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Full Length Article

Chemical Composition, FTIR Studies, Morphological Alterations, and Antifungal Activity of Leaf Extracts of *Artemisia sieberi* from Saudi Arabia

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Abstract

Artemisia sieberi Besser is an herb widely used in traditional medicine in Middle East to treat various ailments. Present study evaluated various solvent extracts of leaves of *A. sieberi* growing in Saudi Arabia, against a wide array of phytopathogenic fungi. All the extracts showed significant inhibition. Methanol and dichloromethane extracts showed strong inhibition of mycelial growth of *Alternaria alternata*, *Fusarium moniliforme*, *F. solani*, *F. oxysporum* and *Macrophomina phaseolina.* Scanning and transmission electron microscopy of treated fungal cells revealed the damaging effects of extracts on fungi. Scanning electron microphotographs of treated cells show shriveled and twisted mycelium with small vesicles, misshapen and ruptured conidia with heavy scaling and leakage of cell contents. The microphotographs from TEM show heavy vacuolation, proliferation of lipid bodies, septal damage, and undeveloped conidia. GC–MS and FTIR analysis revealed the presence of some important bioactive compounds like Isophorone, Cis–9–Tetradecen–1–ol, Phenol,5–methyl–2–(1–methyl ethyl)–acetate, Phenol, 2–methyl–5 (1–methylethyl), Phenol, 2,4,5–trimethyl, coumarin, camphor, 3–bromo–d–, physostigmine and oxacyclododecen–2–one. The functional groups like phenols, flavonoids, polyphenols, aromatic compounds and ketones were identified from the IR spectrum. The rich and diverse chemical composition of *A. seiberi* could be responsible for the strong antifungal activity. Hence, leaves of *A. sieberi* can serve as an ecofriendly substitute for the harmful synthetic fungicides used during harvest and postharvest period. © 2019 Friends Science Publishers

Keywords: *Artemisia sieberi;* Distorted conidia and mycelia; GC–MS; FTIR; SEM; TEM

Introduction

Artemisia is considered as one of most important and largest genera belonging to family Asteraceae (Joan *et al.*, 2011). This genus comprises about 474 species. Most of the plants are annuals or perennials and grow as herbs or shrubs (Koul *et al.*, 2017). Various species of this genus play a vital role in traditional medicine and is also used in cooking. Previous researches have shown the antiparasitic (Bora and Sharma, 2011) antimicrobial, anticandidal and antioxidant activities of oils and aerial extracts of different species of *Artemisia* (Seddiek *et al.*, 2011; Lee *et al.*, 2013; Altunkaya *et al*., 2014; Arbi *et al*., 2017). Due to presence of diverse phytochemicals, this genusis attracting attention from researchers all over the world.

The soil of Saudi Arabia harbors numerous species of plants exhibiting its rich and diverse flora in spite its arid conditions. Recent inventory has shown that among 393 species screened, 61 species were of medicinal use, most of them are herbs while a small number were shrubs and trees (Atiqur Rahman *et al*., 2004).

Several species of *Artemisia* are widely distributed in the Kingdom of Saudi Arabia. *A. sieberi* is a well–known species of family Asteraceae and has many medicinal properties. It grows in the deserts of Saudi Arabia and has been reported from Northern and Central region predominantly, in scattered populations especially in Wadis (Al Badr *et al*., 2012; Arbi *et al*., 2017). It is a bushy shrub with grey green shoot and its leaves are feathery. In, Middle East it is used in traditional medicine as antidote to poison from mushrooms. Its extracts and oil have shown antibacterial and antifungal activity against dermatophytes, it is used as an emmenagogue, in treating malaria and to treat stubborn ulcers (Kamal *et al*., 2007; Rad *et al*., 2008; Mahboubi, 2014, 2017).

Plants constitute a very crucial part of our Universe. Plant products like fruits and vegetables suffer huge losses due to plant pathogens as they attack various parts of plant and their products, in different phases of their growth, during storage and transport. However, amongst all the pathogens, fungal pathogens cause major losses to crops affecting both their quality and quantity of produce.

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Additionally there is a threat to human life due to the mycotoxins produced by fungi. Another major concern is the fungicidal residues present on the fruit surfaces. It has been proved lately that both the mycotoxins and fungicides are potent carcinogenic. Hence, our research aims to screen *A. sieberi* leaf extracts for its chemical composition, FTIR studies, *in vitro* antifungal activity, further SEM and TEM studies of fungi treated with extracts to understand the extent of damage caused both in their morphology and ultra–structure. These studies will help in evaluating and concluding the role of extracts in control of some important fungal phytopathogens as an alternative to harmful synthetic fungicides. To our knowledge, this the first report showing *in vitro* control of some important postharvest fungal pathogens with *A. sieberi* leaf extracts.

Materials and Methods

Plant Material

Plant samples of *A. sieberi* was collected from Buraydah and identified by Dr Mona Al Wahibi, Taxonomy Department of King Saud University. Leaves were separated from the stem, washed in running tap water followed by sterile distil water. Leaves were then allowed to dry in shade for two weeks. Dry leaves were finely powdered and stored for further use.

Plant Extracts

Different solvents (methanol, ethanol, ethyl acetate, dichloromethane, water) were used for extraction purpose. Briefly, 10 g of dry powdered leaves were added to 100 mL of various solvents and distilled water separately and left on the rotatory shakerat 150 rpm for 24 h. The extracts were filtered through Whatman's (No. 1) filter paper and the filtrates were dried in rotary evaporator. After evaporation the residue was reconstituted with mother solvent and different concentrations (0.5, 1.0, 2.5 and 5 mg/mL) were prepared. Reconstituted aqueous extracts were passed through Millipore bacterial filters (0.45 µm) before using them for *in vitro* assay.

Fungal Isolates

The phytopathogens used in this research were procured from the Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, Riyadh, KSA. The following fungi were screened in the present study: *Helminthosporium sativum, Macrophomina phaseoloni, Fusarium moniliforme, F. solani*, *F. oxysporum* and *Alternaria alternata.* All the fungal strains were maintained on Potato dextrose agar.

Fungal Growth Inhibition

In vitro antifungal activity of crude solvent extracts was evaluated by following the Poison food technique (Fakruddin *et al.,* 2012). To 9 cm petri plate, 1 mL of the extract was added followed by 19 mL of molten potato dextrose agar and mixed gently by swirling. The modified agar was allowed to solidify, after which a mycelial plug (6 mm) was placed in the center of the plate. The mycelial plug was removed from the periphery of 9 days old actively growing colony. The above–mentioned experiments was carried out aseptically in a laminar air flow. After inoculation, petri plates were incubated at 25± 2°C. All fungal strains were subjected to different concentrations of solvent extracts in triplicates. Results were noted by measuring the diameter of mycelial growth when the control plate showed full plate growth. and percentage growth inhibition was calculated as follows:

$$\% \text{ inhibition} = (AC - AT)/AC \times 100$$

Where AC= Colony diameter in control plate and AT is the diameter of the colony in treated Petri plates.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) studies was carried out on mycelium showing maximum inhibition when treated with extracts. To have a fair comparison the mycelia from control (untreated) plates were subjected to SEM studies. Small discs (6 mm) were aseptically removed from the inhibited regions of the colony and placed in a sterilized container and fixed in glutaraldehyde (2.5%) in PBS–0.1 M (pH 7.4). This suspension was centrifuged after 48 h, rinsed thrice and the discs were subjected to series of dehydration from 60–100% with ethanol. After dehydration the samples were freeze dried with critical point dryer and mounted on stubs coated with gold. Finally, SEM model (JSM–6060LV–JEOL) Japan–LTD was used to scan and take microphotographs.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was done following the same procedure (fixation and dehydration) as mentioned above for SEM. After dehydrating, the sample was embedded in a resin mixture and thin sections were cut (70–80 nm) and loaded on copper grid. The samples were stained by uranyl acetate and lead citrate and observed under TEM MODEL –Jeol–1011)

GC–MS

GC–MS was performed on the extracts showing strongest inhibition of test fungi. Helium gas with a flow of 1 mL per min served as carrier gas. Instrument was GC–coupled with MS (7890A, 5975C) Agilent Technologies, Santa Clara, USA. Phenyl methyl siloxane column was used (30 m × 250 µm × 0.25 µm). Following conditions were maintained during the GC run: time (90min), volume (1 µL),

temperature (280°C; 250°C), ion source and split ratio (20:1). Further, the temperature was held for 5 min at 40°C to begin with then it was increased to 280°C @ 10°C/min and at this temperature it was maintained for another 5min. An electron impact (70–eV) was generated for mass spectroscopy with a scan range 35 to 780 (m/z). The mass spectrum library of National Institute of Standards and Technology was used for identification.

FTIR

Leaf extract with strongest antifungal activity was subjected to FTIR analysis, to identify the important functional groups present. The spectrometer (Nicolet–6700, Thermo scientific–USA) possessing a beam splitter and a detector (DTGS) equipped with an OMNIC software was used to collect and analyze the spectrum in the scan range of 500–4000 cm–1. The IR spectrum thus obtained was used to interpret the functional groups present in the methanol extract.

Statistical Analysis

All experiments were carried out thrice (triplicates) and results are means of ±S.D.

Results

Antifungal Activity of various Solvent Extracts

A. sieberi leaf extracts showed significant inhibition of the fungi tested. All the phytopathogens were strongly inhibited by methanol extracts. Besides methanol, ethanol and dichloromethane extracts also inhibited the pathogens effectively. However, aqueous and ethyl acetate extracts were inhibitory in a variable manner. Amongst all the tested pathogens, *F. moniliforme* showed maximum inhibition with dichloromethane extracts (93% inhibited). Interestingly, all the *Fusarium* species (*F. moniliforme, F. solani, F. oxysporum*) tested showed strong inhibition of mycelial growth by methanol extracts (89, 86 and 83%, respectively). *M. phaseoloni* and *A. alternata* were sensitive to methanol and dichloromethane extracts (Table 1; Fig. 1–3).

SEM Analysis

The microscopic studies showed significant inhibition of both mycelium and conidial structures. Plates showing strong inhibition were chosen for the microphotographs. It is clear from the microphotographs that the treated mycelium was twisted, shriveled, broken, distorted, and also showed the presence of vesicles on the surface. While the microphotographs of both *Alternaria* and *Fusarium* species shows that the methanol extracts damaged the conidia drastically by altering its shape and prominent protrusions are on the conidia. The cell membrane was disrupted, which lead to leakage of cytoplasmic and cellular contents. Fungi treated with dichloromethane extracts showed the absence of conidia in the microphotographs indicating total suppression of conidial structures. The microphotographs of conidia and mycelium from control plate which was not treated with extracts, showed smooth, regular, and well–defined mycelium and conidia without any distortion (Figs. 4–5).

TEM Analysis

Untreated *A. alternata* and *Fusarium* cells showed regular

Table 1: Antifungal activity of various solvent extracts of *A. sieberi* and the percentage mycelial inhibition

Fungi tested	Concentrations	Percentage mycelial inhibition					
		F. moniliforme	*F. oxysporum*	*F. solani*	*A. alternata*	*M. phaseoloni*	*H. sativum*
Aqueous	0.5	0	38.1±1.71	41.11±0.00	11.85±1.28	35.00±0.00	16.00±0.00
	1	21.20±0.31	40.36±1.28	44.44±1.11	15.55±0.00	38.00±0.00	22.22±0.19
	2.5	20.00±0.00	48.14±0.64	44.44±0.00	22.22±0.00	42.96±0.64	24.00±1.00
	5	66.06±0.50	48.14±0.64	55.92±0.64	42.22±1.11	46.66±0.00	54.44±1.11
Methanol	0.5	44.44±1.07	60.36±1.28	61.85±0.64	52.06±0.87	64.07±0.64	44.81±0.64
	1	50.22±1.07	65.92±0.64	71.84±1.70	58.88±0.00	78.88±0.00	48.88±1.11
	2.5	82.77±0.83	72.22±0.00	74.07±1.28	72.96±1.28	88.62±0.54	58.51±1.69
	5	89.62±0.64	86.66±0.00	83.7±0.64	76.29±0.64	88.00±0.00	74.81±0.64
Ethanol	0.5	34.74±0.65	55.00±0.00	50.70±0.61	33.70±0.64	61.11±0.00	38.88±0.00
	1	45.39±0.53	57.4±0.64	62.22±1.11	41.11±1.11	68.14±1.28	44.00±1.00
	2.5	52.06±0.87	67.84±0.13	66.66±0.00	45.92±0.64	73.33±1.11	54.44±0.00
	5	71.56±0.49	76.66±0.00	79.62±0.64	54.81±0.64	84.44±0.00	67.00±1.00
Ethylacetate	0.5	38.44±0.50	54.81±1.28	37.4±1.28	39.62±0.64	35.51±0.50	33.33±0.00
	1	40.74±0.00	61.00±0.00	42.22±1.92	40.00±0.00	36.66±0.57	38.88±0.64
	2.5	27.51±0.44	64.81±0.64	44.07±064	40.37±0.64	42.18±1.16	38.00±0.00
	5	46.43±0.51	66.29±0.64	46.66±0.00	41.11±0.00	46.00±1.00	41.00±0.00
Dichloromethane	0.5	21.20±0.52	50.11±1.16	50.00±0.00	35.92±0.64	63.59±1.28	30.00±1.00
	1	60.66±0.70	51.44±0.50	53.70±0.64	42.22±1.92	66.66±0.00	33.22±0.19
	2.5	71.95±0.92	77.77±0.00	72.22±1.28	65.55±1.11	77.40±0.64	67.55±0.77
	5	93.00±1.00	81.40±1.22	78.29±0.64	71.11±0.00	88.14±1.28	79.62±0.64
Control	C+M	100±0.00	96.06±0.50	89.62±0.62	88.00±1.00	87.00±1.00	90.00±0.00

Values shown in the table are means (± SD) of triplicates.M-Mancozeb, C- Carbendazem

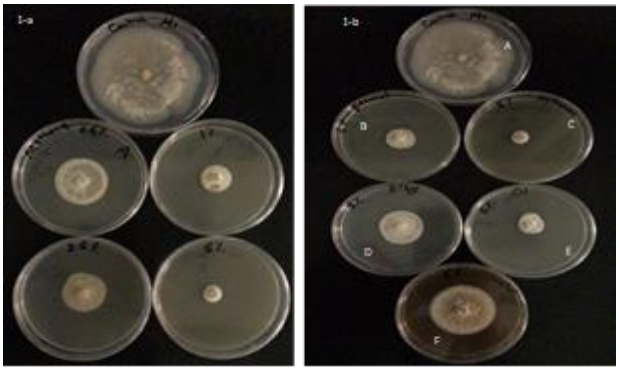

Fig. 1: (a) *In vitro* mycelial inhibition of *F. oxysporum* with methanol extracts (0.5–5%) (b) Inhibition of *F. oxysporum* at 5% concentration by all the solvent extracts (A-Control, B-ethanol, C-methanol, D-ethyl acetate, E-Dichloromethane, F-water

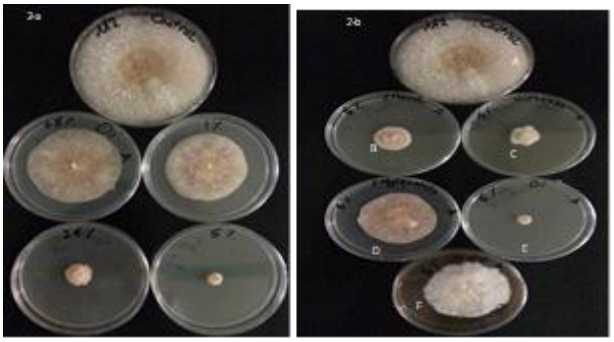

Fig. 2: (a) *In vitro* mycelial inhibition of *F. moniliforme* with Dichloromethane extracts (0.5–5%). (b) Inhibition of *F. moniliforme* at 5% concentration by all the solvent extracts (A-Control, B-ethanol, C-methanol, D-ethyl acetate, E-Dichloromethane, F-water

cell wall and cytoplasmic membrane. Presence of electron dense coating on surface was very prominent. However, in contrast the treated fungal cultures (methanol and dichloromethane extracts) led to alterations in the structure and integrity of cell wall, cytoplasmic membrane and cellular organelles. The cell wall was irregular, disrupted, its surface displayed fragment deposition, excess vacuolization, septal damage, cytoplasmic disintegration, and proliferation of lipid bodies. Many single undeveloped cells were observed further septal formation was incomplete or lacking. (Figs. 6–7)

GC-MS

GC–MS analysis of methanol extracts of *A. sieberi* showed the presence of several peaks corresponding to important phytochemicals (Fig. 8). The identified constituents are listed in Table 2. Some of the important compounds are Isophorone, Cis–9–Tetradecen–1–ol, Phenol, 5–methyl–2–(1–methyl ethyl)–acetate, Phenol, 2–methyl–5 (1 methyl ethyl), Phenol, 2,4,5–trimethyl , Coumarin, 8–acetyl–7–hydroxy–4–methyl, 1–Tetradecanol, Oxacyclododecen–2–one, Phenethylamine, 3,4,5–trimethoxy–alpha–methyl, 4A, 7–Methano–4aH–napth (1,8a–b) oxirene, octahydro–4,4,8,8–tetramethyl, Camphor, 3–bromo–d–, Pregn–4–ene–3, 20–dione, 11–hydroxy–, (11,alpha), 1,2,3,4,5,6–Hexahydro–1,1,5,5–tetramethyl–2, 4a–methanonaph–7(4aH)–one Physostigmine, Eseridine, Triaziquone, Daniquidone

FTIR

Methanol extracts were further subjected to FTIR analysis to ascertain the functional groups present in comparison to GC–MS. Broad peak at 3330 cm–1 corresponds to OH vibrations while at 2920 cm–1is due to CH asymmetry stretching. Peaks at 1711 cm–1 and 1511 cm–1 is from the C=O stretching, aromatic skeletal stretches at 1604 cm–1, peak at 1367 cm–1 is from the CH bending, while the peaks at 1175 cm–1 and 1047 cm–1 are due to C–O–C stretches. The aromatic ring shows a small peak at 834 cm–1 (Table 3; Fig. 9).

Discussion

Fungal resistance to fungicides and pesticides, fear of hazardous effect of fungicides persistence in soils and deleterious effect on human health due to mycotoxins has impelled researchers to look for safer, economical yet effective fungicides derived from plants and its products. Even though many plants have been researched for their medicinal properties to date, still a large number are unexplored for their role in controlling pathogens. The genus *Artemisia* comprises of many species, most of them are used in traditional medicine to treat various disorders. Presence of important chemical compounds with therapeutic value has prompted many researchers to screen the genus *Artemisia* for its biological activities. Our findings show that leaves of *A. sieberi* has the ability to inhibit some important phytopathogens. Recently, essential oils from some species of *Artemisia* have been screened for their antimicrobial activity and have shown promising results, however there are noreports that show the antifungal activity of *A. sieberi* leaf extracts against fungal phytopathogens.

Previous studies show the inhibitory activity of the aerial parts of *A. herba–alba* against *Candida albicans* (Mahboubi *et al*., 2008; Naeinia *et al*., 2009). Oil from stem and leaves of *Artemisia* grown in Iran, showed marked inhibition of *F. solani, F. moniliforme, Rhizoctonia solani and T. phaseolina* with MIC ranging between 500µg/mL to 2000µg/mL (Farzaneh *et al*., 2006). Another study shows *A. sieberi* oil to suppress the formation of conidia and spores of *Aspergillus niger, Zygorrhycus* and *Penicillum italicum* (Tantaoui–Elaraki *et al*., 1999).

Table 2: Chemical composition of methanol extracts of *Artemisia sieberi* leaves

Name of the compound	Molecular weight	Molecular formula
Isophorone	138	C_9H_4O
Cis-9-Tetradecen -1-ol	212	$C_{14}H_{28}O$
Methyl furoate	126	$C_6H_6O_3$
Phenol,4-methyl-2-nitro	153	$C_8H_{11}O_2$
4,5-Dimethyl-ortho-phenylenediamine	136	$C_8H_{12}N_2$
Phenol,5-methyl-2-(1-methyl ethyl)-acetate	192	$C_{12}H_{16}O_2$
Phenol,2-methyl-5(1 methyl ethyl)	150	$C_{10}H_{14}O$
Phenol,2,4,5-trimethyl	136	$C_9H_{12}O$
2,4,Dimethyl phenol	122	$C_8H_{10}O$
1-H-indole,7-methyl-	131	C_9H_9N
Coumarin,8-acetyl-7-hydroxy-4-methyl	218	$C_{12}H_{10}O_4$
1-Tetradecanol	214	$C_{14}H_{30}O$
Oxacyclododecen-2-one	184	$C_{11}H_{20}O_2$
Phenethylamine,3,4,5-trimethoxy-alpha-methyl	225	$C_{12}H_{19}NO_3$
4A,7-Methano-4aH-napth(1,8a-b) oxirene, octahydro-4,4,8,8- tetramethyl	220	$C_{15}H_{24}O$
Camphor,3-bromo-d-	231	$C_{10}H_{15}BrO$
Vitamin A aldehyde	284	$C_{20}H_{28}O$
Pregn-4-ene-3,20-dione,11-hydroxy-,(11,alpha)	330	$C_{21}H_{30}O_3$
1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-2,4a-methanonaph- 7(4aH)-one	218	$C_{15}H_{22}O$
Physostigmine	275	$C_{15}H_{21}N_3O_2$
Eseridine	291	$C_{15}H_{21}N_3O_3$
Triaziquone	231	$C_{12}H_{13}N_3O_2$
Daniquidone	249	$C_{15}H_{11}N_3O$

Table 3: Functional groups present in *A. sieberi* leaves extracts (methanol)

Peak values(cm-1)	Functional groups
3330	OH-stretching vibrations of hydrogen bonded hydroxyl groups.
2920	CH asymmetry stretching
1711	C=O stretch
1604	C-C ring aromatic skeletal stretching
1511	C=O stretch
1367	CH bending
1175	C-O-C stretching band
1047	C-O-C stretching band

A recent study shows the antifungal activity of *A. sieberi* oil against *Malassezia* the causative agent of atopic dermatitis. A large number of Malassezia isolates (130) were isolated from atopic lesions of dogs, all the isolates showed significant inhibition at an MIC ranging between 80–250 µg/mL (Khosravi *et al*., 2016). In another study clinical isolates of dermatophytes were tested with *A. sieberi* oils. However, dermatophytes showed variable inhibitory activity. *Microsporum gypseum* was highly sensitive, while *M. canis*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. schoenleinii* and *T. verrucosum* were least sensitive as they exhibited poor inhibition (Mahboubi and Kazempour, 2015). Incontrast to the above mentioned findings, Khosravi *et al*. (2013) in a separate study reported that all the clinical isolates of dermatophytes showed significant inhibition with *A. sieberi* oils at an MIC ranging between 0.25–1 mg/mL.

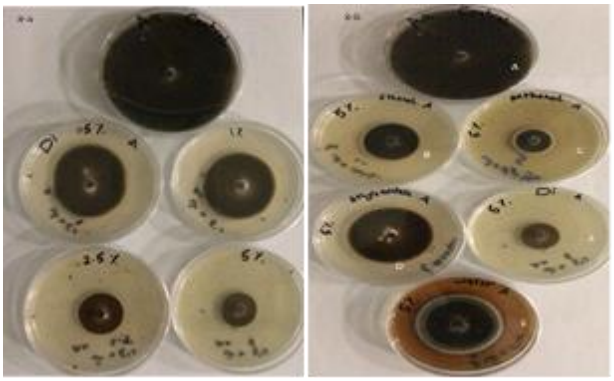

Fig. 3: (a) *In vitro* mycelial inhibition of *A. alternata* with Dichloromethane extracts (0.5-5%). (b) Inhibition of *A. alternata* at 5% concentration by all the solvent extracts. (A-Control, B-ethanol, C-methanol, D-ethyl acetate, E-Dichloromethane, F-water

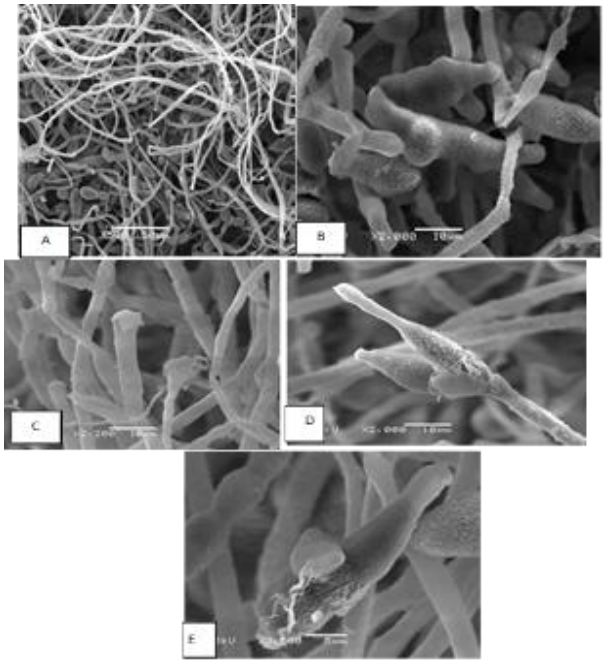

Fig. 4: Microphotographs of *A. alternata*. (A)Untreated cells show intact smooth mycelium and conidia (B-D) Treated cells with methanol (B-C), Dichloromethane (D-E). The mycelium of treated cells show vesicles on the mycelium, completely distorted and shriveled mycelium (A, C) conidia is distorted and completely damaged. Blebbing and cell content leakage is clearly seen (B, D, E)

Various phytocompounds like camphor, 1, 8 cineole, α– thujone, β–thujone from *A. sieberi* oil were tested against abroad spectrum of fungi which included different species of *Candida*, *Aspergillus* and dermatophytes. It was observed that species of *Aspergillus* was resistant to the oil treatment while *Candida* and dermatophytes exhibited significant inhibition (Abu–Darwish *et al*., 2015).

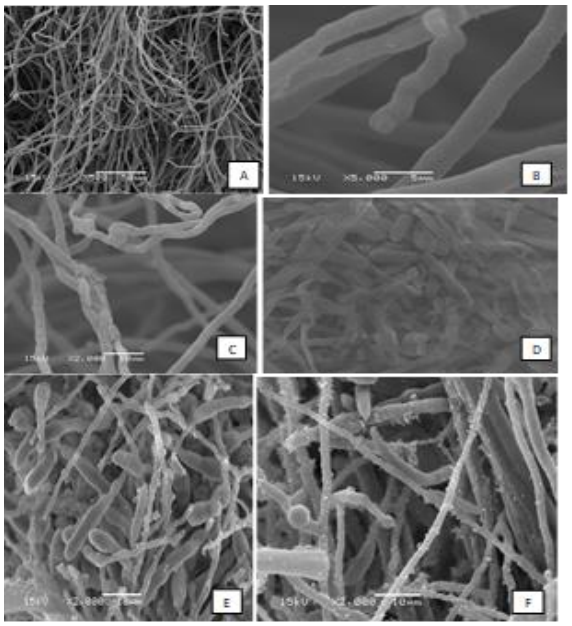

Fig. 5: Microphotographs of *F. oxysporum* (A, B, C) and *F. moniliforme* (D, E, F). A- Untreated cells show intact smooth mycelium and conidia development. B-D- Treated cells with methanol (A-C), Dichloromethane (D-F). The mycelium of treated cells show twisted hyphae, deposition of extruded material and shrivelled mycelium. While the conidia exhibit distorted shape, shorter, deposition of extruded material and completely damaged as seen in D

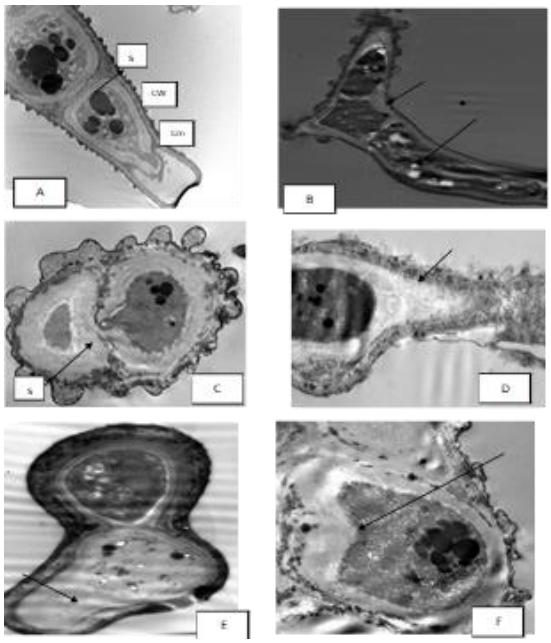

Fig. 6: Transmission electron microscopy micrographs of A. alternata (A) control without treatment shows an electron-dense cell wall, cell membrane (CW, CM), vacuole (V) mitochondria (M), and septum (S). (B-F) treated hyphae with methanol and dichloromethane extract of *A. sieberi* leaves. It can be noted that the protoplasm has receded from the cell membrane, Septal damage, shrinkage of protoplasm, damaged cell wall and membrane, heavy vacuolation and lipid bodies can also be seen, and the microphotograph shows cytoplasm in a collapsed state with electron-dense granules (E, F)

The GC–MS of the methanol extracts in our study showed the presence of camphor which has been reported earlier from *A. sieberi* oils (Mahboubi and Kazempour, 2015). Oxygenated sesquiterpenes, polyphenols, alkaloids, alcohols, phenols, ketones and coumarins were also present in the methanol extracts. Similarly, the FTIR studies showed the presence of important functional groups like aromatic compounds, phenols, esters and ketones. No data are available to make comparisons from other studies, however the composition of oil from different species of *Artemisia*, showed major chemical compounds to be that 1,8 cineole, α– thujone, β–thujone and camphor (Moufid and Eddouks, 2012; Kumlay *et al*., 2015; Majid *et al*., 2016). Similarly, all these constituents have been reported from oils extracted from four species of *Artemisia* (*A. sieberi, A. monosperma*, *A. judaica* and *A. scoparia*) growing in Saudi Arabia (Arbi *et al*., 2017). Our findings also revealed that isophorone and other acids were present in the extracts of *A. sieberi*.

Antibacterial activity of various solvent extracts of the genus *Artemisia* has shown that extracts caused variable inhibition (high to negligible) of phytopathogenic bacteria and some fungi (Ahameethunisa and Hopper, 2010, Komal*et al*., 2015). Recently, Adoui*et al*. (2018) screened aqueous extracts of *A. herba–alba* against *L. monocytogenes M. leteus* and *E. feacalis* strains and reported significant inhibitory activity.

SEM and TEM studies revealed the damaging effect of *Artemisia* extracts on mycelia and conidia. These effects could be due to the presence of diverse bioactive chemical constituents present in the extracts. Phenols, flavonoids, sesquiterpenes and coumarins cause disturbance in cellular organization. The antifungal activity of sesquiterpene is due to its alkylating property (Ivanescu*et al*., 2015). Similarly, phenols and flavonoids cause strong fungal growth inhibition, being lipophilic in nature they diffuse easily through the cell membrane and target the stability by interfering with the synthesis of ergosterol, glucans and mannans (Ahmad *et al*., 2011; Samara *et al*., 2012; Nazzaro *et al*., 2017). This interference causes alterations in cell structure by damaging the membranes, which further results in instability and disintegration, which eventually leads to cell death (Chen *et al*., 2014; Hua *et al*., 2014). Similar to our finding excess vacuolation, cytoplasmic necrosis and formation of unicellular conidia has been reported earlier when *A. alternata* was exposed to a fungicide dicarboximide (Kim *et al*., 2017).

Conclusion

Methanol and dichloromethane extracts of *A. sieberi* leaves are rich source of medicinally important chemical

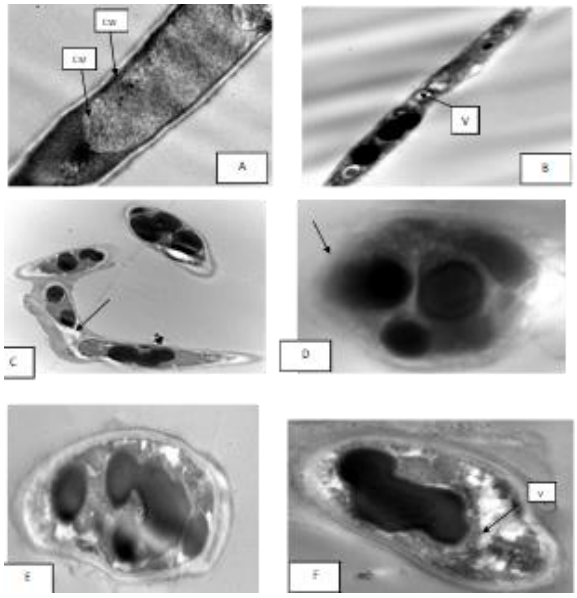

Fig. 7: Transmission electron microscopy micrographs of *F. oxysporum* (A) control without treatment shows an electron-dense cell wall, cell membrane (CW, CM), vacuole (V). Treated hyphae with methanol extract (B-F) show membrane damage, heavy vacuolation, cytoplasmic disintegration and cell wall dissolution leading to distorted shape

compounds. It is clear from our study that these extracts can damage the cell structure by disrupting the cell membrane, which eventually leads to cell death. Hence, they can serve as an excellent substitute for the harmful synthetic fungicides. Further work to understand the mode of action of each compound and their synergistic antifungal activity will help formulate an ecofriendly bio fungicide.

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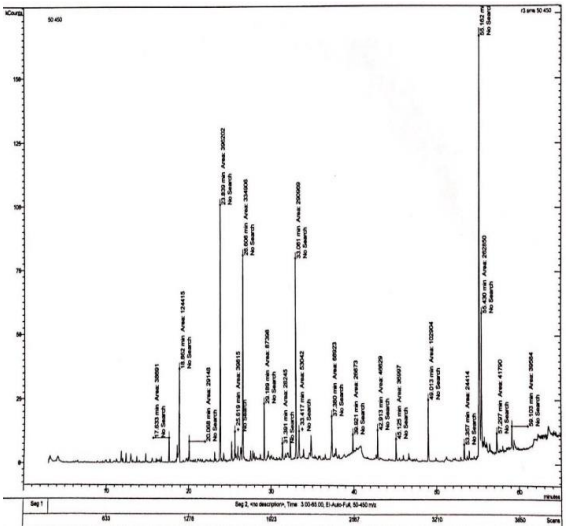

Fig. 8: GC-MS spectrum of methanol extracts of *A. sieberi* leaves

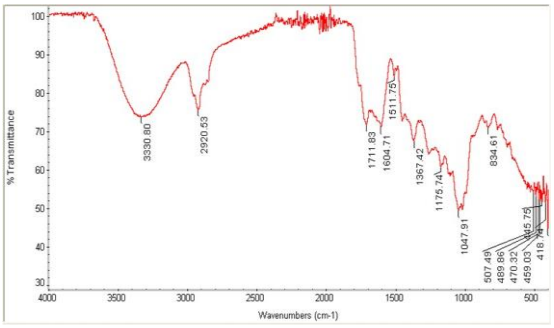

Fig. 9: FTIR spectrum of the methanol extract of *A. sieberi*

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