed to a number of factors such as inadequate or inadequate antibiotic therapy, selective pressure and high prevalence of other diseases, and the lack of ability to launch surveillance or prevention programs[22]. As a result, the development of antimicrobials from natural products could play an important role in meeting the demand for new drugs for microbial infections.

2.7. Mechanism of liver damage associated with antitubercular drug.

Tuberculosis (TB) is one of the major communicable diseases and nearly 2 million people die every year and ranked seventh among all disease[73]. Anti-TB drugs are considered as potentially hepatotoxic and can induce liver injury. Isoniazid (INH) and rifampicin (RIF) are the two major regimens currently used for the treatment of TB for a period of 4 to 6 months[9], which may induce hepatotoxicity[74]. The co-administration of INH and RIF generates metabolic and morphological changes in hepatic tissue because of the fact that the liver is the primary detoxifying organ for the anti-TB drugs[75]. The incidence of hepatic dysfunction is more, when INH and RIF are used in combination and anti-tuberculosis drug induced hepatotoxicity ranges from nonspecific elevation of transaminases to fulminant of liver failure[76]. The liver dysfunction is due to the synergistic effect of INH and RIF. Hydrazine (HYZ) a metabolite of INH is converted to toxic compound by CYP-450, which leads to hepatotoxicity. RIF, aggravates hepatotoxicity by inducing CYP-450, as a result more toxic metabolites are generated from hydrazine[77]. In addition HYZ depletes the reserved glutathione (GSH) level in the liver, resulting in oxidative stress and cell death. Since oxidative stress is one of the major attributing mechanisms for anti-tuberculosis drug induced hepatotoxicity and liver damage, the present study is designed to study the effect of *A. africanus* root extract hepatoprotective as supplementary agent for INH and RIF induced liver dysfunction in rats.

2.8. Liver and liver injury

The liver is the largest organ in the body, contributing about 2 percent of the total body weight or about 1.5 kg in the average adult human. The various functions of the liver are carried out by the liver cells or hepatocyte[78]. Chronic liver diseases are one of the world's leading health issues, with liver cirrhosis and drug-induced liver damage responsible for the ninth leading cause of death in western and developing nations. The liver is the second largest organ in the human body, with over 5,000 distinct body functions, including bile development and excretion, protein synthesis (e.g. albumin, prothrombin, etc.), toxic material detoxification, etc. (e.g. alcohol, drugs etc.) and biochemical production essential for digestion and synthesis as well as metabolism of small, complex molecules, helping to coagulate the blood, clearing the blood of toxins, turning food into

nutrients to regulate hormone levels, battling infections and diseases, regenerating after injury, metabolizing and regulating cholesterol, glucose, iron levels[79]. Liver cell damage can be caused by viral hepatitis, alcohol poisoning or other toxic substances; Hemochromatosis is an inherited disorder that causes iron accumulation in the body, leading to long-term liver damage; Benign tumors such as adenoma, focal nodular hyperplasia. There are many pediatric liver diseases. Proper liver function may be tested by a number of specialized clinical studies, which measure the presence or absence of typical enzymes, metabolites or substances associated with regular activities[79, 80]. General mechanism of drug induced liver injury were described in the (Figure **9)** below.

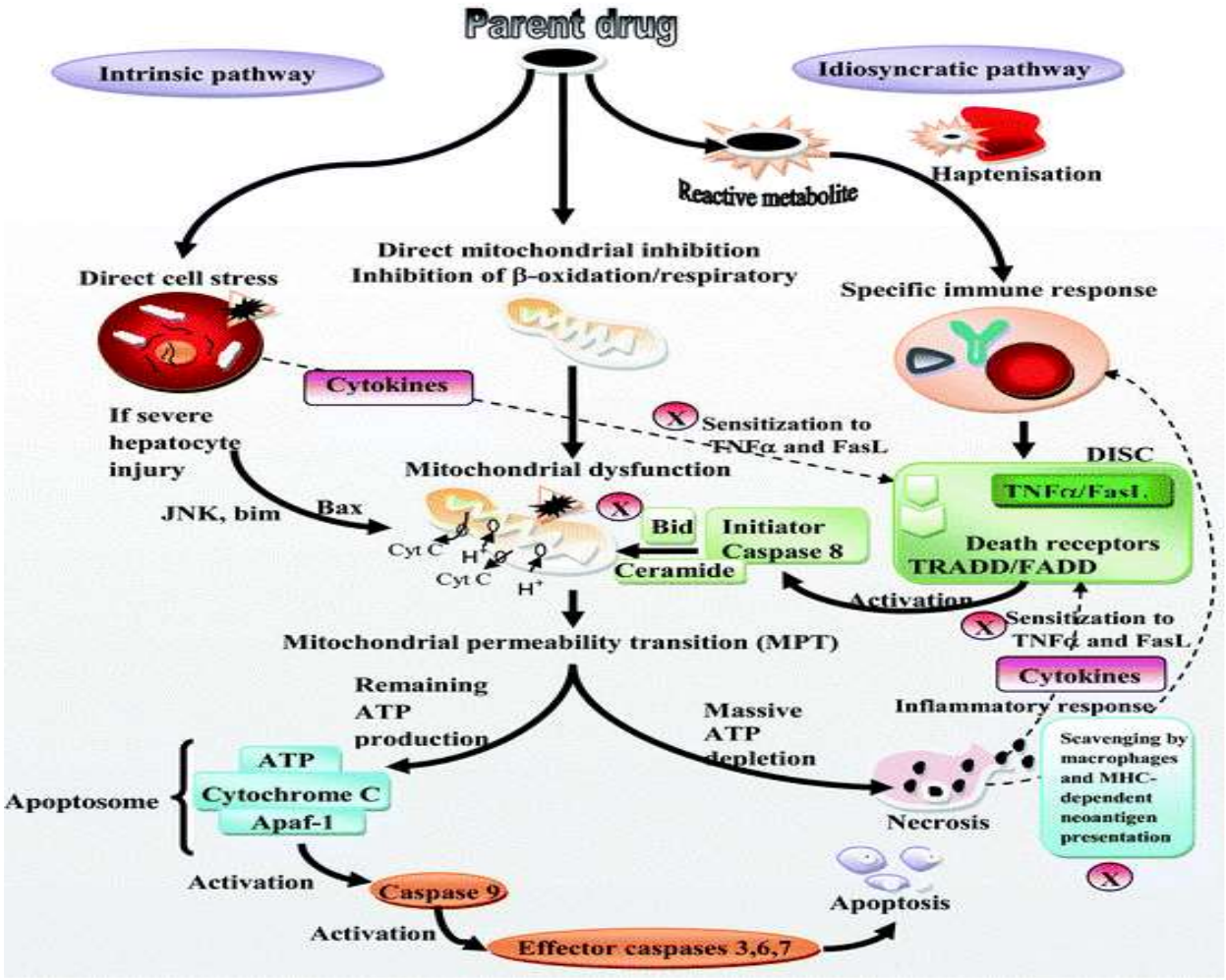


Figure 9. Three-step mechanism of drug induced liver injury.

2.9. Serum biomarkers for liver function tests

Table 6. Major liver biomarker and its functions

Biomarker	Definition	Ref
ALT	Cytoplasmic enzyme found in hepatocytes, found in other cells, such as skeletal muscle, renal and red blood cells in smaller amounts, mainly expressed in the liver and its increased level is indicator of liver injury, the most liver specific of the liver enzymes, catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate, pyruvate in the presence of NADH and lactate dehydrogenase (LDH) is reduced to L-lactate, in addition, NADH is oxidized to NAD+.	(81,82)
AST	Cytoplasmic and mitochondrial enzyme, The enzyme was determined by kinetic method, Evaluation of the level of AST indicates the magnitude of acute liver damage, Increases in AST activity generally parallel those of ALT and is less specific for liver injury than ALT.	(82,83)
ALP	Catalyzes the hydrolysis of phosphate esters, Found mainly in the liver and bones, Marked increase in serum ALP levels has been associated with malignant biliary obstruction, primary biliary cirrhosis, hepatic lymphoma and sarcoidosis, and several bone diseases, Enzyme found in hepatocytes that line the bile canaliculi and It is released into the circulation during intra- or extra-hepatic cholestasis.	(81,83)
Bilirubin	Is the result of degradation heme portion of hemoglobin of dead red blood cells which are normally removed by the liver and excreted via bile, a yellow-pigmented molecule and becomes bound to albumin and transported to the liver where hepatocytes conjugate the bilirubin with glucuronic acid	(84)
Albumin	Synthesized in the liver and is an indicator of liver function, Decreased by trauma, inflammatory conditions and malnutrition, Protein that is produced exclusively in the liver, Albumin constitutes about 60% of the total serum protein in normal and its concentration is considered marker of synthetic function of the liver.	(85,86)

2.10. Role of medicinal plants in management of liver damage

A significant number of medicinal plants have been tested for active principles with curative properties against a variety of diseases and have been found to contain them. Plants contain a number of chemical constituents that have demonstrated hepatoprotective efficacy, such as phenols, coumarins, lignan, essential oil, carotenoids, glycosides, flavonoids, organic acids, lipids and alkaloids. In laboratory animals, for example, a variety of conventional remedies of plant are tested for their potential antioxidant and hepatoprotective effects against various chemical-induced liver damage. CCl_4-induced, paracetamol-induced, alcohol-induced, antitubercular drug-induced, nitrobenzene-induced, hepatotoxicity model induced by bromo benzene, lead-induced etc. Therefore damage to the liver inflicted by hepatotoxic substances is of grave consequences. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid per oxidation and other oxidative damages. Therefore a large number of plants and formulations have been claimed to have hepatoprotective activity so the development of plant based hepatoprotective drugs has been given importance in the global market[87].

For a long historical time, traditional uses of herbal medicines have been documented and are commonly accepted in the mainstream scientific-based health care system as safe and effective and recognized as a form of alternative medicine[87]. In recent decade, Complementary and complementary approaches to medicine using medicinal plants for the prevention and treatment of diseases have increase. Medicinal plants demonstrate effectiveness in treating a number of diseases not otherwise cured by traditional medicines. In some chronic diseases, herbal medicines are increasingly evolving as safer alternatives/adjuncts, and this has been demonstrated following modern clinical methods in certain inflammatory disorders. A large number of medicinal plants have been used traditionally for immunomodulation and hepatoprotection and can be an important source of hepatoprotective drugs[87, 88].

Table 7. Medicinal plants and its Hepatoprotective effect[88, 89, 90 and 91].

S. N	Scientific name	Part used	Extract	Active constituent	Experimental model	Hepatoprotective activity
1	*Allium cepa*	Fresh bulbs	Aqueous extract	Polyphenolic, tannins, saponin, flavonoids	Ethanol induced liver damage in male rats	Significantly decreased AST, ALT, ALP and TB.
2	*Alocasia indica*	Tuber	Ethanolic 80% and Aqueous extract	Alkaloids, flavonoids, glycosides, saponin and tannins	CCl4 induced hepatic injuries in male Albino Wister rats	Recovery percentage of serum ALT and AST
3	*Antrodia Cinnamomea*	Fruit and Mycela	Aqueous and Ethanolic extract 90%	Benzenoids, diterpenes, triterpenoid, steroids succinic acid derivatives	CCl4 induced liver injury and ethanol induced liver injury in rats	Suppression of ethanol and CCl4 induced elevation of expression of hepatic mRNAs
4	*Ficus carica*	Leaves, Fruit and Roots	P. ether, Aqueous and Methanol extract	Phenolic organic acids and volatile compounds	Rifampicin induced liver damage in male rats	Significant reversal of biochemical, histological changes.
5	*Terminalia arjuna*	Bark	Aqueous extract	Flavonoids, tannins oligomeric, Proanthocyanidin	Cadmium induced toxicity in Albino Rats	Significantly elevated ALT, AST and ALP
6	*A. retrofractus*	Roots	Aqueous and Ethanolic extract 90%	Flavonoids, tannins and steroids	Thioacetamid Induced Liver Damage in rats	Significantly decreased serum level of SGOT, SGPT, ALP, TB
7	*A. racemosus*	Roots		Flavonoids, tannins and steroids, saponin	CCl4induced and isoniazid-induced hepatotoxicity in male albino rats	Significantly decreased serum levels of ALT, AST and ALP
8	*A. albus*	Roots	Aqueous extract	phenols, tannins Condensed, flavonoids	CCl4 induced liver injury rats	Significantly protected against increased ALT and AST

3. Material and methods

3.1 Study area and study period

Extraction and isolation of plant based product were carried in Postgraduate Organic Chemistry Laboratory, College of Natural Sciences, Department of Chemistry. The antimicrobial activity tests were carried out at Microbiology Postgraduate Laboratory, College of Natural Sciences Department of Biology. For the care of animals and administration of test medicinal products prepared in a Biotechnology Laboratory, College of Agriculture and Veterinary medicine (July 8 to August 7, 2020). The isolated compound were characterized at Addis Ababa University, College of Natural and computational Sciences, Department of Chemistry Addis Ababa, Ethiopia. The serum analysis and histopathological study were carried out at Awetu (diagnostic laboratory) and Jimma University medical center, respectively.

3.2. Chemicals and drugs

The chemicals such as hexane (95%), petroleum ether (98%), acetone (99.5%), ethyl acetate (99.8%)) methanol (99.99%) all from (LOBACHEMIE, Ltd, India) and chloroform (99%) from (Blulux Laboratories Ltd, India) were used for extraction and isolation of the compounds. Diethyl ether (98%) (Blulux Laboratories Ltd, India) was used for anesthetizing the mice. Ethanol, formalin, DNPH (dihydro phenyl hydrazine) and sodium hydroxide were used for serum analysis. Silica gel 60-120 mesh size were used for as a stationary phase of column chromatography. Mueller hinton agar as culture media, Dimethyl sulfoxide (98%) (Blulux Laboratories Ltd India), Gentamycin, Clotrimazole, Isoniazide, Rifampicin and Silymarin were used as a negative and positive controls during the antimicrobial and hepatoprotective study. A deuterated chloroform ($CDCl_3$, 99.8%) solvent was used for recording NMR spectra. All the chemicals and reagents used in this study were analytical grade.

3.3. Apparatus and instruments

Apparatus such as rotary evaporator (Labo Rota 4000, Heidolph, USA) for solvent evaporation, oral gavage feeding syringe for blood collection and plastic cage for experimental animal grouping, tissue slides for liver histopathology analysis, oven for drying purpose, glass column chromatography for separation of pure compound, silica gel coated TLC for solvent selection and purity of isolated compound and UV-Tec (254 and 365 nm) for detection of the spot on TLC were used. NMR Spectral recording was done using Bruker 400 MHz advance ^{1}H NMR and 100 MHz for ^{13}C NMR spectra with TMS as internal standard. Bunsen burners, Vertical Laminar Flow Cabinet CLB-201-14 and incubator were used during bioassay tests study.

3.4. Plant collection and preparation

The root of *A. africanus* was collected in August, 2019 from southwestern Ethiopia, Bench-Maji Zone, Maji woreda which is 568 km away from Addis Ababa. Identification of the plant species was made by botanist and a voucher specimen was deposited as **WM-01** at Addis Ababa University Herbarium Addis Ababa. The collected root of *A. africanus* was washed, sliced and air-dried under shade. It was powdered using an electrical grinder to improve subsequent extraction by rendering the sample more homogenous, increasing surface area and facilitating the penetration of the solvent into the cell.

3.5. Extraction

The powdered plant material (1kg) was sequentially extracted with petroleum ether (2.5L) for (1x72 h), chloroform 2.5 L for (3x72 h), acetone 2.5 L for (2x72 h) and methanol 2.5 mL for (2x72h) at room temperature by using a maceration technique The extracts of each solvent were filtered and concentrated using a rotary evaporator (Laborota-4000) at a temperature of 40 °C with the speed of 90 rpm. The resulting extracts were stored in desiccators until complete drying. The dried crude extracts were weighed and percentage yield were calculated as 4.0 g (0.4%), 13 g (1.3%), 8 g (0.8%) and 50 g (5%) for petroleum ether, chloroform, acetone and methanol, respectively.

$$\% \text{ yield} = \frac{\text{weight of the crude extract(g)}}{\text{weight of the dried sample used(g)}} \text{X100} \quad (1)$$

3.6. Phytochemical screening

The qualitative phytochemical screening of petroleum ether, chloroform, acetone and methanolic crude extracts of the root of *A. africanus* Lam. were carried out by standard methods to evaluate the presence of saponin, alkaloids, steroid, tannins, flavonoids, phenolic, terpenoids, glycosides and quinones with some modification. All tests were done in replicates.

Phenols (ferric chloride test): In the test tub 0.2 g of plant extract was taken with 1 mL of water in a test tube and 2 drops of Iron III chloride ($FeCl_3$) was added. A blue, green, red or purple color is a positive test[92].

Saponins (Foam test): In the test tub 0.1 g of plant extract was added and diluted with 20 mL distill water and agitated in a graduated cylinder for 15 minutes. The formation of a 1cm layer of foam indicates the presence of saponin[93].

Alkaloids (Wagner's test): A plant extract (0.2 g) was added in test tube and 3 drops of Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 mL of water] added the presence of alkaloids indicated by the formation of a reddish brown precipitate [93,94].

Tannins (Braymer's test): In test tube 1mL 5% ferric chloride ($FeCl_3$) was added to solvent free 0.2 g extract. The presence of tannin is indicated by the formation bluish black or greenish precipitate[92 ,94].

Flavonoids (Sodium Hydroxide Test): In test tube 0.2 g of test solution, a few drops of dilute sodium hydroxide (NaOH) were added, an intense yellow color was produced in the plant extract which becomes colorless on the addition of a few drops of dilute HCl. Indicates the presence of the flavonoid[94].

Terpenoids (Lieberman bur chard test): In a test tube 0.2 g of test solution 2mL of chloroform and 3ml of concentrated sulfuric acid (H_2SO_4) was added to form a layer. The yellow color in a lower layer indicates the presence of terpenoids[92,93,94].

Steroids (Salkowski test): A plant extract (0.2 g) dissolved in chloroform, in the different test tube and a few drops of concentrated sulfuric acid were added to the solution, and appeared dark pink/red color indicating presence of steroids[92,94].

Quinones: In test tube a (0.2 g) of plant extract was treated with a cone. HCl. Formation of a yellow precipitate (or coloration**)** confirms presence of quinines[92,93].

Glycoside: In test tube at (0.1 g) of sample was dissolved in 5mL of water and then aqueous 0.5 ml NaOH solution was added formation of yellow color indicates the presence of a glycoside[93,94].

3.7. Isolation and identification of compounds

After preliminary phytochemical and TLC analysis, the methanol and the combined chloroform a nd acetone extracts were adsorbed on silica gel (with 1:2 silca to sample ratio), and separately applied to column chromatography. The column for methanol extract was then eluted with chloroform containing increasing amounts of methanol (100:0, 99:1, 98:2 and 97:3 up to70:30%) respectively. A total of 40 fractions each with a volume of 100 mL were collected. Each fraction were monitored by TLC with different solvent systems (chloroform: methanol, 9:1, 8:2 and 7:3) respectively after concentrated under reduced pressure using a rotary evaporator at 45°C. Based on the TLC profile similar fraction were pooled in to two major fractions F14-28 a pure compound (50 mg, Rf value of 0.75 with 20% of methanol in chloroform) labeled as **AA-1** and fraction (F29-40) consisted of three spots (chloroform: methanol 7:3) on the TLC, then fractionated using a separator funnel with various solvent systems, such as chloroform, acetone and methanol sequentially, and formed a two layers after chloroform was applied. The chloroform layer were collected and add acetone. After removing the chloroform layer the acetone layer was further purified by Sephadex LH-20 (gel filtration) obtained as a pure compound, 30 mg, with an Rf value of 0.57 in 30% methanol in chloroform labeled as **AA-2** (Figure **10**).

On the other hand, the column for combined crude extracts for chloroform (10 g) and acetone (6 g) was eluted with 100% petroleum ether and then increasing amounts of ethyl acetate (100:0, 99:1, 98:2 and 97:3 up to 0:100%). The fractions were constantly collected at a volume of 100 mL and a total of 100 fractions were collected and each fraction was monitored by TLC with different solvent systems (petroleum ether: ethyl acetate) after concentrated under reduced pressure using a rotary evaporator at 45°C. The fractions containing similar TLC profiles (Rf value) were combined, and resulted twelve major fractions. Four major fractions, F(2-13, 32-39, 40-47 and 56-65), were selected based on the yield of the combined fraction, then further purified using Sephadex LH-20 gel filtration to give four pure compounds labeled as **AA-3** (90 mg, Rf value of 0.52 with 4% ethyl acetate), **AA-4** (10 mg, Rf value of 0.50 in 18% Ethyl acetate), **AA-5**(25 mg, Rf value of 0.40 with 25% ethyl acetate) and **AA-6**(40 mg, Rf value of 0.45 with 30% ethyl acetate) in petroleum ether respectively.

$$Rf \text{ value} = \frac{\text{Distance traveld by solute}}{\text{Distancetravelle by solvent}} \text{X}100 \qquad (2)$$

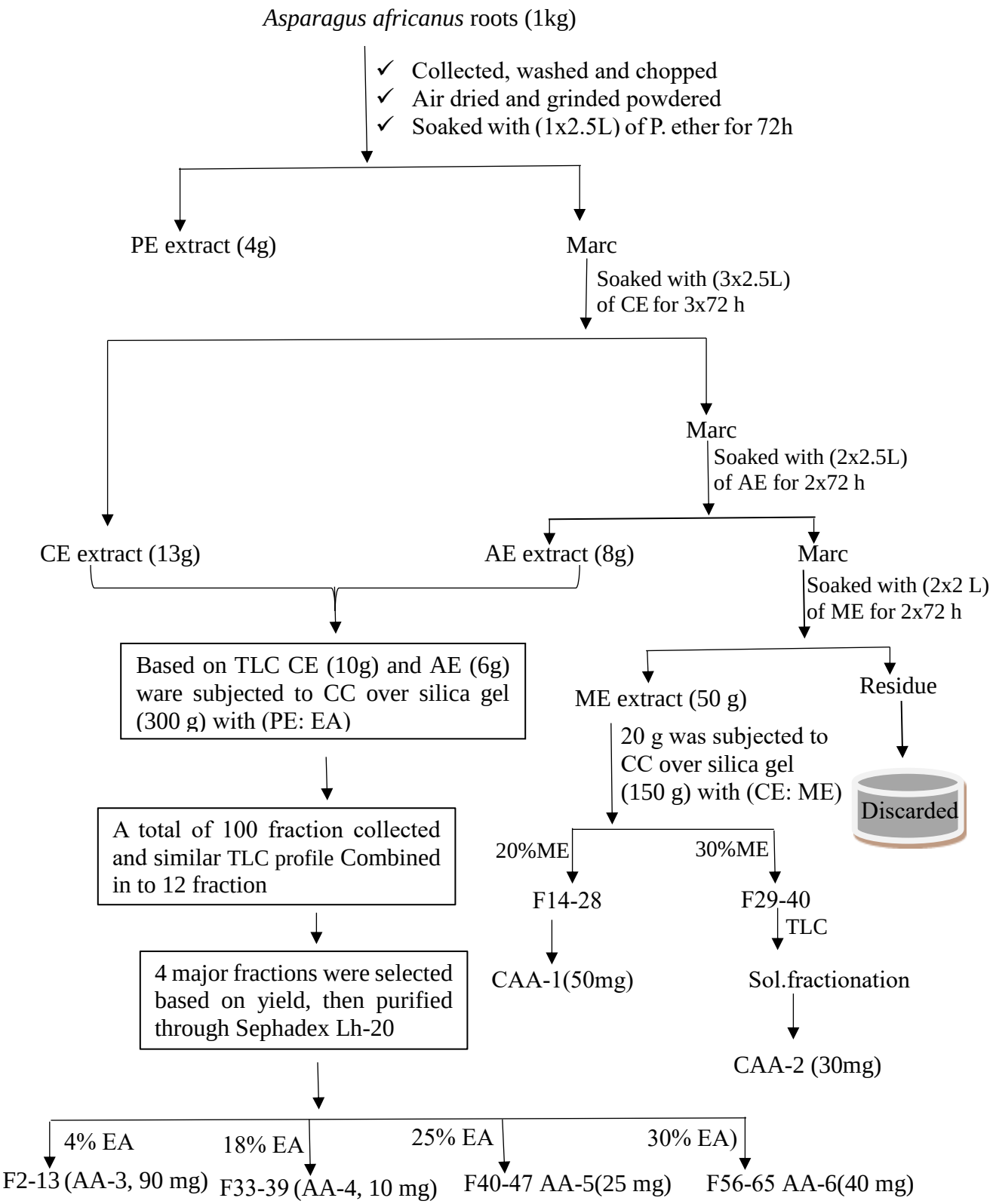


Figure 10. Extraction and isolation of compound from root crude extract of *A. africanus.* (PE= p. ether, CE= chloroform, AE= acetone, ME= methanol) extracts respectively.

3.8. Antimicrobial activity tests

The antimicrobial activity of all the solvent crude extracts and the isolated compounds were evaluated against four bacterial and one fungal strains using disk diffusion method.

3.8.1. Preparation of test solutions

The test solution of both plants extracts and isolated compounds were prepared individually by dissolving 2 g and 0.01 g respectively in 1 mL of dimethyl sulfoxide to prepare 200 and 10 mg/mL stock solution of the test samples respectively. The standard drug for antibacterial (Gentamicin 10 mg/mL) and antifungal (Clotrimazole 25 mg/mL) taste were used[8].

3.8.2. Test strains

Two gram negative bacteria (*Escherichia coli*, ATCC-25922 and *Salmonella thyphimurium,* ATCC-13311) and two gram positive bacteria (*Staphylococcus aureus*, ATCC-25923 and *Bacillus subtilus*, ATCC-6633) and fungal strains (*Candida albicans*, ATCC-14053) were used to carry the tests by disk diffusion method. The disc measuring (6 mm) diameter was prepared from Whatman No.1 filter paper sterilized by dry heat at 180 °C for 1h. The stock solution was prepared by dissolving the crude extracts and isolated compounds in 1mL DMSO. All the strains were obtained from Jimma University, Department of Biology (Microbiology) laboratory.

3.8.3. Antibacterial activity

Agar disk diffusion method was adopted to evaluate the antibacterial and antifungal activities of both crude extracts and isolated compounds. Briefly, the bacteria stock cultures were maintained on the nutrient agar slants which were stored at 4 ℃ in refrigerator until use. The nutrient agar 14 g dissolved in 0.5 L of distilled water. The culture media was boiled in distilled water to dissolve the media and autoclaved at 121°C for 2 h and poured into sterile Petri dishes. The nutrient agar 15 mL were poured in to petri dish and agar cultures of the test microorganisms were prepared according to manufacture instruction. Freshly, grown liquid culture of the test pathogen solution

of having similar turbidity with 0.5 McFarland were seeded over the Mueller-Hinton Agar medium with a sterile swab. Sterile Whatman filter paper discs (6 mm) were soaked with stock solution of plant extract and isolated compounds in DMSO at a concentration of (12.5, 25, 50, 100 and 200) and 10 mg/mL respectively, then placed over the seeded plates at equidistance. The plates were then inverted and incubated at 37 ℃ for 24 h. Gentamicin (10 mg/mL) and DMSO were taken as positive and negative control respectively[8,95]. The clear zones formed around each disc were measured in millimeter. The test assays were done twice for each treatment and control groups and results were presented as Mean ± SD with the help of Microsoft Excel 2013 version.

3.8.4. Antifungal activity

For the antifungal activity of both the crude extract and isolated compounds, similar method were adopted as antibacterial activity with some modification. In this activity the plates were incubated at 37 ℃ for 48 h and Clotrimazole (25 mg/mL) and DMSO were taken as positive and negative control respectively[96 78, 97].

3.9. Hepatoprotective activity test

3.9.1. Study animal

A total of twenty five male Swiss albino mice an average body weight of 38.5 g and 10-12 weeks were obtained from Tropical and Infectious Disease Research Center, Sokoru, Jimma, Ethiopia. They were brought to Postgraduate Biotechnology Laboratory College of Agriculture and Veterinary Medicine, Biotechnology laboratory and free to have access to food and distilled water in accordance with the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals[98]. The mice were allowed to share the same environmental condition and acclimatized for one week before the beginning of the experiment.

3.9.2. Treatment protocol

Twenty five male Swiss albino mice were divided randomly into five groups that contain five in each cage. Each mouse in the given group was differentiated by giving a number on its tail by permanent marker. At the beginning of experiment, mice body weight were measured.
Group **I**-(Normal control group) - were fed normal basal diet and which were received 1mL/kg of distilled water orally for a period of 28 days.

Group **II**-Representing animals (male albino mice) administered orally INH 75 mg/kg and RIF 150 mg/kg body mass by gavage for a period of 28 days.

Group **III**-(Positive control group) – representing animals (male albino mice) administered orally INH 75 mg/kg, RIF 150 mg/kg and Silymarin 100mg/kg body mass for a period of 28 days.

Group **IV-**(Treatment group one) representing the feed combination of basal diet *A. africanus* root extract at dose of 200 mg/Kg and INH 75 mg/kg and RIF 150 mg/kg for a period of 28 days.

Group **V-**(Treatment group two) - representing the feed combination of basal diet, *A. africanus* root extract at dose of 400 mg/Kg, INH 75 mg/kg and RIF 150 mg/kg.

All groups were fed the above mentioned diets daily for a period of 28 day. The isoniazid and rifampicin model were used for scheduling the regimen of dose 75 and 150 mg/kg body weight, respectively. The solutions were prepared separately in sterile distilled water once a day to induce liver damage[99]. The mice were treated with INH, co-administered with RIF for 28 days by oral route. For the hepatoprotective model 200 and 400 mg/kg per day of freshly prepared methanolic crude root extract of *A. africanus* homogenate along with isoniazid and rifampicin solution were administered orally and Silymarin was administered at a dose of 100 mg/kg. The dose of plant extract and the drug were calculated based on the body weight and it's administered orally.

3.9.3. Determination of serum biochemical parameters

The weight of the mice were taken at the interval of a week to control the body weight change in all groups of animals during the study period. At the end of the study period 2-3 mL of blood were taken from each mice through cardiac puncture after anesthetized with diethyl ether and the blood were collected by a plain tube containing serum separator tube (SST) gel by a trained laboratory technician. The serum was separated through centrifugation with a speed of 3000 rpm at room temperature for 10 minutes and put in ice box finally it was analyzed for liver function tests. The mice were sacrificed by cervical dislocation after the blood collected and the liver were taken from each mice, washed with normal saline and fixed in formalin for histopathology investigation[100].

3.9.4. Determination of serum ALT

The serum sample was added in buffered solution containing DL-alanine and α-ketoglutarate (pH = 7.4) and incubated for 30 min at 37 °C. Then, 1.0 mM DNPH was added followed by addition of 0.4 M NaOH and then absorbance was read by a spectrophotometer (UV-VIS 4000; ORI, Germany) at 500 nm. The enzyme activity was determined by a standard curve by using absorbance of test samples[99].

3.9.5. Determination of serum AST

The serum sample was added in buffered solution containing L-aspartate and α-oxaloglutarate (pH = 7.4) and incubated for 1 h at room temperature. Then, 0.1 mL of 3 mM nicotinamide adenine dinucleotide hydride (NADH) was added followed by addition of 0.1 mL of malate dehydrogenase (MDH). The absorbance was measured by a spectrophotometer (UV-VIS 4000; ORI, Germany) at 334 nm. The rate of absorbance is directly proportional to the AST activity that was determined by a standard curve samples[99].

3.9.5. Determination of serum ALP

0.5 mL of ALP substrate was put in test tubes and equilibrated for 3 min at 37 °C. At time interval, 0.05 mL of each standard, control and sample was added to respective test tubes, mixed gently and

then incubated for 10 min at 37 °C; then ALP (2.5 mL) was added and absorbance was read at 590 nm by a spectrophotometer (UV-VIS 4000; ORI, Germany)[99].

3.9.6. Determination of serum TB

0.1 mL of serum was added in 3.9 mL of 50% methanol, then 0.1 mL of diazo reagent was added and solution was mixed well. The reaction mixture was allowed to stand for 30 min at 25 °C and then absorbance was measured at 540 nm by a spectrophotometer (UV-VIS 4000; ORI, Germany) against reagent blank[101].

3.9.6. Determination of serum albumin

10 µL of the serum 1 mL of albumin reagent is added and incubated for 1 minute at 37 °C. The principle involved in this reaction is that the albumin binds with Bromocresol green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The Blue green color formed is proportional to the concentration of albumin[102].

3.10. Histopathological study

The hepatotoxicity effect of isoniazid and rifampicin with counter effect of root extracts of *A. africanus* were evaluated based on histopathological examination of the liver tissue. After the mice were sacrificed and dissection done from the neck to the pubis and the peritoneum was opened by using sterile scissor and part of the liver was excised and taken by blunt forceps to preserving tissue can which contain 10% of buffered neutral formalin. Then the tissue was taken out from the preservative and washed by ethanol subsequently with different /concentration starting from 70 to 95% and xylene solution was used for removing ethanol from the tissue and replace this ethanol with fluid that readily miscible with paraffin wax which enhance the tissue to embed easily with the wax to form tissue blocks. Then after, the tissue block was sectioned by microtome and that section was immersed in a water bath at 40 ºC and the unfolded section was taken and dried by putting in an oven at 56 ºC for 15 minutes. Light microscopic examination of liver was done after Hemotoxylin and Eosin (H & E) stain sections[103,104].

3.11. Data analysis procedures

Data were entered to the epi-data software version 3.1 and then exported to SPSS version 25.0 for the analysis expressed as Mean ± SD. Statistical data analysis were done using one-way ANOVA post-hoc multiple comparisons followed by Tuckey test (Annex-**18**) and $P < 0.05$ considered as statistically significant. The histopathological study were analyzed by pathologist and the results were presented by tables and figures. The result of antimicrobial were analyzed using Excel sheet and origin software version 8, then reported in the table and figure respectively.

3.12. Ethical considerations

The research was conducted after getting ethical approval obtained from the Ethical Review Committee, College of Natural Sciences Jimma University ref. no **RPG/67/2020**. Support letter to Department of Veterinary Sciences was obtained from Department of Chemistry. All experimental activities were carried out in accordance with the code of ethics of experimental animal which comply with scientific and ethical guidelines National Institutes of Health Guidelines NIH, 2016 and WHO, 2010.

4. Results and discussion

4.1. Percentage yield of the crude extracts

The root of *A. africanus* (1kg) was sequentially extracted with petroleum ether, chloroform, acetone and methanol by maceration (Figure **10**). The crude root extracts were weighted after completely removed the solvents and obtained as petroleum ether (4 g), chloroform (13 g), Acetone (8 g) and methanol (50 g), respectively. The percentage yield of crude extracts were calculated using (**Eq. 1**) and the obtained results and the physical appearance of the extracts are given in (Table **8**). As the polarity of extracting solvent increases, the mass (percentage yield) of the extract was increased except for the acetone extract. This result indicates the plant root is rich in polar compounds. Similar reports are also available for the same genus *A. racemosus* root extracts, which are rich in polar compounds[57,58].

Table 8. Percentage yields of *A. africanus* Lam. root extracts.

Solvent extraction	Crud extract (g)	Percentage yield (100%)	Color
Petroleum ether	4	0.4	Yellowish gum
Chloroform	13	1.30	Brown gum
Acetone	8	0.8	Brown gum
Methanol	50	5.0	Yellowish gum

4.2. Phytochemical screening of *A. africanus* root crude extracts

This study showed that petroleum ether, chloroform, acetone and methanolic extracts of the root *A. africanus* have a range of secondary metabolite such as saponin, steroids, phenol, terpenoids, flavonoids, quinones, alkaloids, glycosides and tannins (Table **9**). The methanolic extract contains tannins, terpenoids, saponin, flavonoids, phenols, alkaloids, steroids and glycoside. Whereas saponin, terpenoids and tannin were present in all crude extracts. The acetone extract was also showed a positive result for phenol, terpenoids and tannin tests with strong intense color, but negative results were obtained for quinones and steroids tests. The results suggested that the methanol and acetone are suitable solvents for extraction of polar secondary metabolites from

plants.

Table 9. Phytochemical screening of crude root extract of *A. africanus* Lam.

No	Phytochemicals	Test	Color expected	Crude extracts			
				PE	CE	AE	ME
1	Alkaloid	Wagner test	Reddish brown	-	-	+	+
2	Phenol	Ferric chloride test	Deep blue or black	-	+	+ +	++
3	Flavonoids	Sodium hydroxide test	Yellow to colorless	-	++	+	++
4	Saponin	Foam test	persistent foam	+	+	+	++
5	Terpenoids	Salkowski test	reddish brown ppt	+	++	++	+
6	Quinone	Hydrochloric acid test	yellow ppt	-	+	-	-
7	Steroid	Liebermann's Burchard test	dark pink or red	-	+	-	++
8	Glycoside	Sodium hydroxide test	yellow	-	-	+	++
9	Tannin	Braymer's test	Blue or greenish	+	+	++	++

Key: (PE: petroleum ether, CE: chloroform, AE: acetone, ME: methanol) extracts, - = absence, +: presence, ++ = present in high concentration.

In general, the various solvent crude extracts of the root of *A. africanus* have shown rich in secondary metabolites and may contain potential candidate for different biological and pharmacological activity. The study reported by Sahalie, N and his co workers[105], indicated that the phytochemicals; tannin, terpenoids and saponin are potential candidates for antimicrobial, anthelmintic and antidiarrheal activities. The phytochemical results of the plant root extracts may have promising medicinal applications since tannin, terpenoids, saponin, phenols, and flavonoid are among the major phytochemicals of the plant root extracts. Similar results were reported by Al-snafi and its co-workers[29], that steroidal saponin is the major bioactive component of *Asparagus* species. Saponins have therapeutic applications including antimicrobial, insecticidal, hepatoprotective, anti-inflammatory and decrease cholesterol levels[106]. Terpenoids, phenolic compounds, flavonoid and tannin have a broader biological activity[107]. In the same genus *A. albus* L. leaves and *A. racemosus* root extracts were showed remarkable hepatoprotective and antioxidant activities which can be described to the high content polyphenols, flavonoids and condensed tannin of the extracts[10, 103].

4.3. Structural elucidation of the isolated compounds

A total of six compounds were isolated, of which only two compounds (**AA-5** and **AA-6**) were characterized by ^{1}H and ^{13}C NMR spectroscopy. The remaining four compounds (**AA-1**, **AA-2**, **AA-3** and **AA-4**) were not characterized due to budget and time constraints. The chemical shifts are given in ppm (δ) and were referenced relative to $CDCl_3$ (δ_H 7.28 and δ_C 76-77) ppm for ^{1}H and ^{13}C-NMR spectra, respectively. By comparing the observed spectra with the recorded data of those compounds in the literature, the structural elucidation was performed. The physical data of all the isolated compounds were summarized on the table **10** below.

Table 10. Some of the physical properties of the isolated compounds

P. properties	**AA-1**	**AA-2**	**AA-3**	**AA-4**	**AA-5**	**AA-6**
Color	White amorphous solid	Yellow Oily	White amorphous solid	Yellowish crystal	White crystal solid	yellowish gum
Mass	50 mg	30 mg	90 mg	10 mg	25 mg	40 mg
Fraction no	23-28	29-40	2-13	33-39	40-47	1- 20
Ratio	20% ME	30% ME	4% EA	18% EA	25% EA	30% EA
Mpt	264 -265 ºC	-	273-275 °C	-	-	-
Solubility	Methanol	Methanol	Chloroform	Acetone	Chlorofor	Chlorofor
Rf. Value	0.75	0.70	0.52	0.50	0.40	0.45

4.3.1. Characterization of compound AA-5

Compound **AA-5** was isolated as a white crystalline solid (25 mg) with an Rf value of 0.40 (25% ethyl acetate in petroleum ether as an eluent). The ^{1}HNMR spectrum of compound **AA-5** showed methyl proton peaks at δ_H 0.69 (3H, CH_3-18), 0.96 (3H, CH_3-19), 1.02 (3H, CH_3-21), 0.84 (3H, CH_3-26), 0.71 (3H, CH_3-27), 0.82 (3H, CH_3-29), respectively **(**Appendix **4)**. The peaks at δ_H1.04, 1.85 (2H, H-1), 1.52, 1.82 (2H, H-2), 2.30, 2.25 (2H, H-4), 1.52, 2.01 (2H, H-7), 1.30, 1.52 (2H, H-11), 1.17, 2.01 (2H, H-12), 1.09, 1.52 (2H, H-15), 1.27, 1.84 (2H, H-16), 1.17, 1.30 (2H, H-28) are corresponding to CH_2 groups. The peaks at δ_H 1.88(1H, *m*, C-8), 0.93 (1H, *m*, C-9), 1.10 (1H, *m*, C-14), 1.13 (1H, *m*, C-20), 1.52(1H, *m*, C-24) and 1.84 (1H, *m*, C-25). The peak at δ_H3.53

indicate proton at C-3 attached to hydroxyl group (i.e., 1H, OH on C-3) of sterol moiety.[108] The ^{1}H NMR spectra also showed a downfield signals at δ_H5.36 (1H, *d*, *J*=4.0 Hz, H-6), 5.15 (1H, *dd, J*=8.0, 16.0 Hz, H-22) and 5.01(1H, *dd*, *J*=8.0, 16.0 Hz, H-23) correspond to olefienic protons of stigmasterol at C-6, C-22 and C-23, respectively[108, 109]. The ^{13}C NMR spectra of compound **AA-5** showed a total of 29 carbons signal in the structure (Table **11**, Appendix **5**). The peaks related to six methyl, nine methylene, eleven methine and three quaternary carbons atoms. The peaks at δ_C140.7 (C-5), 121.7 (C-6), 138.3 (C-22) and 129.3(C-23) in the ^{13}C NMR spectrum are corresponding to olefienic carbon[47]. A peak at δ_C71.8 (C-3) indicated the electro negative atom (oxygen) bonded to carbon and its characteristics signals for sterol[109, 110]. The peaks at δ_C (37.3 (C-1), 31.6 (C-2), 42.3 (C-4), 21.2 (C-11), 39.8 (C-12), 24.4 (C-15), 29.0 (C-16) and 25.4 (C-28) represent the methylene (CH_2) groups of stigmasterol[108]. The four downfield signals at δ_C (56.8, 56.0, 51.3 and 50.1) indicates the CH group of C-14, C-17, C-24 and C-9, respectively. Also the peaks at δ_C (29.7, 31.9 and 40.5) are CH groups and assigned to C-25, C-8 and C-20 respectively. The ^{13}C NMR spectrum has also showed the presence of three quaternary signal at δ_C140.7 (C-5), 36.5 (C-10) and 42.2 (C-13) (Table **11**). There are peaks at δ_C 12.3 (C-18), 19.4 (C-19), 22.7 (C-21), 21.1(C-26), 19.0 (C-27) and 12.1(C-28) that correspond to signals of CH_3 groups of stigmasterol[110]. Similarly, stigmasterol also isolated and reported in the same genus *A. racemosus* (**14**)[47]. The ^{1}H and ^{13}C NMR spectral data of compound **AA-5** (Table **11**) is very similar to the spectral data of stigmasterol[108,109], which supports the proposed structure for the isolated compound **AA-5** with the molecular formula of $C_{29}H_{48}O$ and the index of hydrogen deficiency become six (Figure **11**).

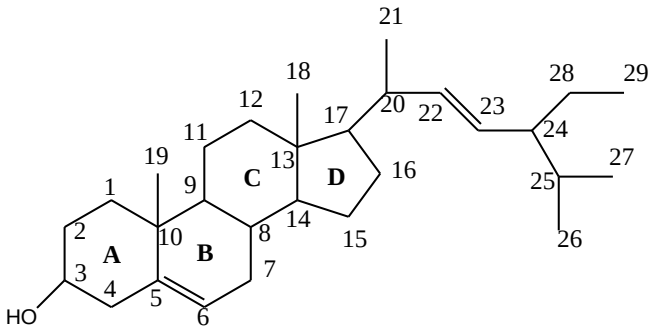


Figure 11. Proposed Structure of compound **AA-5 (**Stigmasterol **16**)

Table 11. Comparison of ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compound **AA-5** with reported data of stigmasterol (500 MHz) (in $CDCl_3$).

	Compound AA-5		**Stigmasterol**[109, 110]		
Position	**δ_H ppm (*m*, *J* in Hz)**	**δ_C ppm**	**δ_H ppm (*m*, *J* in Hz)**	**δ_C ppm**	**M**
1	1.04, 1.85 (2H, *m*)	37.3	1.04, 1.85(2H, *m*)	37.3	CH_2
2	1.52, 1.82 (2H, *m)*	31.6	1.50, 1.84(2H, *m*)	31.7	CH_2
3	3.53 (1H, *m*)	71.8	3.52 (1H, *m*)	71.8	CH
4	2.30, 2.25 (2H, *m)*	42.3	2.34, 2.28 (2H, *m*)	42.3	CH_2
5	-	140.7	-	140.8	C
6	5.36 (1H, *d*, 4.0)	121.7	5.35 (1H, *brd,* 4.72)	121.7	CH
7	1.52, 2.01 (2H, *m*)	31.6	1.53, 1.99 (2H, *m*)	31.9	CH_2
8	1.88(1H, *m*)	31.9	1.99(1H, *m*)	31.9	CH
9	0.93(1H, *m*)	50.1	0.92 (1H,*m*)	50.1	CH
10	-	36.5	-	36.5	C
11	1.30, 1.52 (2H, *m*)	21.2	1.54 (2H, *m*)	21.1	CH_2
12	1.17, 2.01 (2H,*m*)	39.8	1.18, 2.0 (2H, *m*)	39.8	CH_2
13	-	42.2	-	42.2	C
14	1.10 (1H, *m*)	56.8	1.11 (1H, *m*)	56.9	CH
15	1.09, 1.52 (2H, *m*)	24.4	1.07, 1.51 (2H, *m)*	24.4	CH_2
16	1.27, 1.84 (2H, *m*)	29.0	1.28, 1.85 (2H, *m*)	28.9	CH_2
17	1.13 (1H, *m*)	56.0	1.13 (1H, *m*)	56.0	CH
18	0.69 (3H, *s*)	12.3	0.69(3H, *s*)	12.5	CH_3
19	0.96, (3H, *s*)	19.4	1.01 (1H, *s*)	19.4	CH_3
20	1.30, (1H *m*)	40.5	1.41(1H, *m*)	40.5	CH
21	1.02 (3H, *d*, 8.0)	22.7	1.02 (3H, *d*, 7.72)	21.2	CH_3
22	5.15 (1H, *dd*, 8.0, 16.0)	138.3	5.16 (1H, *dd*, 8.5, 15.1)	138.3	CH
23	5.01 (1H, *dd*, 8.0,16.0)	129.3	5.01 (1H, *dd*, 8.6, 15.1)	129.3	CH
24	1.52 (1H, *m*)	51.3	1.54(1H, *m*)	51.2	CH
25	1.84 (1H, *m*)	29.7	1.83(1H, *m*)	31.9	CH
26	0.84(3H, *d*, 8.0)	21.1	0.83 (3H, *d*, 7.32)	21.1	CH_3
27	0.71(3H, *d*, 8.0)	19.0	0.79 (3H, *d*, 6.92)	19.0	CH_3
28	1.17, 1.30 (2H, *m*)	25.4	1.16, 1.43 (2H, *d,* 3.8)	25.4	CH_2
29	0.82(3H, *t,* 8.0)	12.1	0.80 (3H, *t*, 7.1)	12.3	CH_3

s= singlet, *d*, doublet, *m*, multiplet, M, multiplicity

4.3.2. Characterization of compound AA-6

Compound **AA-6** was isolated as yellowish gum (40 mg) with an Rf value of 0.45 with 30% ethyl acetate in petroleum ether as an eluent. ^{1}HNMR spectrum showed signals for fourteen protons (Table **12**, Appendix **6**), where eight of them are aromatic protons of AA′BB′ system at δ_H 7.14 (2H, *d*, 8.0 Hz, H-2', H-6'), 7.10 (2H, *d*, 8.0 Hz, H-2", H-6"), 6.81(2H, *m,* H-3', H-5') and 6.80 (2H, *m*, H-3", H-5"), suggesting the presence of two para-*di* substituted benzene rings in the structure of the compound[111]. The peak at δ_H 4.51(1H, *dd*, 10.0, 5.8 Hz), 5.63(1H, *dd*, 12.0, 8.0 Hz), 6.00(1H, *m*, 16.0, 12.0 Hz) and 6.46(1H, *d,* 12.0 Hz) are the CH group of H-3, H-2, H-4 and H-1, respectively. The peak at δ_H 5.15 (2H, *m*) indicate the CH_2 group at H-5 (Table **12**, Appendix **7**). The ^{13}C NMR spectrum displayed **13** carbon signals, where the two carbon signals at δ_C (156.4 and 155.82) indicate the presence of two quaternary carbons attached to the electronegative atom oxygen at (C-4' and C-4") (Table **12**, Appendix **7**). Four carbon signals at δ_C 131.4 (C-2', C-6'), 128.8 (C-2", C-6"), 115.6 (C-3", C-5") and 115.4 (C-3', C-5') CH appeared to be of double intensity[112,113]. The peak at δ_C 47.2 indicates CH bonded to aromatic ring (C-3). There are additional two quaternary carbons at δ_C 134.5 (C-1") and 130.1 (C-1') (Appendix **7**). A peak at δ_C114.4 indicates CH_2 of (C-5). The DEPT-135 spectra indicated that the presence of one CH_2 group at δ_C114.4 and eight CH groups at δ_C(128.7 (C-1), 130.1 (C-2), 47.2 (C-3), 141.4 (C-4), 131.4 (C-2',6'), 115.4 (C-3',5'), 128.8 (C-2",6"), 115.6 (C-3",5")) (Appendix **8**). Based on these spectroscopic data, compound **AA-6** was identified as 4-((Z)-1-(4-hydroxyphenyl) penta-1, 4-dien-3-yl) phenol, trivial name nyasol, previously reported from root of *A. cochinchinensis*[111]. (Figure **12**)

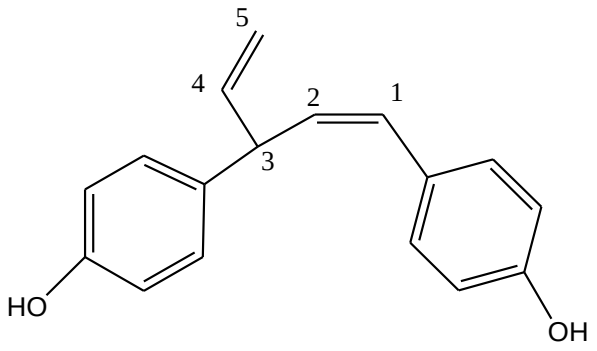


Figure 12. Proposed structure of compound **AA-6 (**Nyasol-**30)**

Table 12. Comparison of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **AA- 6** with reported data (500 MHz) (in $CDCl_3$).

	Compound AA-6			**Nyasol**[111, 113]		
Position	**δ_H ppm (*m*, *J* in Hz)**	**δ_C ppm**	**δ ppm DEPT-135**	**δ_H ppm (*m*, *J* in Hz)**	**δ_C ppm**	**M**
1	6.46(1H, *d,* 12.0)	128.7	128.7	6.54(1H, *d*, 12.0)	128.6	CH
2	5.63(1H, *dd*, 12.0, 8.0)	130.1	131.4	5.70(1H,*dd*, 11.5, 10)	131.7	CH
3	4.51(1H,*dd*,10.0, 5.8)	47.2	47.2	4.51(1H,*dd*, 10, 5.8)	46.8	CH
4	6.00(1H, *ddd*, 16.0,12.0, 4.0)	141.4	141.4	6.03 (1H, *ddd*,17.0, 10.8, 6)	140.7	CH
5	5.15(2H, *m*)	114.4	114.4	5.15(2H, *m*)	115.0	CH2
1'	-	130.1	-	-	129.8	C
2', 6'	7.14(2H, *d*, 8.0)	131.4	131.4	7.19 (2H, *d*, 8)	130.0	CH
3', 5'	6.81(2H, *m*)	115.4	115.4	6.81(2H, *d*, 8)	115.1	CH
4'	-	156.4	-	-	154.6	C
1"	-	134.5	-	-	135.5	C
2", 6"	7.10(2H, *d*, 8.0)	128.8	128.8	7.12 (2H, *m*)	128.9	CH
3", 5"	6.80(2H, *m*)	115.6	115.6	6.79 (2H, *m*)	115.4	CH
4"	-	155.8	-	154.1	-	C

s= singlet, *d*, doublet, *m*, multiplet, M, multiplicity

The compound have one chirality center and have two stereo isomer, but the genus *Asparagus produced only the cis isomer and* *J* value of the 1, 2-double bond in the 1, 4-pentadiene system at δ_H 6.46 Hz (H-1) and 5.63 Hz (H-2) is 12.0 Hz this is the lower *J* value of trans isomer, but indicate the highest *J* value of a cis-stereoisomer (Figure **12**). The genus Asparagus produce the cis isomer[62] and similar compound were isolated from the same genus *A. cochinchinensis* with the *J* value of 12 MHz and the cis isomer was reported as the structure of the compound[111,112].

4.4. Antimicrobial activity of crude extracts and isolated compounds from *A. africanus*

4.4.1. Antimicrobial activity of the crude extracts

Various solvent extracts, such as petroleum ether, chloroform, acetone and methanol were tested for antimicrobial activity against four bacterial (*S. aureus, S. thyphimurium, E. coli, B. subtilis*) and one fungal (*C. albicans*) strains using a disk diffusion method. The inhibition zone of the extracts was compared with standard positive control Gentamycin for antibacterial and Clotrimazole for antifungal activity. All the crude extracts were showed a promising activity against all tested bacterial and fungal strains. The zone of growth inhibition for all the crude extracts against bacterial strains and fungal strains are described in (Table **13**, Appendix **11**).

Table 13. Zone of inhibition of the crude root extracts of *A. africanus* Lam.

Zone of inhibition (in mm) Mean ±SD						
		Gram (-ve) bacteria		**Gram (+ve) bacteria**		**Fungus**
C. extract	Coc.(mg/mL)	*E.coli*	*S. typhi*	*S. aureus*	*B. subtilis*	*C. albicans*
PE	200	7.0±0.0	8.5±0.5	7.5±0.5	9.50±0.5	7.0±0.0
CE	200	8.0±0.0	7.50±0.5	7.0±0.0	7.5±0.5	9.0±2.0
AE	200	12.5±0.9	8.50±0.5	7.5±0.5	11.8±0.8	11.8±0.7
ME	200	12.5±0.7	10.0±0.8	10.0±1.0	11.8±0.8	12.5±1.0
G	10	20.5±0.5	17±0.5	20.0±1.0	19.0±1.0	-
C	25	-	-	-	-	13.0±0.8
DMSO	1(mL/disc)	NI	NI	NI	NI	NI

***Key**:* NI= No inhibition, DMSO = Dimethyl sulfoxide G= Gentamycin C=Clotrimazole, PE= Petroleum ether, CE= Chloroform, AE=Acetone, ME=Methanol.

The petroleum ether and chloroform extracts showed lower activity against all the tested strains compared to the acetone and methanol extracts, this indicated that the bioactive phytochemicals present in these solvent extracts are in lesser amount. For petroleum ether extract the maximum zone of inhibition was observed against *B. subtilus* (9.50±0.5 mm) and for the chloroform extract the maximum zone of inhibition was observed on fungal strain *C. albicans* (9.0±2.0 mm). The acetone extract showed a greater zone of inhibition against *E. coli* (12.5±0.9 mm), *B. Subtilus* (11.8±0.8 mm) and *C. albicans* (11. 8±0.7 mm) respectively. The methanolic extract also exhibited

the highest zone of inhibition against *E. coli* (12.5±0.7mm), *B. Subtilus* (11.8±0.8mm) and *C. albicans* (12.5±1.0). The result were compared with the positive standard Gentamycin for *E. coli* (20.5±0.5 mm), *B. Subtilis* (19.0±1.0 mm) and Clotrimazole for *C. albicans* (13.0±0.8 mm), this result indicated that the presence of more bioactive phytochemicals in this solvent extract. Similar outcomes were found on the same genus *A. racemosus* methanolic extract has shown the maximum zone of inhibition against *E. coli* and for the acetone extract the maximum zone of inhibition against *B. subtilis*[114,115].

4.4.1.1. Dose dependent effect of methanolic and acetone extracts

Based on the activity of the solvent crude root extracts, methanol and acetone extracts were evaluated to compare the efficiency of the plant extract with various concentrations (100, 50, 25 and 12.5 mg/mL) against two bacterial (*E. coli* and *B. Subtilus*) and one fungal (*C. albicans*) strains. In comparison, acetone extract was displayed a significant antimicrobial activity than methanolic extract after 200 mg/mL. Various concentrations of the methanolic and acetone extract results were depicted in the following (Figure **13**, Appendix **13)** below.

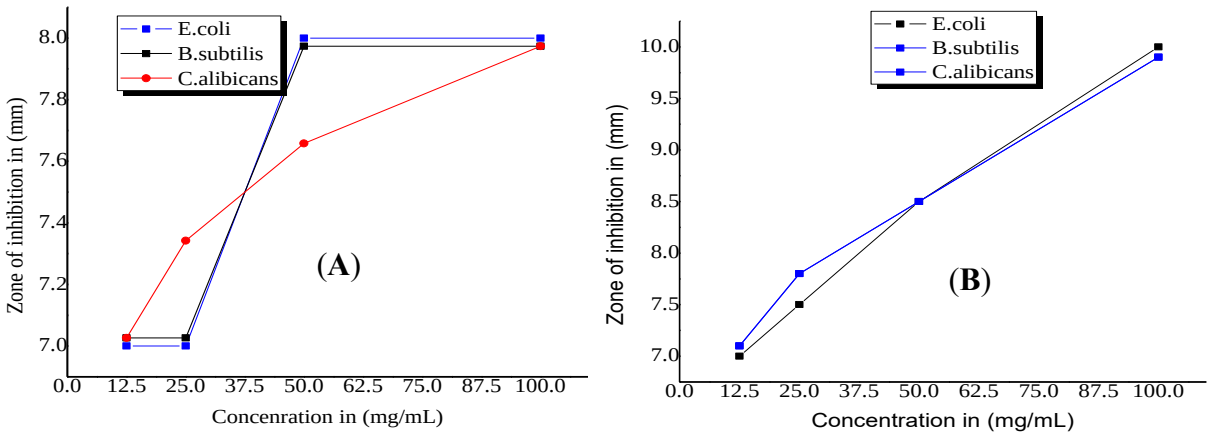


Figure 13. Effect of methanolic (**A**) and acetone (**B**) extract in various concentration

Decrease the concentration of crude extract decrease the zone of inhibition in all the tested strain, but after 100 mg/mL the difference is not significant, this indicated that the higher dose of the plant extract was needed to get the promising zone of inhibition against the tested bacterial and fungal strains. The methanolic extract at a concentration of 100 mg/mL was showed a zone of inhibition

of 8.0±1.0 mm against *E.coli*, but a similar zone of inhibition was observed against *B. subtilus* and *C. albicans* 8.5±0.5 mm. The acetone extract also showed a marginal zone of inhibition against *E.coli* (10.0±1.0), *B. subtilus* (9.0±0.0) and *C.albicanus* (9.0±0.0) at a concentration of 100 m/mL. On the other hand, a smaller dose of methanolic and acetone extracts, showed a little zone of inhibition, this indicated that the MIC of the plant extracts less than 12 mg/mL against *E.coli, B. subtilus* and *C. albicans*. The differences in antimicrobial activity of medicinal plants are obviously related to differences in their active components of phytochemicals. The recent study reported by Mahomoodally, M and its co workers[95], tends to show that phenolic compounds such as flavonoids are responsible compounds for the antimicrobial activities in higher plants. Moreover, it is also claimed that secondary metabolites such as tannins, saponin, terpenoids, steroids and other compounds of phenolic nature are classified as active antimicrobial compounds[116]. Fortunately, phytochemicals screening of this study revealed that the plants possess at least four of the following classes of secondary metabolites: tannins, phenols, flavonoids and saponin. Therefore, in this study the presence of those phytochemicals could to some extent justify the observed antimicrobial activities.

4.4.2. Antimicrobial activities of isolated compounds

In vitro antimicrobial activity tests for the isolated compounds similar procedures were adopted as crude extracts. Some of the isolated compounds were showed a promising activity against all tested bacterial and fungal strains and zone of growth inhibition described in (Table **14**, Appendix **12**). Compound **AA-1** were lower activity against all the tested bacterial and fungal strains with the inhibition zone of 7.0 mm while, compound **AA-2** and **AA-4** were marginal zone of inhibition against all bacterial and fungal strains. Compound **AA-2** have a better activity against, *S. thyphimurium, B. Subtilis* and *C. albicans* with similar zone of inhibition 11.0 mm. compound **AA-3** was little or no inhibitory activity against all the tested strains except on the *B. subtilus* (8.0 mm). However compound **AA-4** showed marginal zone of inhibition against *E.coli*, *S. thyphimurium*, *S. aureus*, *B. subtilus* and *C.albicanus* with the zone of inhibition were 16.0, 13.0, 14.0, 15.0 and 16.0 mm compared with the reference drug, Gentamycin 22.0, 19.0, 20.0, 20.0 and 13.0 mm respectively and also have marginal zone of inhibition compared to crude extract against all the tested strains.

Table 14. Zone of inhibition of the isolated compounds from *A. africanus* root extract.

	Zone of inhibition (in mm)				
	Gram (-ve) bacteria		**Gram (+ve) bacteria**		**Fungus**
Compound(10mg/mL)	*E. Coli*	*S. typhi*	*B. subtilis*	*S. aureus*	*C. albicans*
AA-1	7.0	7.0	7.0	7.0	7.0
AA-2	10.0	11.0	11.0	8.0	11.0
AA-3	7.0	7.0	8.0	NI	NI
AA-4	16.0	13.0	14.0	15.0	16.0
AA-5	8.0	9.0	7.0	8.0	10.0
AA-6	8.0	8.0	7.0	7.0	8.0
G	22.0	19.0	20.0	20.0	-
C (25 mg/mL)	-	-	-	-	13.0
DMSO(1mL/disc)	NI	NI	NI	NI	NI

Key: NI= No inhibition, DMSO = Dimethyl sulfoxide G= Gentamycin C=Clotrimazole, **AA-1-AA-6** = Isolated compound **1-6** respectively.

This result indicated that the plant root have a bioactive metabolites used as antimicrobial drug development. Compound **AA-5** (stigmasterol) showed a marginal zone of inhibition against *E. coli, S. thyphimurium, B. Subtilis, S. aureus* and *C. albicans* with the inhibition zone of the bacterial and fungal strains were 8.0, 9.0, 7.0, 8.0 and 10.0 mm respectively (Table **14**). The present study consistent with the previous report that, stigmasterol (50 mg/mL) showed moderate antibacterial activities against three tested bacterial strains (*S. aureus*, 12.0 mm, *P. aeruginosa*, 11.0 mm and *S. thyphimurium*, 13.0 mm)[117]. The difference in zone of inhibition may be due to dose variation. In addition to this, compound **AA-6** was also having a moderate zone of inhibition against *E. coli* (8.0 mm), *S. thyphimurium* (8.0 mm), *B. Subtilis* (7.0 mm), *S. aureus* (7.0 mm) and *C. albicans* (8.0 mm) compared to the positive standard control Gentamicin (22.0 mm) for *E. coli* and *S. thyphimurium* and (20.0 mm) for *S. aureus* and *B. Subtilis*, but for the fungal strain (*C. albicans*) positive standard Clotrimazole was (13.0 mm). There is a disagreement with the literature reported by[112], that nyasol have anti-Oomycete activity, effectively inhibited the mycelial growth in a range of (1-50 µg/mL), but did not affect the growth of bacteria and yeast, this may due to the dose variation. The crude extracts have good antimicrobial activity compared to the isolated compounds, this may be due to the antagonistic activity of different phytochemical present in it, but compound **AA-4** and **AA-2** are highest zone of inhibition compared to all the isolated compounds and crude extracts.

4.5. Hepatoprotective effect of crude extract

After calculation of the percentage yield and phytochemical analysis, the methanolic extract was selected for the hepatoprotective activity against INH and RIF drug induced hepatotoxicity. The main objective of this study with related to experimental animal was that, methanolic extract 200 mg/kg and 400 mg/kg dose administration in male Swiss albino mice were associated with decreasing the incidence of antitubercular drug hepatotoxicity.

4.6. Effect of methanolic extract on liver and body weight

4.6.1. Effect of crude extract on liver weight

After dissecting the mice, the liver weight of each mice were evaluated. The average liver weight of mice between the groups there is no significant difference, but insignificantly elevated the mean liver weight compared to the control group-I. In this study isoniazide and rifampicin treated mice did not show any significant reduction either in body or in liver weight compared between the groups **(**Figure **14**).

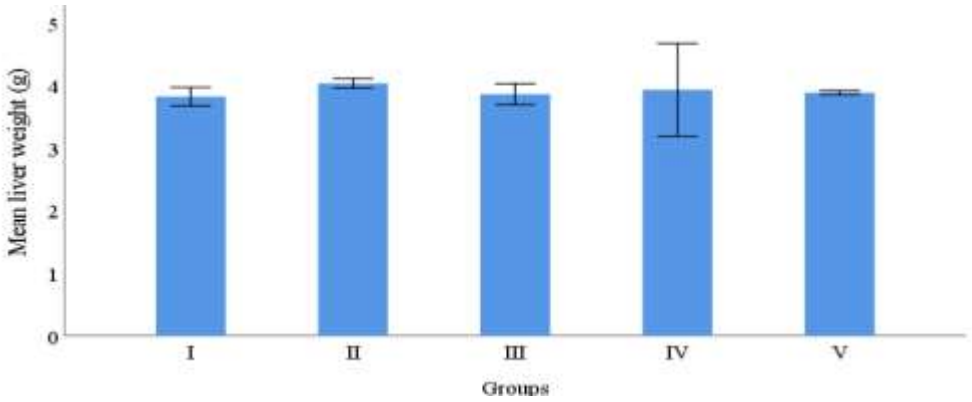


Figure 14. Effect of methanolic extract on the liver weight

In general mean liver weight of group-II (4.04±0.04)>group-IV (3.93±0.37)>group-V (3.89±0.02) > group-III (3.86±0.08)> group-I (3.83±0.1) (P >0.05), this indicate that there is no fat accumulation either drug induced or plant extract treated mice liver. Similar study were reported by[118], that INH and RIF treated animals did not show any significant reduction either in body or in relative liver

weight. Also the mice treated with the plant extract and the reference drug (Silymarin) statistically not significant compared to the normal control group and the toxicant control. Supplementation of carotenoids and garlic have no effect on the body or liver weight of animals with oral administration of isoniazide and rifampicin induced hepatotoxicity[118,119].

4.6.2. Effect of methanolic extract on change in body weight

The mean body weight of each mice were taken within the interval of a weak to prepare the dose of plant extract and toxicant drug (Figure **15**). The mean body weight change of all mice in each weak between the groups were statistically not significant at P>0.05, but the body weight of all mice in the groups were insignificantly elevated from initial body weight to weak-1 and weak-2. After weak-2 the body weight of all mice were increase rapidly. This indicate that, the mice may adapt the drug and the body weight become increase.

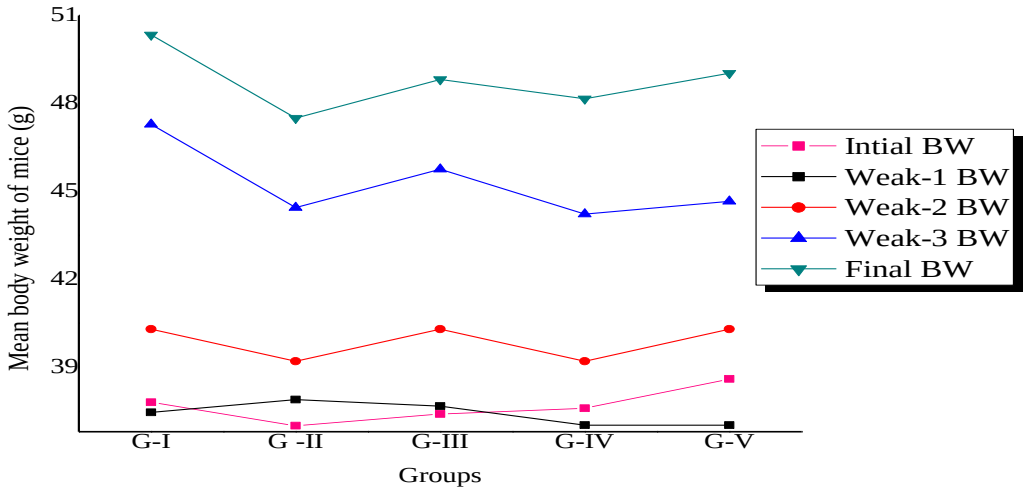


Figure 15. Effect of the methanolic extract on mean body weight change.

The results expressed as mean ± SD. Analyzed by Analysis of variance (ANOVA) followed by Tukey test. n=5, BW=body weight. The result are statistically significant (P < 0.05).

In this study there is a parallel increments of body weight observed either antitubercular drug induced mice or the normal control groups, this indicate that the INH and RIF doesn't affect the food intake of the mice. There were no treatment related mortality, abnormal clinical signs and

remarkable body weight changes with heptoplus to isoniazide and rifampicin induced liver damage in rat[73,119].

4.7. Effect of methanolic extract on liver biomarkers

In this study all the liver biochemical parameter of normal control and the reference standard statistically insignificant (P>0.05). Alanine amino transferase (ALT) is an enzyme found mainly in the liver that is released into the blood stream when the liver is inflamed[120, 11]. The serum ALT level of group-II (118.16±0.34 IU/L) were significantly increase than group-I (41.60±2.63IU/L), but there is no significant difference between group-III (43.10±0.96 IU/L) and group-V (45.84±2.02) compared to the control group (P>0.05). Group-IV (64.5±3.7 IU/L) and group-V (45.8±2.02) decrease significantly the serum ALT level compared to Group-II (118.16±0.34IU/L) (P<0.05). This result indicate, the mice induce hepatotoxicity and the plant extract maintain the elevation of ALT to ward normal control group (Table **15**).

Table 15. Effect of oral administration of methanolic crude root extract of *A.africanus* on serum enzymes level.

		Liver biomarkers	
Groups	**ALT(U/L)**	**AST(U/L)**	**ALP(U/L)**
Group-I	41.60±2.63	55.90±2.60	113.80±6.00
Group-II	118.16±0.34[a]	167.36±4.31[a]	239.20±3.68[a]
Group-III	43.10±0.96[b]	58.00±2.53[bc]	116.90±1.86[b]
Group-IV	64.50±3.70[ab]	100.84±2.40[ab]	199.84±12.70[ab]
Group-V	45.84±2.02[b]	97.42±1.93[ab]	134.74±3.84[ab]

The results were expressed as mean ± SD. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey multiple comparison test, n=5. a= significant different compared to Group-I, b= significant different compared to Group-II, (P < 0.05) statistically significant.

The greater dose 400 mg/kg oral administration of methanolic extract was significantly decrease the serum ALT level than 200 mg/kg (the lower dose) indication of the hepatoprotective effect of the plant and with dose dependent manner. AST of Group-II (167.36±4.31 IU/L) were significantly elevate the serum AST level compared to group-I (55.90±2.60 IU/L) (P<0.05). There is no

significant difference between the serum AST level of group-III (58.00±2.53 IU/L) and group-I (55.90±2.60 IU/L) (P>0.05) also group-IV (100.84±2.40 IU/L) and group-V (97.42±1.93 IU/L) were statistically not significant (P>0.05), but both are significantly decrease the serum AST level of mice compared to group-II (167.36±4.31 IU/L) (P<0.05) (Table **15**). The root extract 200 and 400 mg/kg shows significantly decrease the serum AST level compared to group-II with dose independent manner. Our findings showed a significant increase in the level of serum ALT and AST of group-II antitubercular drugs administered mice as compared to group-I. This result is showed that methanolic extract a potential role to protect liver toxicity. Aspartate aminotransferase (AST) is found in high concentrations in liver, heart, skeletal muscle and kidney and increase AST level is often a sign of liver damage[74, 75]. The serum ALP level of group-II (239.20±3.68 IU/L) were significantly elevated compared to group-I (113.80±6.00 IU/L) (P<0.05). There is no significant difference between the serum ALP level of group-I (113.8±6.00 IU/L) and group-III (116.90±1.86 IU/L) (P>0.05). Group-IV (199.84±12.7 IU/L) and group-V (134.74±3.84 IU/L) have significantly decrease the serum ALP level compared to group-II (239.20±3.68 IU/L) and both are statistically significant at (P<0.05), but increase significantly compared to group-III (116.90±1.86 IU/L) (Table **15**). High values usually indicate that the bile is not flowing normally through the gall bladder and bile duct into the intestine and causes biliary cirrhosis, fatty liver and liver tumor[121].

4.8. Effect of methanolic extract on serum total bilirubin and Albumin

The serum albumin level of group-II (2.97±0.24 g/dL) was decrease significantly compared with group-I (4.42±0.48 g/dL) (P<0.05). Group-III (4.20±0.58 g/dL) and group-V (4.14±0.32g/dL) significantly increase the serum albumin compared with group-II (2.97 ±0.24 g/dL), but group-IV (3.48±0.24 g/dL) and group-II (2.97±0.24 g/dL) were statistically not significant P>0.05. There is no significant different between the two treatment groups (group-IV (3.48±0.24 g/dL) and group-V (4.14±0.32)) and the reference drug Silymarin (group-III (4.20±0.58 g/dL)) (P>0.05) described as follows in (Figure **16**). The plasma albumin level of group-II was significantly decrease compared to group-I, this indicate that INH and RIF reduce the level of synthesis albumin in the liver, but group-V increase the albumin concentration on the plasma near to the normal control group of mice and there is insignificant difference with the reference drug (group-III). Those

results showed that the methanolic extract was a probably safe and non-toxic. The amount of albumin in the plasma treated with the smaller dose of the plant extract is increase compared with group-II, but statistically not significant (P>0.05) (Figure **16**).

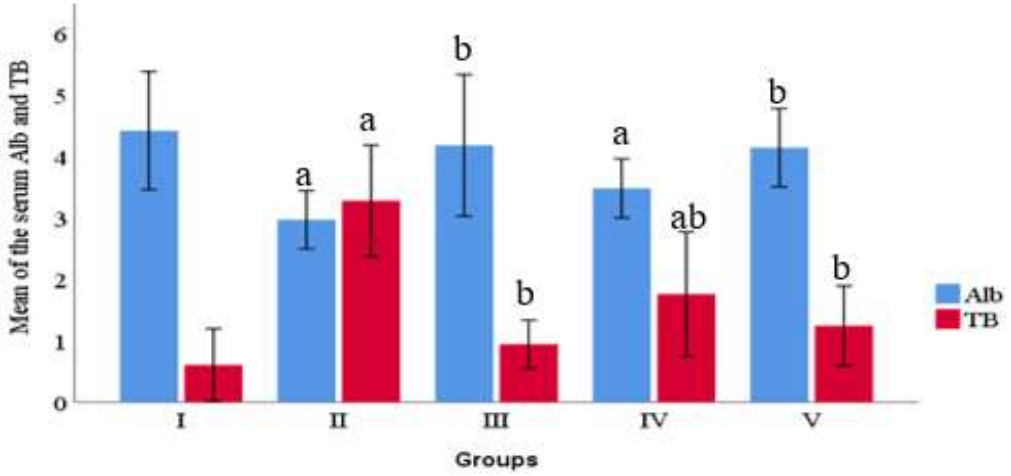


Figure 16. Effect of methanolic extract on serum total bilirubin and albumin

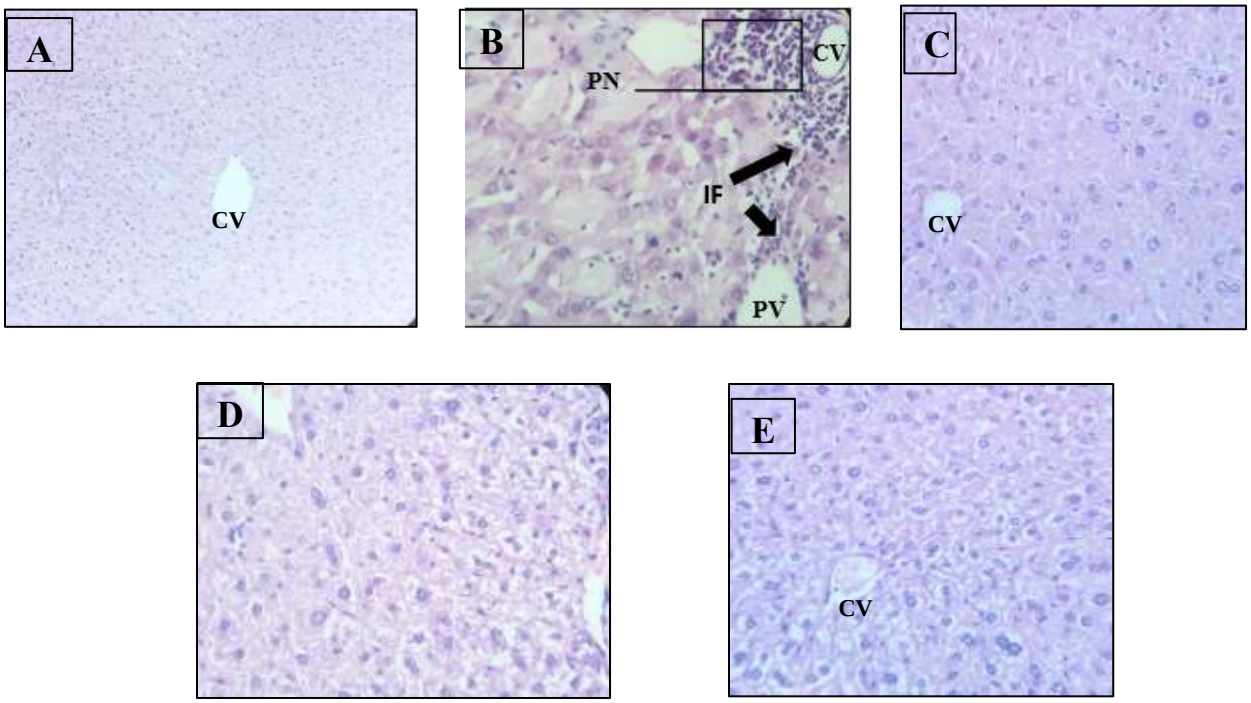
The results were expressed as mean ± SD. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey multiple comparison test, n=5. a= significant different compared to Group-I, b= significant different compared to Group-II, (P < 0.05) statistically significant.

Albumin is predominantly synthesized in the liver and advanced cirrhosis is associated with a decrease in plasmatic albumin. Patients with cirrhosis have impaired hepatocellular function and reduced albumin synthesis[86]. The total bilirubin level of group-II (3.28±0.45 mg/dL) was statistically significant compared to the control (group-I; 0.60±0.29 mg/dL) P<0.05, but there is no significant difference between the total bilirubin level of normal control group and the reference drug Silymarin (group-III; 0.94±0.20 mg/dL). The total bilirubin level of both treatment group (group-IV; 1.76±0.51 mg/dL) and group-V (1.24±0.32 mg/dL) were significantly decrease the serum total bilirubin compared to group-II (3.27±0.45 mg/dL) (P<0.05) (Figure **16**). Also, there is not a significant difference between the treatment (group-V; 1.24±0.32 mg/dL) and the reference (group-III; 0.94±0.20 mg/dL) (P>0.05). The serum ALP and bilirubin were increase on group-II, this due to the induced toxicant (INH and RIF). This result agrees with Sankar, M and it's co-workers[73], that antitubercular drug induced mice increase the serum ALP and total bilirubin in

blood plasma and decrease the serum albumin level and a high concentration of bilirubin in serum is an indication for the cause of high erythrocytes degradation rate due to liver injury when treated with hepatotoxins. In the same genus *A. racemosus* root significantly decrease the levels of total bilirubin in serum which is an indication of hepatoprotective activity[89]. This study confirmed that the plant on the same genus have probably similar effect[39]. The higher dose (400 mg/kg) extract showed reduce the total bilirubin level of the toxicant induced group compared to the lower dose (200 mg/kg) and its statistically insignificant with the reference drug (group-III) $P>0.05$. This result showed that increase the dose of this plant root extract inhibit the total bilirubin level on the blood plasma (Figure **16**). In general, co-administration of high dose group-V (400 mg/kg) *A. africanus* methanolic extract with isoniazide and rifampicin maintained the levels of ALT, AST, ALP, total bilirubin and albumin towards normal as compared to group-IV mice. Therefore, this study presents an attractive and novel idea that a methanolic extract have a hepatoprotective effect against isoniazide and rifampicin induced toxicity in male Swiss albino mice with dose dependent manner and it supporting the traditional claims.

4.8. Histopathological findings

The histological observations basically support the results obtained from serum enzyme assays. In this study, the liver tissues of the control group and Silymarin were normal liver architecture, hepatic parenchymal composed of the hepatocyte, central veins hepatic vessels and bile ducts (Figure **17**; A and C). Histological assessment of the liver sections revealed that injection of isoniazide and rifampicin induced pathological changes such as loss of cellular boundaries, piecemeal necrosis, inflammatory cell, lymphocytic infiltration in hepatic parenchymal, apoptotic hepatocytes and congested central vain, portal tract with portal vain, hepatic vessel and bile duct (Figure **17**; B). The mice treated with 200 and 400 mg/kg of the liver section shows normal liver architecture, composed of the hepatocyte, central veins and hepatic vessels (Figure **17**; D and E) respectively, but 400 mg/kg (Group-V) showed greater hepatoprotective activity compared to group-IV this indicate considerable extent as there was absence of effaced architecture, apoptotic hepatocytes, inflammatory cell, lymphocytic infiltration congested central veins compared to group-II indication of hepatoprotective effect of the extract.



Figures 17. Histopathology of mice liver sections.

A:-Represents the normal control group (Group-I), B:-Represents the INH 75mg/kg and RIF 150 mg/kg (Group-II), C:-Sylimarine (Group-III), D:-MEAA 200 mg/kg (Group-IV) and E:-MEAA 400 mg/kg (Group-V). Hematoxylin-eosin (H&E) stained liver sections magnification 40x.

This study demonstrated that the methanolic extract have a remarkable protective effect against isoniazide and rifampicin induced liver injury in mice and it's related with the phytochemical present in the crude plant extract such as flavonoids, saponin, steroids, phenol and tannin. In the same genus *A. albus* L. leaves and *A. racemosus* root extract improved the structure of hepatic cells, confirming the hepatoprotective effect due to its free radical scavenging and antioxidant activity on account of containing high polyphenols, flavonoids and condensed tannin[12, 103]. The resent study report confirmed that the isoniazide and rifampicin treated mice of the liver signs of inflammatory infiltration, steatosis, vascular endothelial degeneration piecemeal necrosis and focal lobular inflammation were observed[118, 119, 122].

5. Conclusion and recommendations

5.1. Conclusion

In this study the root of *A. africanus* (1kg) were extracted using different solvents such as petroleum ether, chloroform, acetone and methanol. Among this solvents, methanol was the best solvent for extracting bioactive compounds from *A.africanus* since it resulted in the highest extraction yield and the highest content of phytochemicals. The chromatographic separation of the root extract of *A. africanus* led to the isolation of six compounds, among this two of them compounds; compound**AA-5** (stigmasterol) and **AA-6**(nyasol) were characterized using ^{1}H and ^{13}C NMR spectroscopy. The antimicrobial activity of petroleum ether, chloroform, acetone and methanol root extract of *A. africanus* were evaluated against four bacterial (*Escherichia coli*, ATCC-25922 and *Salmonella thyphimurium,* ATCC-13311, *Staphylococcus aureus*, ATCC-25923 and *Bacillus subtilus*, ATCC-6633) and one fungal (*Candida albicans*, ATCC-14053) strains. From this methanolic and acetone extracts (200 mg/mL) showed a greater zone of inhibition against *E. coli*, *B subtilus* and *C. albicans* which could be accounted to the synergistic interactions of several compounds present in it and those solvents were evaluated with various concentration (12.5, 25, 50 and 100 mg/mL) against two bacteria (*E. coli*, *B subtilus*) and one fungal (*C. albicans)* strains. Decrease the concentrations of the extract insignificantly decrease the zone of inhibition. There was still a zone of inhibition at 12.5 mg/mL against those tested strain, it can be concluded that the MIC value of the extracts was less than that of the lowest concentration used in this study. The isolated compounds **AA-2** and **AA-4** showed a promising antimicrobial activity against all the tested bacterial and fungal strains, whereas compound**AA-5** and **AA-6** showed an intermediate zone of inhibition against two bacterial (*E. coli*, *S. typhi)* and one fungal strains (*C.albicanus)*. In the *in vivo* hepatoprotective study the methanolic extract of *A. africanus* (200 and 400 mg/kg) decreased the level of ALT, AST, ALP, total bilirubin and increase the level of serum albumin compared to isoniazide and rifampicin drug induced group in mice and revealed that the extract demonstrated a therapeutic potential as a hepatoprotective activity against antitubercular induced hepatotoxicity with a dose dependent manner.

5.2. Recommendations

Based on the present study the following suggestions are forwarded for further work

- The present study used only gravity based column chromatographic techniques with the help of some organic solvent (petroleum ether: ethyl acetate and chloroform: methanol) as an eluent. Further phytochemical investigation on *A. africanus* Lam. should be done using HPLC on the polar extracts of the plants.
- In this study a total of six compounds were isolated, from this only compounds **AA-5**(stigmasterol) and **AA-6** (nyasol) characterized. Further characterization was also recommended for the rest of the isolated compounds.
- Methanol and acetone extracts gave better antimicrobial activities, further study on the MIC and MBC will be required.
- The isolated compounds**AA-2** and **AA-4** were showed to have marginal activity against all the tested bacterial and fungal strains hence, the mechanism of action, the MIC and MBC for compounds should be further studied.
- In this study only use male Swiss albino mice, further study may require on both sex of the mice.
- More biological assay on other strains will be conducted on various extracts to establish the traditional use of the plant.

6. References

(1) Ganesan, R.; Latha, T.; Mangai, S. Phytochemical analysis and antibacterial activity of roots of *A. racemosus* willd. *Asian J Pharm and Clin Res.* **2015**, 3 (4), 132–139.

(2) Giday, K.; Lenaerts, L.; Gebrehiwot, K.; Yirga, G.; Verbist, B.; Muys, B. Ethnobotanical Study of Medicinal Plants from Degraded Dry Afromontane Forest in Northern Ethiopia: Species, Uses and Conservation Challenges. *J. Herb. Med.* **2016**, 6 (2), 96–104.

(3) Kassaye, K.; Amberbir, A.; Getachew, B.; Mussema, Y. A Historical Overview of Traditional Medicine Practices and Policy in Ethiopia. *Ethiop. J. Heal. Dev.* **2007**, 20 (2), 127–134.

(4) Okolie, O. D.; Manduna, I.; Mashele, S. Phytochemical Analysis of *A. africanus* root extracts. A Dissertation. **2019**, 7 (1), 351–354.

(5) Khapli, S. M.; Mangashetti, L. S.; Yogesha, S. D.; Wani, M. R. IL-3 Acts Directly on Osteoclast Precursors and Irreversibly Inhibits Receptor Activator of NF-KB Ligand-Induced Osteoclast Differentiation by Diverting the Cells to Macrophage Lineage. *J. Immunol.* **2003**, 171 (1), 142–151.

(6) Subba, A.; Mandal, P. Pharmacognostic Studies and in Vitro Antioxidant Potential of Traditional Polyherbal Formulation of West Sikkim with *Asparagus* Spp. *Pharmacogn. J.* **2015**, 7 (6), 348–355.

(7) Irungu, S. Isolation and Characterization of Antimicrobial Compounds From the Plants , *E. Abyssinica* and *Chasmanthera Dependens* Hochst. A thesis. **2012**, 1 (1), 1–98.

(8) Manandhar, S.; Luitel, S.; Dahal, R. K. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *J. Trop. Med.* **2019,** 1(5), 2-5,

(9) Ramírez-marroquín, O. A.; Jiménez-arellanes, M. A. Hepato-Protective Effect from Natural Compounds , Biological Products and Medicinal Plant Extracts on Antitubercular Drug-Induced Liver Injuries : *A Systematic Review Medicinal & Aromatic Plants*. **2016,** 5 (8), 339.

(10) Serairi-Beji, R.; Aidi Wannes, W.; Hamdi, A.; Tej, R.; Ksouri, R.; Saidani-Tounsi, M.; Lachaal, M.; Karray-Bouraoui, N. Antioxidant and Hepatoprotective Effects of *A.albus* Leaves in Carbon Tetrachloride-Induced Liver Injury Rats. *J. Food Biochem.* **2018**, 42 (1), 1–11.

(11) Lina, S. M. M.; Ashab, I.; Bhuiya, M. S.; Shahriar, M. Hepatoprotective Activity of *Hedyotis Corymbosa* (Linn.) Lam. Extract Against Anti-Tubercular Drug Induced Hepatic Damage in Sprague-Dawley Rats. *Bangladesh Pharm. J.* **2018**, 21 (2), 131–138.

(12) Wissem, R. S.; Wannes, A. Antioxidant and Hepatoprotective Effects of *A. Albus* Leaves in Carbon Tetrachloride-Induced Liver Injury Rats. *J. Food Biochem.* **2017**, 1 (1)1–11.

(13) Uddin, M.; Ghufran, M. A.; Idrees, M.; Irshad, M.; Ahmad, W.; Malook, I.; Batool, A.; Rashid, A.; Arshad, M.; Naeem, R. Antibacterial Activity of Methanolic Root Extract of *A. Racemosus. J. Public Heal. Biol. Sci.* **2012**, 1 (2), 32–35.

(14) Scandorieiro, S.; de Camargo, L. C.; Lancheros, C. A. C.; Yamada-Ogatta, S. F.; Nakamura, C. V.; de Oliveira, A. G.; Andrade, C. G. T. J.; Duran, N.; Nakazato, G.; Kobayashi, R. K. T. Synergistic and Additive Effect of Oregano Essential Oil and Biological Silver Nanoparticles against Multidrug-Resistant Bacterial Strains. *Front. Microbiol.* **2016**, 7(1) 1–14.

(15) Nair, R.; Kalariya, T.; Chanda, S. Antibacterial Activity of Some Selected Indian Medicinal Plants. *Int. J. Pharm. Sci. Res.* **2012**, 3 (7), 2038–2042.

(16) Erb, M.; Kliebenstein, D. J. Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites. *J Plant Physiol.* **2020**, 184 (1), 39–52.

(17) Khan, T.; Fatima, K.; Ali, M.; Ovais, M.; Talha, A.; Ikram, K.; Raza, A.; Khan, Z.; Idrees, M. Selected Hepatoprotective Herbal Medicines : Evidence from Ethnomedicinal Applications, Animal Models and Possible Mechanism of Actions. *Frontiers in microbiology*. **2017**, 1(1), 1–17.

(18) Gurung, P.; De, P. Spectrum of Biological Properties of Cinchona Alkaloids : A Brief Review. *J Pharmacogn Phytochem.* **2017**, 6 (4), 162–166.

(19) Shara, M.; Stohs, S. J. Efficacy and Safety of White Willow Bark (*Salix Alba*) Extracts. *J Phytother Res*. **2015**, 1116 (1), 1112–1116.

(20) Stien, D. Quassinoids: Anticancer and Antimalarial Activities. *Springer-Verlag Berlin Heidelberg.* **2013**, 1(1), 3775-3801.

(21) Kupchan, S. M.; Britton, R. W.; Lacadie, J. A.; Ziegler, M. F.; Sigel, C. W. The Isolation and Structural Elucidation of Bruceantin and Bruceantinol, New Potent Antileukemic Quassinoids From. *J org chem*. **1975**, 40 (5), 648–654.

(22) Ruddock, P. S.; Charland, M.; Ramirez, S.; López, A.; Towers, G. H. N.; Arnason, J. T.; Liao, M.; Dillon, J. A. R. Antimicrobial Activity of Flavonoids from Piper *Lanceae folium* and Other Colombian Medicinal Plants against Antibiotic Susceptible and Resistant Strains of Neisseria Gonorrhoeae. *Sex. Transm. Dis.* **2011**, 38 (2), 82–88.

(23) Javadzadeh, Y.; Hamedeyaz, S. Floating Drug Delivery Systems for Eradication of Helicobacter Pylori in Treatment of Peptic Ulcer Disease. *Trends Helicobacter pylori Infect.* **2014**, 1 (1), 13.

(24) Turan, A.; Süleyman, D. Anatomical and Ecological Investigations on Endemic *Hyacinthella heldreichii* (boiss .) chouar (asparagaceae) in Turkey. *Ijaeb.org.* **2016**, 1 (01), 66–72.

(25) Chase, M. W.; Reveal, J. L.; Fay, M. F. A Subfamilial Classification for the Expanded Asparagalean Families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Bot. J. Linn. Soc.* **2009**, 161 (2), 132–136.

(26) Galala, A. A.; Ahmad, K. F. M.; Zaghloul, M. G.; Mansour, E. S. A. Phytochemistry Letters Two New Alkaloids from *Asparagus Stipularis* Forssk Roots. *J Phytochem. Lett.* **2015**, 12, 220–223.

(27) Nawaz, T.; Mansoor, H.; Waqar-U-Nisa; Sajid Aqeel Ahmad, M.; Younis, A.; Kanwal, H. Comparative Anatomy of Root and Stem of Some Native and Exotic *Asparagus* L. Species. *Pakistan J. Bot.* **2012**, 44 (1), 153–158.

(28) Nureye, D.; Assefa, S.; Nedi, T.; Engidawork, E. In Vivo Antimalarial Activity of the 80% Methanolic Root Bark Extract and Solvent Fractions of Gardenia *Ternifolia Schumach*. & Thonn. (Rubiaceae) against Plasmodium Berghei. *Evidence-based Complement. Altern. Med.* **2018**, 1(17) 2-33.

(29) Al-snafi, A. E. The Pharmacological Importance of *A. Officinalis* - A Review. *J of Pharma Biology.* **2015***,* 5(2)*,* 93-98.

(30) Getnet, C. Ethnobotanical study of plants used in traditional medicine and as wild foods in and around taragedam and amba remnant forests in libo kemkem woreda, south Gonder Zone, Amhara Region, Ethiopia **2011**, 1 (1), 1-93.

(31) Mesfin, A.; Giday, M.; Animut, A.; Teklehaymanot, T. Ethnobotanical Study of Antimalarial Plants in Shinile District, Somali Region, Ethiopia, and in Vivo Evaluation of Selected Ones against Plasmodium Berghei. *J. Ethno pharma.* **2012**, 139 (1), 221–227.

(32) Madikizela, B.; Ndhlala, A. R.; Finnie, J. F.; Van Staden, J. Antimycobacterial, Anti-Inflammatory and Genotoxicity Eva Luation of Plants Used for the Treatment of Tuberculosis and Related Symptoms in South Africa. *J. Ethno pharma.* **2014**, 153 (2), 386–391.

(33) Terlizzi, M. E.; Gribaudo, G.; Maffei, M. E. UroPathogenic Escherichia Coli (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-Antibiotic Antimicrobial Strategies. *Front J. Microbiol.* **2017**, 1(8), 1566.

(34) Alebie, G.; Urga, B.; Worku, A. Systematic Review on Traditional Medicinal Plants Used for the Treatment of Malaria in Ethiopia : Trends and Perspectives. *Malar. J.* **2017**, 1(1), 1–13.

(35) Ayele, T. T. Organic Chemistry : Current Research A Review on Traditionally Used Medicinal Plants / Herbs for Cancer Therapy in Ethiopia : Current Status , Challenge and Future Perspectives. *Organic Chem Curr Res.***2018**, 7 (2), 1-21.

(36) Worku, A. M. A Review on Significant of Traditional Medicinal Plants for Human Use in Case of Ethiopia. *J Plant Pathology & Microbiology.* **2017** 16 (307), 1–12.

(37) Omwenga, E. O.; Hensel, A.; Shitandi, A.; Goycoolea, F. M. Ethnobotanical Survey of Traditionally Used Medicinal Plants for Infections of Skin , Gastrointestinal Tract , Urinary Tract and the Oral Cavity in Borabu Sub-County, Nyamira County , Kenya. *J. Ethnopharmacol.* **2015**, 176, 508–514.

(38) Bakari A., Mwamba M, Lumbu S, Pierre D, Kahumba B. Ethnobotanical Survey of Herbs Used in the Management of Diabetes Mellitus in Southern Katanga Area/DR Congo. *Pan Afr Med J.* **2018**, 1(2), 86-88.

(39) Negi, J.; Singh, P.; Joshi, G.; Rawat, M.; Bisht, V. Chemical Constituents of *Asparagus*. *J Pharmacogn. Rev.* **2010**, 4 (8), 215–220.

(40) Kregiel, D.; Berlowska, J.; Witonska, I.; Antolak, H.; Proestos, C.; Babic, M. Saponin-Based , Biological-Active Surfactants from Plants. *open access peer review J* **2017** 1(1), 1-12.

(41) Mazza, G. Saponins : Properties, Applications. *Critical Reviews in Food Science and Nutrition*. **2007**, 47(1), 231–258.

(42) Oketch-Rabah, H. A.; Dossaji, S. F.; Christensen, S. B.; Frydenvang, K.; Lemmich, E.; Cornett, C.; Olsen, C. E.; Chen, M.; Kharazmi, A.; Theander, T. Antiprotozoal Compounds from *A.africanus*. *J. Nat. Prod.* **1997**, 60 (10), 1017–1022.

(43) A Shastry, R.; D. Madagundi, S.; V. Habbu, P.; S. Patil, B.; D. Joshi, S.; H Kulkarni, V. Phytochemical Investigation and Antiepileptic Activity of *Asparagus Racemosus* (Wild) Root Extracts in Rodents. *Rajiv Gandhi Univ. Heal. Sci. J. Pharm. Sci.* **2015**, 5 (3), 97–103.

(44) Huang, X.; Lin, Y.; Kong, L. Steroids from the Roots of *A. Officinalis* and Their Cytotoxic Activity. *J Integr Plant Biol.* **2008**, 50 (6), 717–722.

(45) Simin, K.; Ahmad, V. U. U. Chemical Constituents from *A. dumosus*. Fitoterapia, **1999**, 71 (2000), 331–333.

(46) Pichersky, E.; Raguso, R. A. Why Do Plants Produce so Many Terpenoid Compounds *New J, Phytol.* **2017**, 1(1), 692–702.

(47) Ali, M. S.; Mukherjee, S.; Roy, D.; Pal, G.; Makar, S. *Asparagus Racemosus* , a Climbing Ayurvedic Medicinal Plant : Review on Its Cultivation, Morphology and Medicinal Significance. *J pharma*. **2018**, 6 (12), 46–54.

(48) Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects : An Overview. *J of med.***2018**, 5 (93), 2-16.

(49) Ramit, S and Vikas, J. Shatavari (*A .racemosus*): a review on its cultivation, morphology, phytochemistry and pharmacological importance. *IJPSR.* **2014**, 5 (3), 742–757.

(50) Chitrakar, B.; Zhang, M.; Adhikari, B. Trends in Food Science & Technology *Asparagus* (*A. officinalis*): Processing e Ff Ect on Nutritional and Phytochemical Composition of Spear and Hard-Stem Byproducts. *J Food Sci. Technol.* **2019**, 93 (8), 1–11.

(51) Mishra, J. N.; Verma, N. K. *A. racemosus* : Chemical Constituents and Pharmacological. *J Eur. Biomed. Pharm. Sci.* **2017**, 4 (6), 207–213.

(52) Vincken, J.; Heng, L.; Groot, A. De; Gruppen, H. Saponins , Classification and Occurrence in the Plant Kingdom. *J.phytochem*. **2007**, 68(1), 275–297.

(53) Tafesse, G.; Mekonnen, Y.; Makonnen, E. Antifertility Effect of Aqueous and Ethanol Extracts of the Leaves and Roots of *A. Africanus* in Rats. *J Afr. Health Sci.* **2006**, *6* (2), 81–85.

(54) Nabi, S. A.; Kasetti, R. B.; Sirasanagandla, S.; Tilak, T. K.; Kumar, M. V. J.; Rao, C. A. Antidiabetic and Antihyperlipidemic Activity of *Piper Longum* Root Aqueous Extract in STZ Induced Diabetic Rats. *BMC Complement. J Altern. Med.* **2013**, 13(1)1-12.

(55) Hassan, H. S.; Ahmadu, A. A.; Hassan, A. S. Analgesic and Anti-Inflammatory Activities of *Asparagus Africanus* Root Extract. *African J. Tradit. Complement. Altern. Med.* **2008**, 5 (1), 27–31.

(56) Madikizela, B.; Ndhlala, A. R.; Finnie, J. F.; Staden, J. Van. In Vitro Antimicrobial Activity of Extracts from Plants Used Traditionally in South Africa to Treat Tuberculosis and Related Symptoms. *J. Evid Based Integr Med*. **2013,** 1(1), 1-13.

(57) Dikasso, D.; Makonnen, E.; Debella, A.; Abebe, D.; Urga, K.; Makonnen, W. Original Article In Vivo Anti-Malarial Activity of Hydroalcoholic Extracts from *Asparagus A.fricanus* Lam . in Mice Infected with Plasmodium Berghei. *Blood*. *Ethiop. J. Health Dev*. **2006**, 20 (2), 113-118.

(58) Kongkaneramit, L.; Witoonsaridsilp, W.; Peungvicha, P.; Ingkaninan, K.; Waranuch, N.; Sarisuta, N. Antioxidant Activity and Antiapoptotic Effect of *A. Racemosus* Root Extracts in Human Lung Epithelial H460 Cells. *Exp. Ther. Med.* **2011**, 2 (1), 143–148.

(59) El-ishaq, A.; Alshawsh, M. A.; Mun, K. S.; Chik, Z. Biochemical and Toxicological Effects of Methanolic Extract of *Asparagus africanus* Lam in Sprague-Dawley Rats. *PeerJ.* **2020**, 1(1), 2-15.

(60) García, L. G. Unveiling the Biological Role of Stigmasterol Biosynthesis in Tomato Plants. **2019**, 1 (1), 1-161.

(61) Poul, M.D. Medicinal Natural Products. A Biosynthetic Approach Second Edition. John Wiley & Sons. **2002,** 212 (1), 1-506.

(62) Shiro, S.; Toshiaki, U. Biosynthesis of Lignans and Norlignans. *J Wood Sci*. **2007,** 53 (9*)*, 273–284.

(63) Suzuki, S.; Yamamura, M.; Shimada, M.; Umezawa, T. A Heartwood Norlignan, (E)-Hinokiresinol, Is Formed from 4-Coumaryl 4-Coumarate by a Cryptomeria Japonica Enzyme Preparation. *J Chem. Commun.* **2004**, 24(1), 2838–2839.

(64) Hamburg, M. A. Considerations for Infectious Disease Research and Practice. *J Technol. Soc.* **2008**, *30* (4), 383–387

(65) Morens, D. M.; Folkers, G. K.; Fauci, A. S. Erratum: The Challenge of Emerging and Re-Emerging Infectious Diseases *Nature. J Clin. Infect. Dis.* **2010**, 463 (7277), 122.

(66) Mulaudzi, R. B. Pharmacological Evaluation of Medicinal Plants Used by Venda People against Venereal and Related Diseases. *J E pharma*. **2012**. 135 (1), 330–337.

(67) Ryan, K.J., Ray, C.G. Sherris Medical Microbiology 4th Ed. McGraw Hill, London, **2004**.

(68) Lehrnbecher, T.; Frank, C.; Engels, K.; Kriener, S.; Groll, A. H.; Schwabe, D. Trends in the Postmortem Epidemiology of Invasive Fungal Infections at a University Hospital. *J. Infect.* **2010**, 61 (3), 259–265.

(69) Kumar, R.; Shukla, P. K. Amphotericin B Resistance Leads to Enhanced Proteinase and Phospholipase Activity and Reduced Germ Tube Formation in Candida Albicans. *J Fungal Biol.* **2010**, 114 (2–3), 189–197.

(70) Cong, Y.; Yang, S.; Rao, X. Vancomycin Resistant Staphylococcus Aureus Infections: A Review of Case Updating and Clinical Features. *J. Adv. Res.* **2020**, 21(1), 169–176.

(71) Enne, V. I.; Livermore, D. M.; Stephens, P.; Hall, L. M. C. Persistence of Sulphonamide Resistance in Escherichia Coli in the UK despite National Prescribing Restriction. *J Lancet* **2001**, 357 (9265), 1325–1328.

(72) Foulkes, J. G. Elitra Pharmaceuticals: New Paradigms for Antimicrobial Drug Discovery. *J Drug Discov. Today* **2002**, 7 (5),12–15.

(73) Sankar, M.; Rajkumar, J.; Sridhar, D. Hepatoprotective Activity of Heptoplus on Isoniazid and Rifampicin Induced Liver Damage in Rats. *Indian J Pharm Sci.* **2015**, 77 (1), 556–562.

(74) Tayal, V.; Kalra, B. S.; Agarwal, S.; Khurana, N.; Gupta, U. Hepatoprotective Effect of Tocopherol against Isoniazid and Rifampicin Induced Hepatotoxicity in Albino Rabbits. *Indian J. Exp. Biol.* **2007**, 45 (12), 1031–1036.

(75) Santhosh, S.; Sini, T. K.; Anandan, R.; Mathew, P. T. Hepatoprotective Activity of Chitosan against Isoniazid and Rifampicin-Induced Toxicity in Experimental Rats. *Eur. J. Pharmacol.* **2007**, 572 (1), 69–73.

(76) Yue, J.; Peng, R.; Chen, J.; Liu, Y.; Dong, G. Effects of Rifampin on CYP2E1-Dependent Hepatotoxicity of Isoniazid in Rats. *J Pharmacol. Res.* **2009**, 59 (2), 112–119.

(77) Sarkar, S.; Ganguly, A.; Sunwoo, H. H. Current Overview of Anti-Tuberculosis Drugs : Metabolism and Toxicities Mycobacterial Diseases. **2016**, *6* (2), 1–6.

(78) Fatima, G.; Dhobi, M. Plant Based Treatment of Hepatotoxicity. *Indian jpra*.**2018**, 3 (2), 10–23.

(79) Factor, I.; Santosh, R.; Gourishankar, K.; Fulzele, V. B.; Material, P. Hepatoprotective Activity of *A. Retrofractus* (Family- Asparagaceae) Roots against Thioacetamide Induced Liver Damage in Rats. in*t. J. Pharm. Sci.* **2013**, 3 (1), 57–62.

(80) Krishnan, S. Liver diseases-an overview. **2019**. *World J. Pharm. Sci*. 8, (1), 1385-1395.

(81) Arneson, W.; Brickell, J. *A Laboratory Perspective*; **2007**, 1(1), 1-582.

(82) Pratap, A.; Panakanti, R.; Yang, N.; Eason, J. D.; Mahato, R. I. Inhibition of Endogenous Hedgehog Signaling Protects Against Acute Liver Injury After Ischemia Reperfusion. *J Pharm Res.***2010**, 27, 2492–2504.

(83) Nyblom, H.; Berggren, U.; Balldin, J.; Olsson, R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *J Alcohol & Alcoholism*. **2004**, 39 (4), 336–339.

(84) Thapa, B. R.; Walia, A. Liver Function Tests and Their Interpretation. **2007**, *74*, 67–75.

(85) Tiagi, G. K. Liver Function Tests. *J. Indian Med. Assoc.* **1956**, 27 (1), 9–14.

(86) Carvalho, J. R.; Machado, M. V. New Insights About Albumin and Liver Disease. *J Ann. Hepatol.* **2019**, 17 (4), 547–560.

(87) Areefa Shaik, A.; A.Elumalai, A.; Eswaraiah, M. C.; Swathi, S. An Updated Review on Hepatoprotective Medicinal Plants. *J. Drug Deliv. Ther.* **2012**, 2 (2), 99–105.

(88) Gulati, K.; Reshi, M. R.; Rai, N.; Ray, A. Hepatotoxicity : Its Mechanisms , Experimental Evaluation and Protective Strategies. *Am. J. Pharmacol.* **2018**, 1 (1), 1–9.

(89) Fasalu Rahiman O. M, Rupesh Kumar M, Tamizhmani T, Mohamed Niyas K, Pasumarthi Phaneendra, Satya Kumar B. Hepatoprotective Activity of the Alcohol Extract of *A. racemosus* Roots in Carbon Tetra Chloride Induced Hepato-Toxicity in Albino Rats. In*t. J. Pharm. Sci. Rev. Res.* **2015**, 30(1), 169-172.

(90) Palanisamy, N.; Manian, S. Protective effects of *A. racemosus* on oxidative damage in isoniazid-induced hepatotoxic rats. *J. Toxicol Ind Health.* **2012,** 20(1), 28-238.

(91) Neeta, M. P.; Mukta, N.; Bilwa, K. Original Research Article Comparative Qualitative Phytochemical Analysis of *Sesamum Indicum* L . *Int. J. Curr. Microbiol. Appl. Sci.* **2015**, *2* (2), 172–181.

(92) Zohra, F. T. Extraction of Secondary Metabolites, Phytochemical Screening and the Analysis of Antibacterial Activity in Stevia Rebaudiana. *J BRAC Univ.* **2015**, 1(1), 1–55.

(93) Benkova, M.; Soukup, O.; Marek, J. Antimicrobial Susceptibility Testing: Currently Used Methods and Devices and the near Future in Clinical Practice. *J. Appl. Microbiol.* **2020**., 1(1), 1-12.

(94) Rao, U. S. M.; Abdurrazak, M.; Mohd, K. S. Penyaringan Fitokimia, Jumlah Asai Kandungan Flavonoid Dan Fenolik Pelbagai Ekstrak Pelarut Tepal Musa Paradisiaca. *Malaysian J. Anal. Sci.* **2016**, 20 (5), 1181–1190.

(95) Mahomoodally, M. F.; Gurib-Fakim, A.; Subratty, A. H. Antimicrobial Activities and Phytochemical Profiles of Endemic Medicinal Plants of Mauritius. *J Pharm. Biol.* **2005**, 43 (3), 237–242.

(96) Antara S.; Amla B. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *melia azedarach* L. *Int J Curr Pharm Res.* **2012**, 17 **(**4), 67-73.

(97) Mostafa, A. A.; Al-Askar, A. A.; Almaary, K. S.; Dawoud, T. M.; Sholkamy, E. N.; Bakri, M. M. Antimicrobial Activity of Some Plant Extracts against Bacterial Strains Causing Food Poisoning Diseases. *Saudi J. Biol. Sci.* **2018**, 25 (2), 361–366.

(98) Asgedom, S. W.; Teweldemedhin, M.; Gebreyesus, H. Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia : A Systematic Review. *Hindawi J Pathogens.* **2018**, 4(1), 2-8.

(99) Mörsel, J. -Th; Heyer, R. A Colorimetric Method for the Determination of Glycerides. *J Food / Nahrung* **1990**, 34 (3), 287–288.

(100) Khan, S. W.; Tahir, M.; Lone, K. P.; Munir, B.; Latif, W. Protective effect of *saccharum officinarum L.*(sugar cane) juice on isoniazid induced hepatotoxicity in male albino mice. *J Ayub Med Coll Abbottabad*. **2015**, 27 (2), 346–350.

(101) Gornall, A G.; Bardawill, CJ,; David, MM. Determination of serum proteins by means of the biuret reaction. *J of Biol Chem.* **1949**, 177 (2):751-766.

(102) Doumas, B. T.; Ard Watson, W.; Biggs, H. G. Albumin Standards and the Measurement of Serum Albumin with Bromcresol Green. *J Clin. Chim. Acta* **1971**, 31 (1), 87–96.

(103) Gopalakrishnan, S.; Kalaiarasi, T. Hepatoprotective Activity of the Fruits *Asparagus Officinalis*. *Int. J. Pharm. Sci. Rev. Res.* **2013**, 20 (2), 229–234.

(104) Sankar, M.; Rajkumar, J.; Sridhar. Effect of Hepatoplus on Isoniazid and Rifampicin Induced Hepatotoxicity in Liver Cell Lines. *Int. J. Pharm. Pharm. Sci.* **2015**, 7 (5), 215–219.

(105) Sahalie, N. A.; Abrha, L. H.; Tolesa, L. D. Chemical Composition and Antimicrobial Activity of Leave Extract of *Ocimum Lamiifolium* (Damakese) as a Treatment for Urinary Tract Infection. *J Cogent Chem.* **2018**, 4 (1), 1–10.

(106) Moghimipour, E.; Handali, S. Saponin : Properties, Methods of Evaluation and Applications. *Annual Research & Review in Biology.* **2015**, 5 (3), 207–220.

(107) Saxena, M.; Saxena, J.; Nema, R.; Singh, D.; Gupta, A. Phytochemistry of Medicinal Plants. Plants. *J Pharma and Phyto.* **2017**, 1 (6), 168–182.

(108) Malladi, S.; Vn, R.; K, S. B.; Pullaiah, T. Phytochemical Investigation of Caralluma lasiantha: Isolation of Stigmasterol, an Active Immunomodulatory Agent. *Int.J chem sci.* **2014**, 14 (1), 399–407.

(109) Omar, M. N.; Muzammil, A.; Khan, N. T.; Zahid, M. Isolation and Identi Identification Fication of Bioactive Compounds from Twigs of Artocarpusaltilis. *Merit Res. Journals*. **2013**, 1 (8), 147–155.

(110) Hernández-garcía, E.; García, A.; Avalos-alanís, F. G.; Rivas-galindo, V. M.; Delgadillo-puga, C.; Camacho-corona, R. Data in Brief Nuclear Magnetic Resonance Spectroscopy Data of Isolated Compounds from Acacia Farnesiana (L) Willd Fruits and Two Esteri Fi Ed Derivatives. *Data Br.* **2019**, *22*, 255–268.

(111) Tsui, W. Y.; Brown, G. D. (+)-Nyasol from *Asparagus Cochinchinensis*. *J Phytochem.***1996**, 43 (6), 1413–1415.

(112) Park, H. J.; Lee, J. Y.; Moon, S. S.; Hwang, B. K. Isolation and Anti-Oomycete Activity of Nyasol from *Anemarrhena Asphodeloides* Rhizomes. *J Phytochemistry* **2003**, 64 (5), 997–1001.

(113) Fang, Y.; Park, H. Practical Synthesis of (±)-Nyasol. *Synth. Commun.* **2015**, *45* (1), 137–142.

(114) Chavan, J. J.; Yadav, S.; Patil, D. Phytochemical and antimicrobial studies on *A. racemosus*. *W J P R*. **2015**, 4 (9), 1805-1810.

(115) Himanshu, A.; Gyannprakash, A.; Vinod, C. Evaluation of root extracts of *A. racemosus* for antimicrobial activity. *J DDMC*. **2013**, 3(2), 113-119.

(116) Habtamu, Y.; Eguale, T.; Wubete, A.; Sori, T. In Vitro Antimicrobial Activity of Selected Ethiopian Medicinal Plants against Some Bacteria of Veterinary Importance. *African J. Microbiol. Res.* **2010**, 4 (12), 1230–1234.

(117) Edilu, A.; Adane, L.; Woyessa, D. In Vitro Antibacterial Activities of Compounds Isolated from Roots of *Caylusea Abyssinica*. *Ann. Clin. Microbiol. Antimicrob.* **2015**, *14* (1), 1–8.

(118) Pal, R.; Rana, S.; Vaiphei, K.; Singh. Effect of different doses of carotenoids in isoniazid-rifampicin induced hepatotoxicity in rats. *World J, Gastroenterol.* **2008**, 29 (3), 153–159.

(119) Pal, R.; Vaiphei, K.; Sikander, A.; Singh, K.; Rana, S. V. Effect of Garlic on Isoniazid and Rifampicin-Induced Hepatic Injury in Rats. *World J. Gastroenterol.* **2006**, 12 (4), 636-639.

(120) Hussain, T.; Gupta, R. K.; Sweety, K.; Khan, M. S.; Hussain, S.; Hussain, A. Evaluation of Antihepatotoxic Potential of *Solanum Xanthocarpum* Fruit Extract against Antitubercular Drugs Induced Hepatopathy in Experimental Rodents. *Asian Pac J Trop Biomed*. **2012**, 2 (6), 454–460.

(121) Lina, S. M. M.; Ashab, I.; Bhuiya, M. S.; Shahriar, M. Hepatoprotective Activity of *Hedyotis Corymbosa* (Linn.) Lam. Extract Against Anti-Tubercular Drug Induced Hepatic Damage in Sprague-Dawley Rats. *Bangladesh Pharm. J.* **2018**, 21 (2), 131–138.

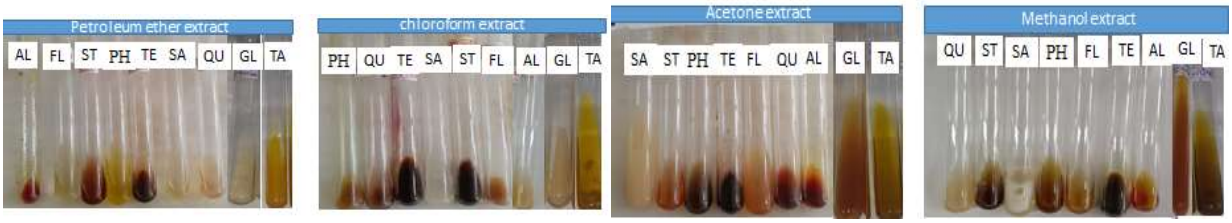
(122) Bais, B.; Saiju, P. Ameliorative Effect of Leucas Cephalotes Extract on Isoniazid and Rifampicin Induced Hepatotoxicity. *Asian Pac. J. Trop. Biomed.* **2014**, 4 (2), 633–638.

Appendix

Appendix 1. Preparation of 0.5 McFarland standard solutions

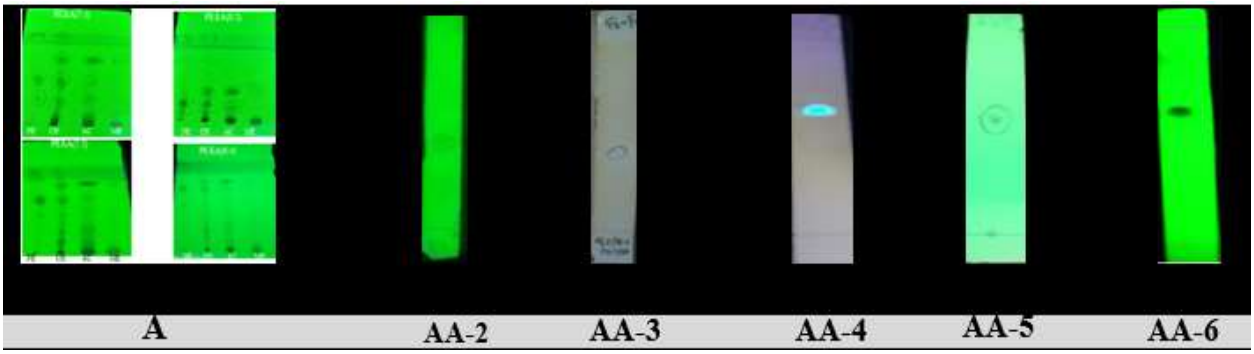
Turbidity standard 0.5 McFarland was prepared by mixing two solutions; solution "A" and solution "B". Solution "A" is 1 % v/v solution of sulphuric acid (H2SO4) and solution "B" is 1 % w/v solution of barium chloride ($BaCl_2$). To get 0.5 McFarland standard, concentration equivalents to cell density of about 107-108 CFUg-1, the amount of 0.5 ml $BaCl_2$ of 1 % solution "A" was mixed with 99.5 ml H_2SO_4 of 1 % solution "B". A small volume of the turbid solution was transferred to a screw-cap bottle of the same types as used for preparing test and control inoculums. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards is used for inoculation of media. The standard was shaken immediately before used; and stored in a well-sealed container in a dark place at room temperature (20 - 28 ºC) when not used.

Appendix 2. Phytochemical screening of *A. africanus* Lam. root crud extract.

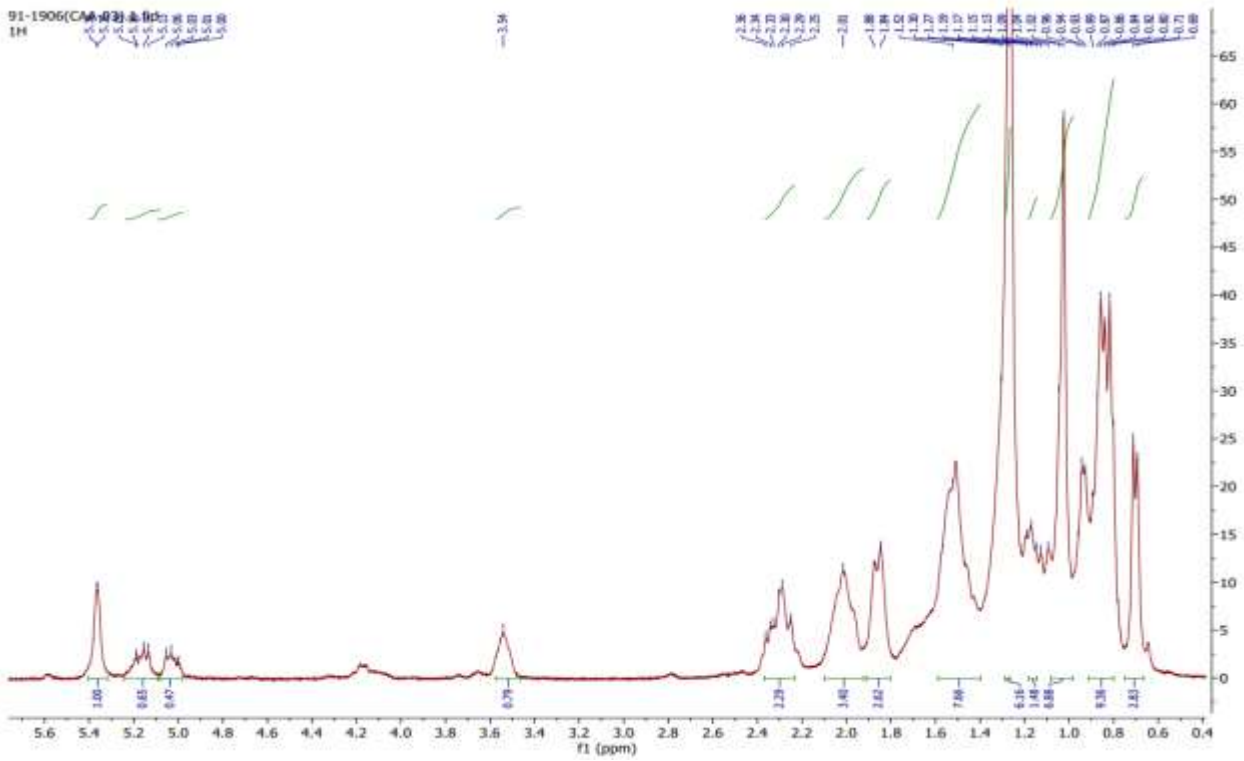


The abbreviation stands for; SA= saponin, ST=steroids, PH= phenol, TE=terpenoids, FL=flavonoids, QU=Qui-nones, AL=alkaloids, GL=glycosides, TA=tannins.

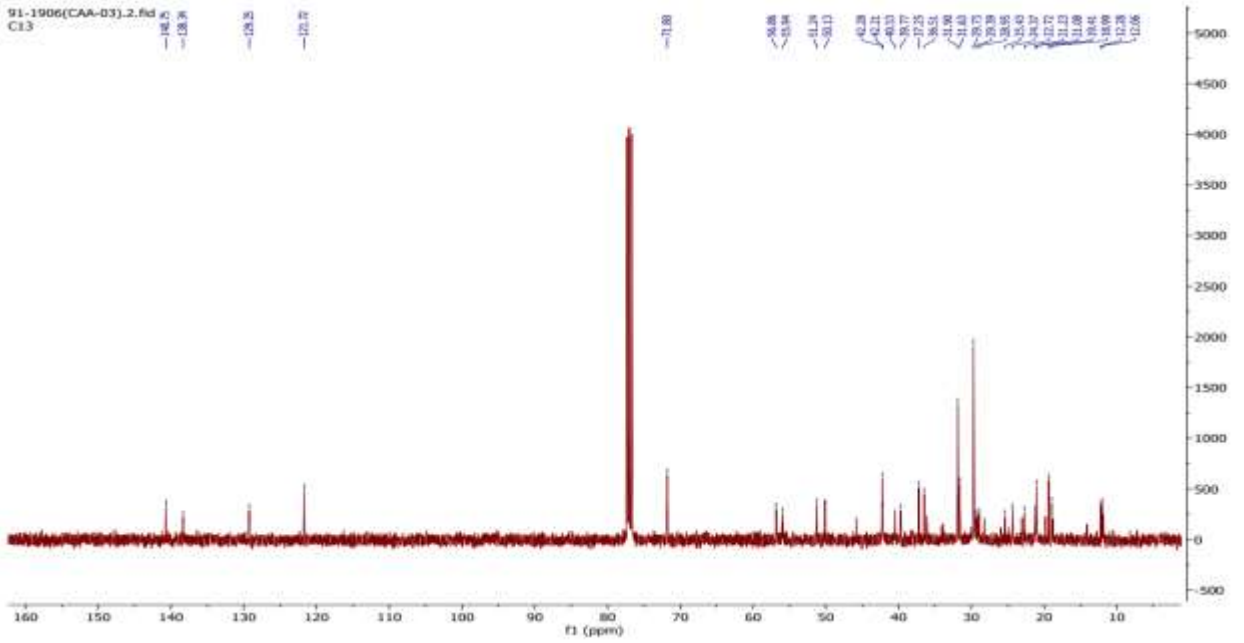
Appendix 3. TLC profile of the crude extracts and isolated compounds



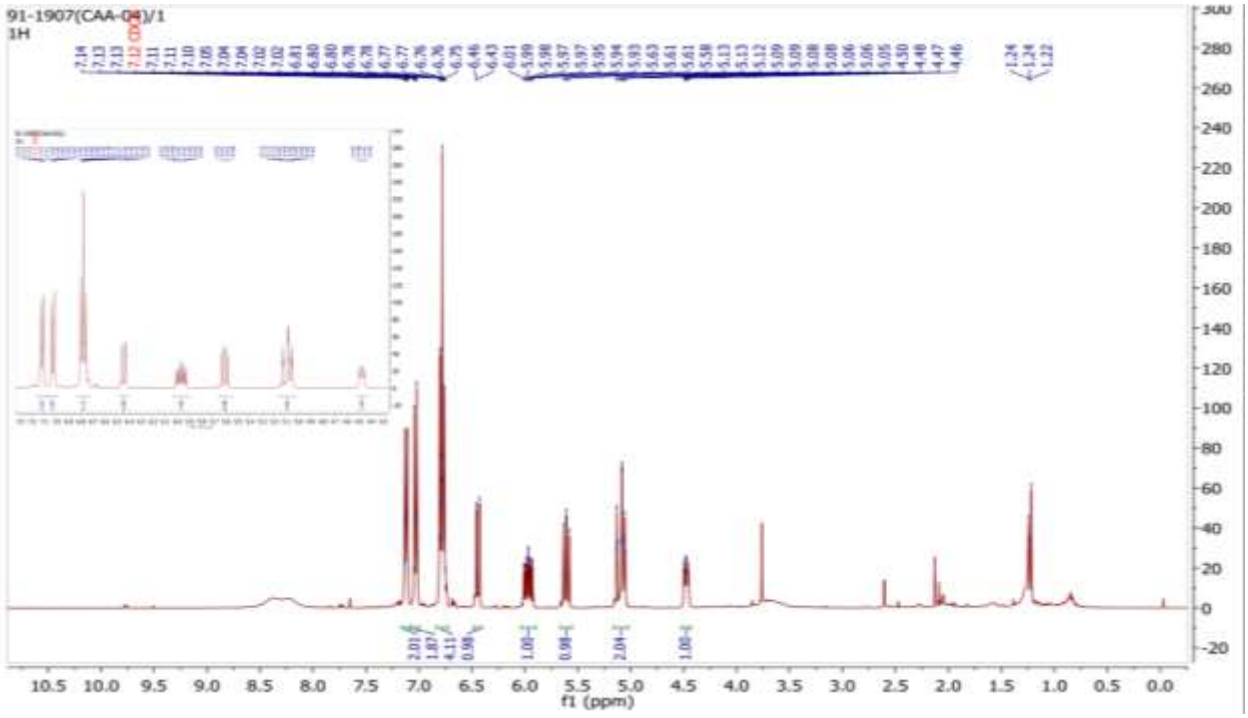
Appendix 4. ^{1}H NMR spectrum of compound **AA-5**, CDCl3, 400 MHz



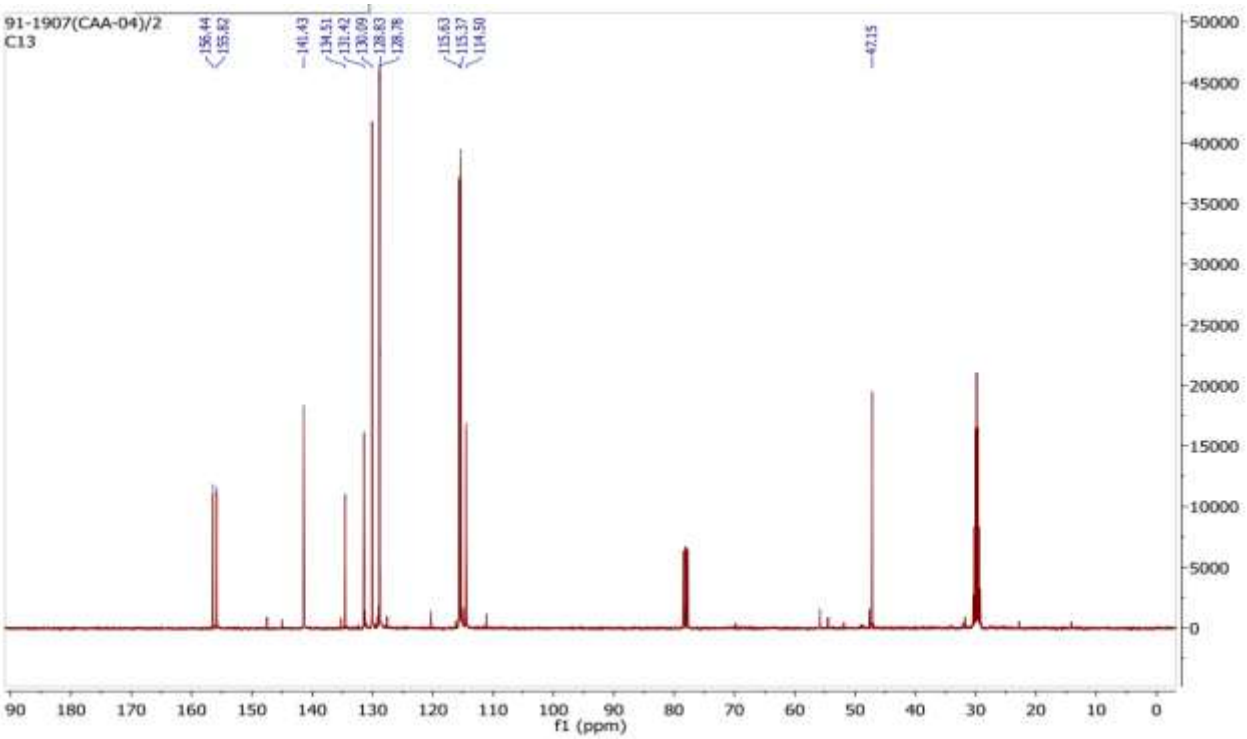
Appendix 5. ^{13}C NMR spectrum of compound **AA-5**, CDCl3, 100 MHz



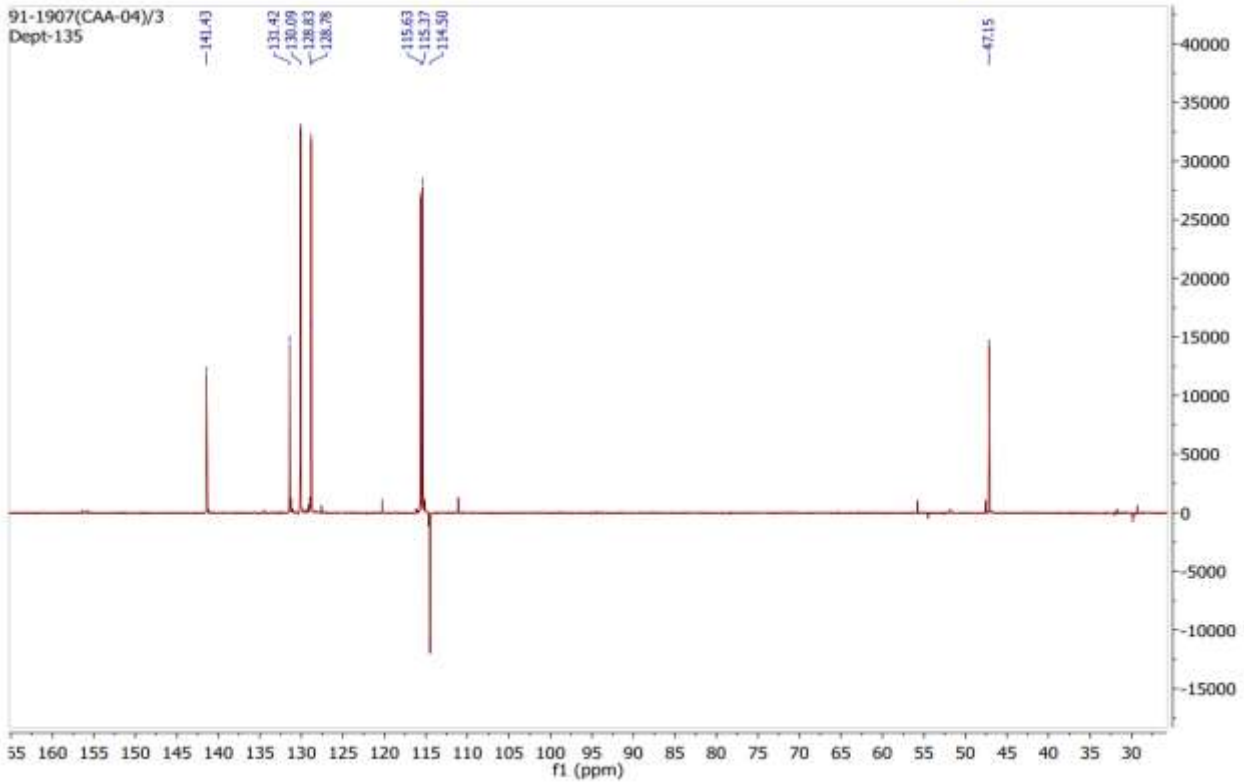
Appendix 6. ^{1}H NMR spectrum of compound **AA-6**, CDCl3, 400 MHz



Appendix 7. ^{13}C NMR spectrum of compound **AA-6**, CDCl3, 100 MHz



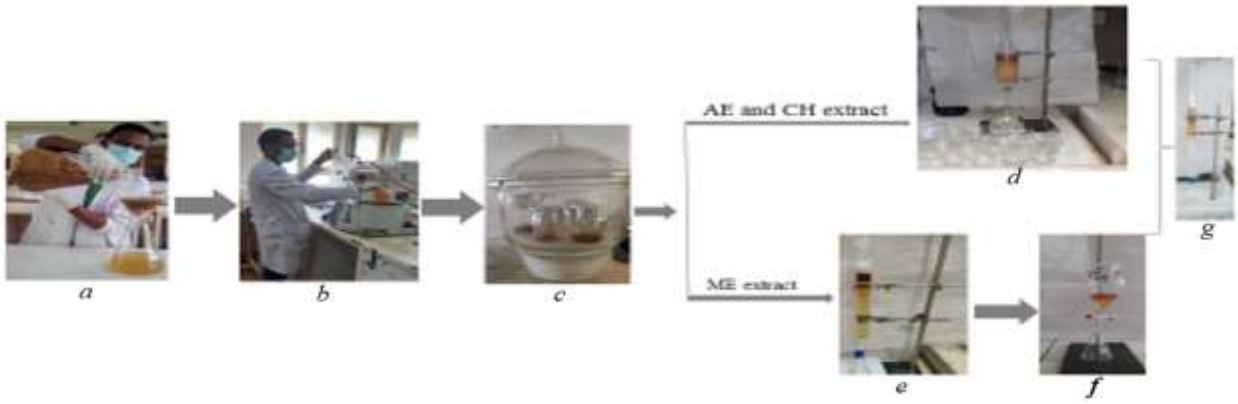
Appendix 8. DEPT-135 spectrum of compound **AA-6**, CDCl3, 100 MHz



Appendix 9. Schematic diagram for preparation of *A. africanus Lam.* for the crude extraction.

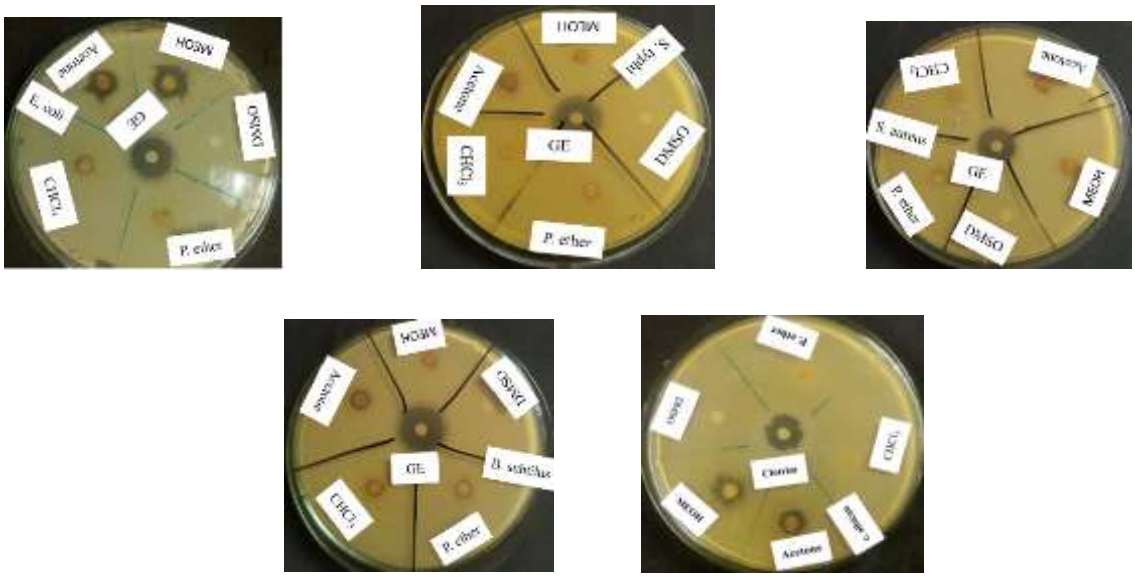
The latter indicate: - a=size reduction, b=dried, c= grinded, d=crude extraction.

Appendix 10. Schematic diagrams for the isolation of compound by column chromatography.

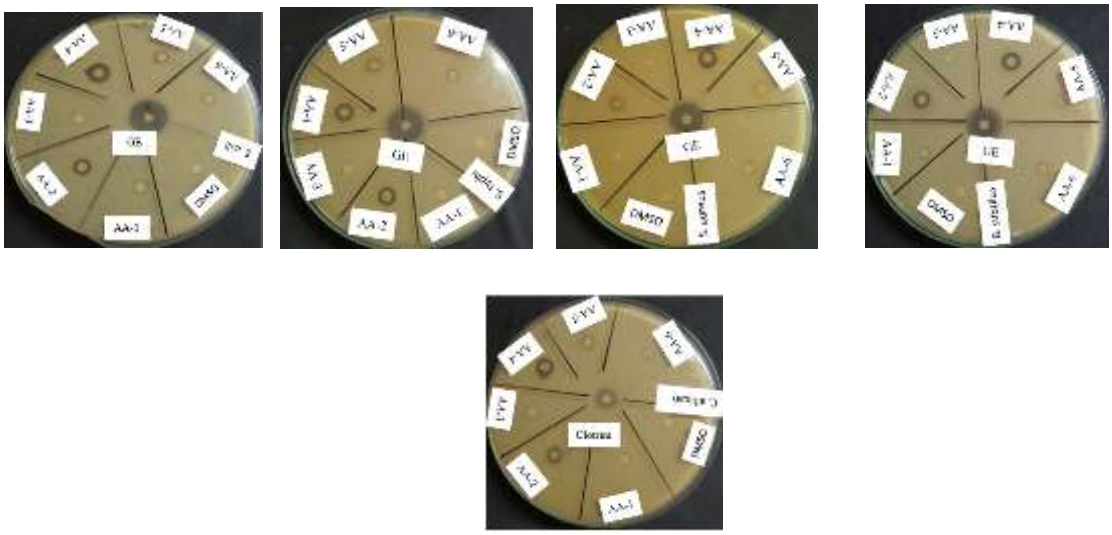


The latter indicate: - a=filtration, b=concentration, c= desecrater, d=1st CC for AE and CE extract,

Appendix 11. Antimicrobial activity of the root crude extract of *A. africanus* Lam.

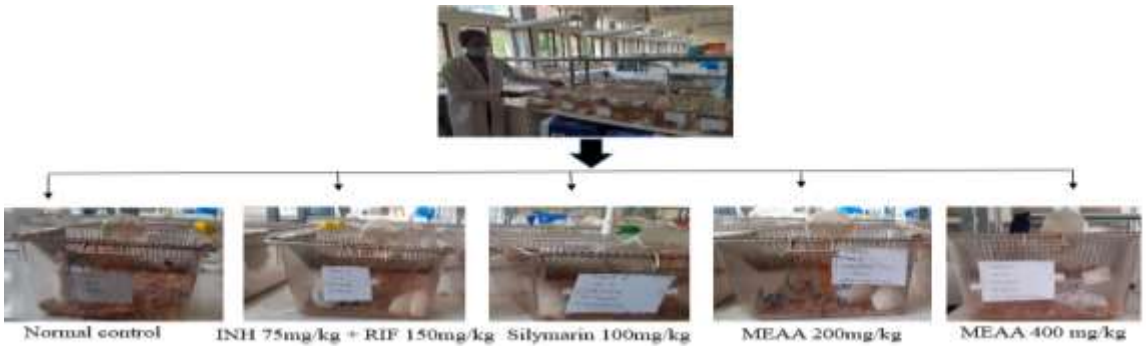


Appendix 12. Antimicrobial activity of isolated compounds from root extract of *A. africanus*

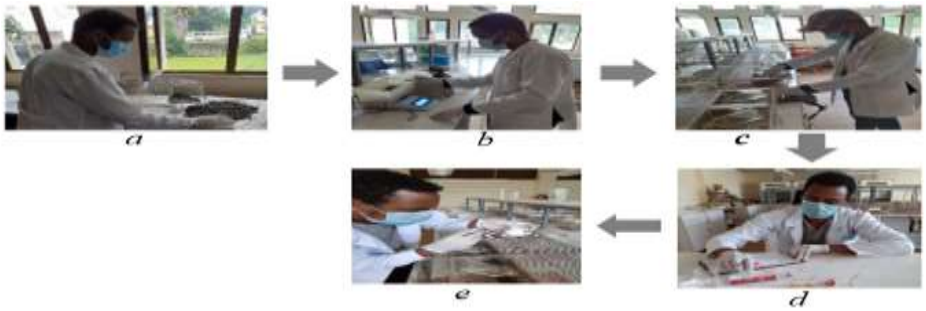


Appendix 13. Dose dependent effect of acetone and methanol crude root extract of *A. africanus*

Appendix 14. Grouping experimental animals and treatment protocol.

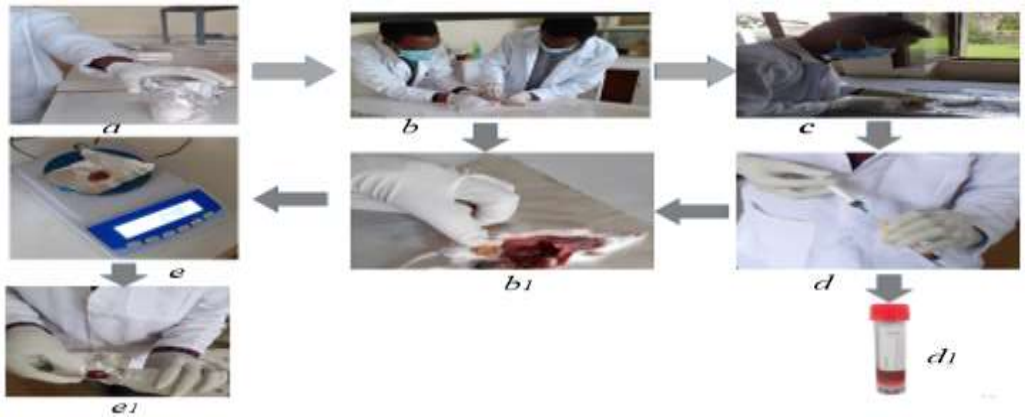


Appendix 15. Schematic diagram for diet preparations, drug dose and oral administration of mice.



The latter indicate: - a, b, and c= preparation, measuring and administration of basal diet, d=oral dose administration and e=preparation of drug dose.

Appendix 16. Blood and liver collections for the serum biomarker and histopathological examination.



The latter indicate: - a= anesthetized with diethyl ether, b & b1=dissecting of the mice, c, d & d1= blood collection and preparation, e and e1=liver weight taken and preservation and

Appendix 17. Body weight follow up checklist

Group	Sex	No.	Initial BW	Week-1	Week-2	Final BW
G-I	Male	1				
		2				
		3				
		4				
		5				
G-II	Male	1				
		2				
		3				
		4				
		5				
G-III	Male	1				
		2				
		3				
		4				
		5				
G-IV	Male	1				
		2				
		3				
		4				
		5				
G-IV	Male	1				
		2				
		3				
		4				
		5				

Appendix 18. Post hoc analysis table one-way ANOVA (Tuckey-test).

Descriptives									
		n	Mean	SD	SE	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
lwt	1	5	3.8260	.07436	.03326	3.7337	3.9183	3.75	3.92
	2	5	4.0360	.03912	.01749	3.9874	4.0846	4.00	4.10
	3	5	3.8640	.08385	.03750	3.7599	3.9681	3.79	4.00
	4	5	3.9340	.37112	.16597	3.4732	4.3948	3.35	4.25
	5	5	3.8860	.01673	.00748	3.8652	3.9068	3.86	3.90
	Tot	25	3.9092	.17552	.03510	3.8367	3.9817	3.35	4.25
I-bwt	1	5	37.8000	1.4832	.66332	35.9583	39.6417	36.00	40.0
	2	5	37.0000	1.0000	.44721	35.7583	38.2417	36.00	38.0
	3	5	37.4000	1.1401	.50990	35.9843	38.8157	36.00	39.0
	4	5	37.6000	.54772	.24495	36.9199	38.2801	37.00	38.0
	5	5	38.6000	1.5165	.67823	36.7169	40.4831	37.00	41.0
	Tot	25	37.6800	1.2151 8	.24304	37.1784	38.1816	36.00	41.0 0
W-1	1	5	38.6000	2.0736	.92736	36.0252	41.1748	36.00	41.0
	2	5	39.0000	.70711	.31623	38.1220	39.8780	38.00	40.0
	3	5	38.8000	.83666	.37417	37.7611	39.8389	38.00	40.0
	4	5	38.200	.44721	.20000	37.6447	38.7553	38.00	39.0
	5	5	38.2000	2.2803	1.01980	35.3686	41.0314	36.00	42.0
	Tot	25	38.5600	1.3868	.27737	37.9875	39.1325	36.00	42.0
W-2	1	5	41.2000	.44721	.20000	40.6447	41.7553	41.00	42.0
	2	5	40.2000	.44721	.20000	39.6447	40.7553	40.00	41.0
	3	5	41.2000	.44721	.20000	40.6447	41.7553	41.00	42.0
	4	5	40.2000	.83666	.37417	39.1611	41.2389	39.00	41.0
	5	5	41.2000	1.0954	.48990	39.8398	42.5602	40.00	43.0
	Tot	25	40.8000	.81650	.16330	40.4630	41.1370	39.00	43.0
W-3	1	5	47.6000	2.0736	.92736	45.0252	50.1748	45.00	50.0
	2	5	45.0000	1.0000	.44721	43.7583	46.2417	44.00	46.0
	3	5	46.2000	1.9235	.86023	43.8116	48.5884	44.00	49.0
	4	5	44.8000	1.3038	.58310	43.1811	46.4189	43.00	46.0
	5	5	45.2000	.83666	.37417	44.1611	46.2389	44.00	46.0
	Tot	25	45.7600	1.7387	.34775	45.0423	46.4777	43.00	50.0
F.bw	1	5	50.4000	2.5099	1.12250	47.2834	53.5166	48.00	53.0
	2	5	47.8000	1.3038	.58310	46.1811	49.4189	46.00	49.0
	3	5	49.0000	.70711	.31623	48.1220	49.8780	48.00	50.0
	4	5	48.4000	.89443	.40000	47.2894	49.5106	47.00	49.0
	5	5	49.2000	1.3038	.58310	47.5811	50.8189	48.00	51.0
	Tot	25	48.9600	1.6196 7	.32393	48.2914	49.6286	46.00	53.0 0

Descriptives

		n	Mean	SD	SE	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
ALT	G-1	5	41.5600	2.63116	1.17669	38.2930	44.8270	38.80	45.40
	G-2	5	118.1600	.33615	.15033	117.7426	118.5774	117.80	118.70
	G-3	5	43.0600	.95812	.42849	41.8703	44.2497	41.80	44.10
	G-4	5	64.4840	3.68948	1.64999	59.9029	69.0651	58.30	68.20
	G-5	5	45.8400	2.02312	.90477	43.3280	48.3520	43.00	48.30
	Total	25	62.6208	29.63802	5.92760	50.3868	74.8548	38.80	118.70
AST	G-1	5	55.9000	2.56515	1.14717	52.7149	59.0851	52.00	58.10
	G-2	5	167.3600	4.31022	1.92759	162.0082	172.7118	160.10	171.20
	G-3	5	58.0000	2.53180	1.13225	54.8564	61.1436	54.10	60.30
	G-4	5	100.8400	2.35648	1.05385	97.9140	103.7660	98.30	104.20
	G-5	5	97.4200	1.93054	.86337	95.0229	99.8171	94.10	99.10
	Total	25	95.9040	41.33787	8.26757	78.8406	112.9674	52.00	171.20
ALP	G-1	5	113.8000	5.99625	2.68160	106.3547	121.2453	107.30	120.20
	G-2	5	239.1800	3.68673	1.64876	234.6023	243.7577	236.00	244.20
	G-3	5	116.9000	1.86682	.83487	114.5820	119.2180	114.10	118.80
	G-4	5	199.8400	12.69854	5.67896	184.0727	215.6073	191.20	222.10
	G-5	5	134.7400	3.83706	1.71598	129.9757	139.5043	129.10	138.10
	Total	25	160.8920	51.36885	10.27377	139.6880	182.0960	107.30	244.20
Alb	G-1	5	4.4200	.48166	.21541	3.8219	5.0181	3.60	4.80
	G-2	5	2.9700	.23675	.10588	2.6760	3.2640	2.66	3.21
	G-3	5	4.1800	.57619	.25768	3.4646	4.8954	3.60	4.80
	G-4	5	3.4820	.24004	.10735	3.1839	3.7801	3.10	3.70
	G-5	5	4.1420	.31909	.14270	3.7458	4.5382	3.80	4.60
	Total	25	3.8388	.65381	.13076	3.5689	4.1087	2.66	4.80
TB	G-1	5	.6040	.29484	.13186	.2379	.9701	.10	.82
	G-2	5	3.2780	.45246	.20235	2.7162	3.8398	2.80	3.80
	G-3	5	.9420	.19524	.08732	.6996	1.1844	.73	1.20
	G-4	5	1.7580	.50727	.22686	1.1281	2.3879	.89	2.20
	G-5	5	1.2440	.32424	.14500	.8414	1.6466	.96	1.60
	Total	25	1.5652	1.01449	.20290	1.1464	1.9840	.10	3.80