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Culturable Fungal Diversity of *Artemisia judaica* Roots and Their Bioactive Compounds

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Abstract: *Artemisia judaica* is recognized for its medicinal properties, yet its associated fungal community, particularly endophytes, remains inadequately studied. This study investigates rhizosphere, rhizoplane, and endophytic fungi from *A. judaica* roots to identify fungal diversity and explore their bioactive compound. Forty root samples were analyzed using various media, revealing differences in colony-forming units (CFUs) among the fungal groups. *Aspergillus* and *Penicillium* dominated the rhizosphere and rhizoplane, while endophytes showed lower yields, with *Aspergillus fumigatiaffinis* and *Aspergillus fischeri* being reported for the first time as *A. judaica* endophytes. A significant finding enhances comprehension of fungal-host interactions medicinal plants. Crude extracts of endophytic fungi exhibited strong antimicrobial activity against Gram-negative and Gram-positive, with notable antifungal effectiveness against *Fusarium solani*. ANOVA revealed significant differences in mean clear zones across bacterial and fungal groups ($p < 0.0001$, $R^2 > 95\%$). Tukey's post-hoc analysis identified *E. coli*, *B. cereus*, and *Fusarium solani* as most susceptible, while *B. subtilis*, *P. aeruginosa*, and *Candida albicans* were least affected. Gas chromatography/mass spectrometry of *A. fumigatiaffinis* extracts identified 28 bioactive compounds, including 14 with high peak areas, demonstrating antimicrobial, cytotoxic, anti-inflammatory, and antioxidant properties.These compounds align with those naturally found in *A. judicata*, revealing an allelopathy relationship and suggesting the fungi's unique ability to synthesize secondary metabolites identical to those in the host plant. This study underscores the fungal diversity in *A. judaica* roots and highlights *Aspergillus fumigatiaffinis* as a promising source of bioactive compounds for pharmacological applications, offering new insights into the potential of endophytic fungi in drug discovery.
Keywords: *Artemisia judaica*, *Aspergillus fumigatiaffinis*, Czapek's, endophytic fungi.

1. Introduction

Artemisia is an enormous and widely spread genus in the family *Astraceae* (Compositae) with approximately 500 species found primarily in the temperate zones of Europe, Asia, and North America. These species include evergreen, seasonal, and annual herbs or tiny trees [1, 2]. Moreover, *Artemisia* constitutes a highly esteemed genus for its abundance of secondary metabolites deemed valuable in various fields, including but not limited to pharmaceuticals and biopesticides. *Artemisia judaica* (L.) (Arabic: Sheeh), an Egyptian therapeutic plant, has been utilized to treat gastroenteritis [3], this species refers to two types of wormwood (common name) utilized by Bedouin tribe communities in Sinai, Egypt, for medicinal and edible purposes. Moreover, it has exhibited a broad set of biological actions, including anti-blastocystis effects as reported by Mokhtar et al. [4] along with anti-inflammatory, painkillers, antipyretic properties as stated by Batanouny et al. [5], and antioxidative action [6]. *Artemisia judaica*'s phytochemical research revealed the presence of several beneficial chemicals, including flavonoids [7, 8], also phenolic acids [9], sesquiterpene lactones [10], and triterpenes [11]. Additionally, supplementation of *Artemisia* could potentially augment the growth efficacy and equilibrium of the intestinal microbiota, with reduced pathogenic bacteria counts and increased beneficial bacteria populations [12]. Furthermore, trace element analysis across various *Artemisia* species indicated optimal hematopoietic complex trace element ratios in *Artemisia vulgaris*, suggesting a unique elemental composition in this plant genus [13]. Scientists are interested in various microbes with which plants interact in complex interactions, including rhizospheric, endophytic, and mycorrhizal organisms. These organisms have flourished in prevalence due to their revealed advantages [14,15].

Endophytes, a type of microorganism that inhabits intercellular and intracellular host plants without causing apparent disease, have important physiological and ecological implications such as growth stimulation and adaptation enhancement [16]. These fungi provide an abundance of new bioactive compounds that can be used in a variety of ways. Many reports have demonstrated that endophytic microbes have a high level of biological diversity and are a rich source of natural products with a broad spectrum of bioactivities [17]. More than 10,000 endophytic strains have been discovered and characterized to date, including bacteria, fungi, and actinomycetes [18]. Furthermore, endophytic moulds provide a critical role in facilitating the production of specific biomolecules that contribute to the growth, immune response, and defense mechanisms of plants [19]. The interaction between endophytes and their host plants can be highly

intimate, to the extent that these microorganisms can produce identical chemical compounds as their plant hosts [20, 21]. Fungal endophytes have shown the ability to synthesize compounds in vitro cultures even in the absence of a plant-host relationship [22, 23], making them beneficial in industrial microbiology applications.

Overall, microbiota in *Artemisia* plants contribute significantly to their growth, health, and bioactivity. The current study aimed at exploring the rhizosphere, rhizoplane and endophytic fungi associated with *A. judaica* root using comparative culturing media and testing the antimicrobial activity of extracts from the most abundant isolated endophytic fungal species. Further the bioactive compound of the most effective extract as antimicrobial agent was explored using gas-chromatography coupled with mass spectrometry analysis

2. Materials and methods

2.1. Collecting Plant Samples

Forty root samples of *Artemisia Judaica* plant were gathered from 'Wadi Abu Shih' in the Red Sea Governorate region, by using a sterile sharp blade. The samples were packed in a clean sterile plastic box, stored with ice packs, and transferred to the laboratory.

2.2. Isolation

2.2.1. Isolation of the Rhizosphere and Rhizoplane Fungi

Isolation of rhizosphere fungi is performed according to *Abdel-Hafez et al.* [24]. Briefly, five grams of root samples were added to sterile distilled water (100 mL) in a 250 mL flask and shaken thoroughly (3 mins, 1/100 dilution was prepared. Only 1 mL of the final dilution was placed on Czapek's-glucose (CzA) and Czapek's-cellulose agar media (CzCA) at pH 8.5.

For the isolation of rhizoplane fungi, the main roots along with lateral branches of *A. judaica* were completely washed with tap water, followed by sterilized distilled water. The cleaned roots then chopped into small pieces, with four segments placed per plate containing the same media CzA & CzCA. The plates were incubated at 26 ± 2.0°C for 5 days.

2.2.2. Isolation of Endophytic Fungi

Root samples were chopped into small fragments with sharp sterile blade. For surface sterility, the chopped fragments were collected soaked in ethanol (70%) for 1 min followed by NaClO (2.5%) for 3 mins., then ethanol (70%) for 30 Sec. The fragments extremely washed with sterile distilled water during the different sterilization stages. Sliced root portions were cleaned twice with sterile distilled water, transferred to sterile dry filter paper, and allowed to dry in a laminar flow. The fragments were then sliced into small pieces (~ 5 x 5 mm) and deposited on the surface of the Petri dishes containing CzA and CzCA media for each plant sample [25].

2.3. Identification of Fungal Isolates

Collected fungi were identified according to Moubasher (1993), Ainsworth (1971), Barron & Peterson (1968), for identifying Hyphomycetes [26 - 28], Gilman (1957), for soil fungi in general [29], and Booth (1977) for Fusarium species [30]. The morphology of purified fungal isolates was examined microscopically. Identification relied on the shape of the fungal colony and the properties of the generated spores.

2.4. Methanol Fungal Extraction of *Aspergillus fumigatiaffinis* & *Aspergillus fischeri*

Two of the endophytically isolated species (*A. fumigatiaffinis* AA-21 and *A. fischeri* AA-41) cultivated in 50 mL of newly sterilized potato dextrose broth (PDB) medium to assess their ability to spontaneously generate antibacterial compounds. Still cultures were kept at 25±2.0°C. After 15 days, the mycelia were extracted and carefully ground in a mortar. The smashed mycelia and fermented broth were extracted with methanol (50 mL); then filtered by Whattman filter paper through anhydrous sodium sulfate. The crude extracts were dried at room temperature. Then dissolved in less amount of methanol.

2.5. Antimicrobial Assay

A total of thirteen pathogenic microorganisms employed for the antimicrobial assays encompassed Gram-positive bacteria (*Bacillus cereus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella*, and *Pseudomonas aeruginosa*), which were graciously supplied by the Sohag Bacteriological Laboratory, Faculty of Science, for the purpose of conducting the antibacterial assessment, as well as opportunistic fungi (*A. flavus* KY609551, *A. fumigatus* MT994683, *A. niger* KY609552 and *A. terreus*), pathogenic fungi such as *Candida albicans* (AUMC No 13507) and *Candida glabrata* (AUMC No.13502), in addition to *Fusarium solani* for the antifungal evaluation. The bacterial strains were cultivated on nutrient agar (NA) medium, while the fungi were grown on Czapek's and Sabouraud media. An aliquot of 10 µL of methanolic extract derived from endophytic fungi was introduced onto Whatman filter paper discs (8 mm). Subsequently, the discs were desiccated and positioned onto the cultured media, incubated at 37°C for 24 hours for the bacterial strains and 72 hours for the fungal strains. The diameters of the zones of microbial growth inhibition were measured to evaluate the antimicrobial efficacy of the samples.

2.6. Analysis Using Gas Chromatography and Mass Spectrometry (GC/MS)

The chemical constituents of the highest antibacterial activity species (*Aspergillus fumigatiaffinis* AA-21) were investigated using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven heat was initially kept at 50°C and then elevated by 5°C min^{-1} to 250°C and held for 2 min, then boosted to the ultimate heat 300°C by 30°C min^{-1} and held for 2 min. The injector and MS transfer line heat were kept at 270, and 260°C respectively. The carrier gas (Helium) was used at a fixed flow speed of 1 mL min^{-1}. The solvent holdup was 4 min and diluted samples

of 1 µL were introduced automatically using Autosampler (AS1300) combined with GC in the splitting mode. Electron-impact ionization mass spectra (EI-MS); in full scan mode, data were gathered at 70 eV ionization voltages spanning the m/z range of 50–650. The temperature of the ion source was set to 200°C. The components were identified by comparing their mass spectra to those from the WILEY 09 and NIST 14 databases [31].

2.7. Statistical analysis:

The triplicate data obtained were subjected to an analysis of one-way ANOVA (Tukey design) using Origin software version 2024b (OriginLab Corporation, Northampton, MA, USA) [32], and $p \leq 0.05$ was considered to indicate statistical significance.

3. Results and Discussion

3.1 Rhizosphere

Fungal diversity isolated from *Artemisia judaica* roots showed distanced differences among rhizosphere, rhizosphere and endophytes, using CzA and CzCA media (**Fig. 1**). The rhizosphere, yielded 13,325 and 9,200 colony fungal forming units per gramme roots (CFUs/g) on CzA and CzCA media, respectively resulted from total 40 root samples. The number of fungal genera and species emerging on CzA medium exceeded nearly 1.5 folds than those emerged on CzCA. *Aspergillus* was the most common genus on both CzA and CzCA media of isolation (**Table 1, Fig. 2**). It was represented by 14 species, of which *A. fumigatiaffinis* S.B. Hong, Frisvad, *A. ficuum* (Reichardt) Thom & Currie, and *A. fumigatus* Fresen. were the most common species. *A. fumigatiaffinis* was the most prevalent species in all isolates, accounting for 27.5% and 25% of the isolates isolated on CzA and CzCA media, respectively. The *Penicillium* genera followed *Aspergillus* and was represented by 8 species **(Table 1).** While other moderate and rare fungal genera were represented by *Alternaria alternata* (Fr.) Keissl, *Myrothecium* species, *Stachybotrys chartarum* (Ehrenb.) S. Hughes, and three *Fusarium* species **(Table 1, Fig. 2).**

Previous studies reported that the predominant fungal species were fungi of phylum: Ascomycetes, with the remaining species attributed to phylum: Hyphomycetes and Zygomycetes. Naim *et al*. (1957); Mostafa & Elwan (1960); Al Mousa *et al*. (2021) documented the presence of endophytic fungi in *A. judaica* [33 -35]. Several investigations found that fungi associated with medicinal plants primarily belong to Ascomycota or their mitosporic fungus, with some also belonging to phylum: Zygomycetes, Oomycetes, and Basidiomycetes [36, 37].

3.2. Rhizoplane Fungi

The rhizoplane fungi were represented mainly by two genera, namely *Aspergillus* and *Penicillium*, and the most widespread genus was *Aspergillus*, accounting for 73.7 and 69.3% of total fungi in rhizoplane samples isolated on both tested media CzA and CzCA, respectively. Also, the number of isolated fungi using CzA medium exceeded the number of isolated fungal colonies on CzCA medium by 1.2 folds. The presence of *Aspergillus fumigatiaffinis* was the highest among all isolates of *Aspergillus* species, accounting for 42.4% and 58.5% in the CzA and CzCA media tested, respectively **(Table 2)**. The current study's findings revealed that the most frequent fungi found in the rhizosphere and rhizoplane were *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium*. These results were The rhizoplane fungi were represented mainly by two genera, namely *Aspergillus* and *Penicillium*, and the most widespread genus was *Aspergillus*, accounting for 73.7 and 69.3% of total fungi in rhizoplane samples isolated on both tested media CzA and CzCA, respectively. Also, the number of isolated fungi using CzA medium exceeded the number of isolated fungal colonies on CzCA medium by 1.2 folds. The presence of *Aspergillus fumigatiaffinis* was the highest among all isolates of *Aspergillus* species, accounting for 42.4% and 58.5% in the CzA and CzCA media tested, respectively **(Table 2)**.

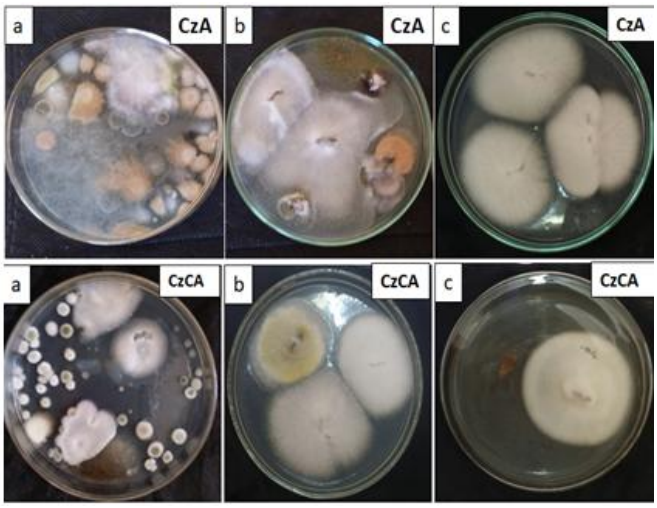


Fig.1: Distribution of (a) rhizosphere, (b) rhizoplane and (c) endophytic fungi according to the types of media used.

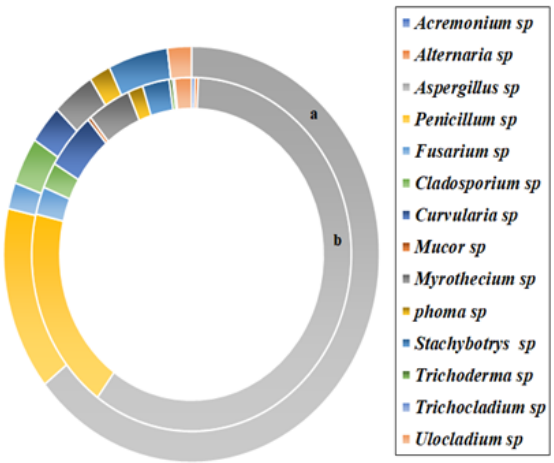


Fig. 2: Distribution of rhizosphere fungi according to the types of media used. The circular chart illustrates the distribution of various rhizosphere fungi across two types of media. The outer ring (a) represents CzA medium, while the inner ring (b) represents CzCA medium. The fungi are color-coded in the chart legend as indicated.

The current study's findings revealed that the most frequent

fungi found in the rhizosphere and rhizoplane were *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium*. These results were consistent with Caruso *et al.* [38] who reported that *Alternaria*, *Aspergillus*, *Fusarium*, *Curvularia*, *Penicillium*, and *Phoma* were the most frequent fungal species linked endophytically with medicinal plants from the *Asteraceae* family.

Table1: Fungal genera and species isolated form rhizosphere of *Artemisia judaica* root on Czapek's–glucose agar medium (CzA) and Czapek's–cellulose (CzCA) agar medium pH=8.5 (1/100 dilute) at 26±2.0℃ for 5 days.

		Fungal species	Rhizosphere -CZA				Rhizosphere -CZCA			
			CFU[a]	CFU%[b]	F[c]	F%[d]	CFU[a]	CFU%[b]	F[c]	F%[d]
Phylum: Ascomycetes		***Aspergillus* group (total count)**	**7,000**	**52.5**	**40**	**100**	**5,480**	**59.5**	**40**	**100**
	1	*A. chevalieri* (L. Mangin) Thom & Church	300	2.25	4	10	480	5.2	4	10
	2	*A. ficuum* (Reichardt) Thom & Currie	1,660	12.4	13	32.5	960	10.4	12	30
	3	*A. flavus* Link	280	2.1	3	7.5	260	2.8	3	7.5
	4	*A. fumigatiaffinis* S.B. Hong, Frisvad & Samson	1,280	9.6	11	27.5	1,160	12.6	10	25
	5	*A. fumigatus* Fresen	780	5.8	6	15	380	4.1	5	12.5
	6	*A. nidulans* (Eidam) G. Winter	240	1.8	2	5	40	0.43	1	2.5
	7	*A. niger* Tiegh	380	2.8	3	7.5	420	4.5	4	10
	8	*A. ochraceus* G. Wilh.	250	1.9	3	7.5	160	1.7	3	7.5
	9	*A. oryzae* (Ahlb.) Cohn	280	2.1	3	7.5	120	1.3	2	5
	10	*A. sydowii* (Bainier & Sartory) Thom & Church	400	3	10	25	480	5.2	12	30
	11	*A. terreus* Thom	780	5.8	8	20	300	3.2	3	7.5
	12	*A. terreus* var. *aureus* Thom & Raper	450	3.3	9	22.5	720	7.8	9	22.5
	13	*A. utus* (Bainier) Thom & Church	40	0.3	1	2.5	-	-	-	-
	14	*A. versicolor* (Vuill.) Tirab	180	1.3	3	7.5	-	-	-	-
		***Penicillium* group (total count)**	**2,200**	**16.5**	**23**	**57.5**	**1,220**	**13.2**	**10**	**25**
	1	*P. chrysogenum* Thom	560	4.2	5	12.5	220	2.4	3	7.5
	2	*P. citrinum* Thom	440	3.3	4	10	200	2.17	2	5
	3	*P. duclauxii* Delacr	900	6.7	8	20	480	5.2	7	17.5
	4	*P. lanosum* Westling	100	0.75	1	2.5	-	-	-	-
	5	*P. griseofulvum* Dierckx	100	0.75	1	2.5	-	-	-	-
	6	*P. oxalicum* Currie & Thom	480	3.6	2	5	260	2.8	3	7.5
	7	*P. palitans* Westling	80	0.6	1	2.5	-	-	-	-
	8	*P. varians* Svilv	100	0.75	1	2.5	60	0.65	1	2.5
Other genera	1	***Acremonium strictum* W. Gams**	40	0.3	1	2.5	-	-	-	-
	2	***Alternaria alternata* (Fr.) Keissl**	1,560	11.7	12	30	640	6.5	16	40
	3	***Cladosporium oxysporum* Berk. & M.A. Curtis**	280	2.1	4	10	320	3.4	4	10
	4	***Curvularia lunata* (Wakker) Boedijn**	620	4.6	5	12.5	260	2.8	6	15
		***Fusarium* (total count)**	300	2.25	7	17.5	180	1.9	4	10
	5	*F. oxysporum* Schltdl	140	1	2	5	100	1.08	2	5
	6	*F. solani* (Mart.) Sacc	260	1.95	4	10	80	0.86	2	5
	7	*F. subglutinans* (Wollenw . & Reinking) P.E. Nelson, Toussoun & Marasas	40	0.3	1	2.5	-	-	-	-
	8	***Mucor hiemalis* Wehmer**	40	0.3	1	2.5	-	-	-	-
	9	***Myrothecium***	520	3.9	5	12.5	320	3.47	4	10
	10	*M. Roridum* Tode	40	0.3	1	2.5	-	-	-	-
	11	*M. Verrucaria* (Alb. & Schwein.) Ditmar	480	3.6	4	10	320	3.4	4	10
	12	***Phoma glomerata* (Corda) Wollenw . & Hochapfel**	180	1.35	3	7.5	160	1.7	3	7.5
	13	***Stachybotrys chartarum* (Ehrenb.) S. Hughes**	320	2.4	2	5	440	4.7	4	10
	14	***Trichoderma aureoviride*** Rifai	40	0.3	1	2.5	-	-	-	-
	15	***Trichocladium griseum* (Traaen) X. Wei Wang & Houbraken**	25	0.18	1	2.5	-	-	-	-
	16	***Ulocladium chartarum* (Preuss) E.G. Simmons**	200	1.5	2	5	180	1.9	2	5
		Total count	13,325	100	40	100	9,200	100	40	100
		No. of genera		14				11		
		No. of species		29				9		

[a] Colony forming unit (CFU), [b] percentage CFUs (CFU%), per total fungi in each sample and per total fungi in all samples for total; [c] frequency of occurrence (F) (F, out of 40 sample), [d] percentage frequency (%F, calculated per 40 samples).

3.3. Endophytic Fungi

The endophytic fungi represented 3,500 and 2,440 CFUs resulting from 600 root segments on CzA and CzCA tested media, respectively. Czapek's–glucose agar medium exceeded CzCA medium in number of isolated fungal colonies by 1.43 folds. The only genus that was isolated is *Aspergillus* and represented of two species *Aspergillus fumigatiaffinis* and *Aspergillus fischeri* only, in percentage 58.9% and 41.1% on CzA and 68% and 32% on CzCA media, respectively **(Table 3)**.

The Family *Asteraceae* justifies the selection of plants for isolating endophytes, as many species of this family have a considerable history of therapeutic usage [39]. Our results revealed that the *Aspergillus* was the most frequent genus in the fungi isolated endophytically from *A. judaica* root. This is inconsistent with Zhang *et al.* [40], who identified *Aspergillus*, *Cephalosporium*, *Fusarium*, and *Mucor* as the main endophytic species isolated from *Artemisia annua*. Also, Al Mousa *et al.* [35] studied endophytic fungi in *Artemisia judaica*, and reported that the most prevalent genera were *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, and *Fusarium*. *Aspergillus* is widely distributed and a cosmopolitan fungus. The great adaptability enables this genus to thrive in a variety of environments, including extreme conditions of high levels of temperature, lack of water activity, fluctuations in pH level, and oxygen concentration in soil [41-43]. In our investigation, *A. fumigatiaffinis* was collected on alkali media only, which is consistent with a prior work which-demonstrated that *A. fumigatiaffinis* was recovered only from the soil of *Talampaya* Park with alkaline pH [44].

The endophytic fungus *A. fumigatiaffinis* was isolated from medicinal plants (Saffron), as stated by Chamkhi *et al.* [45]. To our knowledge, *A. fumigatiaffinis* has not yet been reported to be isolated from *Artemisia* plants. This is the first study to report its isolation from *Artemisia judaica* root. Noteworthy that, the climatic parameters and height above mean sea level of the 'Wadi Abu Shih' area in the Red Sea Governorate region are similar to those of 'Socorro City' (USA), where *A. fumigatiaffinis* was first identified from the soil [46, 47].

3.4. Antibacterial activity of endophytic fungi crude extract

The results showed great effect of both *A. fumigatiaffinis* (AA-21) strain and *A. fischeri* (AA-41) strain crude extract on Gram-negative bacteria (*E. coli*, *Klebsiella*, *Salmonella* and *Pseudomonas aeruginosa*) compared to Gram-positive bacteria (*Bacillus cereus* and *Bacillus subtilis*) **(Fig. 3)**. The maximum zone of inhibition (14.58 mm, 13.8 mm) of both *E. coli and Klebsiella sp*, respectively, followed by *Salmonella* (8.9 mm) and the least zone inhibition was 7.4 mm in case of *Pseudomonas aeruginosa* **(Fig. 4)**. While the methanolic extract of *A. fischeri* (AA-41) exhibited an obvious inhibitory effect on *E. coli* and *Klebsiella* with the diameter of inhibition zone 10.3 and 7.06 mm, respectively. whereas the inhibition zone was 6.12 and 4.8 mm in the case of *Salmonella* and *Pseudomonas aeruginosa*, respectively. However, the crude methanolic extract of both tested fungal strains showed relatively less inhibition effect on Gram-positive bacteria *Bacillus cereus* and *Bacillus subtilis* the inhibitory zone ranged between 11 and 5.8 mm, respectively in the case of using *Aspergillus fumigatiaffinis* extract, whereas when using *Aspergillus fischeri* extract, the size of the inhibition zone for *Bacillus cereus* and *Bacillus subtilis* were 4.8 and 2.9 mm, respectively.

The findings of this study demonstrate the potent antimicrobial activity exhibited by the methanolic crude extract of *Aspergillus fumigatiaffinis* from *A. judaica* roots against *Klebsiella* and *E. coli*, moderate activity against *Pseudomonas aeruginosa* and *Salmonella*, and mild activity against *Bacillus subtilis*. These outcomes align with previous reports by several researchers. Tan et al. highlighted the frequent utilization of *Artemisia* species in treating conditions such as cancer, hepatitis, inflammation, malaria, and diseases caused by

moulds, bacteria, and viruses [48]. Moreover, the endophytic extracts from 11 fungi associated with asymptomatic *A. annua* L. revealed that three strains of *Aspergillus* spp. showed strong antibacterial activity against *E. coli* and *S. aureus* [49]. Since there is an ever-present connection between the plant and endophytic moulds, previous studies have confirmed the ability of isolated fungi endophytically from the *Artemisia* higher plants to affect various types of pathogenic bacteria. Also, *Artemisia thuscula* fungal endophytes have shown antibacterial capabilities against *E. coli* and *S. aureus* according to Zhang *et al*. [40].

The impact of *A. fumigatiaffinis* fungal extract, statistically significant differences in the mean clear zones across the bacterial groups, as demonstrated by the results of the ANOVA analysis ($F(0.57403) = 107.79$, $p < 0.0001$). The elevated R-squared value (0.95737) indicates that 95.73% of the variability in the measurements of clear zones can be attributed to the disparities among these bacterial species. In contrast, the ANOVA results pertaining to *A. fischeri* fungal crude extract yielded ($F(1.552) = 159.393$, $p < 0.0001$). The substantial R-squared value (0.97077) implies that 97.00% of the variance observed in the clear zone measurements is elucidated by the distinctions among these bacterial entities.

The Tukey post-hoc analysis regarding the effect of *A. fumigatiaffinis* fungal crude extract indicated on *E. coli* exhibited the most substantial mean clear zone (14.5 mm), which was significantly different from the other bacterial groups tested ($p < 0.0001$) **(Fig. 5a)**. *Bacillus subtilis* presented the smallest mean clear zone (5.8 mm) and demonstrated significant differences in clear zone size when compared to all other bacterial species **(Fig. 5a)**. It is noteworthy that *E. coli* did not exhibit a significant difference in clear zone size relative to *Klebsiella* ($p = 0.58745$), whereas all other comparisons revealed significant differences at the 0.05 significance level. Levene's test for homogeneity of variances verified that the variances among the groups were equal ($p = 0.71924$). The Tukey post-hoc analysis for *A. fischeri* crude extract indicated that *E. coli* had the largest mean clear zone (10.3 mm), which was significantly different from the other groups ($p < 0.0001$) **(Fig. 5b)**. Furthermore, *Bacillus subtilis* also exhibited the smallest mean clear zone (2.9 mm), which also significantly differed from the clear zone sizes of all other bacterial species **(Fig. 5b)**. Importantly, *Pseudomonas aeruginosa* demonstrated no significant difference in clear zone size when compared to *Bacillus cereus* ($p = 0.99972$), while all other comparisons revealed significant differences at the 0.05 significance level. Levene's test for homogeneity of variances confirmed equal variances among the groups ($p = 0.21134$).

3.5. Antifungal test of methanolic crude extract

The effect of the methanolic crude extract *A. fumigatiaffinis* (AA-21) and *A. fischeri* (AA-41) were tested on saprophytic, opportunistic and pathogenic fungal isolates **(Fig. 6)**. *A.fumigatiaffinis* extract had a significant effect on non-pathogenic fungi *(Fusarium solani)* , where the average clear zone reached (28.4 mm), while when testing it on opportunistic fungi causing aspergillosis, the effect of the fungal extract was variable, where the highest average clear zone (25 mm) was in the case of *Aspergillus terreus*, followed by (24.2, 21.4 mm) for *Aspergillus flavus and Aspergillus fumigatus*, respectively, while *Aspergillus niger* recorded resistance to the fungal extract. The least effect of the fungal extract was for *Candida albicans* and *Candida glabrata*, where the average clear area was recorded at 14, 18.4 mm, respectively. Whereas, the *Aspergillus fischeri* (AA-41) extract had a lesser effect compared to the *Aspergillus fumigatiaffinis* (AA-21) crude extract **(Fig. 7)**, as the largest clear zone (21.1 mm) was in the case of *Fusarium*, while the average clear zone on tested opportunistic fungi were (17.2, 16.2 and 13.2 mm) for *A. terreus*, *A. flavus* and *A. fumigatus*, respectively. As for *Candida albicans* and *Candida glabrata*, the clear zone average was (9 and 10.2 mm), respectively.

When undertaking a systematic investigation into the effects of *A. fumigatiaffinis* fungal extract, statistically significant variations in the mean clear zones were discerned among the evaluated fungal groups, as evidenced by the outcomes of the ANOVA analysis ($F(6.5083) = 391.16$, $p < 0.0001$). The heightened R-squared value (0.98821) suggests that 98.82% of the variability in the quantifications of clear zones can be ascribed to the differences among these fungal species. Conversely, the ANOVA findings related to *A. fischeri* fungal crude extract revealed ($F(4.31558) = 295.80$, $p < 0.0001$). The considerable R-squared value (0.98447) indicates that 98.44% of the variance identified in the clear zone measurements is clarified by the distinctions among these fungal entities.

The Tukey post-hoc analysis indicated that *Fusarium solani* displayed the largest mean clear zone in both *A. fumigatiaffinis* and *A. fischeri* (28.4 and 21.1mm, respectively), significantly differing from other fungal groups ($p < 0.0001$) **(Fig. 8a & b)**. In contrast, *Candida albicans* showed the smallest mean clear zone (14 and 9 mm for *A. fumigatiaffinis* and *A. fischeri*, respectively), also demonstrating significant differences in clear zone size compared to all other fungal species **(Fig. 8a & b)**.

Notably, *A. terreus* did not show a significant difference in clear zone size compared to *A. flavus* ($p = 0.89731$ and 0.57911 for *A. fumigatiaffinis* and *A. fischeri*, respectively), while all other comparisons exhibited significant differences at the 0.05 significance level.

ANOVA demonstrated statistically significant differences in the average of mean clear zones for both tested endophytic methanolic crude extracts among the bacterial and fungal tested group ($p < 0.0001$), accompanied by R-squared values surpassing 95%. The subsequent Tukey's post-hoc analysis delineated *Escherichia coli*, *Bacillus cereus*, and *Fusarium solani* as the most vulnerable organisms, whereas *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* exhibited the least vulnerability **(Fig. 9)**.

The rise of antimicrobial resistance is a pressing global issue, prompting the investigation of new bioactive compound sources [50]. There is an increasing preference for natural products derived from microbial and plant origins. Current research focuses on bioactive compounds from endophytes that

Table 2. Genera and species of isolated fungi form rhizoplane of *Artemisia judaica* root on Czapek's–glucose agar medium (CzA) and Czapek's–cellulose (CzCA) agar medium per 600 root segments out of 40 root samples at 26±2.0℃ for 5 days.

	No.	Fungal species	Rhizoplane -CZA				Rhizoplane -CZCA			
			CFU[a]	CFU%[b]	F[c]	F%[d]	CFU[a]	CFU%[b]	F[c]	F%[d]
Phylum Ascomycota		***Aspergillus* group (total count)**	7360	73.7	40	100	6320	69.3	40	100
	1	*Aspergillus fischeri* Wehmer	1420	17.61	19	47.5	1660	24.7	24	60
	2	*A. flavus* Link	1740	21.5	14	35	420	6.25	5	2.5
	3	*A. fumigatiaffinis* S.B. Hong, Frisvad & Samson	3420	42.4	33	82.5	3940	58.6	38	95
	4	*A. fumigatus* Fresen	340	4.21	5	12.5	100	1.4	2	5
	5	*A. niger* Tiegh	240	2.9	6	15	80	1.2	2	5
	6	*A. terreus* Thom	200	2.4	6	15	120	1.8	2	5
		***Penicillium* group (total count)**	540	6.7	9	22.5	300	4.4	5	12.5
	1	*P. chrysogenum* Thom	80	0.99	1	2.5	60	0.982	1	2.5
	2	*P. citrinum* Thom	100	1.2	3	7.5	120	1.8	2	5
	3	*P. griseofulvum* Dierckx	120	1.4	1	2.5	-	-	-	-
	4	*P. lanosum* Westling	100	1.2	2	5	-	-	-	-
	5	*P. oxalicum* Currie & Thom	140	1.7	2	5	120	1.7	2	5
Phylum Deuteromycota	1	***Gliocladium roseum* Bainier**	160	1.985	3	7.5	100	1.4	1	2.5
		Total count	8060	100	40	100	6720	100	40	100
		No. of genera		3				3		
		No. of species		12				10		

[a] Colony forming unit (CFU) per 600 root segments, [b] percentage CFUs (CFU%), per total fungi in each sample and per total fungi in all samples for total; [c] frequency of occurrence (F) (F, out of 40 sample), [d] percentage frequency (%F, calculated per 40 samples).

Table 3. Endophytic *Aspergillus* species isolated from *Artemisia judaica* root on Czapek's–glucose agar medium (CzA) and Czapek's–cellulose (CzCA) agar medium isolated from 600 root segments belong to 40 root samples at 26±2.0℃ for 5 days.

No.	*Aspergillus* sp.	Endophytic -CzA				Endophytic -CzCA			
		CFU[a]	CFU%[b]	F[c]	F%[d]	CFU[a]	CFU%[b]	F[c]	F%[d]
1.	*Aspergillus fumigatiaffinis* S.B. Hong, Frisvad	2,060	58.9	30	75	1,660	68	21	52.5
2.	*Aspergillus fischeri* Wehmer	1,440	41.1	20	50	780	32	16	40
	Total count	3,500	100	40	100	2,440	100	40	100
	No. of genera		1				1		
	No. of species		2				2		

[a] Colony forming unit (CFU) per 600 root segments; [b] percentage CFUs (CFU%), per total fungi in each sample and per total fungi in all samples for total; [c] frequency of occurrence (F) (F, out of 40 sample), [d] percentage frequency (%F, calculated per 40 samples).

Fig. 3. Effect of methanolic crude extract (a) *A. fischeri exact*, (b) *A. fumigatiaffinis* extract on tested pathogen bacteria.

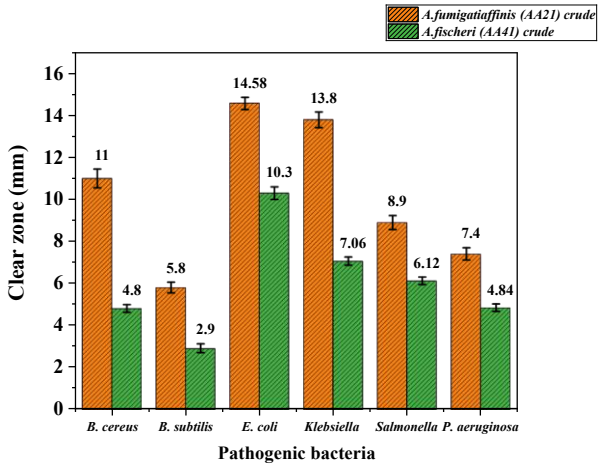


Fig. 4. the antibacterial activity of methanolic fungal extracts from *Aspergillus* fumigatiaffinis and *Aspergillus fischeri* against six bacterial pathogens. The activity is represented as mean inhibition zone diameters (in mm) with standard error bars derived from three replicates. The bacterial pathogens tested include six pathogens.

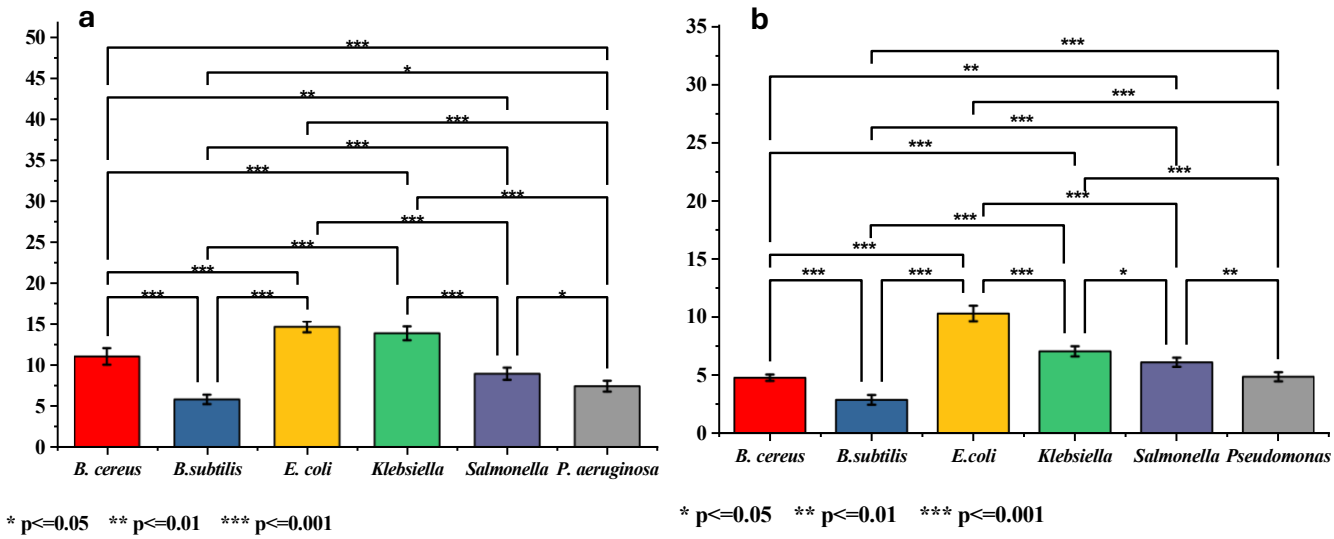


Fig. 5. Tukey post-hoc analysis of methanolic endophytes extract on pathogen Gram-positive and Gram-negative bacteria. (a) *A. fumigatiaffinis* crude extract and (b) *A. fischeri* crude extract

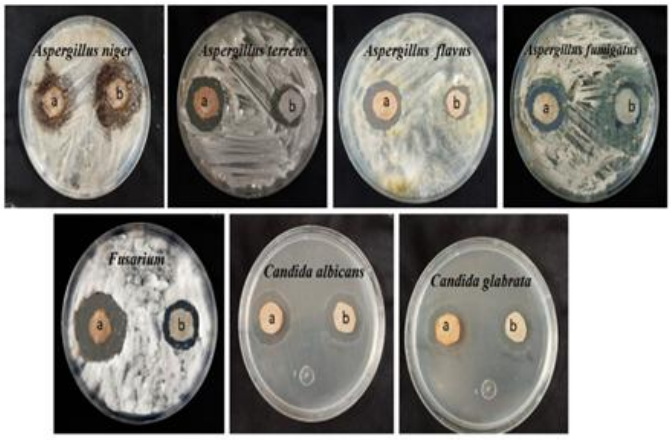


Fig. 6: Effect of methanolic crude extract (a) *A. fumigatiaffinis* methanolic crude extract, (b) *A. fischeri* methanolic crude extract.

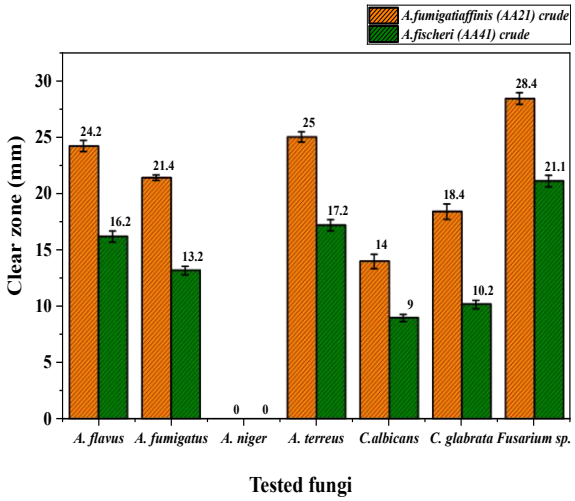


Fig. 7. Antifungal activity of methanolic fungal extract of *A. fumigatiaffinis* AA-21 and *A. fischeri* AA-41 against different types of fungi.

may improve the effectiveness against pathogen microorganism. Endophytic fungi have become a vital source of bioactive compounds with various therapeutic uses [51, 52]. Many antimicrobial agents obtained from endophytes comprise a range of structural types, including alkaloids, peptides, steroids, terpenoids, phenols, quinines, and flavonoids [53]. Our work is the first study of antimicrobial inhibition of extracts from endophytic fungi (*A. fumigatiaffinis* and *A. fischeri*) of the *A. judaica* plant. The extract of the endophytic fungi has proven its effectiveness against 13 isolates of human pathogens. The extract of endophytic fungi had a significant effect on fungi, especially non-pathogenic fungi such as *Fusarium solani* and opportunistic fungi causing aspergillosis, with the exception of *Aspergillus niger*. One-Way ANOVA statistical proved that there is a significant difference between the effect of antifungal and antibacterial of endophytic crude extract. These empirical findings substantiate the antimicrobial properties exhibited by the *Aspergillus* genus. In prior research, the antimicrobial efficacy of the endophytic fungal isolate *Aspergillus* sp. ASCLA, derived from the foliar tissues of the medicinal plant *Callistemon subulatus*, was assessed against *S. aureus*, *Pseudomonas aeruginosa*, *C. albicans*, and *Saccharomyces cerevisiae*, revealing a range of moderate to high antimicrobial activity [54]. The results of our study are consistent with Huang *et al.*; Liu *et al.*; Park *et al.* who reported the effectiveness of endophytic fungi isolated from medicinal plants as antifungals [55 - 57].

3.6. Gas chromatography–mass spectrometry (GC/MS) analysis of *A. fumigatiaffinis* methanolic extract

The Chromatogram of GC/MS investigation for the methanolic extract of *A. fumigatiaffinis* (AA-21), revealed the presence of 28 compounds **(Fig. 10)**, where the highest peak

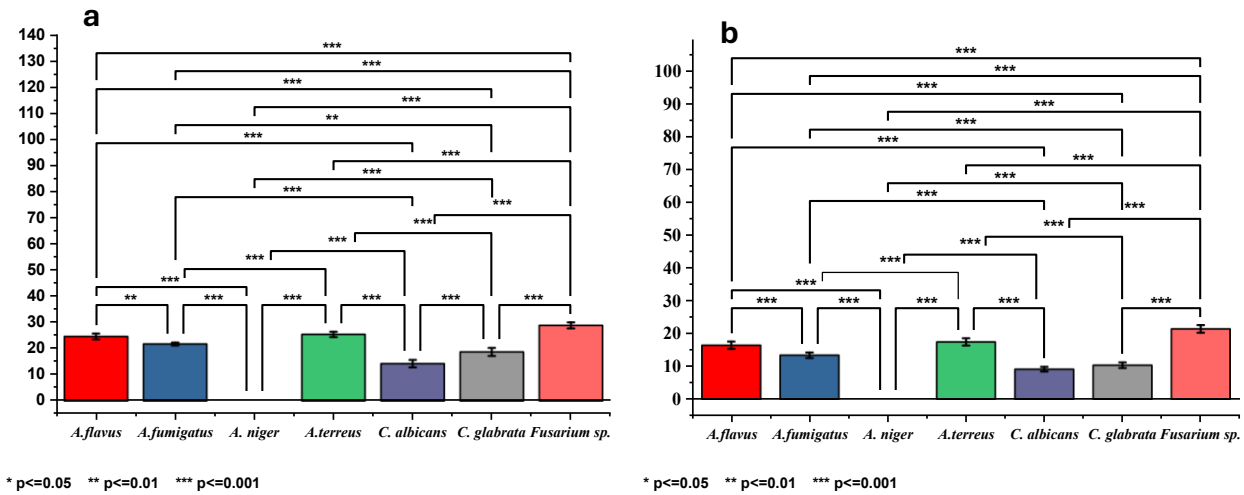


Fig. 8. Tukey post-hoc analysis for antifungal assay activity. (a) *A. fumigatiaffinis* and (b) *A. fischeri*.

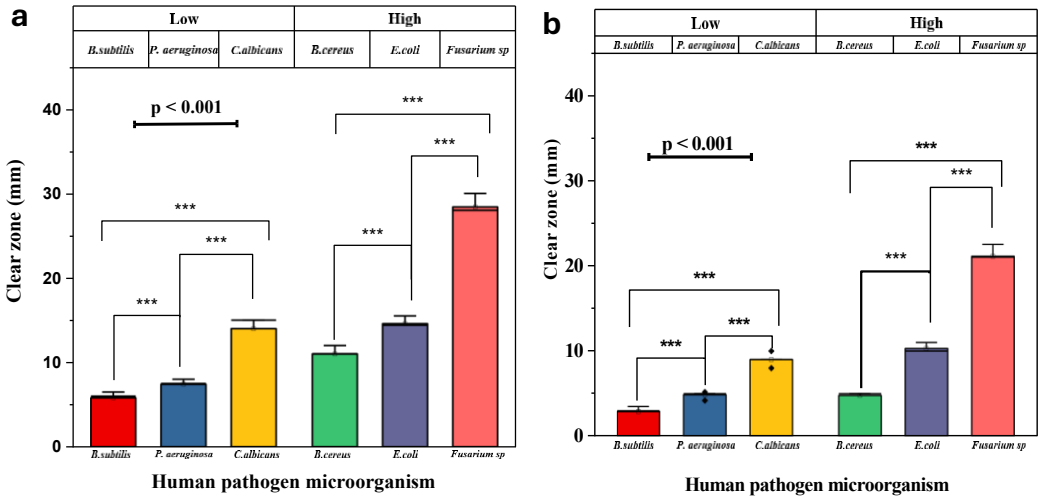


Fig. 9. Tukey post-hoc analysis of antimicrobial activity of endophyte methanolic extract. (a) *A. fumigatiaffinis* and (b) *A. fischeri.*

area appeared in 14 biological compounds that are represented in **Table 4**. Chromatogram of GC/MS investigation for the methanolic extract of *A. fumigatiaffinis* (AA-21), revealed the presence of 28 compounds **(Fig. 10)**, where the highest peak area appeared in 14 biological compounds **Table 4**, where n-hexadecanoic acid, 9, 12-Octadecadienoic acid, 9,12-Octadecadienal, dimethyl acetal and Dodecanoic acid being the predominant compounds, showing highest peak areas.

Recently, there has been an increase in microbial illnesses and the emergence of microbiological resistance to manufactured medications, leading to a renewed interest in the study of natural routes of bioactive chemicals for treating contagious diseases [58]. Endophytic fungus isolated from medicinal plants have long been acknowledged as valuable reservoirs of a diverse array of bioactive molecules, possessing antimicrobial properties, cytotoxic in nature, chemotherapy for cancer, anti-oxidant substances, antimalarial medication, and antiviral properties [59]. Substances, antimalarial medication, and antiviral properties [59]. *Aspergillus* species have been extensively reported in scientific literature as a successful fungal source of novel medicinally potent metabolites, encompassing steroids, pyrones, phenolics, terpenoids, quinones, and nitrogen-containing compounds, demonstrating a vast range of biological activity including phytotoxic, cytotoxic, in addition to antimicrobial properties [60]. It was reported that Dodecanoic acid, also known as 'Lauric acid' which is a medium-chain saturated fatty acid that can be present in coconut and palm kernel oils, has been recognized for its antibacterial and antioxidant properties [61]. Among its various bioactivities, n-hexadecanoic acid, or palmitic acid, has been identified as possessing bioactive characteristics such as anti-inflammatory, antioxidant, and antimicrobial properties [62, 63]. Additionally, according to reports, 9, 12-Octadecadienoic acid (Z, Z) (trans Linoleic acid) has antioxidant, antimicrobial and insecticidal properties.

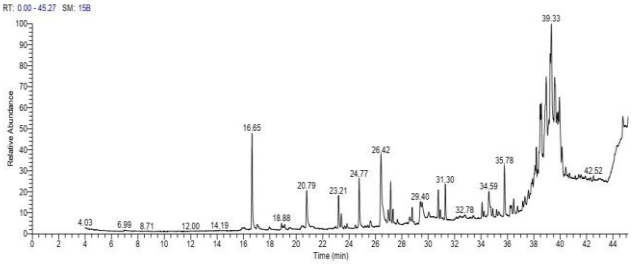
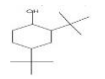
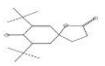
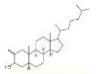
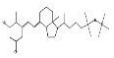
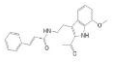
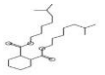
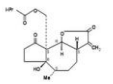


Fig. 10: GC/MS chromatogram of methanolic extract of *Aspergillus fumigatiaffinis* (AA-21).

Table 4. Significant bioactive substances were identified in methanolic extract of *Aspergillus fumigatiaffinis* (AA-21).

No.	Phytochemical compound	RT (min)	Formula	Molecular Weight	Chemical structure	Pharmacological actions
1	2,4-Di-tert-butylphenol	16.65	$C_{14}H_{22}O$	206		Anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal and anticancer [64].
2	1-Eicosanol	18.88	$C_{20}H_{42}O$	298		Anti malaria, anti -fungal and antioxidant [65].
3	1-Docosene	23.21	$C_{22}H_{44}$	308		Antibacterial [66].
4	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	24.77	$C_{17}H_{24}O_3$	276		Antimicrobial, antiviral and anti-inflammatory activities [67].
5	n-Hexadecanoic acid	26.42	$C_{16}H_{32}O_2$	256		Antibacterial activities, Anti -inflammatory, antioxidant [68].
6	Cholestan-3-ol, 2-methylene-, (3á,5à)	28.59	$C_{28}H_{48}O$	400		Anti-inflammatory and cytotoxic activities [69].
7	9,12-Octadecadienoic acid	29.39	$C_{18}H_{32}O_2$	280		Cytotoxic activities [70].
8	1,25-Dihydroxyvitamin D3, TMS derivative	30.04	$C_{30}H_{52}O_3Si$	488		Calcium homeostasis, immunology and cell differentiation [71].
9	2-Acetyl-3-(2-cinnamido)ethyl-7-methoxyindole	34.22	$C_{22}H_{22}N_2O_3$	362		Antifungal and cytotoxic activities [72].
10	9,12-Octadecadienal, dimethyl acetal	34.59	$C_{20}H_{38}O_2$	310		Antimicrobial activities [73].
11	1-Heptatriacotanol	35.18	$C_{37}H_{76}O$	536		Anti-hypercholesterolemic, anticancer, antineoplastic and anti-HIV [74].
12	Diisooctyl phthalate	35.79	$C_{24}H_{38}O_4$	390		Anti-microbial and cytotoxic activities [75].
13	Isochiapin B	40.14	$C_{19}H_{22}O_6$	346		Antimicrobial, antioxidant, antisect and anticancer activities [72].
14	psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	42.52	$C_{42}H_{64}O_2$	600		Antifungal, antibacterial and anti-inflammatory activities [76].

CRediT authorship contribution statement

Conceptualization and supervision, M.B.A and S.S.M; methodology, A.A.Y.; software, A.A.Y.; formal analysis, M.O.; investigation,visualization,and draft manuscripte preparation, A.A.Y.; writing—review and editing, M.O. All authors have read and agreed to the published version of the manuscript.

Data availability statement

All data produced throughout this researsh are incorporated within this published manuscript.

Declaration of competing interest

The authors declare no competing interests.

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