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Original article

Natural products of *Alternaria* sp., an endophytic fungus isolated from *Salvadora persica* from Saudi Arabia

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ABSTRACT

This study is to evaluate the potential of endophytic fungi of *Salvadora persica* for the production of bioactive compounds against pathogenic bacteria and fungi. Forty-two fungal isolates were obtained from 135 young and old stem and 125 root segments. Those 42 isolates representing ten fungi include: *Trichoderma* sp. (the most common), two species of *Alternaria*, *Rhizopus arrhizus* and 6 sterile mycelia. The ten fungi were grown in liquid culture and their crude extracts were tested against pathogenic bacteria and fungi. Nine crude extracts gave positive reactions against pathogenic bacteria of which *Alternaria* sp. (A8) was chosen further study. The fungal isolate was growing as sterile mycelium and was identified by phylogenetic analyses based on LSU rDNA sequence data and it might represent undescribed species of *Alternaria*. Sixty-two bioactive chemical compounds were identified from the ethyl acetate crude extracts of *Alternaria* sp., of which the following were recorded as major compounds in the active sub-fractions. These compounds showed strong antibacterial activity in combination.

1. Introduction

There is a general call for new antibiotics, chemotherapeutic agents, and agrochemicals that are highly effective, possess low toxicity, and have minor environmental impact (Patil et al., 2016). Due to an increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, interest in the use of biogenic drugs has been renewed around the world (Nalawade et al., 2003). During the last twenty years, more than half of the drugs in the market come from biological resources (Vuorela, 2004). Medicinal plants and their endophytes have proved to be a good source of bioactive metabolites (Cai et al., 2004, Newman and Cragg, 2007). Around 420,000 plant species exist in nature (Vuorela, 2004). Medicinal plants have been used to prevent or cure illness due to the presence of bioactive compounds in their tissues and were used in traditional medicines for several millennia (Radad et al., 2006; Jahanpour et al., 2015) (see Fig. 1).

Endophytes are microorganisms that reside in the healthy looking plant tissues and are a promising source of novel bioactive natural metabolites (Sun and Guo, 2012; Patil et al., 2016). Carroll (1986) defined endophytes as mutualists that colonize aerial parts of living plant tissues and do not cause disease symptoms. Petrini (1991) defined endophytes as organisms that colonize internal plant tissues for some time in their life without causing apparent harm to the host. Endophytes are present in every plant on earth including: mosses (Davey and Currah, 2006), ferns (Swatzell et al., 1996), grasses (Su et al., 2010), shrubs (Iannone et al., 2015), deciduous and coniferous trees (Albrectsen et al., 2010; Sun et al., 2011), and even lichens (Li et al., 2007a,b).

Endophytic fungi are mainly Ascomycetes and their asexual stages, while Basidiomycota, Zygomycota and Chytridiomycota are poorly represented (Sinclair and Cerkauskas, 1996). Endophytes can produce various bioactive natural products (Aly et al., 2010; Liu et al., 2011) that promote host growth and resistance to environmental stress (Saikkonen et al., 2010), decompose litter (Purahong and Hyde, 2011; Sun et al., 2011), suppress pathogens, aid in removing contaminants, solubilize phosphate or contribute to nitrogen assimilation for plants (Hallmann et al., 2006).

Medicinal plants produce bioactive compounds capable of preventing or curing illnesses. They also provide a unique

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Fig. 1. *Salvadora persica* at Okair Geological Reserve (26°29′9″N, 48°24′54″E) located 160 km west of Dammam city, Saudi Arabia.

environment for endophytes and have been recognized as a repository of endophytes with novel metabolites of pharmaceutical importance (Wiyakrutta et al., 2004). Endophytes affects the quality and quantity of the crude drugs derived from medicinal plants through a particular fungus-host interaction (Jia et al., 2016). Examples include an anticancer agent isolated from an endophytic taxon growing on the leaves of the Indian medicinal plant *Mimusops elengi* (Deshmukh et al., 2009) and the potent anticancer compound secalonic acid D, which was isolated from a mangrove endophytic fungus and displays high cytotoxicity to HL60 and K562 cells by inducing leukemia cell apoptosis (Zhang et al., 2009).

Fungal endophytes colonize internal healthy plant tissues and persist without causing visible disease symptoms (Hyde and Soytong, 2008; Purahong and Hyde, 2011). They have been isolated from nearly all studied hosts, including *Populus tremula* (Albrectsen et al., 2010), *Pinus halepensis* (Botella et al., 2010), grasses (Su et al., 2010; Ghimire et al., 2011), *Taxus globosa* (Rivera-Orduna et al., 2011), *Vitis vinifera* (Gonzalez and Tello, 2011) and sea grasses (Sakayaroj et al., 2010). Some endophytic fungi are known to produce bioactive metabolites (Xu et al., 2010), and these metabolites may be similar to those produced by the host (Keller et al., 2005). For example, a paclitaxol-producing endophyte, *Taxomyces andreanae*, was successfully discovered from the Pacific yew *Taxus brevifolia* (Stierle et al., 1993). A recent study using a paclitaxel-producing endophytic isolate of *Chaetomium* sp. reported a potential industrial yield of paclitaxel of 1124.34 μg/L using an optimized fermentation process (Jin et al., 2011). A gibberellin-producing endophytic strain of *Penicillium commune* has also been isolated from *Sesamum indicum* (Choi et al., 2005).

Kingdom of Saudi Arabia is gifted with a wide range of flora, consisting of a large number of medicinal herbs, shrubs and trees (Mossa et al., 2000). It is estimated that the flora of Saudi Arabia has a great medicinal species diversity, which is expected to be more than 1200 (over 50%) out of its 2287 species (Rahman et al., 2004). Endophytic fungi were isolated from a few species of medicinal plants in Saudi Arabia (Gashgari et al., 2016). This study aims to isolate and characterize bioactive natural products from endophytic fungi of *Salvadora persica* collected from Okair Geological Reserve, Dammam city, Saudi Arabia.

Salvadora L. (Salvadoraceae) is evergreen trees or shrubs distributed in tropical Africa and Asia (Arora et al., 2014). Species of *Salvadora* are deep rooted mesomorphic xerophytes with high salt tolerance (Khan and Qaiser, 2006). *Salvadoraceae* is a small family comprising of three genera (*Azima* Lam., *Dobera* and *Salvadora*) and 12 species which are distributed mainly in the tropical and subtropical Asia and Africa (Willis, 1973). Salvadoraceae in Saudi Arabia is represented by two genera and two species namely: *Dobera glabra* (Forssk.) Juss. Ex Poir and *S. persica* L. *Salvadora persica* (Arak) has antiurolithiatic properties (Geetha et al., 2010). Due to the presence of benzyl isothiocynate *S. persica* is widely used in an oral hygiene as tooth-cleaning stick (miswak) (Halawany,

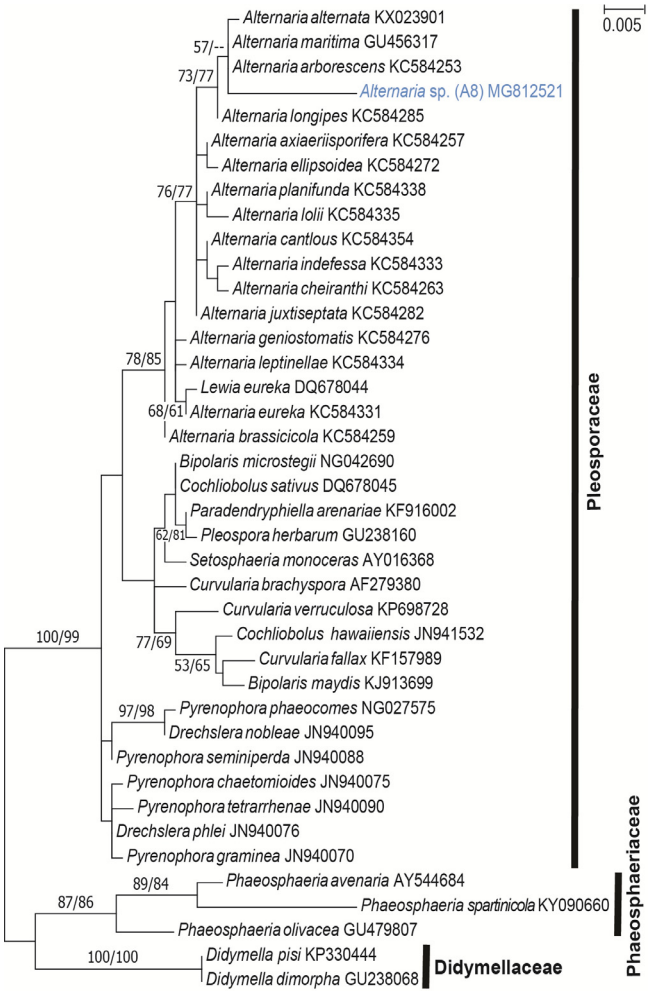


Fig. 2. Phylogenetic relationships of *Alternaria* sp. (A8) with phylogenetically related *Alternaria* species based on the nucleotide sequences of LSU rDNA. The tree is rooted to representatives of Didymellaceae. The maximum likelihood tree (ML) was constructed in MEGA7 (Tamura et al., 2013). Bootstrap support on the nodes represent ML and MP $\geq$ 50% respectively. Newly generated sequence in the present study is in blue.

2012). Although it is a pre-islamice practice, the use of miswak has dramatically increased after the spread of Islamic culture (Bos, 1993). There are 182 plant species suitable for preparing tooth brush sticks, however, miswak harvested from *S. persica* are the most commonly used in different parts of the world (Mohamed et al., 2006). The use of the tooth-cleaning sticks dates back to the Babylonians some 7000 years ago. They were also used by the Greeks, Romans, Jews and the Egyptians.

A few studies have been carried out to study endophytic fungi of *S. persica* (Korejo et al., 2014). Korejo et al., (2014) isolated 5 endophtyic fungi from *S. persica*. These fungi namely: *Aspegillus niger*, *A. flavus*, *Macrophomina phaseolina*, *Penicillium restrictum* and *P. canescens*. Dhankhar et al. (2013a) isolated seventeen endophytic fungi from *Salvadora oleoides* Decne in India and studied their abilities to produce natural products. Isolated endophytic fungi include: 10 *Aspergillus* spp., *Cladosporium herbarum*, *Epicoccum nigrum*, *Fusarium moniliforme*, *Penicillium chrysogenum*, *Phoma* sp. and *Pythium spinosa*. In another study, Dhankhar et al. (2013b) isolated 27 fungal species representing 18 fungal genera from 3750 samples of leaf segment, petiole and stem from five different sites of three seasons were collected and analyzed. In the current study we isolate endophytes from *S. persica*, to investigate endophyte extracts for anti-bacterial activity using human pathogenic

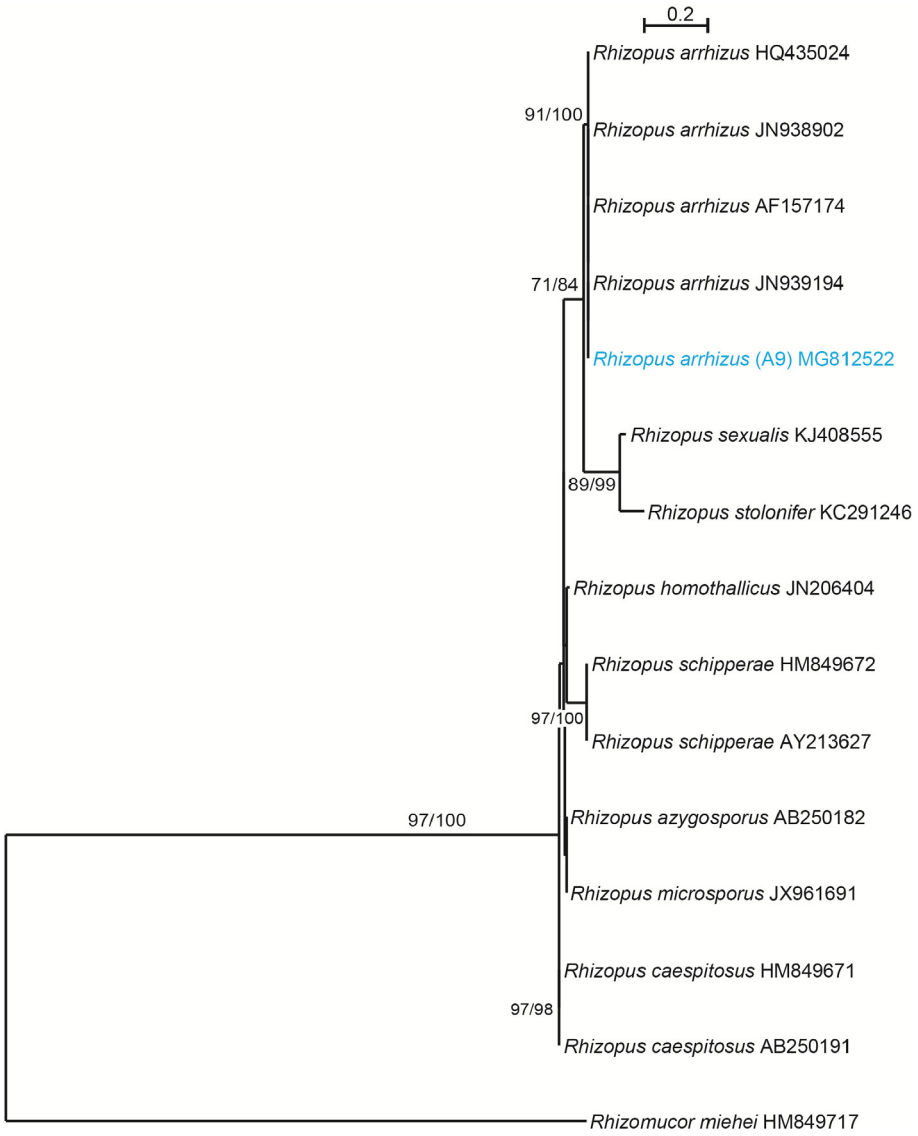


Fig. 3. Phylogenetic relationship of *Rhizopus arrhizus* (A9) along with phylogenetically related fungi in the genus *Rhizopus* based on the nucleotide sequences of LSU rDNA. The maximum likelihood tree (ML) was constructed in MEGA7 (Tamura et al., 2013). Bootstrap support on the nodes represent ML and MP $\geq$ 50% respectively. The tree is rooted to *Rhizomucor miehei*. Newly generated sequence in the present study is in blue.

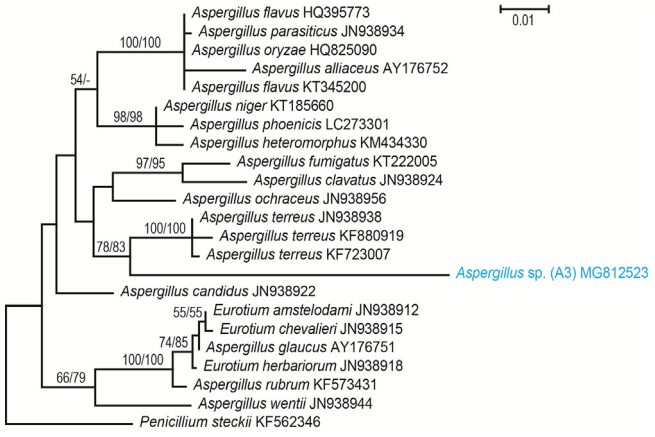


Fig. 4. Phylogenetic relationship of *Aspergillus* sp. (A3) along with phylogenetically related fungi in the genus *Aspergillus* based on the nucleotide sequences of LSU rDNA. The maximum likelihood tree (ML) was constructed in MEGA7 (Tamura et al., 2013). Bootstrap support on the nodes represent ML and MP $\geq$ 50% respectively. The tree is rooted to *Penicillium steckii*. Newly generated sequence in the present study is in blue.

Table 1
Endophytic fungi isolated from 135 stem segments and 125 root segments *Salvadora Persica.*

Fungi	Organ	N	%
Alternaria sp.1 (A4)	stem	2	1.5
# *Alternaria* sp.2 (A8)	stem	3	2.2
# *Rhizopus arrhizus* A. Fisch. (A9)	root	7	5.6
# *Aspergillus* sp. (A3)	stem	5	3.7
Trichoderma sp. (A1)	stem	7	5.2
Sterile mycelium (A2)	stem	4	3
Sterile mycelium (A5)	stem	3	2.2
Sterile mycelium (A6)	stem	1	0.7
Sterile mycelium (A7)	stem	4	3
Sterile mycelium (A10)	root	6	4.8

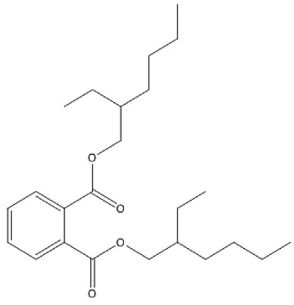
N number of isolates. % Frequency of occurrence. # Supported by molecular data.

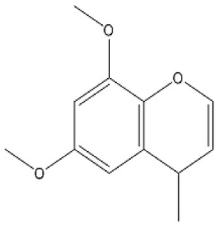
Table 2
Bioassay results of endophytic fungi of *S. persica* (the numbers are the clear zones in centimeter).

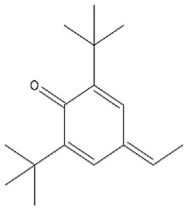
Fungi	*S. aureus*	*E. coli*	*C. albicans*	*A. fumigatus*
Alternaria sp.1 (A4)	0.9	0.9	Negative	Negative
# *Alternaria* sp.2 (A8)	1.3	1.2	Negative	Negative
# *Rhizopus arrhizus* (A9)	1.7	2.3	Negative	Negative
Trichoderma sp. (A1)	0.8	1.1	Negative	Negative
# *Aspergillus* sp. (A3)	0.9	1.1	Negative	Negative
Sterile mycelium (A2)	1.1	Negative	Negative	Negative
Sterile mycelium (A5)	0.9	Negative	Negative	Negative
Sterile mycelium (A6)	1	Negative	Negative	Negative
Sterile mycelium (A7)	Negative	Negative	Negative	Negative
Sterile mycelium (A10)	1.1	1.1	Negative	Negative

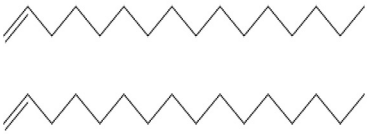
Table 3
Major natural products compounds identified in the ethyl acetate extract from the culture filtrate of *Alternaria* sp. (A8) by GC-MS:

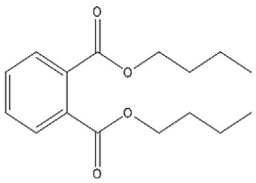
Peak no.	R-Time	Name of the compound	Molecular formula	Molecular weight	Area %	Activity
40	40.92	1,2-Benzenedicarboxylic acid, bis (2- ethyhexyl) ester	$C_{24}H_{38}O_4$	390	51.15	Antimicrobial
10	20.42	6,8-Dimethoxy-4-methyl-4H-chromene			10.87	
20	29.101	2,5-Cyclohexadien-1-one, 2,6-bis (1,1-dimethylethyl)-4-ethylidene-	$C_{16}H_{24}$	232	3.94	
11	21.893	Cetene	$C_{16}H_{32}$	224.43	3.32	Antioxidants
22	29.817	1,2-Benzenedicarboxylic acid, dibutyl ester (CAS) Butyl phthalate	$C_{16}H_{22}O_4$	278	3.23	Antimicrobial, antioxidant, plasticizer, cosmetics
16	26.242	1-Octadecene	$C_{18}H_{36}$	252.48	2.64	Anticancer, antioxidant and antimicrobial activities
4	9.87	Benzeneethanol	$C_8H_{10}O$	122.16	2.47	
27	31.971	1-Octdecene	$C_{18}H_{36}$	252.48	2.21	
23	30.201	Cycloeicosane	C_{20} H_{40}	280.54	1.96	
2	4.03	1-Butanol, 3-methyl-acetate	$C_7H_{14}O_2$	130.18	1.91	
8	16.991	1-Tetradecene	$C_{14}H_{28}$	196.37	1.77	Antimicrobial
5	11.559	Naphthalene	C_{10} H_8	128.17	1.69	Insecticidal
13	24.001	Phenol, 2,4-di-t-butyl-6-nitro	$C_{14}H_{21}$ NO_3	251.32	1.59	Antimicrobial, anticancer

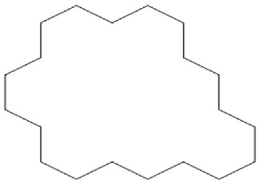

1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester


6,8-dimethoxy-4-methyl-4H-chromene

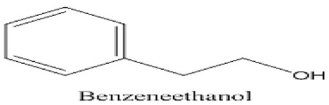

2,5-cyclohexadien-1-one, 2,6-bis (1,1-dimethylethyl)-4-ethylidene-

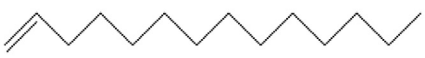

Cetene 1-Hexadecene

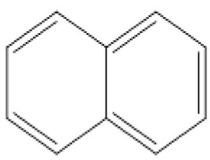

1,2-Benzenedicarboxylic acid, dibutyl ester (CAS) Butyl phthalate


Cyclocicosane


1-Octadecene


Benzeneethanol


1-Tetradecene


Naphthalene

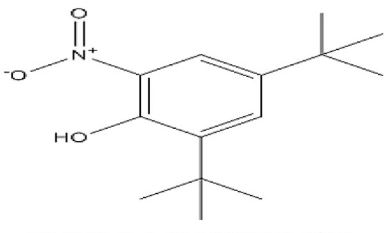
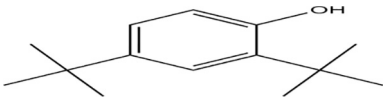
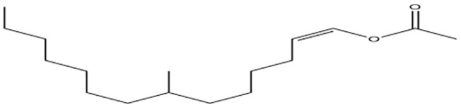
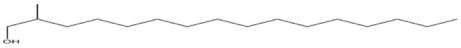
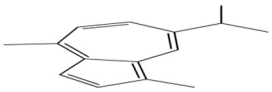
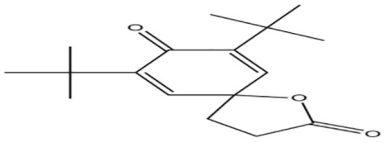
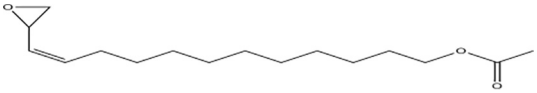
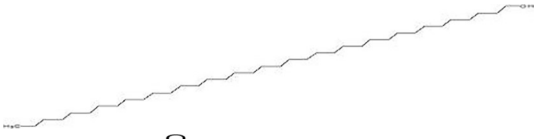
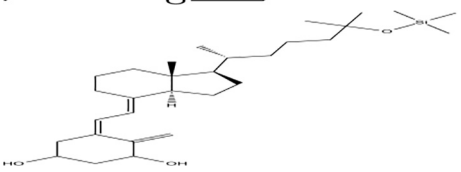

Phenol, 2,4-di-t-butyl-6-nitro

Table 4
Major bioactive compounds identified from ethyl acetate crude of *Alternaria* sp. (isolate No. A8), sub-fraction (A8-1-5):

Bioactive compound	RT (min)	Peak area%	MW	Chemical structure	Pharmacological actions
Phenol, 2,4-bis(1,1-dimethylethyl)-	18.00	26.92	206		Antibacterial and moderate cytotoxic (Teresa et al., 2014, Chuah et al., 2015).
7-Methyl-Z-tetradecen-1-ol acetate	25.62	12.53	268		
1-Hexadecanol, 2-methyl-	27.07	11.43	256		Antimicrobial, antioxidant (Ubaid et al. 2016).
Azulene,1,4-dimethyl-7-(1-methylethyl)	24.05	10.76	198		Antibacterial
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-Diene-2,8-dione (Ketone)	26.19	8.37	276		Antioxidant
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	23.19	7.45	268		
1-Heptatriacotanol	16.3	5.99	536		Antioxidant, anticancer, anti-inflammatory (Kumaradevan et al., 2015).
Propanoic acid, ethyl ester	3.73	5.15	102		Unkown
9,10-Secocholesta-5,7,10(19)-Triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-(Calcitriol)	12.3	1.94	488		Anticancer, regulate calcium in human blood

bacteria, then to use bio-assay guided fractionation to purify the active antibacterial compounds.

2. Materials and methods

2.1. Collection of plant materials and isolation and identification of endophytic fungi

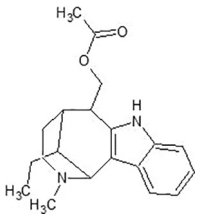
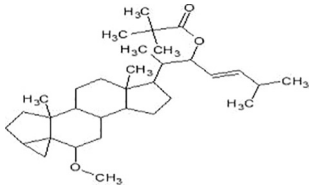
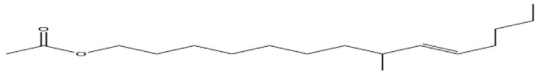
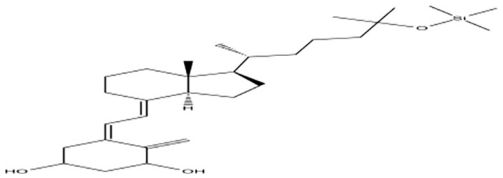
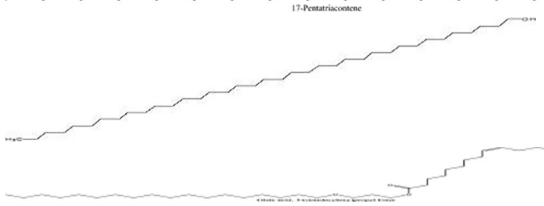
Fresh healthy-looking stem, roots and leaves (both young and old) of *S. persica* were collected on 4 August 2015 from Okair Geological Reserve, 26°29′9″N, 48°24′54″E, located 160 km west of Dammam city, Saudi Arabia. Samples were kept in clean plastic bags in ice bags, returned to the laboratory and processed within 24 h (Abdel-Wahab et al., 2017). The plant species was identified by Dr. Jacob T. Pandalayil, curator of KSU Herbarium, Department of Botany and Microbiology, College of Science, King Saud University and the herbarium material of *S. persica* was preserved at the department's herbarium. Isolation and identification of endophytic fungi using morphological and DNA sequences of ribosomal genes were described in details in Abdel-Wahab et al. (2017). Isolated fungi were grown in liquid cultures and natural products were extracted using ethyle acetate from culture filtrates. Resulted crude extracts were tested against pathogenic bacteria and fungi. Fungal isolated gave positive results were grown on large scale and resulted crude extracts were fractioned using silica gel columns and TLC sheets. Produced fractions were tested again and compounds in the actives sub-fractions were determined using mass spectrometry (GC-MS). Methods of growing fungal isolates, extraction and isolation of natural products, test organisms used, antimicrobial activity and isolation of metabolites were previously described (Abdel-Wahab et al., 2017).

3. Results and discussion

Forty-two fungal isolates were obtained from 135 young and old stem and 125 root segments. Those 42 isolates representing ten fungi include: *Trichoderma* sp. (the most common), two species of *Alternaria* (Fig. 3), *R. arrhizus* (Fig. 3) and *Aspergillus* sp. (Fig. 4) and 6 sterile mycelia. The ten fungi were grown in liquid culture and their crude extracts were tested against pathogenic bacteria and fungi. Twenty leaves (10 young and 10 old) of *S. persica* were processed for the isolation of fungal endophytes. However, no endophytic fungi were isolated from leaf samples. Nine crude extracts gave positive reactions against pathogenic of which two were chosen namely: *Alternaria* sp. (A8) and *R. arrhizus* (A9) for further study (Tables 1 and 2).

A few studies have been carried out to study endophytic fungi of *S. persica* (Korejo et al., 2014). Korejo et al., (2014) isolated eight endophytic fungi (including 2 *Aspergillus* spp., 3 *Penicillium* spp., *Fusarium solani*, *M. phaseolina* and *Rhizoctonia solani*) from 74 root, stem and leaves of *Salvadora* species. In their study, *Aspergillus*

Table 5
Major bioactive compounds identified from ethyl acetate crude of *Alternaria* sp. (isolate No. A8), sub-fraction (A8-1-6):

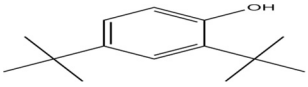
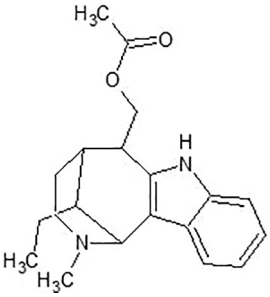
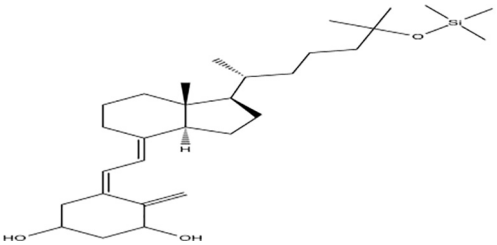
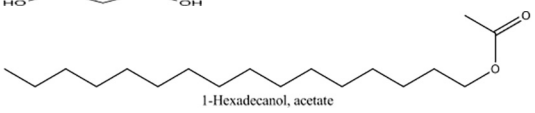
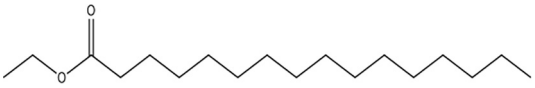
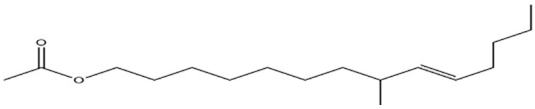
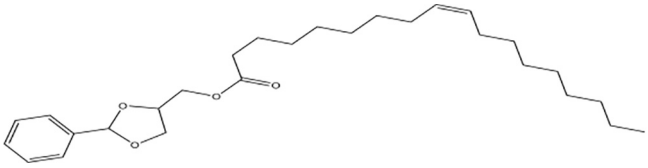
Bioactive compound	RT (min)	Peak area%	MW	Chemical structure	Pharmacological actions
Dasycarpidan-1-methanol, acetate (ester)	24.73	30.33	326		Antimicrobial (Sundar and Pillaiy, 2016).
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	25.41	22.14	498		New compound
E-8-Methyl-9-tetradecen-1-ol acetate	26.19	15.49	268		Insect pheromone (Ramalakshmi and Muthuchelian, 2011).
9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)- (Calcitriol)	12.8	8.32	488		Anticancer, regulate calcium in human blood
17-Pentatriacontene	23.98	6.71	490		Antimicrobial
1-Heptatriacotanol	16.65	6.34	536		Antioxidant, anticancer and anti-inflammatory
Oleic acid, 3-(octadecyloxy) propyl Ester	30.95	5.82	592		Antifungal (Abubacker and Devi, 2014).
7-Methyl-Z-tetradecen-1-o l acetate	26.65	3.25	268		Anticancer, anti-inflammatory, hepatoprotective
Z-5-Methyl-6-heneicosen-11-one	23.09	2.13	322		

niger was the most common fungus followed by *A. flavus* and *Penicillium* spp. Endophtyic fungi reported in the current study are totally different from endophytic fungi recorded in the previous studies from the same host (Korejo et al., 2014). Previous studies used different methods of endophytic fungi isolation where they surface sterilize the plant material and then ground them and use pour plate method. In the pour plate method, fast growing fungi like *Aspergillus* species over grow other slow growing fungi. Also the structure of endophytic fungi communities from different geographical regions in the same host plant are frequently different (Jiang et al., 2010). The previous study has been carried in India while the present study in Saudi Arabia. Dhankhar et al. (2013a) isolated seventeen endophytic fungi from *S. oleoides* Decne in India and studied their abilities to produce natural products. Isolated endophytic fungi include: 10 *Aspergillus* spp., *C. herbarum*, *E. nigrum*, *F. moniliforme*, *P. chrysogenum*, *Phoma* sp. and *Pythium spinosa*. In another study, Dhankhar et al. (2013b) isolated 27 fungal species representing 18 fungal genera from 3750 samples of leaf segment, petiole and stem from five different sites of three seasons (summer, rainy and winter) were collected and analyzed. Antidiabetic and hypolipidemic activities of the crude extracts of the isolated fungi were tested and they obtained positive results from four fungal isolates (see Tables 3–6).

Thirty-seven bioactive chemical compounds were identified from the crude extracts of *Alternaria* sp. (A8) using GC-MS (Figs. 5–8). Thirteen major bioactive compounds were recorded namely: 1,2-Benzenedicarboxylic acid, bis (2-ethyhexyl) ester representing 51.15% of the crude extract, 6,8-dimethoxy-4-methyl-4H-chromene (10.87), 2,5-Cyclohexadien-1-one, 2,6-bis (1,1-dimethylethyl)-4-ethylidene- (3.94%), Cetene (3.32%), 1,2-Benzenedicarboxylic acid, dibutyl ester (3.23%), 1-Octadecene (2.64%), Benzeneethanol (2.47%), 1-Octdecene (2.21%), Cycloeicosane (1.96%), 1-Butanol, 3- methyl-, acetate sopentyl alcohol, acetate (1.91%), 1-Tetradecene (1.77%), Naphthalene (1.69%), Phenol, 2,4-di-t-butyl-6-nitro (1.59%). These compounds showed strong antibacterial activity in combination. The fungal isolate was identified by phylogenetic analyses based on LSU rDNA sequence data and it might represent undescribed species of *Alternaria* (Fig. 2).

1,2-Benzenedicarboxylic acid, bis (2-ethyhexyl) ester also known as di-(2-ethylhexyl) phthalate (DEHP) or bis-(2-ethylhexyl) phthalate (BEHP) was the major compound (repre-

Table 6
Major bioactive compounds identified from ethyl acetate crude of *Alternaria* sp. (isolate No. A8), sub-fraction (A8-3-2):

Bioactive compound	RT (min)	Peak area %	MW	Chemical structure	Pharmacological actions
Phenol, 2,4-bis(1,1-dimethylethyl)-	16.66	20.16	206		Antibacterial and moderate cytotoxic.
Dasycarpidan-1-methanol,acetate (ester)	24.73	17.25	326		Antimicrobial
9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)- (Calcitriol)	12.8	16.02	488		Anticancer, regulate calcium in human blood
1-Hexadecanol, acetate	22.64	15.54	284		
Hexadecanoic acid, ethyl ester (Ethyl Palmitate)	26.96	10.56	284		Anti-inflammatory
E-8-Methyl-9-tetradecen-1-ol acetate	26.19	4.98	268		Insect pheromone
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	29.1	1.56	444		

sented by 51.15%) of the ethyl acetate crude extract of the endophytic fungus *Alternaria* sp. (A8) isolated from the medicinal plant *S. persica*. 1,2-Benzenedicarboxylic acid, dibutyl ester (CAS) Butyl phthalate is among the major compounds recorded in this study and represented by 3.23% of the Crude. Phathalates compounds were reported from *Calotropis gigantean* (Habib and Karim, 2009), *Alchornea cordifolia* (Mavar-Manga et al., 2008) and *Aloe vera* (Lee et al., 2000). 1,2-Benzenediccarboxylic acid, dioctyl ester isolated from the ethyl acetate soluble sub- portion of the unripe fruits of *Nauclea latifolia* showed strong antibacterial activity against *Gram* positive bacteria (Ajoke et al., 2014). Phthalic Acid derivative produced by *Streptomyces bangladeshiensis* showed strong antimicrobial activity (Al-Bari et al., 2006). 6,8-dimethoxy-4-methyl-4H-chromene (10.87%) is the second major compound in the active fraction of the crude. This compound seems to be a new compound and we will do more analytical work on it. 1-Octadecene is among the major compounds recorded in this study and represented by 2.64% of the Crude. Octadecane is an alkane hydrocarbon which possesses various activities such as anticancer, antioxidant and antimicrobial activities (Valderramas et al., 2008, Karmakar et al., 2011). Phenol, 2,4-bis(1,1-dimethylethyl)-(26.92%) was the major compound in this sub-fraction. 2,4-Di-tert-butylphenol (2,4-DTBP) is a natural compounds present in medicinal plants. It is reported to have herbicidal properties. It was produced by *Streptomyces* sp. and showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with mode of action against bacterial cell wall synthesis and also showed moderate cytotoxic activity (Chawawisit et al., 2015). 7-Methyl-Z-tetradecen-1-ol acetate (12.53%) was the second major compound reported in this sub-fraction. 7-Methyl-Z-tetradecen-1-ol acetate was isolated from the medicinal plant *Mentha viridis* (Hameed et al., 2015). 1-Hexadecanol, 2-methyl- (11.43%) was the third major compound recorded in this sub-fraction. 1-Hexadecanol, 2-methyl- was isolated from the methanolic extract of *S. aureus*. And it showed antimicrobial and antioxidant activity (Jaddoa et al., 2016).

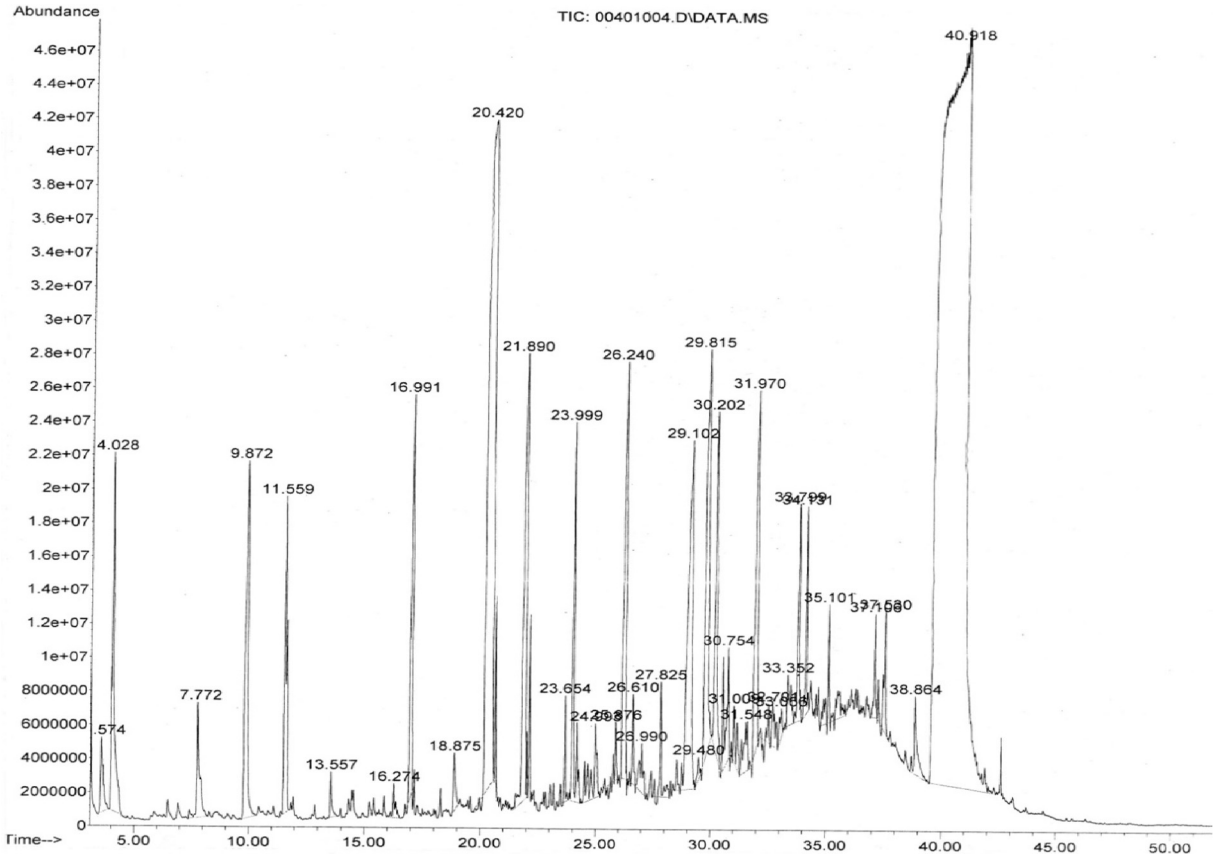


Fig. 5. GC-MS chromatogram of the active fraction of the ethyl acetate of *Alternaria* sp. (A8).

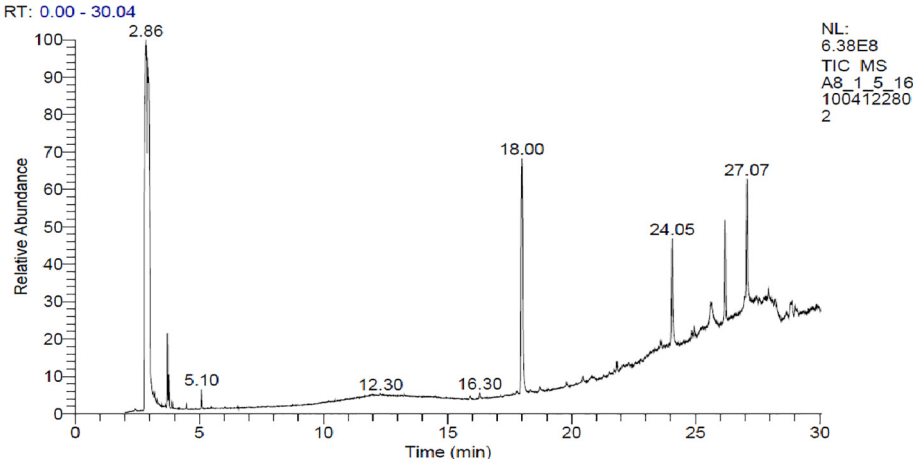


Fig. 6. GC/MS chromatogram of volatile bioactive organic components derived from *Alternaria* sp. (isolate No. A8), sub-fraction A8-1-5.

Azulene,1,4-dimethyl-7-(1-methylethyl)- (10.76%) was the fourth major compound reported in this sub-fraction. Azulene,1,4-dimethyl-7-(1-methylethyl)- was isolated from the methanolic extract of the medicinal plant *Lindera nacusua* and it showed antibacterial activity (Wei et al., 2016). Dasycarpidan-1-methanol, acetate was the major compound in this sub-fraction. Dasycarpidan-1-methanol, acetate (ester) was the main antimicrobial bioactive compound that was isolated from the prickly custard apple (*Annona muricata*) and it was isolated from the methanolic and ethyl acetate crude extract from both peel and pulp and it was identified as best antibacterial agent (Karthikeyan et al., 2016). E-8-Methyl-9-tetradecen-1-ol acetate (15.49%) was the third major compound recorded in this sub-fraction. E-8-methyl-9-tetradecen-1-ol acetate was isolated from the ethanolic extract of the leaves of the medicinal plant *Mallotus tetracoccus* (Ramalakshmi and Muthuchelian, 2011).

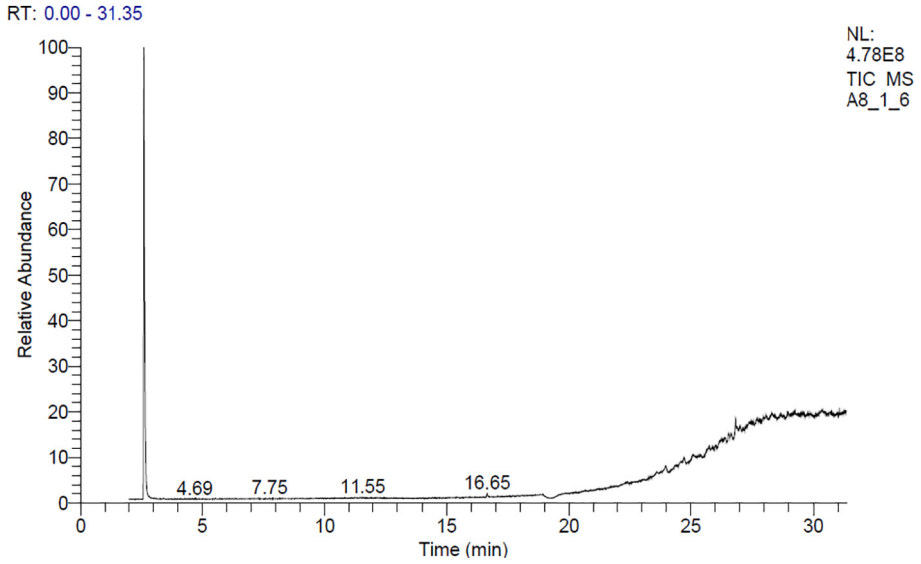


Fig. 7. GC/MS chromatogram of volatile bioactive organic components derived from *Alternaria* sp. (isolate No. A8), sub-fraction A8-1-6.

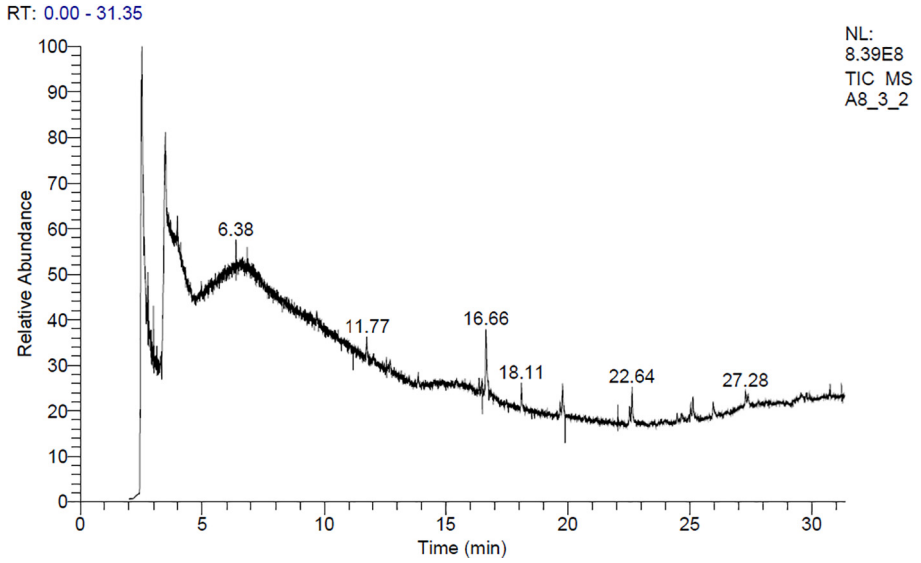


Fig. 8. GC/MS chromatogram of volatile bioactive organic components derived from *Alternaria* sp. (isolate No. A8), sub-fraction A8-3-2.

Conflict of interest

The authors declare that they have no conflicts of interest

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