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Extraction and Identification of saponin extracts from *Lepidium aucheri Boiss* and antifungal properties evaluation

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***Abstract*— The analysis by High-performance liquid chromatography (HPLC) revealed that the saponin extracts contain a significant component called (Hederagenin glycoside). The wavelengths and electronic transitions of the active groups of the saponin extract were also determined using the UV-Vis Spectra. The optimal result was obtained by utilizing the Gemini C18 (5 µm) stationary phase manufactured by Phenomenex. They determine the optimal chromatographic conditions for enhancing LC/MS analysis by evaluating several mobile phases on a reversed-phase C18 column. Illustrates the anticipated patterns of fragmentation observed in the mass spectra of substances. The Antifungal activity against Aspergillus flavus, Aspergillus Niger, and Aspergillus Alternaria was determined. The inhibitory effect in vitro was defined to appear, triterpenoid saponins extract showed maximum growth activity inhibitory of fungi against pathogens like Aspergillus Alternaria and Aspergillus Niger (2 cm), at the concentration (100 ppm) and the minimum growth activity inhibitory was against Aspergillus Alternaria (5 cm) at a concentration (25 ppm) compare with control. The triterpenoid saponins extract its ability to grow activity inhibition of fungi type Aspergillus Alternaria, Aspergillus Niger. On the other hand, the percentage of hemolysis activity in human blood increases with a high concentration of triterpenoid Saponins extract.**

***Keywords*— Plant extracts; Saponins; Antifungal activity; Aspergillus Alternaria**

I. INTRODUCTION

Natural goods are compounds or substances derived from living organisms. These can include a wide range of molecules, such as secondary metabolites, peptides, proteins, nucleic acids, and more, often involved in various biological functions and interactions. Natural products have been a rich source of pharmaceuticals, providing many essential drugs and drug leads. They are often studied for their potential therapeutic properties and roles in ecology, evolution, and other scientific disciplines. Examples of natural products include antibiotics, anticancer agents, immunosuppressants, and plant-derived compounds used in traditional medicine[1]. Medicinal plants are indeed a rich source of natural products with diverse bioactive compounds, including saponins. Saponins are a class of natural compounds found in many plants, and they are known for their various biological activities, including antioxidant, anti-inflammatory, anticancer, and immune-modulating properties [2]. Saponins are also known for their ability to lower cholesterol levels, enhance immune function, and have antimicrobial properties. They are widely studied for their potential therapeutic applications and are found in many medicinal plants used in traditional medicine systems worldwide [3].

Lepidium aucheri Boiss is a small herbaceous plant with a height range of 2-15 cm, characterized by white petals. It is native to Iraq and is mainly found in desert areas and the alluvial plain. As a member of the family Cruciferae (also known as Brassicaceae), *Lepidium aucheri* is one of several species of Lepidium found in Iraq [4]. The conservation and use of medicinal plants like Lepidium aucheri are essential for preserving biodiversity, preserving traditional knowledge, and discovering new potential therapies. The plant's medicinal properties may be attributed to its bioactive compounds, which include flavonoids, phenolic compounds, and glucosinolates [5].

Saponins, present in many plants, including *Lepidium aucheri*, are known for their diverse biological effects. These compounds have been studied for their potential benefits in human and animal nutrition, including their role as antioxidants, cholesterol-lowering agents, and immune system modulators. The biological effects of saponins make them an exciting area of research for their potential therapeutic applications in various health conditions [6].

Saponins, plant-derived secondary metabolites, are used in shampoos and beverages to induce foaming. The sapogenins generated from saponins during acid hydrolysis are frequently employed as initial substances for producing steroidal medicines. Nevertheless, the present techniques incorporate saponin within a dense substance known as "gum," which comprises other contaminants [7]. This gum restricts the availability of saponin, resulting in decreased hydrolysis efficiency and necessitating significant amounts of heat, organic solvents, and labor to retrieve the sapogenin

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[8]. Herbalists have traditionally produced tinctures by immersing plant components in a combination of alcohol and water, allowing them to soak at room temperature for extended periods. Several herbal tinctures contain saponins that are present in solution without any gum [9].

II. Aim of Study

The current study seeks to extract the components of saponins quantified from the *Lepidium aucheri* Boiss plant in the Thi-Qar Governorate of Iraq. It also wants to diagnose the extract and evaluate its biological activity against different types of fungi.

III. Material and Method

Lepidium aucheri boiss was gathered northwest of Nasiriyah City, Iraq. Prof. Hyder Radhi verified the plant's authenticity and categorized the specimen in the University of Thi-Qar Biological Department of Science. The plant was cleaned, given a distilled water wash, allowed to air dry for several weeks in the shade, ground into a powder, and stored in opaque glass jars for later use.

A. Extraction of crude saponin from Lepidium aucheri boiss

100 g of air-dried powdered leaves of Lepidium aucheri boiss were extracted with 70% ethanol (EtOH) (24 hours x 1000 mL) at room temperature. The EtOH solution was concentrated to 300 ml by low-pressure evaporation at 45 °C and then successively extracted with chloroform (24 hours x 100 ml x 3) and n-butanol (24 hours x 100 ml x 3). The n-butanol layer was evaporated to dryness for the saponins extract (10g), The substance is a potent saponin extract with a dark brown hue, and high viscosity, and produces a substantial amount of foam. [10].

B. Detection of Triterpenoids in Plants Extracts

1ml of concentrated sulfuric acid was added to 1ml of extract dissolved in chloroform; the purple-red indicates the presence of triterpenoids [11].

C. Investigation of Saponin Extract by UV-VIS (Spectra)

Using an automatic recording spectrophotometer, the absorption spectra of plant components were measured in a diluted solution against a blank solvent. Distilled water was utilized as the solvent for the UV spectroscopy technique, which was carried out using saponin extract. The absorbance of the sample solution was measured using a twin-beam UV-VIS spectrophotometer (PG Instruments Limited T80) with a scan range of 190 to 800 nm [12].

D. Investigation of Saponin Extract by FTIR Spectra

We recorded using Model Bruker FT-IR affinity spectrophotometer in the Department of Chemistry, College of Science, Thi-Qar University, Iraq. Only principal absorption bands of interest are reported and expressed in cm-1.

E. Investigation of Saponins Extract by HPLC Technique

The sample was analyzed using an HPLC column with ODS particles measuring 50×4.6 mm in diameter. The mobile phase comprised water with 0.1% phosphate buffer (0.01 M) labeled as (A) and acetonitrile with 0.1% phosphate buffer (0.01 M) labeled as (B), flowing at a rate of 1.0 mL/min. Analyzed using the gradient elution method described below: 20% A and 80% B were maintained for 10 minutes, followed by a 5-minute wash with 100% B after each run. UV detection was set at 254 nm. The separation occurred using a Shimadzu 10 AV-LC liquid chromatographic system with a binary delivery pump model LC-10A Shimadzu. The eluted peaks were detected using a UV-Vis 10 A-SPD spectrophotometer [13].

F. Investigation of Antifungal Activity of Saponins Extracts

Microorganisms: Aspergillus flavus, Aspergillus niger, and Aspergillus Alternaria were obtained from the AL-Hussein Teaching Hospital in the Thi-Qar microbiology laboratory.

G. Antifungal activity test

The antifungal activity was tested in vitro using yeast PDA on a Petri dish [14]. The concentrations that were measured matched those previously mentioned. But the PDA medium also received saponin extract added to it. For control tests, sterile water (25, 50, and 100 ppm) was added to the PDA medium. After preparing the Petri dishes, the fungi were infected immediately by inserting a 5 mm diameter sample of the cultivated test fungus' mycelial mass in the center of each plate. This sample was taken from the periphery of the growing cultures on PDA plates prepared as previously mentioned and cut with a sterile cork borer. Measuring the diameter of the mycelial growth, the Petri dishes were kept at 25ºC in the dark. After the mycelial mass control Petri dishes had nearly filled the Petri dish after seven days, the incubation was halted. By averaging the mycelial mass's radial expansion in two orthogonal directions, the diameter of the growth mass was ascertained [15 - 17].

H. Investigation of Saponins Extract by LC- LC-mass Spectra

The HPLC-Mass system was a Water Alliance in the Department of Chemistry at Surrey University in London, UK. It consisted of a quaternary pump, a vacuum solvent micro degasser, and an autosampler with a 100-well tray. The separation was conducted using a Gemini C18 column with 100 x 2.0 mm dimensions and a particle size of 5 µm (Phenomenex). The column's temperature was kept and utilized at room temperature. The mobile phase was a mixture of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) flowing at a rate of 0.5 ml/min. The gradient elution process transitioned from 95% A and 5% B to 80% A and 20% B over specific time intervals: 0 min, 0.40 min, and 2 min. After each run, there was a 3.50-minute wash with 100% solvent B, followed by an equilibration phase where the solvent composition changed from 95% to 5% B over 6 minutes and 95% A to 5% B over 7 minutes. The analyses were conducted using Nitrogen with the capillary temperature set at 350ºC, the capillary voltage at 57 V, and a gas nebulizer flow of 11L/min. Mass spectra of the production were acquired within the m/z range of 100-1000.

IV. Results and Discussion

A. Plant Extracts:

The weight of dry powder material of *Lepidium aucheri boiss* was 100 g. The dry powder product LAB saponin extract was 10 g.

B. UV-Visible Spectra:

Figure (1) illustrate the UV-visible analysis results for the saponin extracts, recorded between 190 and 800 nm. The UV-Vis spectra of saponins have an intensity and a distinct absorption peak at 205 nm for π-π* electronic transitions, with λ max at 205 nm and another peak at 264 nm and 345 nm, respectively. This is because these compounds have multi-double boundaries in their rings. Due to the presence of non-bonding electrons for oxygen atoms in these compounds, n-π* electronic transitions cause the other peak, which was low intensity, to arise at longer wavelengths at 264 and 345 nm.

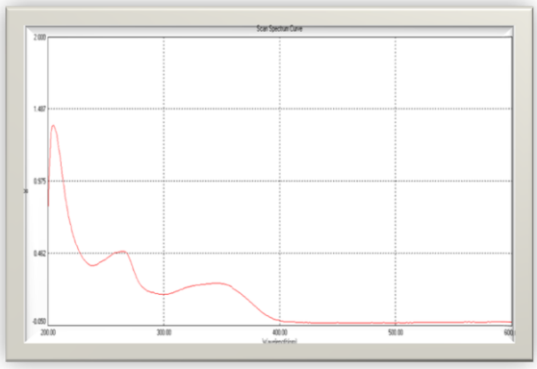


Figure (1): Absorption spectrum of Saponins extract by (UV- SCAN) in water.

C. Characterization of saponins which extract by studies Fourier transform Infrared Spectrophotometry (FTIR)

FTIR spectra were measured to identify saponin extracts in the range (500 - 3500 cm-1) (Fig 2). In general, the bands in the spectra of FTIR were expected to appear as follows:

1- Stretching vibration hydroxyl group (-OH) long and broad peak (3394.24 cm-1).

2- Stretching vibration belongs to the band (C-H) group, which has a robust and sharp intensity between ranges (2933.53 - 2620.50 cm-1).

3- Stretching vibration carbonyl group (C=O) sharp peak in the range (1705.42 - 1600.62 cm-1).

4- Stretching vibration belongs to the band (C=C) group with a medium intensity between (1398.11 - 1384.47 cm-1).

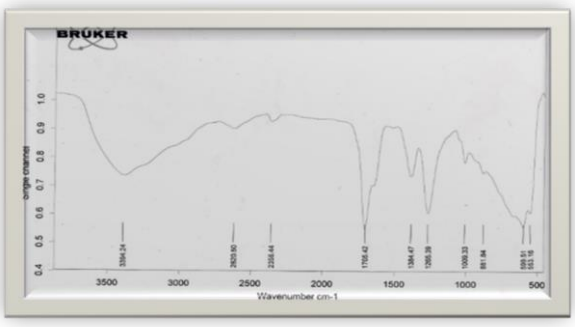


Figure (2): FTIR spectrum of acidic hydrolysis of Saponins extract.

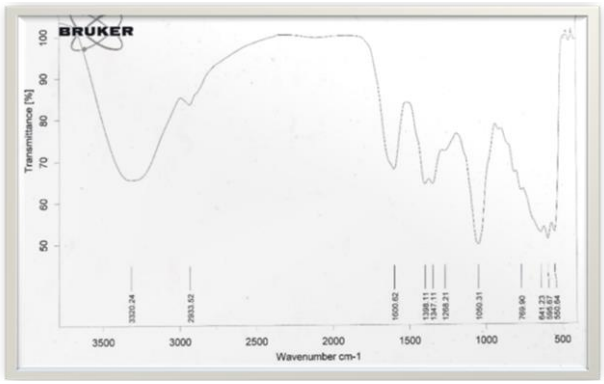


Figure (3): FTIR spectrum of Saponins extract.

D. Identification of Plant Saponins Compounds by HPLC Technique:

Figures (4) and (5) demonstrated a consistent retention time between the sample and the standard for most components in each extract. Tables (1) and (2) displayed the retention time of the standards and Saponins extracts, respectively. The analysis revealed that the saponin extracts contain a significant component called Hederagenin, as indicated by the HPLC results. Figures 4 and 5 depict the presence of this drug, while its structures are presented in Table (3). The peaks observed in the chromatogram indicate the presence of unidentified chemicals, believed to be derivatives of saponins compounds [18]. High-performance liquid chromatography (HPLC) extensively studies saponin compounds [19].

The extracts were examined to determine the concentration of saponin compounds. The chemical was identified by comparing its retention time with pure commercial standards [20]. The HPLC analysis of saponins is influenced by several parameters, such as sample purification, mobile phase composition, column types, and detectors [21].

Table (1): Retention time of standard Hederagenin compound

Seq	Standard	Retention time (min)	Area
1	Hederagenin	1.351	2764644

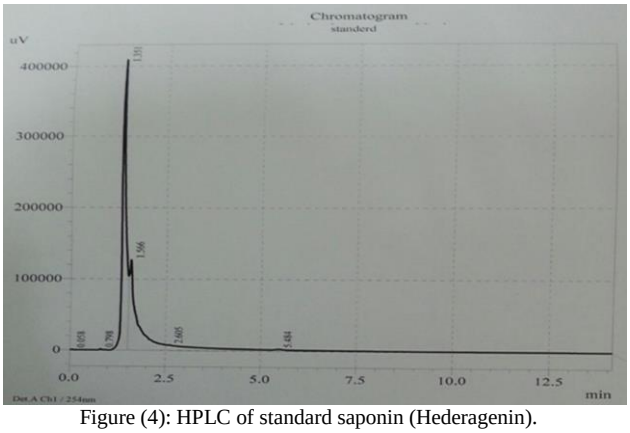


Figure (4): HPLC of standard saponin (Hederagenin).

Table (2): Retention time of Saponins compounds in *L. aucheri Boiss*

Seq	Saponins contents in the extract	Retention time (min)	Area
1	Hederagenin	1.397	2915762

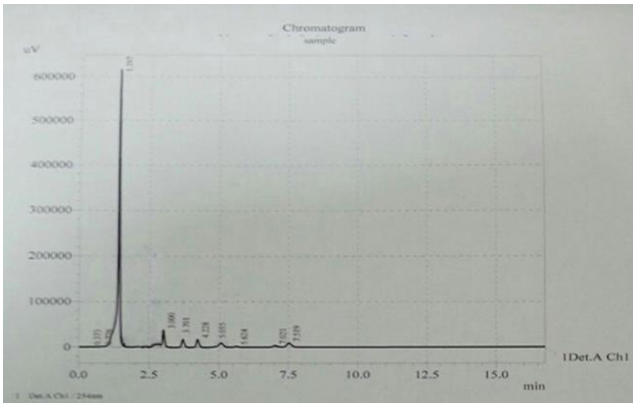
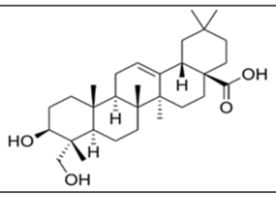


Figure (5): HPLC spectrum of hydrolysis Saponins extract

Table (3): Names and structure of Saponin compound suggested in L. aucheri Boiss

Communally name	Organizational name	**Structure**
Hederagenin	3(***β***)-3,23-Diydroxyolean-12-en-28-oic acid	

E. Characterization of saponins extract by studies (HPLC-MS):

The most favorable outcome was achieved using the Gemini C18 (5 µm) stationary phase from Phenomenex. They identify the best chromatographic conditions for an improved LC/MS analysis by testing various mobile phases on a reversed-phase C18 column. Figure (6) displays the chromatogram of the terpenoids found in L.aucheri boiss samples. This procedure utilized the positive ions of compounds 1-3 collected, as indicated in Table 4. Figure (8) displays the proposed fragmentation patterns seen in the mass spectra of substances.

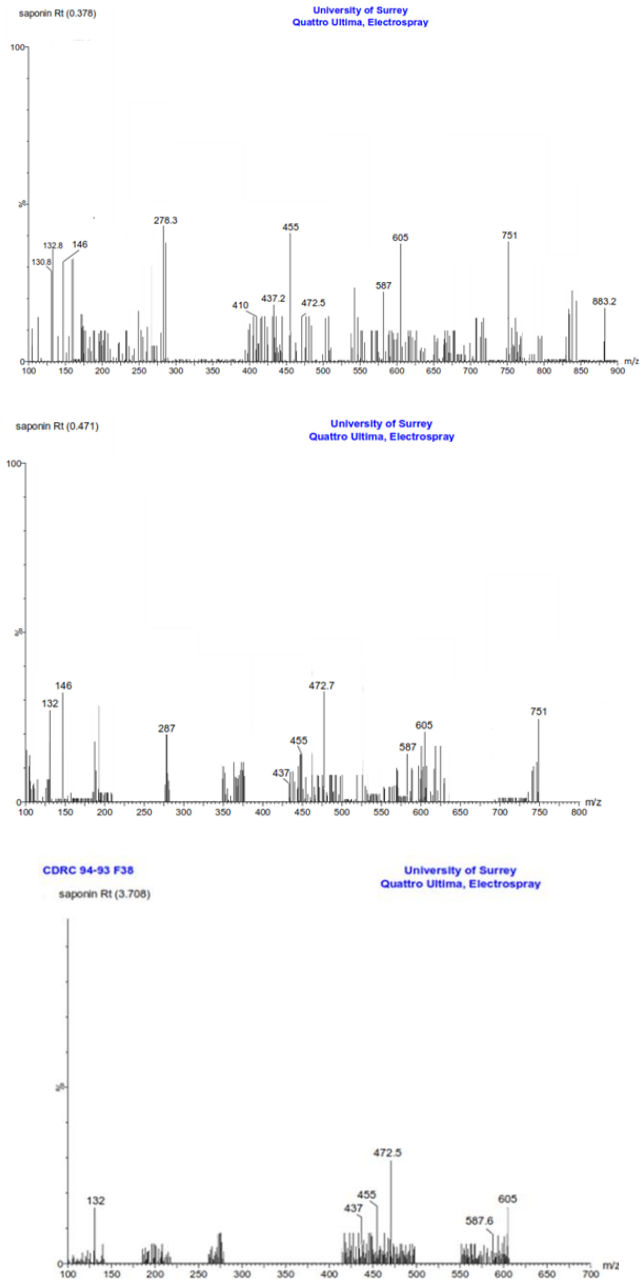


Figure (6): Extracted ion chromatographic and mass spectra of saponins (A compound 1- 3) from L. aucheri boiss

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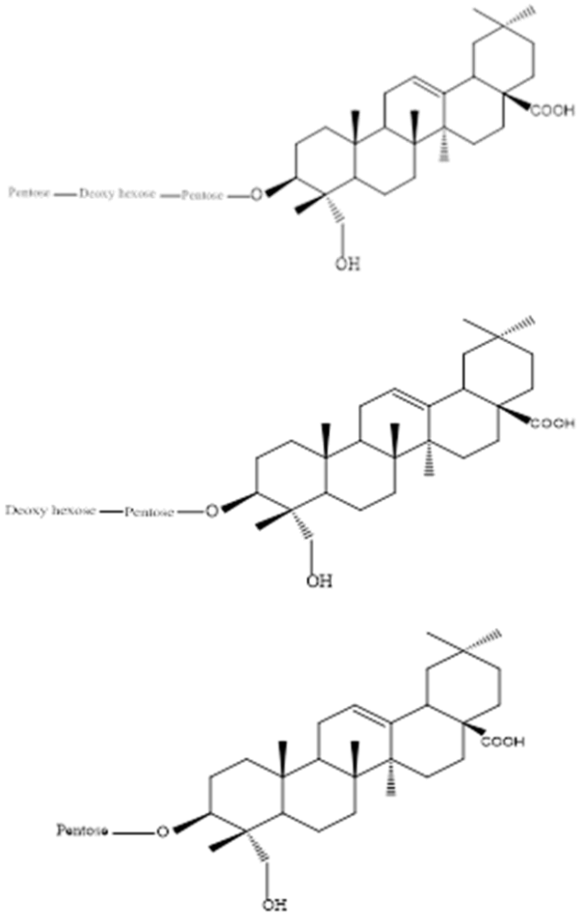

Figure (7): The Stru cture compounds of chemical constituents from *L. aucheri boiss* extract.

F. Antifungal Properties of Saponins Extracts.

Managing fungal infections continues to be a significant concern on a global scale. Over the past few decades, there has been a notable rise in the prevalence of fungal infections, such as superficial mycoses (affecting nails, hair, skin, and mucosal membranes) and systemic mycoses (affecting vital organs)[22]. The prevalence of dermatophytosis is increasing among school children in rural settings, compared to other fungal illnesses.

Saponins are recognized for their diverse biological actions, such as their ability to inhibit the growth of fungi. They can disturb the integrity of fungal cell membranes, resulting in the demise of the cells. Saponins possess the ability to disrupt fungal enzymes and impede their proliferation. Research has demonstrated that saponin extracts derived from many plants display antifungal properties against a wide range of fungus species [23].

The study's tables (5) indicate that the separated saponins exhibit antifungal activities against a range of fungi, such as A. flavus, A.niger, and A. Alternaria. On the contrary, it does not impact the growth of fungi such as C. albicans, C.tropicalis, and C.krusithe. The triterpenoid saponin extract demonstrated the most significant growth inhibition against fungal infections, specifically A. Alternaria and A. niger, with a diameter of 2 cm when applied at a concentration of 100 ppm. Compared to the control, the growth inhibition against A. Alternaria was minimal, measuring just 5 cm at a concentration of 25 ppm. The triterpenoid saponin extract exhibits notable fungicidal activity against A. alternaria and A. niger fungi. As mentioned earlier, the findings were confirmed by other researchers who reported substantial antifungal effectiveness against foodborne illnesses [24-25]. On the other hand, Tables (5) demonstrate that the triterpenoid saponin extract does not successfully hinder the growth of fungi such as C. albicans, C. tropicalis, and C. krusi, with no apparent inhibition. These findings are similar to the results documented by [26-27]. The values of growth inhibitory activities of fungi in this study are shown in Table (5).

Table (4): Peak assignment for the analysis of terpenoid saponins by HPLC-MS Method.

Peak	RT	[M]+	MS fragment ions (m/z)
1	0.378	883.2	883.2 751 [M+¬¬- H - Sugar pentose] 605 [M+ - H - Sugar pentose - Sugar deoxy Hexose] 587 [M+ - H-OH - Sugar pentose - Sugar deoxy Hexose] 455 [M+- H - Sugar pentose - Sugar deoxy Hexose - Sugar pentose] 455 [M+- H - OH - Sugar pentose - Sugar deoxy Hexose - Sugar pentose] 437 [M+- 2H2O - Sugar pentose - Sugar deoxy Hexose - Sugar pentose] 410 [Sugar pentose + Sugar deoxy Hexose + Sugar pentose] 287 [Sugar pentose - Sugar deoxy Hexose] 146 [Sugar deoxy Hexose] 132 [Sugar pentose]
2	0.471	751	605 [M+ - H - Sugar Deoxy Hexose] 587 [M+ - H - OH - Sugar Deoxy Hexose] 472.7 [M+ - H - Sugar Deoxy Hexose - Sugar Pentose] 455 [M+ - H - OH - Sugar Deoxy Hexose - Sugar Pentose] 437 [M+ - H2O - Sugar Deoxy Hexose - Sugar Pentose] 287 [Sugar Deoxy Hexose + Sugar Pentose] 146 [Sugar Deoxy Hexose] 132 [Sugar Pentose]
3	3.708	605	587.6 [M+ - H - OH] 472.5 [M+ - H - Sugar Pentose] 455 [M+ - H - OH - Sugar Pentose] 437 [M+ - 2H2O - Sugar Pentose] 132 [Sugar pentose]

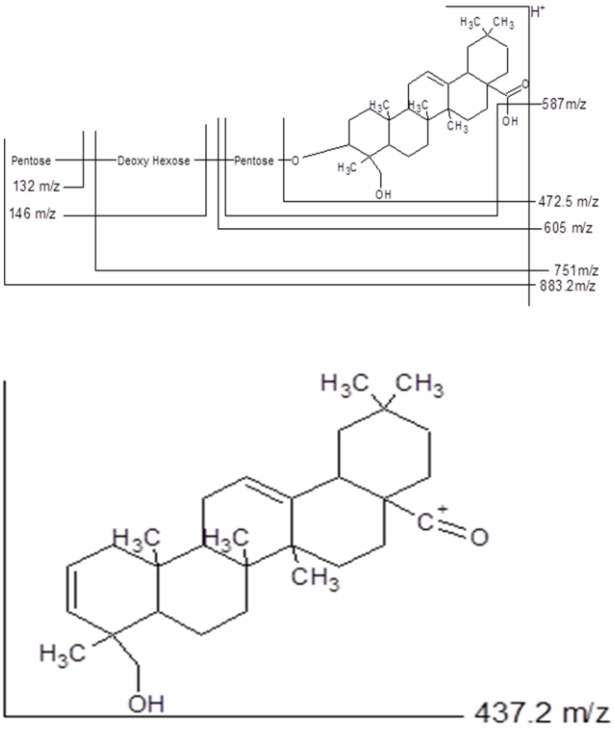

Figure (8): proposed fragmentation of compound 1.

Table (5): The growth inhibitory activities of fungi.

Concentration of triterpenoid saponin	Type of fungi		
	A.alternaria	A.niger	A.flavus
Control	9 cm	9 cm	9 cm
25 ppm	5 cm	3 cm	4 cm
50 ppm	3 cm	2.5 cm	3.5 cm
100 ppm	2 cm	2 cm	3 cm

Table (6): Diameters of inhibition zone (millimeter) for all extracts

Type of Fungi	Zone of inhibition in mm
	Triterpenoid Saponins Extracts
C.albicans	0
C.tropicalis	0
C.krusi	0
H2O	0

Image (1): The control of Aspergillus Alternaria fungi

Image (2): The triterpenoid saponin concentration (25 ppm)

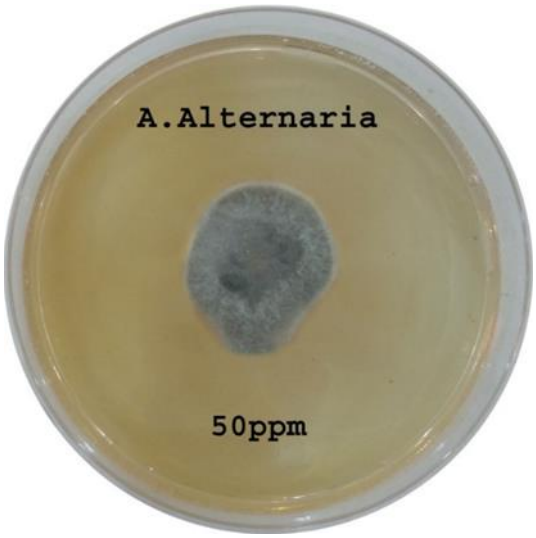

Image (3): The triterpenoid saponin at concentration (50 ppm)

Image (4): The triterpenoid saponin at a concentration (100 ppm**)**

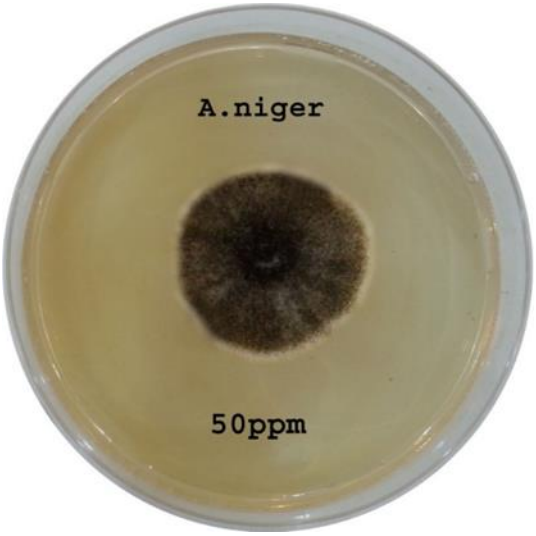

Image (7): The triterpenoid saponin at a concentration of (50ppm

Image (5): The control of Aspergillus niger fungi

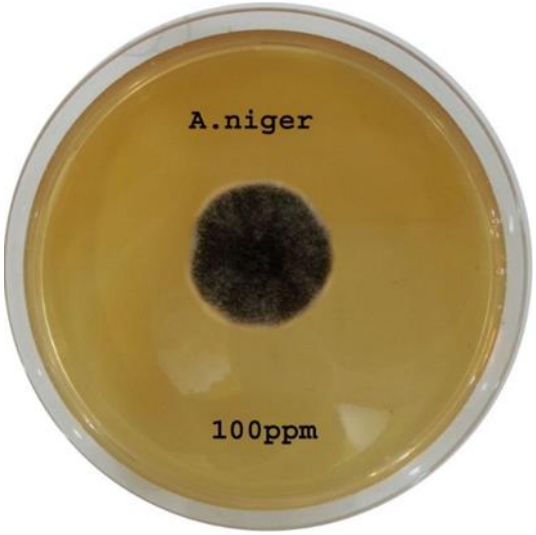

Image (8): The triterpenoid saponin at a concentration (100ppm)

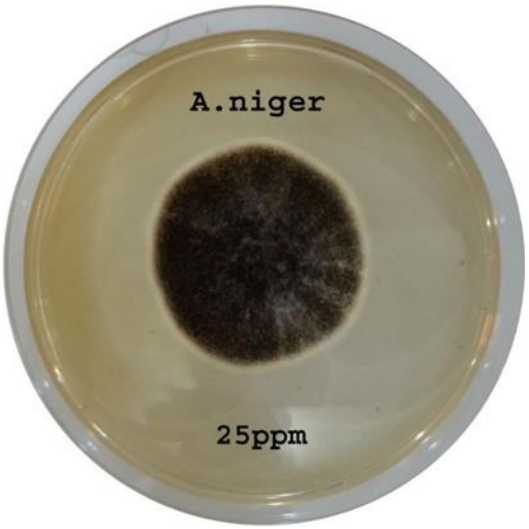

Image (6): The triterpenoid saponin at a concentration (25ppm

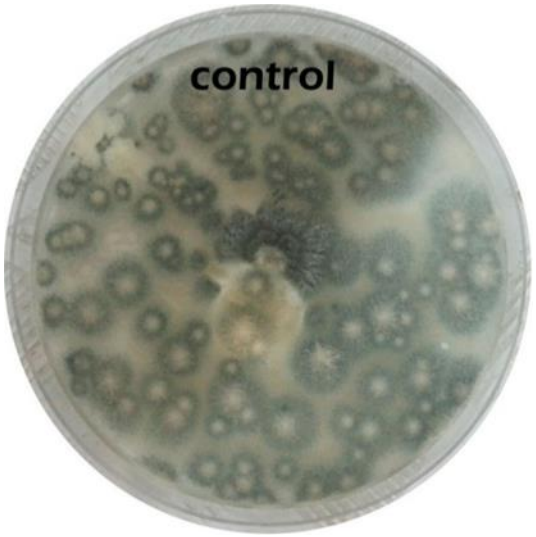

Image (9): The control of Aspergillus flavus fungi

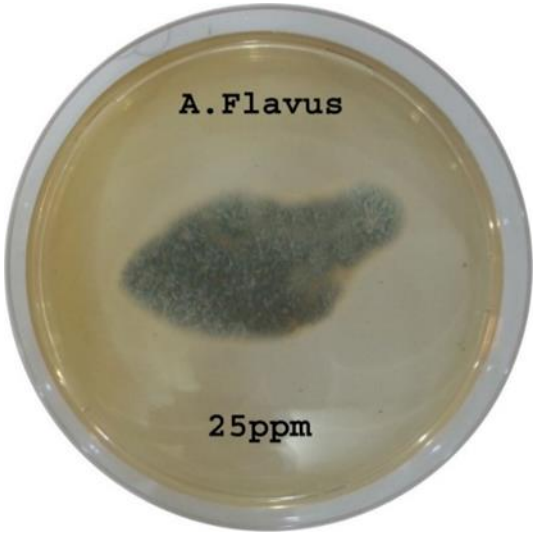

Image (10): The triterpenoid saponin at a concentration (25ppm)

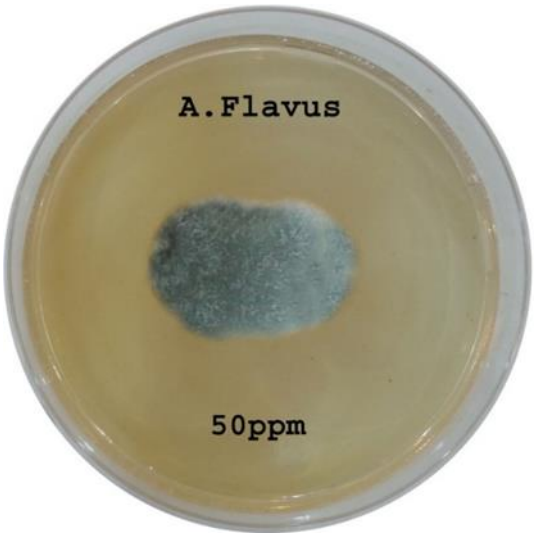

Image (11): The triterpenoid saponin at a concentration (50ppm)

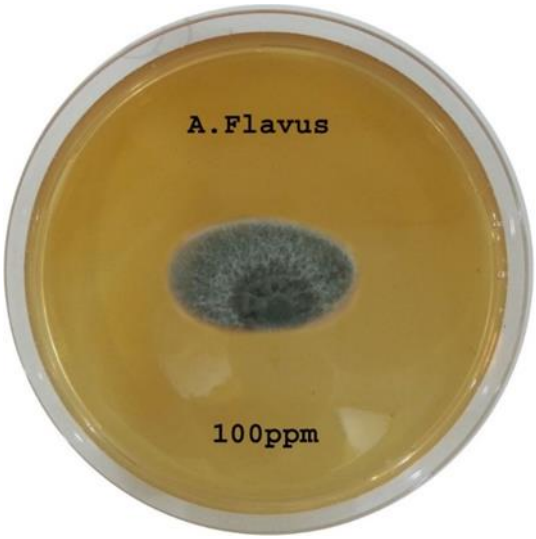

Image (12): The triterpenoid saponin at a concentration (100ppm

Image (13): The activity of Saponins extracts against(C.krusi) fungi.

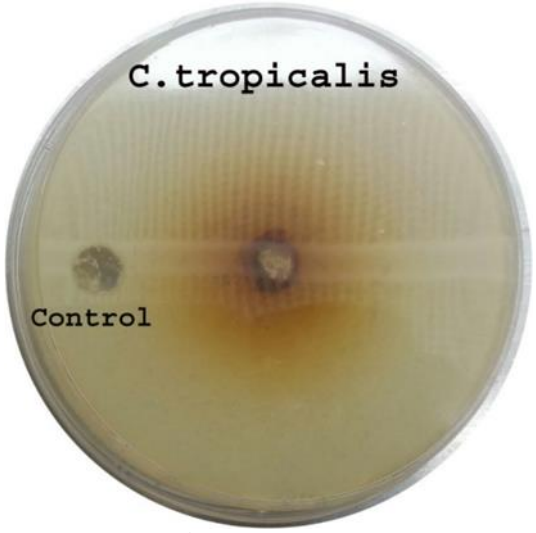

Image (15): The activity of Saponins extracts against(C.tropicalis) fungi

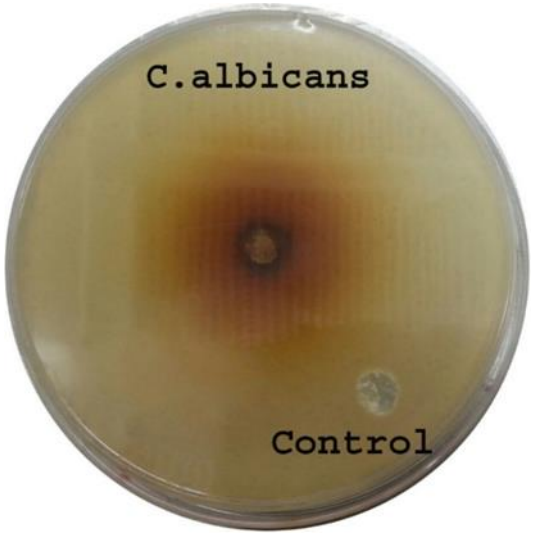

Image (14): The activity of Saponins extracts against(C.albicans) fungi.
Control: H2O

V. CONCLUSION

The following things can be inferred from the conclusion of this study:

a high yield was obtained from the triterpenoid saponin extract derived from L. aucheri boiss.

The identification of compounds present in each extract is accomplished by utilizing a UV-visible spectrum. Moreover, the existence of multiple peaks signifies the existence of triterpenoid saponin.

The compounds included in each extract are determined by analyzing the FTIR spectrum, which reveals the presence of triterpenoid saponin by the observation of several peaks.

The substance was identified using High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) techniques. From the triterpenoid saponin extract of L.aucheri boiss, one compound (Hederagenin) and three glycoside saponin compounds (1-3) were isolated.

The isolated chemicals have been evaluated for their fungistatic properties in comparison to different fungi. These extracts exhibit variable effects on distinct fungi when compared to the control fungi.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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